Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1631–1639

BIOORGANIC & MEDICINAL CHEMISTRY

Development of Novel ^{19}F NMR pH Indicators: Synthesis and Evaluation of a Series of Fluorinated Vitamin B_6 Analogues

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Received 9 January 1998; accepted 7 May 1998

Abstract—We have synthesized a series of novel fluorinated vitamin B_6 analogues (6-fluoropyridoxol derivatives) as potential ^{19}F NMR pH indicators for use in vivo. Modifications included addition of aldehyde, carboxyl or aminomethyl groups at the 4- or 5-ring position, and examination of a trifluoromethyl moiety as an internal chemical shift standard. The variation in chemical shift with respect to acid–base titration showed pK_a values in the range 7.05–9.5 with a chemical shift sensitivity in the range 7.4–12 ppm. Several of the molecules readily cross cell membranes providing estimates of both intra- and extra-cellular pH in whole blood. 6-Fluoropyridoxamine (6-FPAM) exhibits a pK_a = 7.05, which is closer to normal physiological pH than the parent molecule 6-fluoropyridoxol (6-FPOL) (pK_a = 8.2), and should thus, be useful for precise and accurate measurements of pH in vivo. Enhanced spectral resolution for 6-FPAM over 6-FPOL is demonstrated in whole blood and the perfused rat heart.

Introduction

The ^{19}F NMR resonance of 6-fluoropyridoxol (2-fluoro-5-hydroxy-6-methyl-3,4-pyridinedimethanol; 6-FPOL) exhibits exceptional sensitivity to changes in pH with ~10 ppm acid/base shift.[1,2] We have previously demonstrated the use of this molecule to measure simultaneously both intra- and extra-cellular pH in whole blood, and confirmed the measurements using the traditional techniques of ion-electrode polarography and ^{31}P NMR spectroscopy.[1] More recently, we extended the application to measurement of the pH gradient in the perfused rat heart to monitor metabolic and respiratory alkalosis.[3] While a pK_a of 8.2 is appropriate for monitoring pH in the basic range, it is not ideal for measurements under normal physiological conditions. Extensive literature on modifications of vitamin B_6 suggested that the introduction of electron donating or withdrawing groups at the 4- and 5-positions of the pyridoxol ring could significantly alter the pK_a of the 3-phenolic group, and the NMR properties at the 6-position.[4–6] We have synthesized and evaluated a series of derivatives as potential in vivo pH indicators (Schemes 1 and 2). While some of the target molecules have been reported previously,[7] in this work they are evaluated for the first time as ^{19}F NMR pH indicators for in vivo applications.

Results

6-Fluoropyridoxol (6-FPOL) **1**, 6-fluoropyridoxal (6-FPAL) **2**, 6-fluoropyridoxamine (6-FPAM) **4**, **5** and **10** were prepared from pyridoxol hydrochloride according to Korytnyk's methods (Schemes 1 and 2).[7] Given the preferential reactivity of the 4-CH_2OH, modification at the 5-position required protection conveniently provided by a 3, 4 acetonide, as reviewed by others.[8,9] Oxidation of **5** with manganese dioxide gave the aldehyde **10**. The isopropylidene group in **10** was hydrolyzed with hydrochloric acid to give 6-fluoroisopyridoxal **14**. However, the free aldehyde proton NMR peak, which was found for 6-fluoroisopropylidene isopyridoxal **10** at 10.45 ppm in $CDCl_3$ was missing from **14**. Instead, an

Key words: pH; ^{19}F NMR; fluoropyridoxol; vitamin B_6; fluoropyridoxamine.

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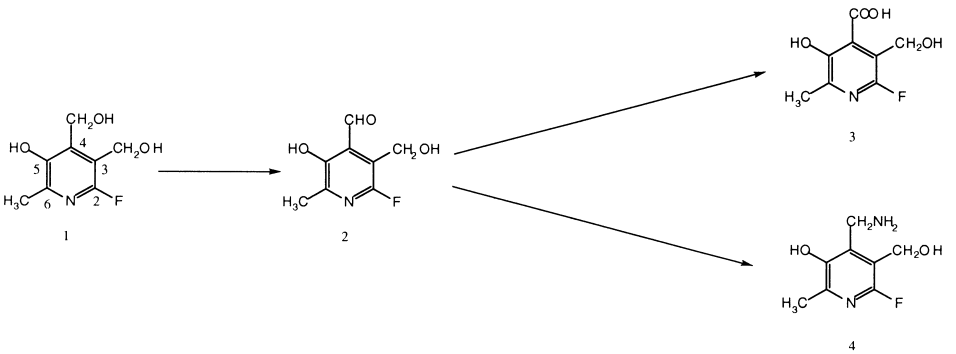
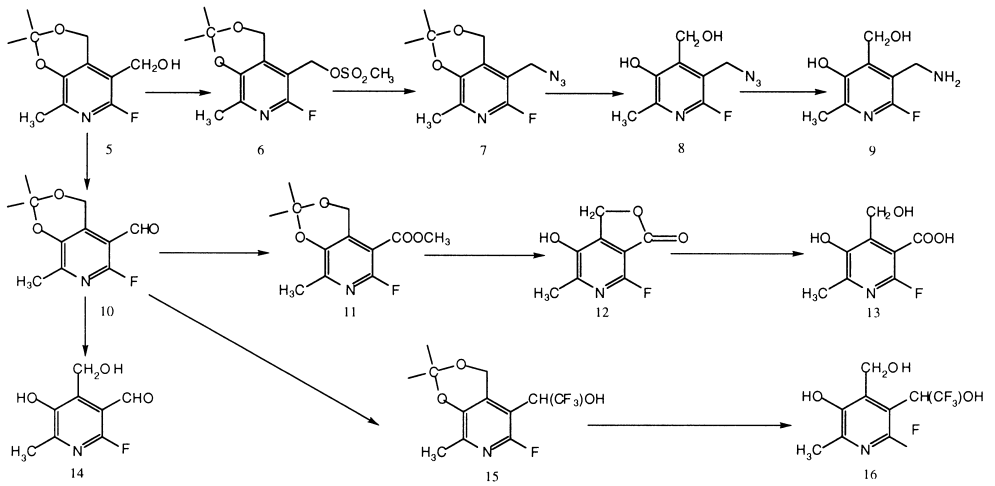
0968-0896/98/$—see front matter
PII: S0968-0896(98)00104-7



