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Optimization of labeling dipicolylamine derivative, *N,N'*-(5-(4-aminobutoxy)-1,3-phenylene)*bis*(methylene)*bis*(1-(pyridin-2-yl)-*N*-(pyridin-2-ylmethyl)methanamine), with three ^{18}F-prosthetic groups as potential imaging agents for metastatic infectious disease

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The aim of this study was to develop ^{18}F-labeled dipicolylamine derivative (compound 1) with three ^{18}F-prosthetic groups, ^{18}F-NFP, ^{18}F-SFB, and ^{18}F-FET, which were synthesized with labeling yields of 72 ± 10%, 75 ± 8%, and 90 ± 8%, respectively. Compound 1 was then conjugated with ^{18}F-NFP, ^{18}F-SFB, and ^{18}F-FET, respectively. Factors such as the amount of 1, reaction temperature, and time were examined to optimize the yields. The optimal labeling conditions were found to be 1 (0.1 mg, 0.17 μmol), room temperature, and 10 min for both ^{18}F-NFP and ^{18}F-SFB; 1 (4 mg, 6.8 μmol), 100 °C, and 10 min for ^{18}F-FET. The total synthesis time, the overall yields, and the average specific activity were 105, 75, and 65 min; 68 ± 9%, 71 ± 11%, and 76 ±13%; 625 Ci/mmol, 853 Ci/mmol, and 3.5 Ci/mmol for ^{18}F-FP-1,^{18}F-FB-1, and ^{18}F-FE-1, respectively (n = 5, decay-corrected based on ^{18}F).

Keywords: ^{18}F-prosthetic groups; dipicolylamine derivative; metastatic infectious disease; PET

Introduction

Non-invasive imaging has significantly augmented the investigation of various metastatic disease processes caused by infection, inflammation or cancer.[1–3] Since the advent of gallium-67 (^{67}Ga) citrate for routine infection imaging, many imaging agents have been developed and evaluated to better localize and detect areas of metastatic infection within the body.[4,5] However, almost all of the commonly used imaging agents such as ^{18}F-FDG localize to areas of inflammation rather than specifically those of infection, making clinical interpretation difficult and unreliable, particularly when the infection requires aggressive therapeutic intervention.[6] Therefore, it is critical to develop a more reliable and more specific imaging agent for metastatic infection.

Biomarkers will be of significant interest if they can be used to map the molecular features of the infected cells. Phosphatidylserine (PS) is the major anionic phospholipid in the plasma membrane, which is sequestered almost completely in the membrane inner leaflet. PS is usually externalized to the outer leaflet of the membrane during cell death, apoptosis, and neutrophil necrosis.[7–9] The appearance of PS on the cell surface is a common indicator for most types of cell death.[10] The resolution of infection, in part, is associated with neutrophil apoptosis, leading to necrosis; thus PS can be used as an excellent target for localizing infectious foci.[11,12] Annexin V, an endogenous human protein with a high affinity to PS, can be used *in vitro* to detect apoptosis. However, radiolabeled annexin V and its derivatives currently have limited *in vivo* applications due to low target-to-background ratios and high liver and kidney accumulations.[13]

Bis(zinc(II)–dipicolylamine) (Zn^{2+}–DPA) coordination complexes are synthetic small molecules that can mimic the apoptosis sensing function of annexin-V. PSVue®794, a commercially available fluorescent Zn^{2+}-DPA coordination complex, was shown to selectively stain the same cells as fluorescently labeled annexin-V in cell culture.[14] Near-infrared fluorescent (NIR) Zn^{2+}-DPA derivatives have been recently developed to effectively target cell death.[15,16] Moreover, several fluorescent dye labeled Zn^{2+}-DPA derivative probes demonstrated high selectivity to the anionic surfaces of bacterial infected cells and can be used for *in vivo*

