Automated radiosynthesis of two ^{18}F -labeled tracers containing 3-fluoro-2-hydroxypropyl moiety, ^{18}F FMISO and ^{18}F PM-PBB3, via ^{18}F epifluorohydrin

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Abstract

Background

[^{18}F]Fluoromisonidazole ([^{18}F]FMISO) and 1-[^{18}F]fluoro-3-((2-((1*E*,3*E*)-4-(6-(methylamino)pyridine-3-yl)buta-1,3-dien-1-yl)benzo[d]thiazol-6-yl)oxy)propan-2-ol ([^{18}F]PM-PBB3 or [^{18}F]APN-1607) are clinically used radiotracers for imaging for hypoxia and tau pathology, respectively. Both radiotracers were produced by direct [^{18}F]fluorination using the corresponding tosylate precursors **1** or **2** and [^{18}F]F[−], followed by the removal of protecting groups. In this study, we synthesized [^{18}F]FMISO and [^{18}F]PM-PBB3 by [^{18}F]fluoroalkylation using [^{18}F]epifluorohydrin ([^{18}F]**5**) for clinical applications.

Results

First, [^{18}F]**5** was synthesized by the reaction of 1,2-epoxypropyl tosylate (**8**) with [^{18}F]F[−] and was purified by distillation. Subsequently, [^{18}F]**5** was reacted with 2-nitroimidazole (**6**) or PBB3 (**7**) as a precursor for ^{18}F -labeling, and each reaction mixture was refined by semipreparative high-performance liquid chromatography and formulation to obtain the [^{18}F]FMISO or [^{18}F]PM-PBB3 injection. All synthetic sequences were performed using an automated ^{18}F -labeling synthesizer. The [^{18}F]FMISO thus obtained showed sufficient radioactivity (0.83 ± 0.20 GBq at the end of synthesis; $n = 8$) with appropriate radiochemical yield based on [^{18}F]F[−] ($26 \pm 7.5\%$ at the end of irradiation with decay-corrected; $n = 8$). [^{18}F]PM-PBB3 thus obtained showed sufficient radioactivity (0.79 ± 0.10 GBq; $n = 11$) with appropriate radiochemical yield based on [^{18}F]F[−] ($16 \pm 3.2\%$ at the end of irradiation with decay-corrected; $n = 11$).

Conclusions

Both [^{18}F]FMISO and [^{18}F]PM-PBB3 injections were successfully synthesized with sufficient radioactivity by [^{18}F]fluoroalkylation using [^{18}F]**5**.

Background

Fluorine-18 (^{18}F , $T_{1/2} = 109.8$ min) is indispensable for the development of positron emission tomography (PET) tracers because its decay characteristic is better than that of carbon-11 ($T_{1/2} = 20.1$ min). As of now, many ^{18}F -labeled tracers containing [^{18}F]fluoroalkyl moieties have been developed. To synthesize these PET tracers, direct [^{18}F]fluorination of the corresponding tosylate or triflate precursor and [^{18}F]F[−] has been a conventional method (Coenen 2007; Hamacher et al. 1986; Snyder and Kilbourn 2003). In addition, [^{18}F]fluoroalkylation is also a useful tool for inserting ^{18}F into target molecules containing nucleophilic hydroxyl and amino functional groups (Zhang and Suzuki 2007). [^{18}F]Fluoroalkylation has some advantages over direct [^{18}F]fluorination. For example, [^{18}F]fluoroalkylation applies to more

accessible and available phenols, carboxylic acids, amines, and amides compounds as precursors for ^{18}F -labeling (Iwata et al. 2002; Wilson et al. 1995; Zhang and Suzuki 2007).

We have synthesized ^{18}F fluoroalkyl agents, such as ^{18}F fluoro-methyl, ethyl, and propyl bromide ($^{18}\text{F}[\text{F}(\text{CH}_2)_n\text{Br}$, $n = 1-3$) (Yanamoto et al. 2009; Yui et al. 2010; Zhang et al. 2002; Zhang et al. 2003; Zhang et al. 2004; Zhang and Suzuki 2007), deuterium-substituted ^{18}F fluoromethyl bromide ($^{18}\text{F}[\text{FCD}_2\text{Br}]$), and its triflate ($^{18}\text{F}[\text{FCD}_2\text{OTf}]$) using an automated ^{18}F -labeling synthesizer (Arakawa et al. 2008; Mori et al. 2019). Using these ^{18}F fluoroalkyl agents, we synthesized dozens of ^{18}F fluoroalkylated tracers starting from the precursors of phenols, carboxylic acids, amines, and amides for PET imaging of receptors, enzymes and transporters in the brain (Zhang and Suzuki 2007). Among these PET tracers, ^{18}F FEDAA1106 (Fujimura et al. 2006), ^{18}F FE-SPARQ (Haneda et al. 2007), ^{18}F FMeNER- d_2 (Arakawa et al. 2008), ^{18}F FEPE2I (Sasaki et al. 2012), and ^{18}F FEDAC (Chung et al. 2018; Xie et al. 2012) have been synthesized for clinical applications in our PET center.

