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Nucleophilic ^{18}F-fluorination of phosphorofluoridates and phosphonofluoridic acids *via* imidazole-activated precursors

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ABSTRACT

^{18}F-Labeled organofluorophosphates are important radiosynthons that have only been previously accessible *via* $^{18/19}$F-isotope-exchange with limited molar activities. Herein, a novel ^{18}F-fluorination methodology has been developed to prepare ^{18}F-labeled phosphorofluoridates and phosphonofluoridic acids *via* the [^{18}F]F$^-$ nucleophilic substitution of imidazole-activated precursors. The efficient one-step ^{18}F-fluorination affords stable products in the presence of Zn(II) with high radiochemical yields and high molar activities. This ^{18}F-fluorination method could be used to prepare various phosphorofluoridate and phosphonofluoridic acid analogs for use as ^{18}F-radiosynthons and potential positron emission tomography tracers.

Introduction

Phosphate and phosphonic acid groups are privileged scaffolds that are widespread in biologically and medicinally active compounds [1]. Additionally, radiolabeled phosphates and phosphonic acids are irreplaceable as radiosynthons and radiotracers for the investigation of phosphate-related physiological processes and diseases [2]. In prior studies, only ^{32}P-labeled phosphates were available to monitor their physiological pathways *in vitro* [3], which could not provide real-time dynamic images *in vivo* due to the nuclide property of ^{32}P (100% β^- decay). Instead, ^{18}F (97% β^+ decay, 635 keV, $t_{1/2} \approx 109.8$ min), the most widely used positron emitting isotope, allows wide bioisosteric replacement and *in vivo* imaging with positron emission tomography (PET) [4]. However, ^{18}F-labeled organofluorophosphates were only previously accessible *via* $^{18/19}$F-isotope-exchange with limited molar activities (A_m, the measured radioactivity per mole of compound, measured in Bq/mol or GBq/μmol) [5,6].

Due to analogous van der Waals radius and valence electron numbers between fluoro and hydroxyl groups, fluorination of the phosphate or phosphonic acid moiety can produce a chemically stable phosphorofluoridate or phosphonofluoridic acid derivative which is isostructural and isoelectronic to the unmodified substrate [7]. Additionally, nucleophilic fluorination on non-phosphorus moieties may involve individual synthesis of the precursors, multistep radiosynthesis [8], and potential change of bioactivity of the substrate [9]. Therefore, the ideal fluorination sites are the terminal positions of the phosphate and phosphonic acid groups. Imidazole-activated phosphates prepared *via* a one-step coupling reaction from phosphates [10] can be fluorinated using aqueous fluoride [11]. This process can be accelerated by the presence of Lewis acids [12], making these promising candidates for precursors. It was hypothesized that the same fluorination and coupling reaction may also be applicable to phosphonic acids.

Herein, a method for the ^{18}F-fluorination of phosphorofluoridates and phosphonofluoridic acids *via* imidazole-activated precursors is described. Zn^{2+} species were determined as the best Lewis acid catalysts among the tested metal cations (e.g. Mg^{2+} and Mn^{2+}) to facilitate the elimination of imidazole *via* activation of the P-N bond [13]. In addition, the *in vitro* and *in vivo* stabilities of the ^{18}F-labeled phosphorofluoridates and phosphonofluoridic acids were evaluated for potential applications as radiosynthons and PET tracers.

Results and discussion

Five imidazole-activated precursors, sodium (1*H*-imidazol-1-yl) phosphonates (**1a**–**4a**) and sodium benzyl (1*H*-imidazol-1-yl)phosphinate (**5a**), were synthesized in 35–82% yield from the corresponding phosphates or phosphonic acid *via* the one-step