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imaging of gram positive as well as gram negative bacterial infection in mice.[17–19] Although NIR labeled Zn^{2+}-DPA complexes have achieved some early success, further applications are limited by the disadvantages of fluorescence imaging in sensitivity and tissue penetration. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging hold great advantages for clinic translational research. Wyffels *et al*.[20] recently developed ^{99m}Tc labeled Zn^{2+}-DPA coordination complexes with two different chelators and performed the preliminary *in vivo* evaluation for the detection of cell death. Results indicated that selective accumulation in a rat model of ischemia-reperfusion injury was detected for two tracers in the apoptotic area with high target-to-background ratios. Although ^{99m}Tc labeled Zn^{2+}-DPA coordination complexes could successfully detect cell death, the results remains preliminary and the application will be only limited to SPECT imaging. PET isotope, ^{64}Cu, was recently used to coordinate with DPA derivative by simply addition of ^{64}Cu directly. Both *in vitro* and *in vivo* PET studies revealed high U87MG tumor retention at all time points.[21] However, high accumulation and retention in the liver were observed as in the case of many other ^{64}Cu labeled tracers.[22] Studies showed that compound **1** is a small molecule and can be modified with various imaging probe.[17–19] indicating the possibility of labeling **1** with a short-lived PET isotope such as ^{18}F. Compared with other metal isotopes, ^{18}F ($t_{1/2}$ = 109.6 min) is currently the most commonly used PET isotope with favorable nuclidic properties, for example, minor disturbance of original biomolecules. The selection of suitable prosthetic groups plays a critical role in the radiolabeling process because ^{18}F cannot be linked directly to the molecules by nucleophilic substitution.[23] In order to obtain an ideal prosthetic group with the smallest steric hindrance, three different ^{18}F-prosthetic groups, 4-nitrophenyl 2-^{18}F-fluoropropionate (^{18}F-NFP), *N*-succinimidyl 4-^{18}F-fluorobenzoate (^{18}F-SFB), and 2-^{18}F-Fluoroethyl toslyate (^{18}F-FET), were selected to label **1**, respectively. Because Zn^{2+}-DPA coordination complexes can be simply prepared by adding two equivalents of zinc nitrate into one equivalent of **1** for 30 min immediately before performing any biological experiment; we chose to focus on optimizing the radiolabeling yields of ^{18}F–**1** by different reaction factors without the Zn complex step.

Results and discussion

Experimental

General

Chemicals. Reagents and solvents were all commercially available and used as received, without further purification. Water for ion chromatography, acetic acid, trifluoroacetic acid (TFA), anhydrous acetonitrile (CH_3CN), anhydrous dimethyl sulfoxide (DMSO), *N,N*-Diisopropylethylamine (DIPEA), tetrabutylammonium hydroxide (40 wt%) in water (TBAH), potassium carbonate, *O*-(*N*-Succinimidyl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TSTU), 2-fluoropropionic acid, 4-fluorobenzoic acid, ethyl 2-bromopropionate, *bis*(4-nitrophenyl)carbonate, ethyl 4-dimethylamino benzoate, methyl triflate, ethylene di(*p*-toluenesulfonate), kryptofix-2.2.2 (K222) and 1-octanol were purchased from Sigma Aldrich. 2-fluoroethyl tosylate was purchased from ABX (Radeberg, Germany). *N,N′*-(5-(4-aminobutoxy)-1,3-phenylene)*bis*(methylene) *bis*(1-(pyridin-2-yl)-*N*-(pyridin-2-ylmethyl)methanamine) (compound 1) was kindly provided by Molecular Targeting Technologies, Inc. (West Chester, PA, USA). Sep-Pak Accell plus QMA plus cartridge and C18 Sep-Pak cartridges were purchased from Waters, Inc. QMA was pretreated with 5 ml 8.4% $NaHCO_3$ and 5 ml water and C18 cartridge with ethanol and water before use.

Analytical methods. For online radioactivity and UV measurements, the HPLC column was connected to a UV-visible detector (Varian Inc., Walnut Creek, CA, USA) and then to a NaI (Tl) scintillation flow-through detector (Bioscan, Washington DC, USA), and the data were recorded and processed by Galaxie software system (Varian Inc., Walnut Creek, CA, USA) for determination of radiochemical yields. The analytical HPLC column used for the identification of standards and purity of radioactive products was Luna μm C18 150 × 4.6 mm (Phenomenex, Torrance, CA, USA). The mobile phase was 40% CH_3CN/0.1% TFA, and the flow rate was 1 mL/min. The semi-preparative HPLC column used for purifying radioactive products was Luna 5 μm C18 250 × 10.00 mm (Phenomenex, Torrance, CA, USA). Flow rate was 5 mL/min. The gradient was 95% solvent A [0.1% TFA in water] and 5% B [0.1% TFA in acetonitrile] (0–2 min) to 60% solvent A and 40% solvent B at 20 min, then 80% B (22–30 min). UV absorbance was monitored at 254 nm. Mass Spectrometry (MS) analysis was performed using the Mass Spectrometry Laboratory at the University of Louisville. ^{1}H NMR spectra were obtained using a Mercury 800-MHz instrument (NMR Instrumentation Facility at the University of Louisville), and the chemical shifts were reported in parts per million on the δ scale relative to an internal Tetramethylsilane (TMS) standard.