The 3- ^{18}F fluoro-2-hydroxypropyl (^{18}F FHP) moiety is used instead of the aforementioned conventional ^{18}F fluoroalkyl moieties, and is a useful to improve *in vivo* stability and pharmacokinetics of ^{18}F fluoroalkyl radiotracers. Many PET tracers containing the ^{18}F FHP moiety have been developed, some of which have been used in clinical studies, such as ^{18}F FMISO (Bruehlmeier et al. 2004; Eschmann et al. 2005), ^{18}F THK-5351 (Harada et al. 2016; Tago et al. 2016), ^{18}F FC1195S (Byun et al. 2017; Lee et al. 2016; Yang et al. 2016), ^{18}F SMBT-1 (Harada et al. 2021), and ^{18}F PM-PBB3 (Tagai et al. 2021) (Fig. 1). Among these PET tracers, ^{18}F THK-5351 (Fig. 1) was shown to improve *in vivo* metabolic stability by containing the ^{18}F FHP moiety, whereas the 2-arylquinoline derivatives containing the ^{18}F fluoroethyl and the ^{18}F fluoropropyl moieties showed slow clearance of radioactivity from blood and high bone uptake of $^{18}\text{F}[\text{F}]^-$ due to defluorination (Tago et al. 2016).

Direct ^{18}F fluorination using a tosylate or triflate precursor and $^{18}\text{F}[\text{F}]^-$ is a widely used method for the introduction of ^{18}F into target molecules to afford a large number of ^{18}F -labeled PET tracers (Coenen 2007; Hamacher et al. 1986; Snyder and Kilbourn 2003). For the current clinical applications in our PET center, ^{18}F FMISO as a PET imaging agent for tumor hypoxia (Oh et al. 2005; Tang et al., 2005), and ^{18}F PM-PBB3 as a PET imaging agent for tau pathology (Kawamura et al. 2021) have been prepared by direct ^{18}F fluorination using tosylate precursors and $^{18}\text{F}[\text{F}]^-$, followed by the removal of the protecting group. This direct ^{18}F fluorination was achieved within the same reaction vessel in an automated synthesizer. Moreover, the one-step radiolabeling technique for ^{18}F -labeled tracers could be readily transferred to other PET centers to perform multisite studies using the same study protocol (Kawamura et al. 2016; Kawamura et al. 2021; Mori et al. 2017). In fact, automated radiosynthesis of ^{18}F PM-PBB3 by direct ^{18}F fluorination has been transferred to a dozen of PET centers in Japan, China, Taiwan, and the USA (Hsu et al. 2020; Su et al. 2020; Weng et al. 2020). On the other hand, tosylated precursors for direct ^{18}F fluorination should be synthesized with at least two steps and are limited to radiosynthesis.

In this study, to determine an effective synthetic route for [^{18}F]FMISO and [^{18}F]PM-PBB3 with sufficient radioactivity and high quality for clinical applications, we synthesized these two PET tracers using [^{18}F]epifluorohydrin ([^{18}F]5) as an ^{18}F -labeling agent by the reaction of easily accessible 2-nitroimidazole (6, Fig. 2) or PBB3 (a phenol precursor; 7, Fig. 3), and compared the synthetic results of [^{18}F]fluoroalkylation using [^{18}F]5 and [^{18}F]fluorination using [^{18}F]F $^{-}$ to evaluate their relative merits.