Scheme 1. Synthetic approaches to 4-position derivatives.



Scheme 2. Synthetic approaches to 5-position derivatives.

AB quartet ($J = 15$ Hz) appeared, which we attribute to the formation of the 4, 5 hemiacetal. This coincided with previous reports for vitamin B_6 showing that 4, 5 aldehyde/alcohol tends to yield the hemiacetal, hence, producing non-equivalence of the methylene protons in the ring.[4,10] Examination of the infrared spectra of **14** and **2** showed an absence of a carbonyl band, which was, however, evident in 6-fluoroisopropylidene isopyridoxal **10** at 1689 cm^{-1}. This indicated that both pyridoxal **2** and isopyridoxal **14** exist in the hemiacetal form.[7]

By analogy to Korytnyk's method,[11] methylation of **10** gave the methyl ester **11**. We attempted to hydrolyze the protecting isopropylidene group selectively to obtain the 5-methyl ester derivative, but even mild hydrolysis gave the lactone **12**. The 5-pyridoxic acid derivative **13** was prepared from the lactone **12** by saponification.

All the fluorinated vitamin B_6 analogues exhibited a single sharp ^{19}F NMR resonance in aqueous solution for the 6-F atom (e.g. 6-FPAM in Figure 1). The acid and base resonances were in fast exchange providing a single resonance although there was some line broadening around the pK_a (line width 27 Hz at pH 7.0 for 6-FPAM **4**). ^{19}F NMR chemical shifts of the compounds were very sensitive to pH with a range of 7.4 to 12.1 ppm between acid and base (Table 1 and Fig. 2). From the titration curves, the pK_a of each molecule was determined (Table 1). Compound **16** exhibited two ^{19}F NMR signals with an intensity ratio of ~3–1 for peaks at ~ −1 ppm (essentially pH invariant) and −4 to −14 ppm depending on pH (Fig. 3). The titrations of each of the peaks is shown with respect to the external TFA standard and also for 6-F relative to the internal standard (Fig. 4).

6-FPOL **1**, the aldehydes **2**, **14** and amines **4**–**9** immediately exhibited two resonances in whole blood, which we attribute to intra- and extra-cellular compartments, respectively, showing that these molecules readily penetrated blood cells (e.g. 6-FPAM **4**, Fig. 5). For **4** in whole blood δ (^{19}F) = −14.793 and −16.935, corresponding to $pH_i = 7.16$ and $pH_e = 7.59$ (Fig. 5). The

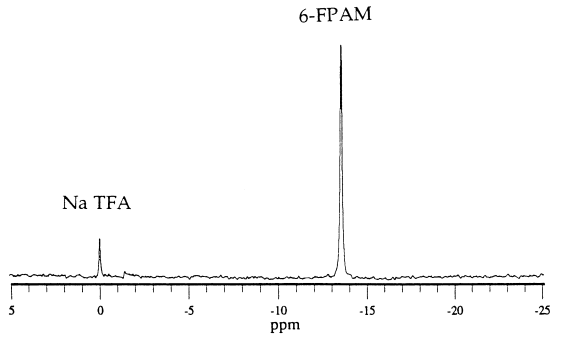


Figure 1. ^{19}F NMR spectrum of 6-FPAM in water at pH 6.9 ($\delta = -13.57$ ppm).

carboxyls **3**, **13** and lactone **12** provided a single signal only, indicating that they do not enter cells and report the extra cellular pH only. **16** showed a single resonance only for each of the CF_3 and 6-F resonances indicating that it did not penetrate red blood cells.

4 was added to a retrograde perfused heart and in common with **1** displayed two signals indicating cellular penetration (Fig. 6). For **4** in the heart δ (^{19}F) = −14.497 and −16.727 ppm, corresponding to $pH_i = 7.11$ and $pH_e = 7.54$. By comparison, pH_i (^{31}P NMR) = 7.15 on the basis of chemical shift of Pi ($\delta = 5.01$ ppm) and $pH_e = 7.75$ (electrode).

Discussion

The pyridine core of vitamin B_6 is readily amenable to many modifications.[7,9,12] As shown by us and others, introduction of a fluorine atom para to the 3 phenolic group provides a titratable ^{19}F NMR signal, which is highly sensitive to changes in pH.[1,6] We have further shown that modification of the 4- and 5-position hydroxymethyl moieties produces modification of the pK_a with relatively minor changes in chemical shift and chemical shift range. The ability to modify the structure is crucial in optimizing the characteristics of a pH indicator. Compound **4** (pK_a 7.05) appears to be the most promising candidate to complement 6-FPOL for in vivo NMR pH measurements.