Synthesis of cold reference standards

3Synthesis of N-(4-(3,5-*bis*((*bis*(pyridin-2-ylmethyl)amino)methyl) phenoxy)butyl)-2-fluoropropanamide (FP-**1**)

2-Fluoropropionic acid (5 mg, 54.3 μmol) in anhydrous CH_3CN (0.5 mL) was added to TSTU (17.6 mg, 58.5 μmol). The reaction mixture was stirred at room temperature for 0.5 h, and **1** (3 mg, 5.1 μmol) and DIPEA (20 μL, 115 μmol) in DMSO (0.1 mL) were added. The mixture was further stirred at room temperature for 2 h and purified using semi-preparative HPLC. The collected fractions were lyophilized and analyzed by MS (calculated: 661.6, found: 662.4) and ^{1}H NMR (800 MHz, d^6-DMSO), selected peaks: δ 8.50 (d, *J* = 4.8 Hz, 4H), 7.75 (t, *J* = 8 Hz, 4H), 7.59 (d, *J* = 8 Hz, 4H), 7.25–7.30 (m, 4H), 7.09 (s, 1H), 6.80 (s, 2H), 5.82–6.90 (m, 1H, CF-H).

Synthesis of N-(4-(3,5-*bis*((*bis*(pyridin-2-ylmethyl)amino)methyl) phenoxy)butyl)-4-fluorobenzamide (FB-**1**).

4-Fluorobenzoic acid (5 mg, 35.7 μmol) in anhydrous CH_3CN (0.5 mL) was added to TSTU (12 mg, 40 μmol). The reaction mixture was heated at 100 °C for 10 min, and the solvent was evaporated. Compound **1** (3 mg, 5.1 μmol) and DIPEA (20 μL, 115 μmol) in DMSO (0.1 mL) were added, stirred at room temperature for 2 h and purified by semi-preparative HPLC. The collected fractions were lyophilized and analyzed by MS (calculated: 709.9, found: 710.3) and ^{1}H NMR (800 MHz, d^6-DMSO): δ 8.68 (d, *J* = 4.8 Hz, 4H), 7.97–7.98 (m, 2H), 7.94 (t, *J* = 8 Hz, 4H), 7.60 (d, *J* = 8 Hz, 4H), 7.48 (t, *J* = 6.4 Hz, 4H), 7.35–7.37 (m, 4H), 7.19 (s, 1H), 7.11 (s, 2H), 4.27 (s, 8H), 4.16 (s, 4H), 4.04 (t, *J* = 6.4 Hz, 2H), 3.40 (q, *J* = 6.4 Hz, 2H), 1.82–1.84 (m, 2H), 1.73–1.75 (m, 2H), 1.30–1.33 (m, 2H).

Synthesis of N-(4-(3,5-*bis*((*bis*(pyridin-2-ylmethyl)amino)methyl) phenoxy)butyl)-2-fluoroacetamide (FE-**1**).

2-Fluoroethyl tosylate (9 mg, 41 μmol), **1** (3 mg, 5.1 μmol), and DIPEA (20 μL, 115 μmol) in DMSO (0.2 mL) were mixed together, stirred at 100 °C for 30 min and purified by semi-preparative HPLC. The collected fractions were lyophilized to afford yellow solid and analyzed by MS (calculated: 647.1, found: 646.6) and ^{1}H NMR (800 MHz, d^6-DMSO): δ 8.51 (d, J = 4.0 Hz, 4H), 7.76 (t, J = 8 Hz, 4H), 7.60 (d, J = 8 Hz, 4H), 7.28 (t, J = 6.4 Hz, 4H), 7.12–7.14 (m, 1H), 6.83–6.84 (m, 2H), 4.73 (td, J = 42, 4.8 Hz, 1H), 3.97 (t, J = 6.4 Hz, 2H), 3.74 (s, 8H), 3.54 (s, 4H), 3.40–3.42 (m, 2H), 1.56–1.70 (m, 4H).