Methods

General

1-*H*-1-(3-Fluoro-2-hydroxypropyl)-2-nitroimidazole (FMISO, Fig. 1) and 2-nitroimidazole (6, Fig. 2) were purchased from ABX (Radeberg, Germany). 1-Fluoro-3-((2-((1*E*,3*E*)-4-(6-(methylamino)pyridine-3-yl)buta-1,3-dien-1-yl)benzo[*d*]thiazol-6-yl)oxy)propan-2-ol (PM-PBB3, Fig. 1), and 2-((1*E*,3*E*)-4-(6-(methylamino)pyridin-3-yl)buta-1,3-dienyl)benzo[*d*]thiazol-6-ol (PBB3; 7, Fig. 3) (Hashimoto et al. 2014; Maruyama et al. 2013) were provided by Shanghai ChemPartner (Shanghai, China). All chemical reagents and the organic solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA), Fujifilm Wako Pure Chemical Co. (Osaka, Japan), and Nacalai Tesque (Kyoto, Japan), and were used without any further purification. Fluorine-18 was produced by the $^{18}\text{O}(\text{p}, \text{n})^{18}\text{F}$ nuclear reaction using a CYPRIS HM-18 cyclotron (Sumitomo Heavy Industry, Tokyo, Japan). A dose calibrator (IGC-3R Curiometer; Aloka, Tokyo, Japan) was used for all radioactivity measurements, unless otherwise stated. An automated multi-purpose synthesizer developed in-house was used for all radiosynthetic runs in this study (Fukumura et al. 2007). Semipreparative high-performance liquid chromatography (HPLC) was performed using a JACSO HPLC system (PU-2080 pump and UV-2075 detector; JACSO, Tokyo, Japan) equipped with a radioactivity detector (Ohyo Koken Kogyo, Tokyo, Japan).

Production of [^{18}F]F $^{-}$

Fluorine-18 as [^{18}F]F $^{-}$ was produced from the cyclotron on > 98% enriched H_2^{18}O (ROTEM Industries, Arava, Israel) and 18 MeV protons (14.2 MeV on the target). The cyclotron-produced aqueous [^{18}F]F $^{-}$ containing H_2^{18}O was passed through a Sep-Pak Accell Plus QMA Plus Light cartridge (130 mg, Waters, Milford, MA, USA), [^{18}F]F $^{-}$ was eluted from the cartridge using a mixture of a solution of potassium carbonate (K_2CO_3 , 16.8 nmol) in water (0.2 mL) and a solution of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane (Kryptofix 222, 7.5 mg) in acetonitrile (CH_3CN , 0.2 mL), and then was transferred to a reaction vessel. The aqueous solution was dried at 120°C for 20 min to remove water and CH_3CN .

Automated radiosynthesis of [^{18}F]FMISO using [^{18}F]5

After the [^{18}F]F $^{-}$ solution was dried, a solution of epoxypropyl tosylate (8, 3.5 mg) in *o*-dichlorobenzene (0.15 mL) was added to the reaction vial containing dry [^{18}F]F $^{-}$ at 130°C. The resulting [^{18}F]5 was distilled from the reaction vial under an atmosphere of nitrogen and was transferred to another reaction vial

containing precursor **6** (2 mg) and 1 mol/L sodium hydroxide solution (NaOH, 18 μ L) in anhydrous *N,N*-dimethylformamide (DMF, 0.25 mL) maintained at -15°C. After 2 min trapping of [18 F]**5**, the reaction mixture was heated at 150°C for 20 min, and then was diluted with 0.5 mL of the semipreparative HPLC eluent. The solution mixture was transferred to the injector for semipreparative HPLC, as described in the general section. The semipreparative HPLC conditions were as follows: XBridge C₁₈ column (5 μ m, 10 mm i.d. $\times$ 250 mm length; Waters), a mixture of ethanol and water (2:98, vol./vol.) as the mobile phase, 5.0 mL/min flow rate, and UV detection at 325 nm. The retention time of [18 F]FMISO was approximately 12 min. The HPLC fraction of [18 F]FMISO was collected in a flask containing polysorbate 80 (75 μ L) in ethanol (0.3 mL) and ascorbic acid injection (25 mg/0.1 mL) was added before radiosynthesis. The solution was subsequently evaporated to dryness, and the residue was dissolved in physiological saline. The resulting solution was passed through a Millex-GV filter (Millipore) to obtain [18 F]FMISO as an injectable solution.