Table 1.

pH indicator	pH	^{19}F NMR (δ)	pH sensitivity ($\Delta\delta$)	pK_a
1	3.2	−9.84	9.72	8.2
	12.6	−19.56		
2	2.0	−8.65	10.65	8.0
	11.4	−19.30		
3	2.0	−11.72	7.44	9.5
	12.0	19.16		
4	3.3	−9.19	10.00	7.05
	10.2	−19.19		
9	2.8	−7.57	11.98	8.0
	11.5	−19.55		
12	2.3	−7.94	12.14	8.0
	11.9	−19.63		
13	2.2	−7.41	10.55	8.5
	11.8	−17.96		
14	1.80	−10.2	8.78	7.4
	12.00	−19.0		
16	2.37	−4.45	9.64	7.6
	11.80	−14.09		

While **1** performs well in the study of tissue alkalosis, the basic pK_a is not ideal for investigations of normal physiology and acidosis. Indeed, it is widely recognized that the pK_a of an indicator should be close to the pH of interest; otherwise, the sensitivity, potential precision, and accuracy are compromised.[13] Therefore, the carboxylic acids **3** and **13** are less suitable since they make the pK_a more basic. The aldehydes **2** and **14** move the pK_a in the appropriate direction, but difficulties with in vivo reactivity with cellular nucleophiles can be anticipated. The 5-NH_2 (**9**) shows some improvement, but the 4-NH_2 (**4**) appears most useful.

In common with the parent molecule **1**, titration of **4** in plasma showed no change compared with distilled

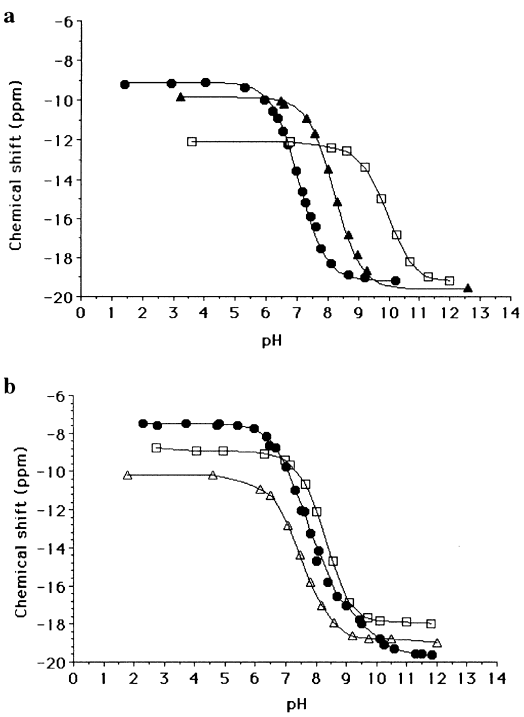


Figure 2. Titration curves of 6-FPOL derivatives. (a) 4-Position ▲ FPOL; ● CH_2NH_2; □ CO_2H. (b) 5-Position △ CHO; ● CH_2NH_2; □ CO_2H.

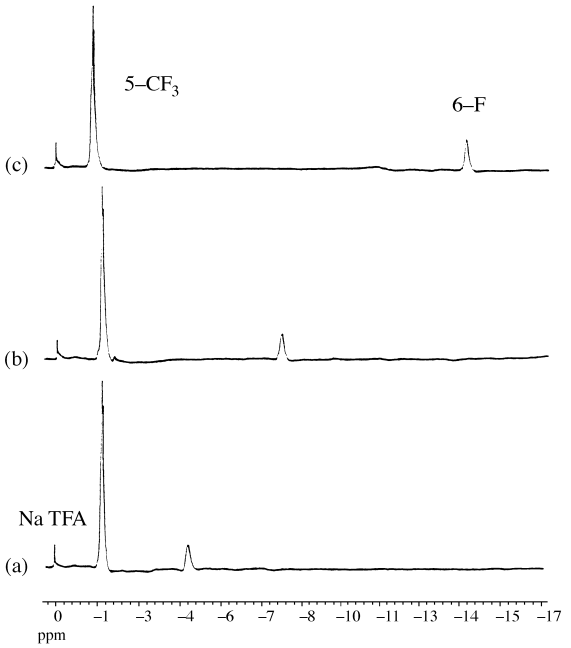


Figure 3. Titration of 5-CF_3-6-FPOL: spectrum at (a) pH = 2.6; (b) pH = 7.5; (c) pH = 11.8.

water. This is important since the cellular environment is not necessarily well defined, particularly in the case of tissue pathologies. This has always been a handicap in the use of endogenous Pi as a ^{31}P NMR pH indicator since the chemical shift is sensitive to ionic strength and the presence of various ions. Errors in pH estimates as high as 0.5 pH units may occur, though some knowledge of the cellular environment should allow determinations with a typical error of ~0.05–0.1 pH units.[14] The sulfonamide ^{19}F NMR pH indicator ZK-150471, developed by Schering, exhibits a chemical shift offset of 0.2 ppm in plasma, complicating interpretation of pH determinations in vivo.[15]