Radiochemistry

Preparation of N-(4-(3,5-*bis*((*bis*(pyridin-2-ylmethyl)amino)methyl) phenoxy)butyl)-2-[^{18}F]-fluoropropanamide (^{18}F-FP-**1**)

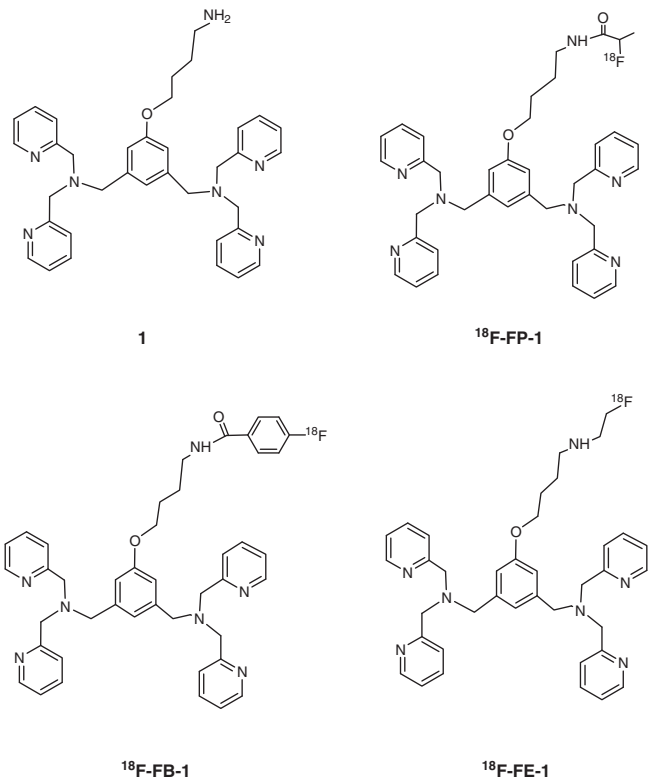
^{18}F-NFP was prepared following reported procedure with minor modification.[24] For a typical run, 15.6 mCi [^{18}F]-fluoride was trapped on a QMA cartridge. The activity was eluted to a v-vial with 0.6 mL 90% acetonitrile containing K222 (30.0 mg, 80 μmol) and potassium carbonate (5 mg, 36 μmol). Azeotropic evaporation was performed at 100 °C with a nitrogen flow, and drying was repeated by addition of CH_3CN (0.5 mL × 3). Ethyl 2-bromopropionate (5 mg, 28 μmol) in anhydrous CH_3CN (0.5 mL) was added and heated at 100 °C for 10 min. Afterwards, TBAH (20 μL,31 μmol) in CH_3CN (0.5 mL) was added and heated at 100 °C for 5 min. The liquid was carefully removed with nitrogen and *bis*-4-nitropenyl carbonate (20 mg, 66 μmol) in dry CH_3CN (1 mL) was added and heated at 100 °C for 10 min. After cooling, the reaction mixture was diluted with 45% CH_3CN/water with 0.1% acetic acid (3 mL) and loaded onto a semi-prep Phenomenex Luna C18 column running at 5 mL/min with 60% CH_3CN/water containing 0.1% TFA as the mobile phase. The product was collected in multiple fractions following radioactive peak at a retention time of 8.7 min. The fraction with the maximum radioactivity was then diluted with water with 0.1% acetic acid (30 mL). The solution containing the desired product was passed through a Sep-Pak plus C18 cartridge. The activity trapped in the cartridge was washed with water (1 mL). The product was eluted with dichloromethane (1 mL). The water layer on the top was carefully removed and the organic layer with product was gently dried with nitrogen at room temperature. For the coupling with 1, the dry residue of ^{18}F-NFP was typically reconstituted in DMSO (0.1 mL), mixed with **1** (0.1 mg, 0.17 μmol) and DIPEA (20 μL, 115 μmol). The mixture was stirred at room temperature for 10 min, then diluted with 5% acetic acid (3 mL) and loaded onto the semi-preparative C18 column. ^{18}F-FP-**1** was collected at 16.2 min.