The radiochemical purity of [18 F]FMISO was determined with the help of analytical HPLC under the following conditions: XBridge C₁₈ column (5 μ m, 4.6 mm i.d. $\times$ 150 mm length; Waters), a mixture of 90% acetonitrile solution and 50 mM ammonium phosphate buffer (pH 9.3) (7:93, vol./vol.) as the mobile phase, 1.0 mL/min flow rate, and UV detection at 325 nm. The retention time of [18 F]FMISO was 5.6 min. The identity of [18 F]FMISO was confirmed by co-injecting it with authentic unlabeled FMISO. The molar activity of [18 F]FMISO was measured using the same analytical HPLC system. The mass (μ mol) of FMISO with a known radioactivity (GBq) was determined with the help of the analytical HPLC by comparing the UV absorbance at 325 nm of [18 F]FMISO with that of known concentrations of unlabeled FMISO.

Automated radiosynthesis of [18 F]PM-PBB3 using [18 F]**5**

After the [18 F] F^- solution was dried, a solution of epoxypropyl tosylate (**8**, 3.5 mg) in *o*-dichlorobenzene (0.15 mL) was added automatically to the reaction vial containing dry [18 F] F^- at 130°C. The resulting [18 F]**5** was distilled from the reaction vial and was transfer to another reaction vial containing precursor **7** (1 mg) and 1 mol/L NaOH (3.5 μ L) in anhydrous DMF (0.25 mL) maintained at -15°C. After 2 min of trapping of [18 F]**5**, the reaction mixture was heated at 130°C for 20 min, and then was diluted with 0.5 mL of the semipreparative HPLC eluent. The mixture solution was transferred to the injector for semipreparative HPLC as described in general section. The semipreparative HPLC conditions were as follows: Capcell Pak C₁₈ column (5 μ m, 10 mm i.d. $\times$ 250 mm length; Shiseido, Tokyo, Japan), a mixture of acetonitrile, water, and triethylamine (40:60:0.1, vol./vol./vol.) as the mobile phase, 5.0 mL/min flow rate, and UV detection at 365 nm. The retention time of [18 F]PM-PBB3 was 14.9 min. The HPLC fraction of [18 F]PM-PBB3 was collected in a flask containing polysorbate 80 (75 μ L) in ethanol (0.3 mL) and ascorbic acid injection (25 mg/0.1 mL) was added before radiosynthesis. The solution was subsequently evaporated to dryness, and the residue was dissolved in physiological saline. The solution of [18 F]PM-PBB3 was passed through a Millex-GV filter to obtain [18 F]PM-PBB3 as an injectable solution. The HPLC

separation and formulation were performed under UV-cut light (< 500 nm wavelength cutoff, ECOHiLUX HES-YF; Iris Oyama, Sendai, Japan) without fluorescent light to prevent the photoisomerization of [^{18}F]PM-PBB3.

The radiochemical purity of [^{18}F]PM-PBB3 was determined by analytical HPLC under the following conditions: Atlantis T3 column (5 μm , 4.6 mm i.d. $\times$ 150 mm length; Waters), a mixture of acetonitrile and 50 mM ammonium acetate (40:60, vol./vol.) as the mobile phase, 1.0 mL/min flow rate, and UV detection at 365 nm. The retention time of [^{18}F]PM-PBB3 was 12 min. The identity of [^{18}F]PM-PBB3 was confirmed by co-injecting it with authentic unlabeled PM-PBB3. The molar activity of [^{18}F]PM-PBB3 was measured using the same analytical HPLC system. The mass (μmol) of [^{18}F]PM-PBB3 with a known radioactivity (GBq) was determined by comparing the UV absorbance at 365 nm of PM-PBB3 with that of known concentrations of unlabeled PM-PBB3. All the above analytical processes were conducted in the absence of fluorescent light to prevent the photoisomerization of [^{18}F]PM-PBB3.

Results

Automated radiosynthesis of [^{18}F]FMISO using [^{18}F]5

We synthesized [^{18}F]FMISO under various reaction conditions for the [^{18}F]fluoroalkylation of 2-nitroimidazole precursor **6** and [^{18}F]5 using an automated ^{18}F -labeling synthesizer. With an increase in the amount of **6** from 0.5 to 4 mg, the radiochemical yield of [^{18}F]FMISO gradually increased to 36% from 0.5 to 2 mg, and marginally increased up to 42% from 2 to 4 mg (Fig. 4(A)). In addition, increasing the reaction temperature from 90°C to 150°C increased the radiochemical yield of [^{18}F]FMISO by up to 40% (Fig. 4(B)). Furthermore, the radiochemical yield obtained by using sodium hydroxide (36%) as a base for the reaction was slightly higher than that obtained using sodium carbonate (22%) or potassium hydroxide (24%). From these results, we determined that the radiosynthesis of [^{18}F]FMISO using [^{18}F]6 is as follows: 2 mg of precursor **6**, 18 μmol of sodium hydroxide as a base for the reaction, a temperature of 150°C and 20 min for the reaction time. After completion of the reaction, semipreparative HPLC for the reaction mixture was performed to efficiently separate [^{18}F]FMISO from the mixture, affording the radiochemically and chemically pure product as an injectable solution (Fig. 5(A)). No significant UV peak corresponding to unreacted **6** and its decomposition component was observed in the analytical HPLC chromatogram of the final product solution (Fig. 5(B)).