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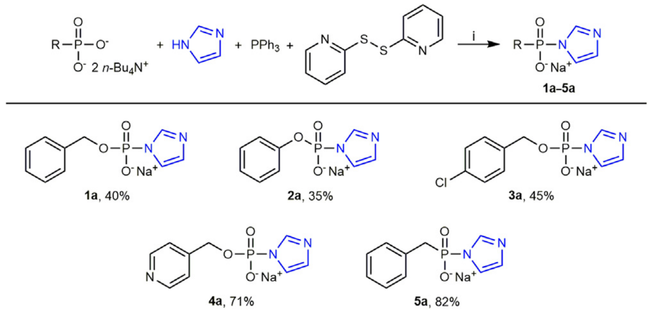
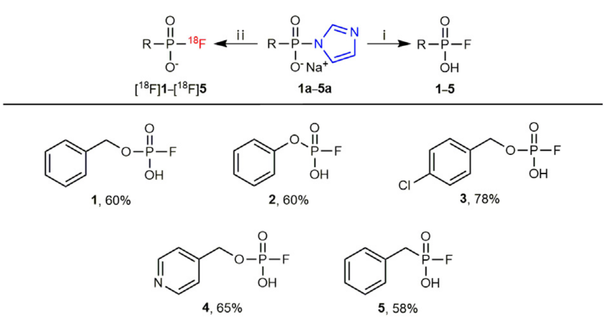
coupling with imidazole in the presence of triphenylphosphine and 2,2′-dithiodipyridine [10] (Scheme 1). Phosphorofluoridates (**1**–**4**) and benzyl phosphonofluoridic acid (**5**) were synthesized as reference compounds in 58–78% yield from **1a**-**5a** by $ZnCl_2$-mediated nucleophilic fluorination [12] (Scheme 2).



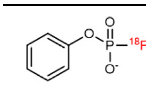
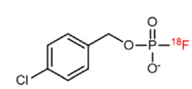
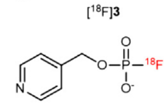
Scheme 1. General synthetic route for imidazole-activated precursors **1a**–**5a**. Reagents and conditions: (i) (a) phosphate or phosphonic acid (1.0 equiv.), imidazole (8.0 equiv.), PPh_3 (4.0 equiv.), 2,2′-dithiodipyridine (4.0 equiv.), DMF, RT, 24–30 h; (b) NaI (8.0 equiv.), acetone, 0 °C, 2 h.



Scheme 2. General synthetic route for phosphorofluoridates and phosphonofluoridic acids **1**–**5** and the radiosynthetic route for [^{18}F]**1**–[^{18}F]**5**. Reagents and conditions: (i) (a) **1a**–**5a** (1.0 equiv.), $ZnCl_2$ (8.0 equiv.), TBAF (3.0 equiv.), THF, 60 °C, 12 h; (b) 732 strong acid cation exchange resin, H_2O, RT, 0.5 h; (ii) **1a**–**5a**, [^{18}F]KF/K_{222}, Zn(II) salts ($Zn(OAc)_2$, $ZnCl_2$, or $Zn(NO_3)_2$), DMSO.

A solution of **1a** and various Zn(II) salts ($Zn(OAc)_2$, $ZnCl_2$, or $Zn(NO_3)_2$) dissolved in anhydrous DMSO were shaken with an azeotropically dried [^{18}F]KF/K_{222} complex, which was prepared by mixing [^{18}F]F$^-$, K_{222} ([2.2.2]-cryptand), and K_2CO_3, in order to determine the optimized reaction conditions. A time-based study (Fig. 1b) showed that the ^{18}F-fluorination reaction was almost complete after 20 min and afforded 67.5 ± 1.2% radiochemical yield (RCY). The RCY increased with an increase in temperature, and the effect of increased temperature on RCY became negligible beyond 70 °C (Fig. 1c). In addition, the optimum precursor amount was 0.2 mg (Fig. 1d), and the optimal amount of the Zn(II) salt was ≥ 5.0 equiv. with respect to the precursor (Fig. 1e). Although 86.9 ± 0.9% and 82.0 ± 0.9% RCYs were respectively obtained in the presence of $Zn(NO_3)_2$ and $ZnCl_2$ for [^{18}F]**1**, it is safer to use $ZnCl_2$ as a catalyst because $Zn(NO_3)_2$ is a hazardous chemical which can easily explode [14]. The A_m of [^{18}F]**1** was determined as 7.5 GBq/μmol and the characterization of [^{18}F]**1** was performed