Preparation of N-(4-(3,5-*bis*((*bis*(pyridin-2-ylmethyl)amino)methyl) phenoxy)butyl)-4-[^{18}F]fluorobenzamide (^{18}F-FB-**1**)

^{18}F-SFB was prepared following reported procedure with minor modification.[25,26] Typically, a solution of ethyl 4-(trimethylammonium triflate) benzoate (5 mg, 31 μmol) in CH_3CN (0.5 mL), which was prepared following reported procedure,[23] was added to 10 mCi of dry ^{18}F/K222/K_2CO_3 residue and heated at 100 °C for 10 min. Afterwards, TBAH (20 μL, 31 μmol) in CH_3CN (0.5 mL) was added and heated at 100 °C for 5 min. The liquid was removed with nitrogen and TSTU (10 mg, 33 μmol) in dry CH_3CN (1 mL) was added and heated at 100 °C for 5 min. After cooling down, 5% acetic acid (8 mL) was added and loaded onto a Sep-Pak plus C18 cartridge, which was then washed with 10 ml water and 10 ml 10% CH_3CN. The product was eluted with 1 mL of CH_2Cl_2 and dried with a flow of nitrogen. For the coupling with **1**, the dry residue of ^{18}F-SFB was reconstituted in DMSO (0.1 mL), mixed with **1** (0.1 mg, 0.17 μmol) and DIPEA (20 μL, 115 μmol). The mixture was stirred at room temperature for 10 min, then diluted with 5% acetic acid (3 ml) and loaded onto semi-preparative C18 column.^{18}F-FB-**1** was collected at 18.4 min.

Preparation of N-(4-(3,5-*bis*((*bis*(pyridin-2-ylmethyl)amino)methyl) phenoxy)butyl)-2-[^{18}F]fluoroacetamide (^{18}F-FE-**1**)

^{18}F-FET was prepared following reported procedure with minor modification.[27] For a typical run, ethylene di(*p*-toluenesulfonate) (5 mg,13 μmol) was added to dry ^{18}F/K222/K_2CO_3 residue and heated at 100 °C for 10 min. After cooling, the reaction mixture was diluted with 45% CH_3CN/water (3 mL) with 0.1% acetic acid and loaded onto a semi-prep Phenomenex Luna C18 column running at 5 mL/min with 60% CH_3CN/water containing 0.1% TFA as the mobile phase. The product was collected in multiple fractions following radioactive peak with a retention time of 9.5 min. The fraction with the maximum radioactivity was then diluted with water with 0.1% acetic acid (30 mL). The solution containing the desired product was passed through a Sep-Pak plus C18 cartridge, and the activity trapped in the cartridge was washed with water (1 mL). The product was eluted with dichloromethane (1 mL). The water layer on the top was carefully removed, and the organic layer with product was gently dried with nitrogen at room temperature. For the reaction with **1**, dry residue of ^{18}F-FET was reconstituted in DMSO (0.1 mL),



Scheme 1. Chemical structures of **1**, ^{18}F-FP-**1**, ^{18}F-FB-**1**, and ^{18}F-FE-**1**.

mixed with **1** (4 mg, 6.8 μmol) and DIPEA (20 μL, 115 μmol). The mixture was heated at 100 °C for 10 min. ^{18}F-FE-**1** was collected at 14.3 min.