Table 1 summarizes the results of the automated radiosynthesis of [^{18}F]FMISO by [^{18}F]fluoroalkylation using **6** and [^{18}F]5 for clinical applications. We successfully synthesized [^{18}F]FMISO using [^{18}F]5, with sufficient radioactivity (0.83 ± 0.2 GBq, $n = 8$) for clinical applications. The decay-corrected radiochemical yield of [^{18}F]FMISO based on [^{18}F]F $^-$ was 26 ± 7.5 % at the end of the synthesis ($n = 8$). All the results of quality control for the [^{18}F]FMISO injection complied with our in-house quality control and quality assurance specifications (Table 1).

Table 1
Radiosynthetic results of [^{18}F]FMISO by [^{18}F]fluoroalkylation using precursor **6** and [^{18}F]**5**

		Results ^a
Cyclotron-produced [^{18}F]F ⁻ (GBq)		5.2 ± 0.20
Radioactivity (GBq) ^b		0.83 ± 0.20
Radiochemical yield (%) ^c		26 ± 7.5
Radiochemical purity (%)	EOS	99 ± 0.50
	3 h after EOS	> 95
Synthetic time (min)		75 ± 4.0
Molar activity (GBq/μmol) [#]		110 ± 20
^a <i>n</i> = 8; ^b At the end of synthesis (EOS); ^c Decay-corrected to the end of irradiation		

Automated radiosynthesis of [^{18}F]PM-PBB3 using [^{18}F]**5**

We synthesized [^{18}F]PM-PBB3 by the [^{18}F]fluoroalkylation of precursor **7** and [^{18}F]**5** (Fig. 3), according to the reaction conditions previously determined for the reaction of a conventional phenol precursor with [^{18}F]**5** (Fujinaga et al. 2018). After trapping [^{18}F]**5** for 2 min, the [^{18}F]fluoroalkylation of **7** and [^{18}F]**5** was performed at 130°C for 20 min. The reaction mixture was then separated using semipreparative HPLC (Fig. 6(A)) to produce radiochemically and chemically pure [^{18}F]PM-PBB3 as an injectable solution (Fig. 6(B)).

Table 2 summarizes the automated radiosynthesis results of [^{18}F]PM-PBB3 by [^{18}F]fluoroalkylation using precursor **7** and [^{18}F]**5** for clinical applications. We successfully synthesized [^{18}F]PM-PBB3 using [^{18}F]**5**, with sufficient radioactivity (0.79 ± 0.1 GBq, *n* = 11) for clinical applications. The decay-corrected radiochemical yield of [^{18}F]PM-PBB3 based on [^{18}F]F⁻ using [^{18}F]**5** was 16 ± 3.2 % at the end of synthesis (*n* = 11). All the results of quality control for the [^{18}F]PM-PBB3 injection complied with our in-house quality control and quality assurance specifications (Table 2).

Table 2
Radiosynthetic results of [^{18}F]PM-PBB3 by [^{18}F]fluoroalkylation using precursor **7** and [^{18}F]**5**

		Results ^a
Cyclotron-produced [^{18}F]F ⁻ (GBq)		7.4 ± 0.20
Radioactivity (GBq) ^b		0.79 ± 0.10
Radiochemical yield based on [^{18}F]F ⁻ (%) ^c		16 ± 3.2
Radiochemical purity (%)	at EOS	99 ± 0.50
	at 3 h after EOS	> 95
Synthetic time (min)		78 ± 4.0
Molar activity (GBq/μmol) ^b		330 ± 140
^a <i>n</i> = 11; ^b At the end of synthesis (EOS); ^c Decay-corrected to the end of irradiation		