Table 1
Substrate scope for the synthesis of ^{18}F-labeled phosphorofluoridates.[a]

Compound	Additives (mg)	RCY (%)[b]
 [^{18}F]**2**	K_{222} (0.8 mg) K_2CO_3 (0.1 mg)	57.1 ± 2.5
	K_{222} (4.0 mg) K_2CO_3 (0.5 mg)	1.9 ± 0.4
 [^{18}F]**3**	K_{222} (0.8 mg) K_2CO_3 (0.1 mg)	69.1 ± 11.4
	K_{222} (4.0 mg) K_2CO_3 (0.5 mg)	10.2 ± 2.3
 [^{18}F]**4**	K_{222} (4.0 mg) K_2CO_3 (0.5 mg)	90.7 ± 4.7

[a] Reagents and conditions: **2a**–**4a** (0.2 mg), $ZnCl_2$ (5.0 equiv.), DMSO, 70 °C, 20 min.
[b] RCYs were determined by radio-TLC (n = 2–3).

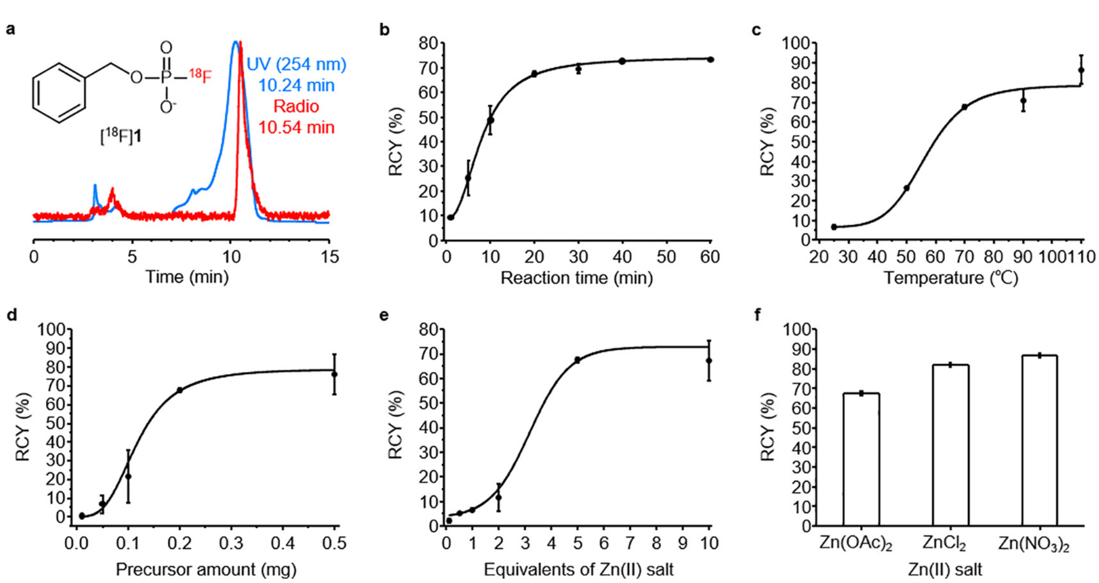


Fig. 1. Optimization of the ^{18}F-fluorination conditions for [^{18}F]**1**. (a) Radio-HPLC characterization of [^{18}F]**1** *via* co-injection of [^{18}F]**1** and **1**; (b) effect of the reaction time: **1a** (0.2 mg, 0.77 μmol), $Zn(OAc)_2$ (5.0 equiv.), 70 °C; (c) effect of the temperature: **1a** (0.2 mg, 0.77 μmol), $Zn(OAc)_2$ (5.0 equiv.), 20 min; (d) effect of the precursor amount: $Zn(OAc)_2$ (5.0 equiv.), 70 °C, 20 min; (e) effect of the equivalents of Zn(II): **1a** (0.2 mg, 0.77 μmol), $Zn(OAc)_2$, 70 °C, 20 min; (f) effect of the utilized Zn(II) salt: **1a** (0.2 mg, 0.77 μmol), Zn(II) (5.0 