Results and discussion

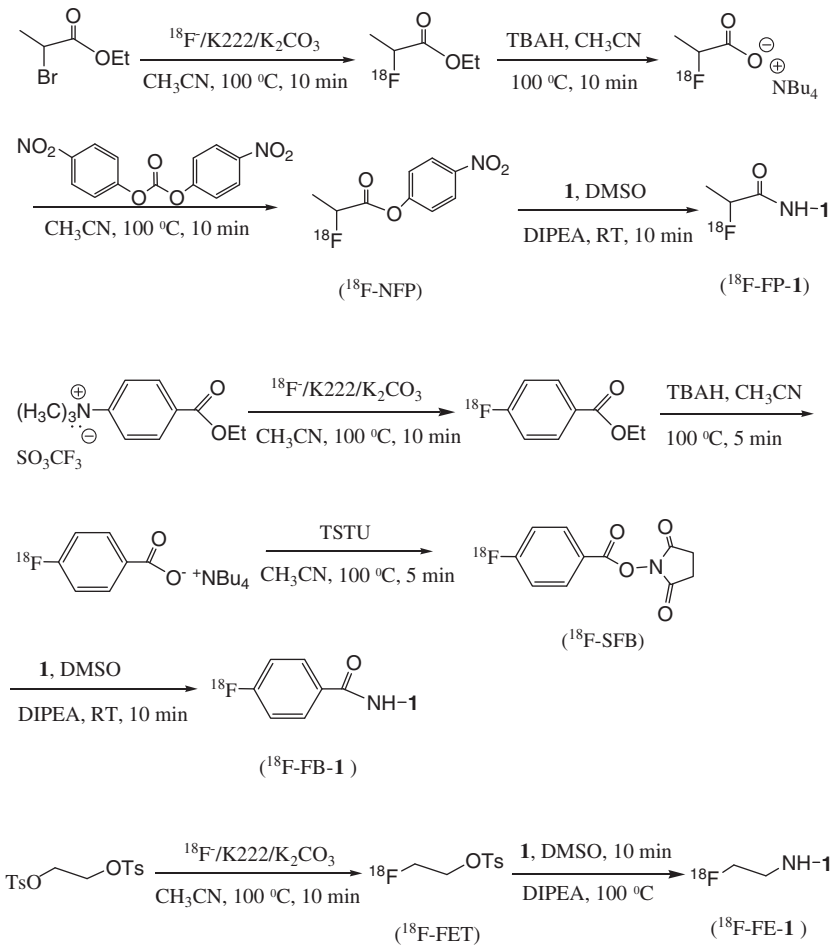
Chemistry

The chemical yields for three cold reference standards, FP-**1**, FB-**1**, and FE-**1** were 95%, 90%, and 65%, respectively. Structures of three compounds were confirmed with MS and ^{1}H NMR. All these three reference compounds were used to verify the identification of the corresponding labeled radiotracers by co-injection in the HPLC analysis.

Radiochemistry

Structures of **1** and three ^{18}F-labeled radiotracers were shown in Scheme 1. The reaction schemes for radiosyntheis of ^{18}F-FP-**1**, ^{18}F-FB-**1**, and ^{18}F-FE-**1** were shown in Scheme 2. Experimental results indicated that all the injected radioactivity was eluted out of the analytical HPLC column for all the experiments (data not shown); therefore, analytical HPLC can be used to determine the yields and purity. All three labeled tracers correlated well with their corresponding cold references by co-injection in the HPLC analysis.

Three prosthetic groups, ^{18}F-NFP, ^{18}F-SFB, and ^{18}F-FET, were selected to conjugate with **1**, respectively. ^{18}F-NFP was synthesized in a three-step and one-pot reaction with HPLC purification in the end. The total synthesis time was about 60 min with an average yield of 72 ± 10% (decay-corrected, $n = 5$). To saponify the ester efficiently in a shorter time, TBAH (20 μL, 115 μmol) was used instead of high volume potassium hydroxide as reported in the literature,[24] which is less toxic and faster to evaporate the solvent for the following step. After HPLC purification, ^{18}F-NFP was processed with a C18 cartridge and eluted with 1 ml of CH_2Cl_2. Because of the highly volatility and hydrolysis nature of ^{18}F-NFP, evaporation needs to be performed at room temperature with low stream of gas in a short time (<10 min). ^{18}F-SFB was synthesized in a three-step and one-pot method using one C18 cartridge for purification at the end. The total synthesis time for ^{18}F-SFB was about 40 min with an average yield of 75 ± 8% (decay corrected, $n = 5$). TBHA was also used to simplify reaction by avoiding one C18 cartridge purification step for the acid intermediate,[25–27] which reduced the reaction time for at least 15 min and avoided at least 20% radioactivity loss. ^{18}F-FET was prepared in one step with HPLC purification. The synthesis time was about 30 min with an average yield of 90 ± 8% (decay corrected, $n = 5$). The specific activity for ^{18}F-NFP, ^{18}F-SFB, and ^{18}F-FET was 8.4–13.5 mCi/nmol,



Scheme 2. Reaction schemes for ^{18}F-FP-**1**, ^{18}F-FB-**1**, and ^{18}F-FE-**1** using three prosthetic groups, ^{18}F-NFP, ^{18}F-SFB, and ^{18}F-FET, respectively.

