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Journal of Labelled Compounds and Radiopharmaceuticals

Received 1 December 2011, Accepted 8 December 2011 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1958

Fully automated synthesis and purification of 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl)-*p*-[^{18}F]fluorobenzamido]ethylpiperazine

Kazutaka Hayashi,[a,b]* Kenji Furutsuka,[a,c] Takehito Ito,[a,c] Masatoshi Muto,[d] Hatsumi Aki,[e] Toshimitsu Fukumura,[a] and Kazutoshi Suzuki[a]

We have developed an efficient synthesis method for the rapid and high-yield automated synthesis of 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl)-*p*-[^{18}F]fluorobenzamido]ethylpiperazine (*p*-[^{18}F]MPPF). No-carrier-added [^{18}F]F^- was trapped on a small QMA cartridge and eluted with 70% MeCN(aq) (0.4 mL) containing Kryptofix 222 (2.3 mg) and K_2CO_3 (0.7 mg). The nucleophilic [^{18}F]fluorination was performed with 3 mg of the nitro-precursor in DMSO (0.4 mL) at 190 °C for 20 min, followed by the preparative HPLC purification (column: COSMOSIL Cholester, Nacalai Tesque, Kyoto, Japan; mobile phase: MeCN/25 mM $AcONH_4$/AcOH = 200/300/0.15; flow rate: 6.0 mL/min) to afford *p*-[^{18}F]MPPF (retention time = 9.5 min). *p*-[^{18}F]MPPF was obtained automatically with a radiochemical yield of 38.6 ± 5.0% (decay corrected, *n* = 5), a specific activity of 214.3 ± 21.1 GBq/μmol, and a radiochemical purity of >99% within a total synthesis time of about 55 min.

Keywords: 5-HT_{1A} receptor; *p*-[^{18}F]MPPF; [^{18}F]fluoride; automated synthesis; preparative HPLC separation

Introduction

The serotonin receptors have been divided into at least seven classes (5-HT_{1-7}), depending on their structures and pharmacological characteristics.[1,2] These serotonin receptor subtypes are involved in various physiological processes and diseases, such as sleep, sexual disorders, depression, anxiety, schizophrenia, migraine, and Alzheimer's disease. The major interest in the development of radiopharmaceuticals in this field has focused on the 5-HT_{1A} and 5-HT_{2A} receptor subtypes.[3,4]

[Carbonyl-^{11}C]WAY-100635, the first effective radiopharmaceutical for the serotonergic system, has been widely used for selective imaging of 5-HT_{1A} receptors with positron emission tomography.[5,6] Furthermore, the fluoro analog of WAY-100635, 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl)-*p*-fluorobenzamido] ethylpiperazine (*p*-MPPF), was developed.[7] The first attempt to label *p*-MPPF with ^{18}F was reported in 1997, and *p*-[^{18}F]MPPF was synthesized from the nitro-precursor, 4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl)-*p*-nitrobenzamido]ethylpiperazine (*p*-$MPPNO_2$; 4 mg), in DMSO at 140 °C for 20 min, which gave a radiochemical yield of 10% end-of-bombardment (EOB) in a total synthesis time of 90 min.[8] Le Bars *et al.* synthesized *p*-[^{18}F]MPPF with 10 mg of *p*-$MPPNO_2$ in DMSO by microwave heating at 500 W for 3 min instead of conventional heating, with improved HPLC separation of *p*-[^{18}F]MPPF from *p*-$MPPNO_2$, which gave *p*-[^{18}F] MPPF at a radiochemical yield of 25% end-of-synthesis (EOS) in a total synthesis time of 70 min.[9]

Generally, the major problem with ^{18}F-labeled radiopharmaceuticals prepared with a nitro-precursor is the difficulty of HPLC separation between the nitro-precursor and the ^{18}F-labeled compound[9–12] because the retention time of the ^{18}F-labeled compound is often close to that of the nitro-precursor. The preparative HPLC conditions were examined previously, aiming at quick and efficient separation of the ^{18}F-labeled compound from the nitro-precursor.[9–11] *p*-[^{18}F]MPPF could be separated efficiently in a comparatively short time by HPLC (column: SymmetryPrep C18, 10 mm i.d. × 300 mm, 7 μm; Waters, Milford, MA, USA; mobile phase: MeOH/THF/50 mM NaOAc buffer (pH 5) = 27/18/55; flow rate: 5 mL/min; wavelength: 274 nm), as described by Le Bars *et al.*[9] The retention times of *p*-[^{18}F]MPPF and *p*-$MPPNO_2$ were 15 and 24 min, respectively.