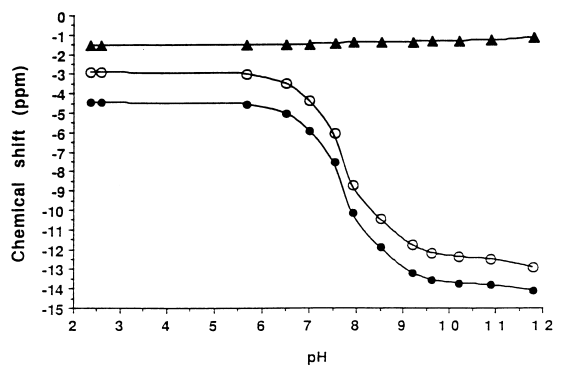


Figure 4. Titration of 5-CF_3-FPOL with respect to intramolecular CF_3: O 6-F; or with respect to external standard (TFA): ● 6-F, ▲ CF_3.

Importantly, **4** rapidly enters red blood cells in whole blood and the perfused heart. Indeed, several of the indicators were observed to enter red blood cells, suggesting either diffusion of the molecules across the cell membrane or implying promiscuity of the transport process. This is important since many exogenous pH indicator molecules experience difficulty entering cells and require precursors to facilitate cellular loading.[16] Other exogenous indicators are restricted to the extracellular compartment only.[15,17] 6-FPAM shows a single resonance in solution implying fast exchange between acid and base forms; however, two signals were observed in the presence of cells, indicating a slow exchange between the compartments. It is reassuring that the pH estimates derived from **4** coincide closely with the independent traditional techniques of ^{31}P NMR and electrode polarography.

The change in ^{19}F chemical shift with pH in the physiological range in these molecules is considered to be mainly due to protonation and deprotonation of the 3-phenolic OH.[2,6] This affects the electronic environment around fluorine at the 6-position, which is particularly pronounced due to para location. It is apparent from the Table that introduction of an aminomethyl group at the 4- or 5-position causes the pK_a to shift towards the acidic range, while the electron withdrawing carboxyl group causes a shift towards base. Comparison of the pK_a for specific substituents at each position shows that changes show the same trends for both positions, though they are substantial for the 4 aminomethyl and carboxyl groups.

An internal chemical shift reference would increase the versatility of the molecules. In the past we attached **2** to polymer carriers, which were also labeled with pH invariant CF_3 groups.[18] While this provided a pH chemical shift reference, the molecules had very high molecular weight and were restricted to the extra cellular compartment. Others have attached nontitrating CF_3[15] or ^{19}F groups directly to small pH indicator molecules. We have now added a CF_3 group at the 5-position of the pyridoxol ring. Figure 4 shows that the 6-F still titrates, while the CF_3 is largely unchanged. However, the molecule no longer penetrates cells. Given the ease with which many pyridoxol analogues enter cells, we envision that minor structural modification should facilitate transmembrane transport. In the future we will evaluate a reference on **4**. We note that the chemical shift range of the lactone **12** is 12 versus 10 ppm for **4**, suggesting that there is room for improvement through further molecular modification. Another approach may be replacement of the 6-F by 6-CF_3 by analogy with the work of Jones et al.[20] The chemical shift range would be expected to decline, but the increased SNR could enhance precision of pH estimates in vivo.