Discussion

We successfully synthesized [^{18}F]FMISO and [^{18}F]PM-PBB3 by [^{18}F]fluoroalkylation using [^{18}F]**5** with sufficient radioactivity for clinical applications. For [^{18}F]FMISO, the radiochemical yield of [^{18}F]fluoroalkylation using **6** and [^{18}F]**5** was 26 ± 7.5% (*n* = 8, Table 1), while the yield by the direct [^{18}F]fluorination of **1** with [^{18}F]F⁻ was approximately 40% (from the average result in our routine radiosynthesis). For [^{18}F]PM-PBB3, the radiochemical yield of [^{18}F]fluoroalkylation using **7** and [^{18}F]**5** was 16 ± 3.2% (*n* = 11, Table 2), while the yield obtained by the direct [^{18}F]fluorination of **2** with [^{18}F]F⁻ was 25 ± 6.0% (*n* = 53) (Kawamura et al. 2021). The reason for the difference in radiochemical yields between the two methods is the relatively lower reactivity of the corresponding precursors toward [^{18}F]**5** as a radiolabeling agent than that toward [^{18}F]F⁻. Moreover, the reactivity of [^{18}F]**5** seemed to be lower than that of conventional [^{18}F]fluoroalkyl agents, such as [^{18}F]fluoroethyl bromide and [^{18}F]fluoroethyl iodide, toward the same phenol precursor. Recently, we found that the use of some Lewis acids could increase the reactivity of [^{18}F]**5** with aniline analogs (Fujinaga et al. 2019), and expected that the radiochemical yield of PET tracers containing the [^{18}F]FHP moiety could be increased by [^{18}F]fluoroalkylation using phenol or amine and [^{18}F]**5**, catalyzed by Lewis acid.

On the other hand, for direct [^{18}F]fluorination, the tosylate precursors **1** and **2** should be synthesized with at least two steps from **6** and **7**, respectively, and were limited to only the radiosynthesis of [^{18}F]FMISO and [^{18}F]PM-PBB3. For [^{18}F]fluoroalkylation, imidazole precursor **6** and phenol precursor **7** were available and accessible. In particular, precursor **7** (PBB3) is an authentic unlabeled compound of [^{11}C]PBB3, which is a clinically used radiotracer for PET imaging of tauopathy in the human brain (Hashimoto et al. 2014;

Hashimoto et al. 2015; Maruyama et al. 2013). Moreover, **6** or **7** could be used to react with [^{18}F]**5** as well as other radiolabeling agents, such as [^{11}C]methyl iodide and [^{18}F]fluoroalkyl agents, to produce diverse PET tracer candidates. A structure-activity relationship study is helpful for finding PET tracers with improved *in vitro* properties and *in vivo* behaviors by reacting the same precursor with diverse radiolabeling agents. This strategy has been applied to develop PET tracers in our group and to explore the best version from a series of candidates with the same chemical skeleton (Fujinaga et al. 2012; Zhang et al. 2003; Zhang et al. 2004).

In this synthesis, the resulting [^{18}F]**5** radiolabeling agent was purified by distillation from the [^{18}F]fluorinated mixture of epoxytosylate **8** with [^{18}F]F $^-$, and was used for [^{18}F]fluoroalkylation (Fujinaga et al. 2018). The distillation procedure removed all non-volatile impurities, such as metal ions from the cyclotron target, unreacted **8** and [^{18}F]F $^-$, and the phase transfer reagent Kryptofix 222 and K $_2\text{CO}_3$. Because of the utilization of purified [^{18}F]**5**, only a small amount of precursor **6** (2 mg) or **7** (1 mg) was used for the [^{18}F]fluoroalkylation, resulting in a clear [^{18}F]fluoroalkylated reaction mixture. As shown in the respective HPLC separation charts for the reaction mixtures, in addition to the unreacted [^{18}F]**5**, only the desired product corresponding to [^{18}F]FMISO (Fig. 5(A)) or [^{18}F]PM-PBB3 (Fig. 6(A)) peak was obtained from the reaction. Because of the large difference in the retention times of [^{18}F]**5** and [^{18}F]FMISO or [^{18}F]PM-PBB3, HPLC separation was easily conducted to obtain two radiochemically and chemically pure products (Figs. 5(B) and 6(B)). Moreover, after [^{18}F]fluoroalkylation, the reaction mixture did not require deprotection with acid, directly resulting in [^{18}F]FMISO or [^{18}F]PM-PBB3.