Recently, we have developed an efficient and fully automated synthesis method to achieve a high radiochemical yield of

[a]*Radiopharmaceutical Production Team, Molecular Probe Group, Molecular Imaging Center, National Institute of Radiological Sciences (NIRS), 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan*

[b]*Molecular Imaging Integration Unit, RIKEN Center for Molecular Imaging Science (CMIS), 6-7-3 Minatojima-minamimachi, Chuo-ku, Hyogo 650-0047, Japan*

[c]*SHI Accelerator Service Co., Ltd., 1-17-6 Osaki, Shinagawa-ku, Tokyo 141-0032, Japan*

[d]*Tokyo Nuclear Service Co., Ltd., 1-3-5 Taito, Taito-ku, Tokyo 110-0016, Japan*

[e]*Department of Pharmaceuticals, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-80, Japan*

**Correspondence to: Kazutaka Hayashi, Molecular Imaging Integration Unit, RIKEN Center for Molecular Imaging Science (CMIS), 6-7-3 Minatojima-minamimachi, Chuo-ku, Hyogo 650-0047, Japan.*
E-mail: hayashik@riken.jp

[^{18}F]fluoroazomycin arabinoside ([^{18}F]FAZA) with a small amount of precursor and base.[13]

In this study, we investigated the optimal ^{18}F-labeling conditions for automated synthesis with a small amount of *p*-MPPNO$_2$ and developed an efficient synthesis method, as well as an improved preparative HPLC separation method, for rapid and high-yield synthesis of *p*-[^{18}F]MPPF with the National Institute of Radiological Science (NIRS) synthesis system.

Results and discussion

Automated synthesis of *p*-[^{18}F]MPPF

The automated synthesis procedure included the separation of [^{18}F]F$^-$ from the irradiated [^{18}O]H$_2$O, azeotropic drying of [^{18}F]F$^-$, nucleophilic [^{18}F]fluorination with *p*-MPPNO$_2$, preparative HPLC separation of *p*-[^{18}F]MPPF, and formulation of *p*-[^{18}F]MPPF.

Generally, ^{18}F-labeling conditions of *p*-[^{18}F]MPPF starts from *p*-MPPNO$_2$ and requires high temperature (140–150 °C) and long reaction time (20 min).[8,9] In a preliminary study, we have used 50% MeCN(aq) containing Kryptofix 222 (K.222; 7.5 mg) and potassium carbonate (K_2CO_3; 2.3 mg) as the eluent. We performed the automated synthesis of *p*-[^{18}F]MPPF with precursor (3 mg), K.222 (7.5 mg), and K_2CO_3 (2.3 mg) at 130 °C for 20 min or at 150 °C for 10 min. At reaction temperature of 130 °C for 20 min, we obtained 14.7 ± 3.2% radiochemical yield of *p*-[^{18}F]MPPF. Then, at reaction temperature of 150 °C for 10 min, we obtained 18.6 ± 3.6% radiochemical yield of *p*-[^{18}F]MPPF. As shown in Table 1, in the [^{18}F]fluorination reactions with precursor (3 mg), K.222 (7.5 mg), and K_2CO_3 (2.3 mg), the best radiochemical yield of *p*-[^{18}F]MPPF was 23.4 ± 2.6% by heating at 150 °C for 20 min. From these results, we decided to use the starting ^{18}F-labeling conditions (150 °C, 20 min).

In this study, we investigated the effects of the reaction temperature and the amount of base on a radiochemical yield of *p*-[^{18}F]MPPF. Two different base concentrations were used, K.222 (2.3 mg)/K_2CO_3 (0.7 mg) in 70% MeCN(aq) (0.4 mL) and K.222 (7.5 mg)/K_2CO_3 (2.3 mg) in 50% MeCN(aq) (0.4 mL). The former base concentration was used in our previously reported method,[13] and the latter base concentration has been used for the routine production of various ^{18}F-labeled radiopharmaceuticals at the NIRS.

The [^{18}F]fluorination reactions were performed with 3 mg of *p*-MPPNO$_2$ in DMSO (0.4 mL) at 150, 170, or 190 °C for 20 min. The results are shown in Table 1. The [^{18}F]fluorination yield strongly depended on the amount of base and reaction temperature. The use of K.222 (7.5 mg)/K_2CO_3 (2.3 mg) led to a lower radiochemical yield along with increased reaction temperature. At reaction temperatures of 150 and 170 °C, we obtained 23.4 ± 2.6% and 13.8 ± 3.0% radiochemical yields of *p*-[^{18}F]MPPF, respectively. However, at 190 °C, the peak corresponding to *p*-[^{18}F]MPPF could not be detected by preparative HPLC separation. On the other hand, the use of K.222 (2.3 mg)/K_2CO_3 (0.7 mg) led to a higher radiochemical yield along with the reaction temperature. At reaction temperatures of 150, 170, and 190 °C, we obtained *p*-[^{18}F]MPPF at 22.4%, 28.7 ± 3.1%, and 38.6 ± 5.0% radiochemical yields, respectively. We could achieve the best result of 38.6 ± 5.0% of *p*-[^{18}F]MPPF by the reaction at 190 °C with K.222 (2.3 mg)/K_2CO_3 (0.7 mg) and 3 mg of *p*-MPPNO$_2$ in DMSO (0.4 mL) for 20 min. The present work showed a quite different tendency of *p*-[^{18}F]MPPF yield along with the reaction temperature of the two different base concentrations, which was probably due to the decomposition of *p*-MPPNO$_2$ in the presence of excess K.222/K_2CO_3 at high reaction temperature. The residual amount of *p*-MPPNO$_2$ was <1% with K.222 (7.5 mg)/K_2CO_3 (2.3 mg) and >50% with K.222 (2.3 mg)/K_2CO_3 (0.7 mg), respectively, after a 20-min reaction at 190 °C. Furthermore, this might have been the result of the decomposition of the produced *p*-[^{18}F]MPPF itself in the presence of excess K.222/K_2CO_3 at high reaction temperature, as in the case of [^{18}F]flumazenil.[15,16]