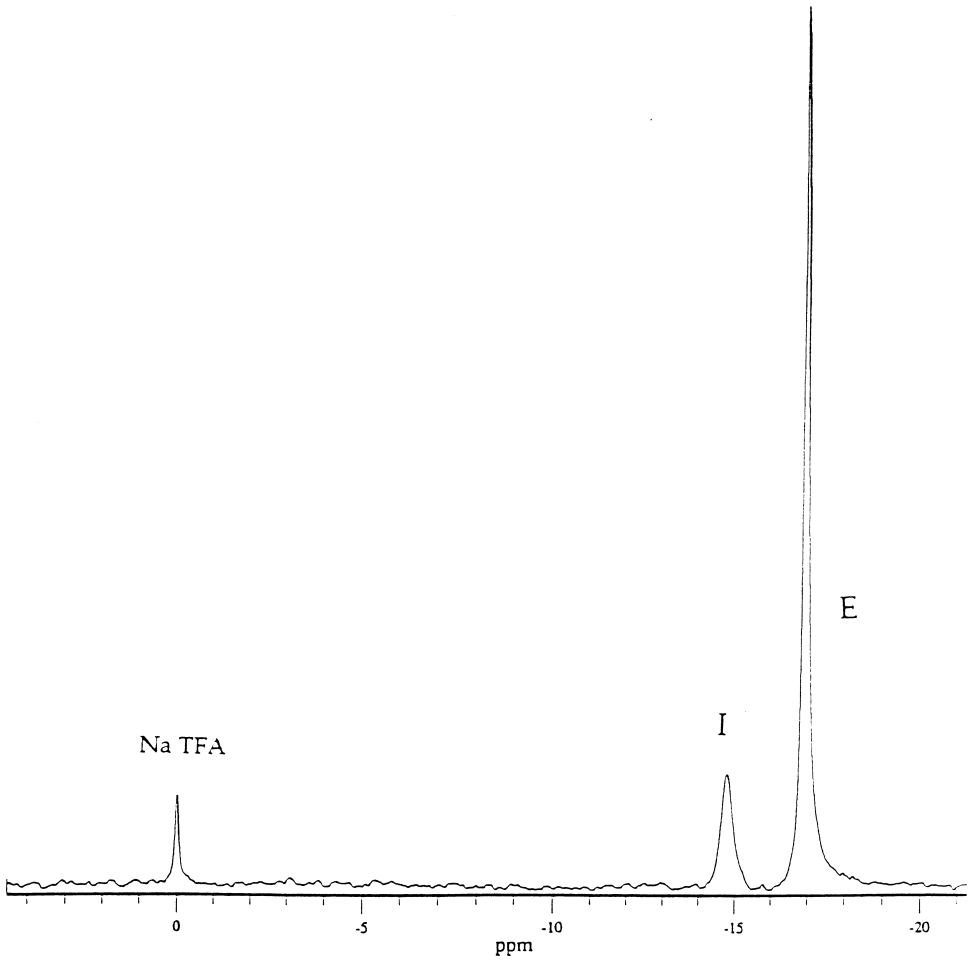


Figure 5. Spectrum of 6-FPAM in whole rabbit blood. Two signals are observed for 6-FPAM attributed to intra ($\delta = -14.793$ ppm) and extra ($\delta = -16.935$ ppm) cellular compartments corresponding to pH 7.16 and 7.59, respectively. A capillary of sodium TFA served as external chemical shift reference.

The relative merit of **4** and **1** in normal retrograde perfused rat hearts under baseline conditions is shown in Figure 6. In each case pH_i ~7.1 and pH_e ~7.6 with a transsarcolemmal pH gradient ~0.5. It is apparent that the chemical shift difference of the two resonances is greater for **4** ($\Delta\delta$ 2.2 ppm versus 1.1 ppm for **1**) providing better spectral resolution. Moreover, the pH_i is close to the pK_a and should provide better precision in the pH estimate. Furthermore, any acidification such as might accompany ischemia will cause a pH shift towards the pK_a of **4** and away from **1**.

Conclusion

^{19}F labeled molecules exploit the high visibility of fluorine, the great sensitivity of ^{19}F to the environmental milieu, and the lack of background signal. However, widespread application of such molecules was, heretofore, hindered by low response to changes in pH,[16] a pK_a far from the physiological range[21] or difficulty loading the indicators into cells.[22] **4** overcomes each of these problems and shows great promise for future investigations in perfused organs and in vivo. Given the extensive evidence that cellular pH and the trans membrane pH gradient are involved in many regulatory processes,[23–26] the application of **4** in vivo promises to provide insight into developmental processes and prognostic information regarding therapeutic outcome.

Experimental

NMR spectra were obtained using either a 7 T vertical bore spectrometer under control of a Tecmag console (^1H 300 MHz; ^{19}F 282 MHz) or a 9.4 T GE Omega system