equiv.), 70 °C, 20 min. HPLC conditions: column: Nacalai Tesque Cosmosil 5C18-MS-II column (4.4 μm, 4.6 mm × 250 mm); elution: isocratic, 70% phosphate buffered saline (0.01 mol/L, pH = 7.40) and 30% CH_3OH; flow rate: 1.0 mL/min. RCYs were determined by radio-TLC (n = 2–3).

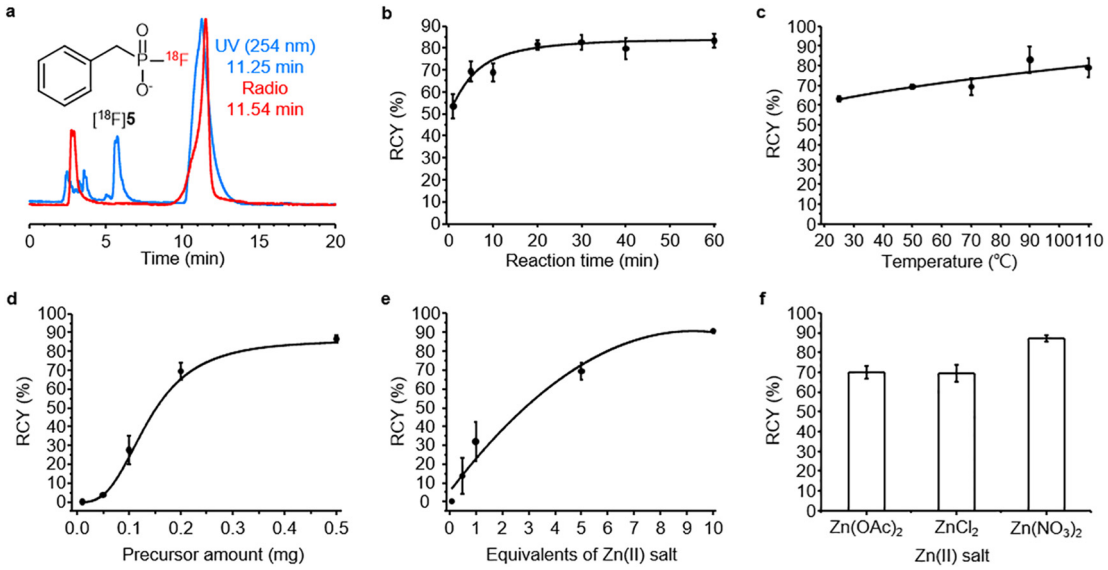


Fig. 2. Optimization for the ^{18}F-fluorination conditions for [^{18}F]**5**. (a) Radio-HPLC characterization of [^{18}F]**5** *via* co-injection of [^{18}F]**5** and **5**; (b) effect of the reaction time: **5a** (0.2 mg, 0.82 μmol), $ZnCl_2$ (5.0 equiv.), 70 °C; (c) effect of the temperature: **5a** (0.2 mg, 0.82 μmol), $ZnCl_2$ (5.0 equiv.), 5 min; (d) effect of the precursor amount: $ZnCl_2$ (5.0 equiv.), 70 °C, 5 min; (e) effect of the equivalents of Zn(II): **5a** (0.2 mg, 0.82 μmol), $ZnCl_2$, 70 °C, 5 min; (f) effect of the utilized Zn(II) salts: **5a** (0.2 mg, 0.82 μmol), Zn(II) (5.0 equiv.), 70 °C, 5 min. HPLC conditions: column: Nacalai Tesque Cosmosil 5C18-MS-II column (4.4 μm, 4.6 mm × 250 mm); elution: isocratic, 80% phosphate buffered saline (0.01 mol/L, pH = 7.40) and 20% CH_3OH; flow rate: 1.0 mL/min. RCYs were determined by radio-TLC (n = 2–3).