For direct [^{18}F]fluorination, the tosylate precursor **1** or **2** is not stable in the presence of excess K $_2\text{CO}_3$ and Kryptofix 222 at high temperatures; therefore, a relatively large amount of **1** (5 mg) (Tang et al. 2005) or **2** (2 mg) (Kawamura et al. 2021) was required for the [^{18}F]fluorination with [^{18}F]F $^-$ in order to produce sufficient radioactivity for clinical applications. The unreacted precursors and decomposed chemical components made the HPLC purification inconvenient (Supplemental information: Fig. S1 and S2). The synthesis of [^{18}F]FMISO by [^{18}F]fluorination using **1** and [^{18}F]F $^-$, after removal of the tosyl group in [^{18}F]**3** by treating the reaction mixture with HCl, *p*-toluenesulfonic acid (TsOH) was obtained. Only HPLC separation of the reaction mixture could not remove TsOH perfectly, and part of it would be left in the final product solution. Therefore, in our laboratory, after semipreparative HPLC for the reaction mixture of [^{18}F]**3** with HCl, the HPLC fraction was passed through a Sep-Pak cartridge (Cl $^-$ form) to delete TsOH. In addition, [^{18}F]FMISO was obtained as a chemically and radiochemically pure injectable solution.

Conclusions

In this study, we successfully synthesized [^{18}F]FMISO and [^{18}F]PM-PBB3 by [^{18}F]fluoroalkylation using [^{18}F]**5**, although the radiochemical yields of [^{18}F]fluoroalkylation using [^{18}F]**5** were relatively lower than those of the corresponding direct [^{18}F]fluorination using [^{18}F]F $^-$. Furthermore, we obtained relatively high chemical and radiochemical purity of [^{18}F]FMISO or [^{18}F]PM-PBB3 injection by radiosynthesis with

[¹⁸F]fluoroalkylation using [¹⁸F]**5**. Radiosynthesis using [¹⁸F]**5** is expected to be widely used to develop and produce useful PET tracers containing the [¹⁸F]FHP moiety.

Abbreviations

[¹⁸F]FMISO: [¹⁸F]Fluoromisonidazole; [¹⁸F]PM-PBB3: 1-[¹⁸F]Fluoro-3-((2-((1*E*,3*E*)-4-(6-(methylamino)pyridine-3-yl)buta-1,3-dien-1-yl)benzo[d]thiazol-6-yl)oxy)propan-2-ol; [¹⁸F]**5**: [¹⁸F]Epifluorohydrin; HPLC: high-performance liquid chromatography; PET: Positron emission tomography; [¹⁸F]FHP: 3-[¹⁸F]Fluoro-2-hydroxy)propyl; PBB3: 2-((1*E*,3*E*)-4-(6-(Methylamino)pyridin-3-yl)buta-1,3-dienyl)benzo[d]thiazol-6-ol; K₂CO₃: Potassium carbonate; Kryptofix 222: 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane; CH₃CN: Acetonitrile; NaOH: Sodium hydroxide; DMF: *N,N*-Dimethylformamide; TsOH: *p*-Toluenesulfonic acid; EOS: The end of synthesis

Declarations

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Authors' contribution

TO designed the study, performed synthesis, and analyzed the data; YK, MO, and NN operated the radiosynthesizer; MF, WM, KK, and MH assisted with the synthesis; KF and HH analyzed quality control data; KK summarized the study, and wrote the manuscript; MRZ designed the study and reviewed the manuscript; all authors read and approved the final manuscript.

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no potential conflicts of interest with respect to the authorship or publication of this article.

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Figures

Figure 1

Chemical structures of PET tracers containing (3-[^{18}F]fluoro-2-hydroxy)propyl ([^{18}F]FHP) moiety used in clinical applications. [^{18}F]FMISO (Bruehlmeier et al. 2004; Eschmann et al. 2005); [^{18}F]THK-5351 (Harada et al. 2016; Tago et al. 2016); [^{18}F]FC1195S (Byun et al. 2017; Lee et al. 2016; Yang et al. 2016); [^{18}F]SMBT-1 (Harada et al. 2021); [^{18}F]PM-PBB3 (Tagai et al. 2021).