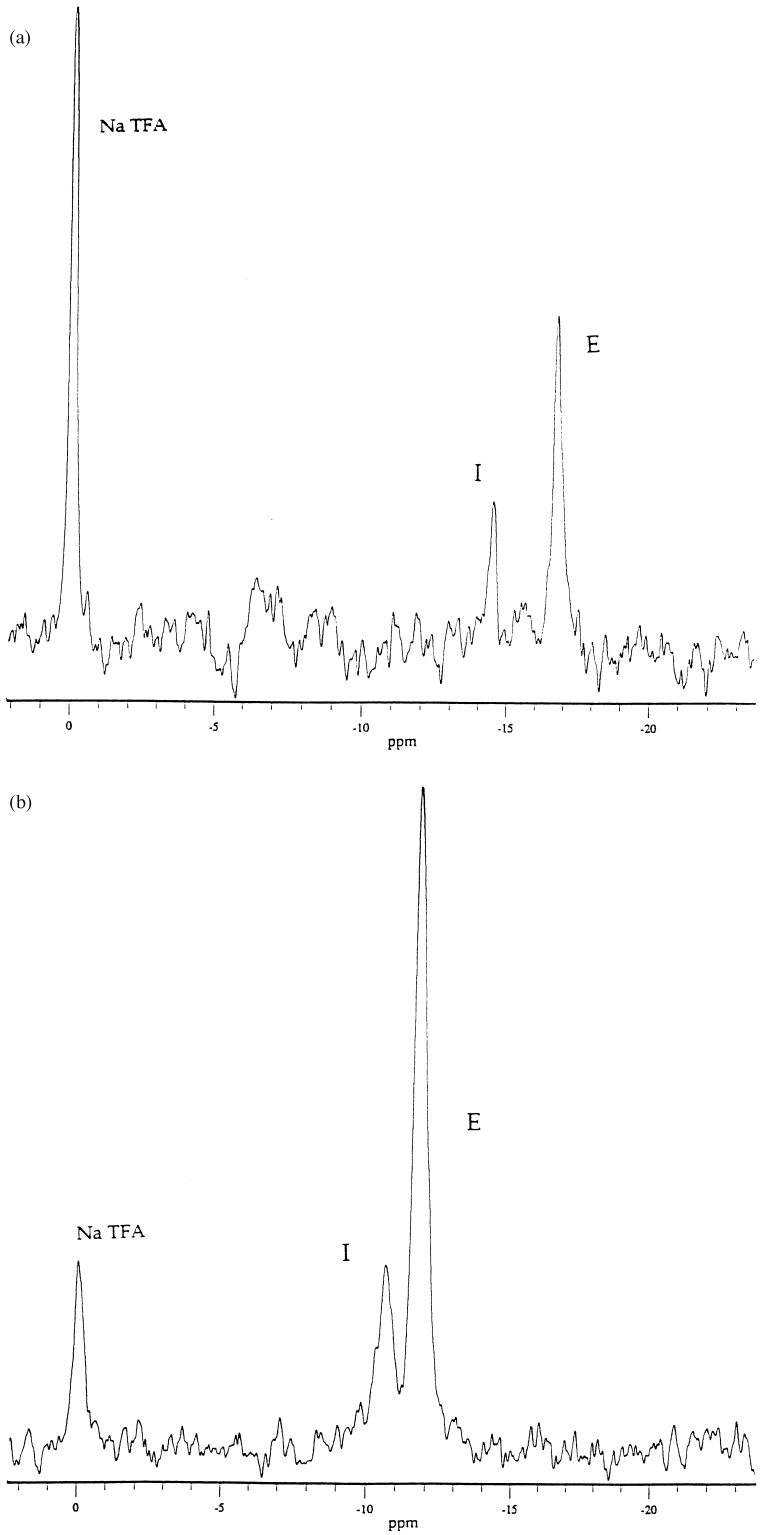


Figure 6. ^{19}F NMR spectra of fluorinated pH indicator in Langendorff perfused rat heart. (a) 6-FPAM **4** indicating $pH_i = 7.11$ ($\delta -14.497$ ppm), $pH_e = 7.54$ (-16.727 ppm), $\Delta pH = 0.43$; $\Delta\delta = 2.23$ ppm; (b) 6-FPOL **1** indicating $pH_i = 7.15$ ($\delta -10.644$ ppm), $pH_e = 7.59$ (-11.762 ppm) $\Delta pH = 0.44$; $\Delta\delta = 1.1$ ppm.

(^{1}H 400 MHz; ^{19}F 376 MHz; ^{31}P 121 MHz). ^{1}H chemical shifts are referenced to TMS, ^{19}F to a dilute solution of sodium trifluoroacetate (NaTFA) in a capillary, and ^{31}P to cellular phosphocreatine. Spectra of solutions and blood were generally obtained at room temperature. Heart perfusion was performed at 37 °C.

A small quantity of respective derivatives was dissolved in distilled water and a capillary of sodium trifluoroacetate added, as external chemical shift reference ($\delta = 0$ ppm). ^{19}F NMR spectra were acquired, and the pH varied to assess the titration curve. Fresh whole blood was drawn from the lateral ear vein of New Zealand white rabbits and stored chilled in the presence of heparin (up to 1 week) prior to NMR observation. Typically, 1 to 12 mg pH indictor were dissolved in ~500 μL water, plasma, or whole rabbit blood for NMR studies. A small quantity of each derivative was added to fresh whole rabbit blood and ^{19}F NMR spectra were obtained to assess cellular penetration. When only a single resonance was observed, the pH was altered to verify that coincidence represented failure to cross the cell membranes and not a lack of signal resolvability.

The pH of each sample was measured in the NMR tube using a combination pH electrode (Wilmad, Buena, NJ) attached to a pH meter (Corning 220, Sudbury, UK). Shimming was performed on the water signal and ^{19}F NMR spectra were typically obtained in 1 min by averaging 32 transients with 4K data points across ±5000 Hz. Data were apodized with a 20 Hz exponential line broadening prior to Fourier transformation. In each case the chemical shift end points and pK_a were determined. ^{31}P NMR spectra were obtained with 512 transients and 4K data points across ±4000 Hz. In this case a 25 Hz line broadening was applied. For titration curves the pH was altered by addition of aliquots of NaOH or HCl. Whole blood samples were also centrifuged and both the supernatant and pellet re-examined to verify intra- and extra-cellular signals.

Heart perfusion

Compounds **1** or **4** (200 mg, respectively) were added to the perfusate reservoir of a rat heart undergoing retrograde perfusion, as described in detail previously.[3] A balloon in the left ventricle was filled with dilute NaTFA as a chemical shift reference and connected to a pressure transducer to allow continual monitoring of heart rate and developed pressure. ^{19}F and ^{31}P NMR spectra were acquired both before and after addition of the pH indicator.

Syntheses

Chemicals were of general reagent grade and purchased from Aldrich, Sigma, or Fluka. Infrared spectra (IR) were recorded in KBr on a Mattson Galaxy 2000 FT-IR spectrometer. Mass spectra were obtained at the Nebraska Center for Mass Spectrometry or the Howard Hughes Medical Institute at UT-Southwestern.

6-Fluoropyridoxol (1). 6-Aminopyridoxol 35 g (0.19 mol) was dissolved in HF/pyridine (100 mL) at 0–5 °C and $NaNO_2$ (16 g) added slowly with stirring (1 h), followed by stirring at 60 °C (1.5 h). 5 N NaOH solution was added at 0 °C until pH 5–6 and the solution extracted with ether. Extract was washed with water, dried (Na_2SO_4), and concentrated to give 17.5 g (50%) 6-fluoropyridoxol; mp 154–156 °C (lit. mp 155–156 °C).

6-Fluoropyridoxal (2). MnO_2 (3.9 g) was added to a solution of 6-fluoropyridoxol (1 g, 5.3 mmol) in water (100 mL), through which N_2 was bubbled. The mixture was stirred (2.5 h), filtered and concentrated. Recrystallization ($CHCl_3$) gave 0.6 g (61%) 6-fluoropyridoxal; mp 131–133 °C (lit. mp 133 °C).