12.7–21.5 mCi/nmol, and 22.9–25.8 mCi/nmol, respectively. Theradiochemical purity for three tracers was > 99.5% as determined by analytical HPLC.

For the following experiments, factors such as the amount of **1**, reaction time, and reaction temperature were studied to optimize the labeling yield of **1** using three different prosthetic groups.

Effect of the amount of 1 on yield

As shown in Figure 1, the yield with ^{18}F-NFP was > 90% in the range of 50 μg–1 mg (0.085 μmol–1.7 μmol), demonstrating the high reactivity of ^{18}F-NFP. Lower amount than that was not evaluated. For the other two prosthetic groups, the amount of **1** highly affected the yield. For ^{18}F-SFB, the yield was less than 10% at 50 μg (0.085 μmol) but rapidly increased to over 95% at =0.1 mg (0.17 μmol), whereas the yield for ^{18}F-FET was < 30% even at 1 mg (1.7 μmol) and about 85% at 4 mg (17 μmol).

Effect of reaction time on yield

The yields gradually increased with time for all the three groups approximately by 10% from 5 to 30 min, and slowly achieved the plateau (Figure 2). Considering the short half-life of ^{18}F, 10 min was selected as the optimal reaction time for all three prosthetic groups.

Effect of reaction temperature on yield

The reaction temperature significantly affected the ^{18}F-FET yield. When **1** (1 mg,1.7 μmol) was used and reacted for 10 min, only 0%–5% of yields were obtained at 25–80 °C and increased by ~25% at 100 °C. For the other two groups, there was no noticeable change with temperature (Figure 3).

Results in this study indicated that although slightly more compound **1** is required for ^{18}F-SFB than ^{18}F-NFP in order to obtain similar range of yields; both prosthetic groups had similar labeling conditions, reactivity, and labeling yields, whereas ^{18}F-FET required harsh labeling conditions, such as high temperature, organic solvent, and high amount of **1**. The reason was probably because of the different mechanisms involved in the reaction of **1** with ^{18}F-FET. The conjugations of both

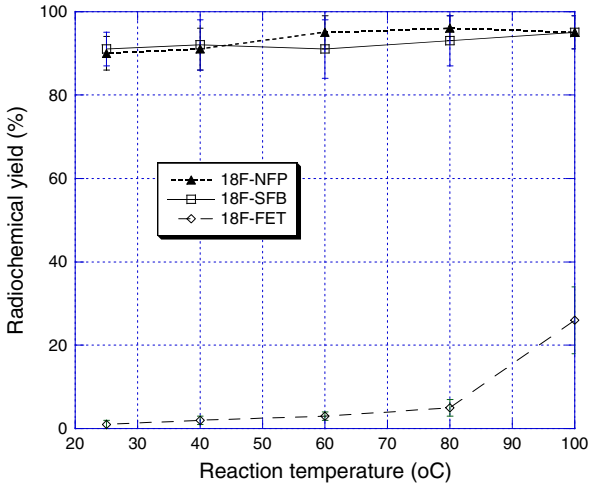


Figure 2. Effect of reaction temperature (°C) on labeling yield with three prosthetic groups ($n = 3$). **1**(0.1 mg, 0.17 μmol) was used for ^{18}F-FP-**1** and ^{18}F-FB-**1**, and **1**(1 mg, 1.7 μmol) for ^{18}F-FE-**1**. Reaction time: 10 min.

^{18}F-NFP and ^{18}F-SFB with **1** involves the formation of a bond between carboxylic group and amine group that can be successfully performed at room temperature in aqueous or organic solvents in high yields. However, the reaction of ^{18}F-FET with **1** is an S_N2 reaction, which usually requires harsh conditions to obtain reasonable yield; for example, anhydrous aprotic solvent and high temperature.

Although the coupling reaction of amino group and carboxyl group can be performed in both organic and aqueous conditions, experimental results showed that the coupling efficiency in anhydrous organic solvent was much higher than that in aqueous or organic solvent with some portion of moisture. In our case, we observed that the coupling is more efficient in anhydrous organic solvents than aqueous solvents; the presence of trace amounts of moisture reduced the yields by about 30%.