Previous study

Present study

Figure 2

Radiosynthesis of $[^{18}\text{F}]\text{FMISO}$ by $[^{18}\text{F}]\text{F}^-$ and $[^{18}\text{F}]\text{fluoroalkylation}$ using $[^{18}\text{F}]\text{epifluorohydrin}$ ($[^{18}\text{F}]\text{5}$). In this study, after the $[^{18}\text{F}]\text{F}^-$ solution was dried, a solution of **8** (3.5 mg) in o-dichlorobenzene (0.15 mL) was added to a reaction vial containing dry $[^{18}\text{F}]\text{F}^-$. The resulting $[^{18}\text{F}]\text{5}$ was distilled under an atmosphere of nitrogen, and transferred to another reaction vial containing **6** (2 mg) and 1 mol/L NaOH (18 μL) in anhydrous DMF (0.25 mL) maintained at -15°C . After 2 min of trapping of $[^{18}\text{F}]\text{5}$, the reaction mixture was heated at 150°C for 20 min to obtain $[^{18}\text{F}]\text{FMISO}$.

Previous study

Present study

Figure 3

Radiosynthesis of $[^{18}\text{F}]\text{PM-PBB3}$ by $[^{18}\text{F}]\text{F}^-$ and $[^{18}\text{F}]\text{fluoroalkylation}$ using $[^{18}\text{F}]\text{epifluorohydrin}$ ($[^{18}\text{F}]\text{5}$). In this study, after the $[^{18}\text{F}]\text{F}^-$ solution was dried, a solution of **8** (3.5 mg) in o-dichlorobenzene (0.15 mL) was added to the reaction vial containing the dry $[^{18}\text{F}]\text{F}^-$. The resulting $[^{18}\text{F}]\text{5}$ was distilled from the reaction vial and was transferred to another reaction vial containing **7** (1 mg) and 1 mol/L NaOH (3.5 μL) in anhydrous DMF (0.25 mL) maintained at -15°C . After 2 min of trapping of $[^{18}\text{F}]\text{5}$, the reaction mixture was heated at 130°C for 20 min to obtain $[^{18}\text{F}]\text{PM-PBB3}$.

Figure 4

Radiochemical yields of [18F]FMISO via [18F]5 in varied precursor dose (A) and reaction temperature (B). [18F]5 was distilled from the reaction vial and was transferred to the next reaction vial containing 6 (0.5, 1.0, 2.0 and 4.0 mg) and 1 mol/L NaOH (4.5, 9.0, 18 and 36 μ L) in anhydrous DMF (0.25 mL) maintained at -15°C. After 2 min of trapping of [18F]5, the reaction mixture was heated at 90, 110, 130, and 150°C for 20 min to obtain [18F]FMISO.

Figure 5

Semipreparative HPLC (A) and analytical HPLC (B) chromatograms of $[^{18}\text{F}]FMISO$ by $[^{18}\text{F}]$ fluoroalkylation using $[^{18}\text{F}]5$. The semipreparative HPLC conditions were as follows: XBridge C18 column (5 μm , 10 mm i.d. $\times$ 250 mm length; Waters), a mixture of ethanol and water (2:98, vol./vol.) as the mobile phase, 5.0 mL/min flow rate, and UV detection at 325 nm. The retention time of $[^{18}\text{F}]FMISO$ was approximately 12 min (A). The analytical HPLC conditions were as follows: XBridge C18 column (5 μm , 4.6 mm i.d. $\times$ 150 mm length; Waters), a mixture of 90% acetonitrile solution and 50 mM ammonium phosphate buffer (pH 9.3) (7:93, vol./vol.) as the mobile phase, 1.0 mL/min flow rate, and UV detection at 325 nm. The retention time of $[^{18}\text{F}]FMISO$ was 5.6 min (B).

Figure 6

Semipreparative HPLC (A) and analytical HPLC (B) chromatograms of $[^{18}\text{F}]PM-PBB3$ by $[^{18}\text{F}]$ fluoroalkylation using $[^{18}\text{F}]5$. The semipreparative HPLC conditions were as follows: Capcell Pak C18 column (5 μm , 10 mm i.d. $\times$ 250 mm length; Shiseido), a mixture of acetonitrile, water and triethylamine (40:60:0.1, vol./vol./vol.) as the mobile phase, 5.0 mL/min flow rate, and UV detection at 365 nm. The retention time of $[^{18}\text{F}]PM-PBB3$ was 14.9 min (A). The analytical HPLC conditions were as follows: Atlantis T3 column (5 μm , 4.6 mm i.d. $\times$ 150 mm length; Waters), a mixture of acetonitrile and 50 mM ammonium acetate (40:60, vol./vol.) as the mobile phase, 1.0 mL/min flow rate, and UV detection at 365 nm. The retention time of $[^{18}\text{F}]PM-PBB3$ was 12 min (B).

Supplementary Files

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