2-Fluoro-5-hydroxy-3-(hydroxymethyl)-6-methyl-4-pyridinecarboxylic acid (3). MnO_2 (15 g, 172 mmol) was added to a solution of **2** (1.3 g, 7 mmol) in alcoholic KOH (1.3 g in 130 mL EtOH). The mixture was stirred at ambient temperature for 24 h. 30% H_2O_2 (5 mL) was added to reoxidize the manganic salt. The mixture was filtered, washed with 0.1 N alcoholic KOH, neutralized to pH 7 with 1 N HCl, and the precipitate filtered. The solution was further acidified to pH 4 and the precipitate filtered again, then concentrated. The crude product was purified by column chromatography (SiO_2, EtOAc/MeOH, 95:5) to give 0.8 g (57%) of **3**. ^{1}H NMR (CD_3OD) δ 2.35 (s, 3H, CH_3), 4.87 (s, 2H, CH_2); Electrospray MS m/z 245.95 $(M + Na_2\text{-}H)^+$; calcd for $C_8H_8FNO_4$ 201.0437.

6-Fluoro-α^4,α^5-*O*-isopropylidenepyridoxol (5). *p*-Toluenesulfonic acid (7.6 g, 80 mmol) was added to 6-fluoropyridoxol (3.75 g, 40 mmol) in Me_2CO (300 mL, dried over $CaSO_4$) and stirred for 24 h. Solvent was substantially removed under reduced pressure, then cold saturated $NaHCO_3$ solution (100 mL) added with stirring. Following extraction with EtOAc and drying (Na_2SO_4), solvent was removed under reduced pressure and product purified by column chromatography (SiO_2, 1% MeOH in DCM) to give a yellow oily product 2.55 g (28%) of **5**.

Compound 6. Methane sulfonyl chloride (0.337 mL, 4.39 mmol) was added to a solution of **5** (500 mg, 2.20 mmol), Et_3N (1 mL), DMAP (catalytic amount) in dry DCM (10 mL) and stirred for 2 h at 0 °C. Water (10 mL) was then added and the mixture extracted with DCM. The combined organic layers were concentrated and purified by column chromatography (SiO_2, EtOAc/

hexane) to give **6**, as a pure solid; ^{1}H NMR ($CDCl_3$) δ 1.56 (s, 6H, $C(CH_3)_2$), 2.36 (s, 3H, $ArCH_3$), 2.61 (s, 3H, SO_2CH_3), 4.82 (s, 2H, CH_2), 4.98 (s, 2H, CH_2).

Compound 7. Sodium azide (100 mg) was added to a solution of **6** (500 mg, 1.6 mmol) in DMF (10 mL). The reaction mixture was stirred at ambient temperature for 12 h. Column chromatography (SiO_2, EtOAc/hexane) gave 300 mg (74%) of **7**; ^{1}H NMR ($CDCl_3$) δ 1.56 (s, 6H, $C(CH_3)_2$), 2.35 (s, 3H, $ArCH_3$), 4.26 (s, 2H, CH_2N_3), 4.87 (s, 2H, CH_2OH).

2-Fluoro-3-(azidomethyl)-5-hydroxy-6-methyl-4-pyridinemethanol (8). A mixture of **7** (300 mg, 1.2 mmol) and 1 N HCl (1 mL) in 1,4-dioxane (5 mL) was heated at 80 °C for 4 h. The mixture was neutralized with 1 M $NaHCO_3$, extracted with EtOAc, concentrated and purified by PTLC to give 200 mg (78%) of **8** as a solid; ^{1}H NMR (acetone-d_6) δ 2.31 (s, 3H, CH_3), 4.26 (s, 2H, CH_2N_3), 4.98 (s, 2H, CH_2OH), 5.45 (br, H, α^4-OH), 8.96 (s, H, ArOH); HREIMS *m*/*z* 212.0704; calcd for $C_8H_9FN_4O_2$, 212.0709.

2-Fluoro-3-(aminomethyl)-5-hydroxy-6-methyl-4-pyridinemethanol (9). To a solution of **8** (200 mg, 0.9 mmol) in EtOH (10 mL) was added 10% Pd/C (10 mg). The reaction mixture was stirred at ambient temperature under H_2 (25 lb) for 12 h. The catalyst was filtered and EtOH evaporated to give **9**: mp 158-160 °C; ^{1}H NMR (CD_3OD) δ 2.34 (s, 3H, CH_3), 3.87 (s, 2H, CH_2NH_2), 4.88 (s, 2H, CH_2OH); HREIMS *m*/*z* 186.0801; calcd for $C_8H_{11}FN_2O_2$, 186.0804.

6-Fluoro-α^4,3-*O*-isopropylideneisopyridoxic acid methyl ester (11). A solution of 6-fluoro-α^4,3-*O*-isopropylideneisopyridoxal **10** (100 mg, 0.44 mmol) in MeOH (10 mL) was added to a mixture of activated MnO_2 (505 mg), NaCN (73 mg) and glacial acetic acid (26 mg) in MeOH (20 mL). The reaction mixture was stirred at ambient temperature for 12 h and filtered under reduced pressure. The filtrate was concentrated, the residue dissolved in water and extracted by ether. The combined organic layers were washed with saturated $NaHCO_3$, dried (Na_2SO_4) and concentrated. Column chromatography (SiO_2, EtOAc/hexane) gave 80 mg (71%) of **11** as a solid: mp 70–75 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 6H, $C(CH_3)_2$), 2.40 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 5.08 (s, 2 H, CH_2).