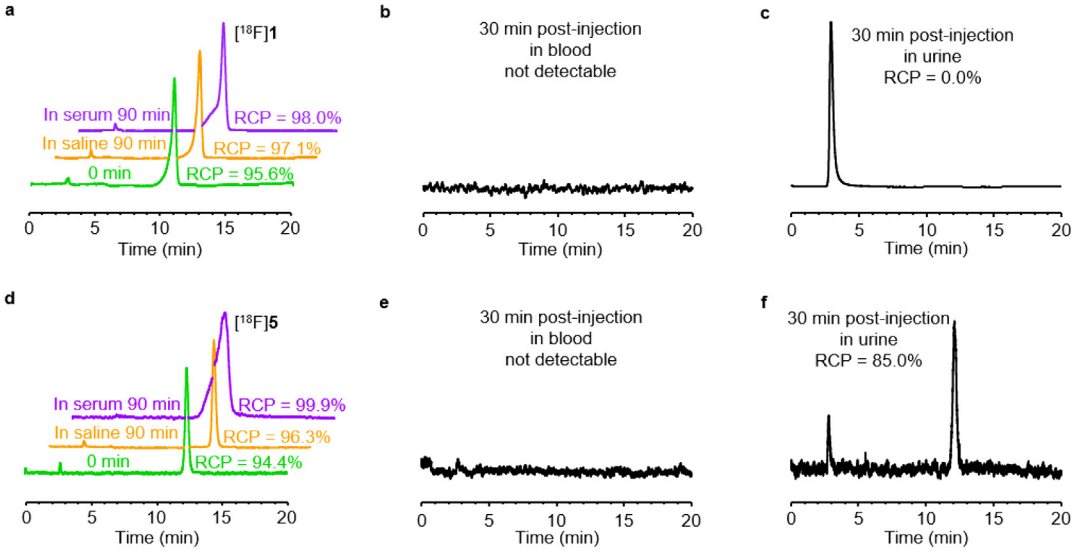


Fig. 3. Stabilities of [^{18}F]**1** and [^{18}F]**5** *in vitro* and *in vivo*. (a) Radio-HPLC analysis of [^{18}F]**1** after 90 min incubation in saline and mouse serum; (b) radio-HPLC analysis of [^{18}F]**1** in mouse blood at 30 min post-injection; (c) radio-HPLC analysis of [^{18}F]**1** in mouse urine at 30 min post-injection; (d) radio-HPLC analysis of [^{18}F]**5** after 90 min incubation in saline and mouse serum; (e) radio-HPLC analysis of [^{18}F]**5** in mouse blood at 30 min post-injection; (f) radio-HPLC analysis of [^{18}F]**5** in mouse urine at 30 min post-injection. HPLC conditions for the analysis of [^{18}F]**1** and [^{18}F]**5** are consistent with those used in Fig. 1 and Fig. 2, respectively.

using radio-high-performance liquid chromatography (radio-HPLC) (Fig. 1a).

As shown in Table 1, precursor **2a** was ^{18}F-fluorinated in 57.1 ± 2.5% RCY. Compound **3a** with chlorine substitution at the *para*-position of the benzene ring afforded 69.1 ± 11.4% RCY. Heterocyclic substituted precursor **4a** was also suitable for this transformation and afforded 90.7 ± 4.7% RCY. However, the RCYs for [^{18}F]**1**–[^{18}F]**3** decreased significantly when the amounts of K_{222} and K_2CO_3 were increased five fold (Table 1 and Table S1) due to a change in the pH caused by K_2CO_3. Excess $ZnCl_2$ is used to ensure weakly acidic pH conditions, which are important for this nucleophilic fluorination. The necessary addition of 0.1 mg K_2CO_3 for [^{18}F]F^- activation affords a favorable pH (6.0–6.5). Nevertheless, excess K_2CO_3 can result in a weakly alkaline pH (8.0–8.5), leading to low RCYs.