In addition, ^{18}F-FE-**1** was not completely separated from original compound **1** because of close retention time (<0.5 min). Our results showed that at least 30% of unreacted **1** was not removed

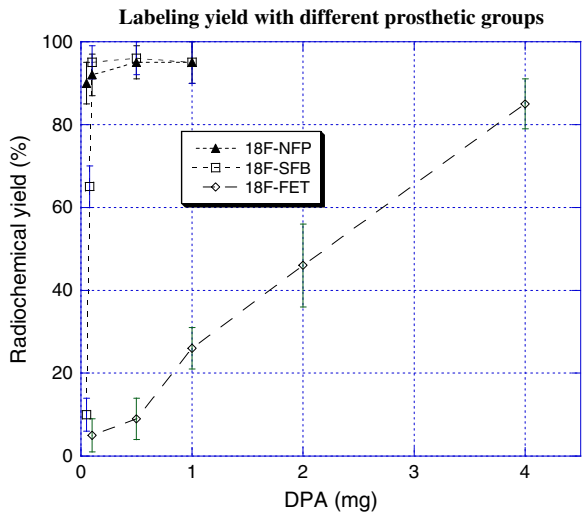


Figure 1. Effect of amount of **1** on labeling yield with three prosthetic groups ($n = 3$). 25 °C was used for ^{18}F-FP-**1** and ^{18}F-FB-**1**. 100 °C was used for ^{18}F-FE-**1**. Reaction time: 10 min.

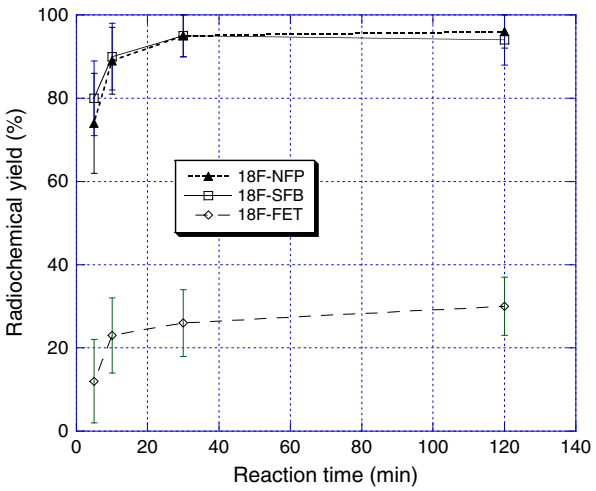


Figure 3. Effect of reaction time on labeling yield with three prosthetic groups ($n = 3$). **1** (0.1 mg, 0.17 μmol) and 25 °C were used for ^{18}F-FP-**1** and ^{18}F-FB-**1**, and **1** (1 mg, 1.7 μmol) and 100 °C were used for ^{18}F-FE-**1**.

from the labeled **1**; thus, the specific activity of ^{18}F-FE-**1** was highly reduced. But the potential advantage for ^{18}F-FET was the smaller size of the ^{18}F-ethyl-group than ^{18}F-propyl-group and ^{18}F-benzyl-group, which could potentially bring less steric hindrance to the original molecule of **1** and thus could better keep the intactness of **1**. Studies have shown that zinc complexation is necessary to ensure **1** binds to PS with high affinity and specificity.[17–19] Therefore, it is critical to reconstitute ^{18}F-**1** with Zn^{2+} prior to performing any biological evaluation.

Conclusions

Compound **1** was successfully labeled with three different prosthetic groups. The optimal labeling conditions were 0.1 mg (0.17 μmol) of **1**, room temperature, 10 min for both ^{18}F-NFP and ^{18}F-SFB; 4 mg (6.8 μmol) of **1**, 100 °C and 10 min for ^{18}F-FET. The total synthesis time, the overall yields, and the average specific activity were 105, 75, and 65 min; 68 ± 9%, 71 ± 11%, and 76 ±13%; 625 Ci/mmol, 853 Ci/mmol, and 3.5 Ci/mmol for ^{18}F-FP-**1**,^{18}F-FB-**1**, and ^{18}F-FE-**1**, respectively ($n = 5$, decay-corrected based on ^{18}F).

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Conflict of Interest

The authors did not report any conflict of interest.

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