2-Fluoro-5-hydroxy-4-(hydroxymethyl)-6-methyl-3-pyridinecarboxylic acid lactone (12). A mixture of **11** (80 mg, 0.31 mmol) and 1 N HCl (0.5 mL) in 1,4-dioxane (1 mL) was heated at 80 °C for 4 h. The mixture was then neutralized with 0.5 M $NaHCO_3$, extracted with EtOAc, concentrated and purified by PTLC to give 57 mg (85%) of **12**: mp 147–149 °C; ^{1}H NMR (acetone-d_6) δ 2.34 (s, 3H, CH_3), 5.29 (s, 2H, CH_2); HREIMS *m*/*z* 183.0332, calcd for $C_8H_6FNO_3$ 183.0331.

2-Fluoro-5-hydroxy-4-(hydroxymethyl)-6-methyl-3-pyridinecarboxylic acid (13). A mixture of **12** (50 mg, 0.23 mmol) and 0.1 N NaOH (2 mL) was stirred at ambient temperature for 30 min. The mixture was then neutralized with 0.1 N HCl and concentrated. Residue was dissolved in DCM/methanol (8:2) to give **13** as a pure solid: ^{1}H NMR (CD_3OD) δ 2.34 (s, 3H, CH_3), 4.86 (s, 2H, CH_2); FABMS *m*/*z* 201 (M^+), 224 $(M+Na)^+$; calcd $C_8H_8FNO_4$ 201.0437.

2-Fluoro-5-hydroxy-4-(hydroxymethyl)-6-methyl-3-pyridinecarboxaldehyde (14). A mixture of **10** (100 mg, 0.44 mmol) and 1 N HCl (0.5 mL) in 1,4-dioxane (1 mL) was heated at 80 °C for 4 h. The mixture was neutralized with 0.5 M $NaHCO_3$, extracted with EtOAc, concentrated and purified by PTLC to give 69 mg (85%) of **14**: mp 117–119 °C; ^{1}H NMR (CD_3CN) δ 2.28 (s, 3H, CH_3), 4.99 (d, J = 15 Hz, 1H, CH_2), 5.16 (d, J = 15 Hz, 1H, CH_2), 6.44 (s, 1H, CH); HREIMS *m*/*z* 185.0487, calcd for $C_8H_8FNO_3$ 185.0488.

6-Fluoro-α^4,3-*O*-isopropylidene-α^5-trifluoromethyl-pyridoxol (15). To a mixture of **10** (50 mg, 0.22 mmol) and TMS-CF_3 (trimethylsilyl trifluoromethane, 46 mg, 0.324 mmol) in THF (5 mL) at 0 °C was added TBAF (tetrabutylammonium fluoride, catalytic amount). Yellow color instantaneously developed with the initial evolution of fluorotrimethylsilane. The mixture was allowed to come to ambient temperature and stirred for 1 h, then 0.5 N HCl (2 mL) added and subsequently stirred for 1 h to hydrolyze the trifluoromethylated siloxy compound. The reaction mixture was neutralized with 0.5 N Na_2CO_3, concentrated, dissolved in ethyl ether, and washed with water. Concentration and purification by column chromatography (SiO_2, EtOAc/hexane) gave 52 mg (80%) of **15**; ^{1}H NMR ($CDCl_3$) δ 1.56 (s, 6H, $C(CH_3)_2$), 2.37 (s, 3H, CH_3), 3.10 (dd, J = 7 Hz, 1H, OH), 4.97 (d, J = 14 Hz, 1H, 4-CH_2), 5.17 (d, J = 15 Hz, 1H, 4-CH_2), 5.33 (quintet, J = 7 Hz, 1H, CH); HREIMS *m*/*z* 295.0823, calcd for $C_{12}H_{13}F_4NO_3$ 295.0831.

2-Fluoro-5-hydroxy-6-methyl-α^5-trifluoromethyl-3,4-pyridinedimethanol (16). A mixture of **15** (50 mg, 0.17 mmol) and 1 N HCl (0.5 mL) in 1,4-dioxane (1 mL) was heated at 80 °C for 4 h. The mixture was neutralized with 0.5 M $NaHCO_3$, extracted with EtOAc and concentrated. PTLC gave 29 mg (67%) of **16** as a solid: ^{1}H NMR (CD_3CN) δ 2.33 (s, 3H, CH_3), 4.34 (br, 1H, OH), 4.90 (d, J = 7 Hz, 1H, OH), 5.04 (d, J = 14 Hz, 1H, CH_2), 5.14 (d, J = 14 Hz, 1H, CH_2), 5.44 (quintet, J = 7 Hz, 1H, CH), 8.87 (s, 1 H, ArOH); HREIMS *m*/*z* 255.0511, calcd for $C_9H_9F_4NO_3$ 255.0518.

Acknowledgements

The authors are grateful to Dr Evelyn Babcock and Dr Anca Constantinescu for valuable discussions. This work was supported in part by the Texas Higher Education Coordinating Board (ATP 003660-032) (V.D.M.), and The Whitaker Foundation (R.P.M.). NMR experiments were performed at the Mary Nell and Ralph B. Rogers NMR Center, an NIH Biotechnology Research Center #5-P41-RR02584.

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