The ^{18}F-fluorination of sodium benzyl (1*H*-imidazol-1-yl)phosphinate (**5a**) was also explored. A RCY of 53.5 ± 5.5% was observed after only one minute at 70 °C in the presence of $ZnCl_2$ (5.0 equiv.), and the ^{18}F-fluorination reaction was almost complete after 20 min (Fig. 2b). A RCY of 63.1 ± 1.2% was obtained after 5 min at room temperature (RT), which increased by only 15.8% when the temperature was increased to 110 °C (Fig. 2c). Additionally, a RCY higher than 69.4 ± 4.4% was observed for [^{18}F]**5** when ≥ 0.2 mg of **5a** and ≥ 5.0 equiv. (with respect to **5a**) of $Zn(Cl)_2$ were used. (Fig. 2e). [^{18}F]**5** was synthesized from **5a** (0.2 mg) in the presence of $Zn(NO_3)_2$ (5.0 equiv.) at 70 °C for 5 min with 87.0 ± 1.6% RCY

(Fig. 2f) and 2.2 GBq/μmol A_m. The characterization of [^{18}F]**5** was performed using radio-HPLC (Fig. 2a).

The stabilities of ^{18}F-labeled phosphorofluoridates and phosphonofluoridic acids were also evaluated *in vitro* and *in vivo*. Model compounds [^{18}F]**1** and [^{18}F]**5** (radiochemical purity (RCP) ≥ 95.0% after purification) were incubated with saline and mouse serum at 37 °C for 90 min, and the stabilities were analyzed using radio-HPLC. All the stability studies were repeated 3 times, and the utilized mice were ICR female mice. No defluorination was observed after 90 min in saline or serum (Fig. 3a and Fig. 3d), indicating the strong *in vitro* stabilities of [^{18}F]**1** and [^{18}F]**5**. The *in vivo* metabolic stabilities of [^{18}F]**1** and [^{18}F]**5** were measured by analyzing the radio-metabolites in mice blood and urine at 30 min post-injection. For [^{18}F]**1**, the radioactivity in the blood could be barely detected and no parent compound could be detected in mice urine (Fig. 3b and Fig. 3c). This indicated that [^{18}F]**1** was cleared from the blood after 30 min, presumably due to its good water-solubility, and the radiolabeled product was hydrolyzed during circulation. After 30 min circulation of [^{18}F]**5**, the radioactivity in the blood could barely be detected. However, a maximum 85.0% and an average 65.6 ± 16.2% (n = 3) of the radioactive compound detected in the urine was [^{18}F]**5** (Fig. 3e and Fig. 3f), confirming less defluorination than that of [^{18}F]**1**. It is hypothesized that the replacement of the O—P bond by the C—P bond in [^{18}F]**5** can reduce the recognition of phosphonofluoridic acid by phosphatases and therefore strengthen the *in vivo* metabolic stabilities [15].

Conclusion

In summary, an efficient method for the ^{18}F-fluorination of phosphorofluoridates and phosphonofluoridic acids has been developed *via* imidazole-activated phosphate and phosphonic acid precursors in the presence of Zn(II). This method affords >50% RCYs for the phosphorofluoridate analogs and >80% RCYs for thebenzyl phosphonofluoridic acid as well as high A_m. The ^{18}F-labeled benzyl phosphorofluoridate and benzyl phosphonofluoridic acid show sufficient *in vitro* stabilities, and the latter exhibits better *in vivo* metabolic stability. This nucleophilic ^{18}F-fluorination method could be applied to prepare various phosphorofluoridate and phosphonofluoridic acid analogs as ^{18}F-radiosynthons and potential PET tracers.

Ethical statement

All experimental animal procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Utilization Committee of Xiamen University.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.152917.

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