Table 1. Effect of reaction temperature and base concentration on *p*-[^{18}F]MPPF yield

	Radiochemical yield*	
	Concentration of K.222/K_2CO_3	
Temperature (°C)	K.222 (2.3 mg)/K_2CO_3 (0.7 mg)	K.222 (7.5 mg)/K_2CO_3 (2.3 mg)
150	22.4% (n = 1)	23.4 ± 2.6% (n = 3)
170	28.4 ± 3.1% (n = 3)	13.8 ± 3.0% (n = 3)
190	38.9 ± 5.0% (n = 5)	0% (n = 2)≅

p-[^{18}F]MPPF injection yield after decay corrected.
≅*p*-[^{18}F]MPPF was not detected by preparative HPLC separation.

Preparative HPLC separation of *p*-[^{18}F]MPPF

Before, we used the NIRS sample injection unit (NIRS-SX.01) to inject the reaction mixture onto an HPLC column, which allowed injection of all the reaction mixture, even if it contained bubbles. The disadvantage of this method was that the reaction mixture was injected onto the column through the HPLC pump from the reservoir after slight dilution with an eluent, which might have broadened the corresponding radioactivity peak. The peak broadening is disadvantageous here because the preparative HPLC separation is relatively difficult.

In this study, a new injection method applying the principle of the air-lock phenomenon was used to stop liquid transfer when a pressurized gas reached the filter surface,[17] in which the reaction mixture was directly transferred to an HPLC injection loop through a Millex-LG filter (Millipore, Billerica, MA, USA; 0.20 μm). The injector valve was turned on after the liquid flow was stopped by the air lock on the filter, and then the preparative HPLC separation was carried out with a Cholester column. The remaining radioactivity in the Millex-LG filter was 3.1 ± 1.1% (decay corrected, on the basis of starting [^{18}F]F$^-$, n = 5). The major advantages of this method were its simplicity, rapidity, and reliability, without broadening the corresponding radioactivity peak. In addition, it prevented air bubbles from entering the preparative HPLC column.

In the preparative HPLC separation, C18 columns have been used most widely. However, C18 columns might provide insufficient separation of compounds with similar hydrophobicity because the main separation mechanism of the C18 column is based on hydrophobic interaction. The separation of such compounds might be improved by using different packing materials that retain compounds on the basis of a secondary interaction in addition to a hydrophobic interaction. We used a COSMOSIL Cholester column (Nacalai Tesque, Kyoto, Japan), which is a new silica-based reversed-phase column. This column could

provide better selectivity for closely related compounds, particularly for geometrical or positional isomers, than traditional C18 columns.[18,19] The preparative HPLC chromatograms of the crude product are shown in Figure 1. With the use of the traditional C18 column (COSMOSIL MS-II, 10 mm i.d. × 250 mm, 5 μm), the retention times of *p*-[^{18}F]MPPF and *p*-MPPNO$_2$ were 9.6 and 10.4 min, respectively, and the contamination of *p*-MPPNO$_2$ in the final product was minimal (1.1–3.1 nmol/mL; Figure 1(a)). In contrast, with the use of the COSMOSIL Cholester column, the retention times of *p*-[^{18}F]MPPF and *p*-MPPNO$_2$ were 9.5 and 11.1 min, respectively, and the contamination of *p*-MPPNO$_2$ in the final product was negligible (below the detection limit; Figure 1(b)). Compared with the traditional C18 column, the use of the COSMOSIL Cholester column could avoid the contamination of *p*-MPPNO$_2$ in the final product.

Le Bars *et al.*[9] reported that the preparative HPLC conditions described by Shiue *et al.*[8] (Spherisorb ODS, 10 mm i.d. × 250 mm, 5 μm; Waters; mobile phase: MeCN/4 mM $(NH_4)_2HPO_4$ = 70/30; flow rate: 7.5 mL/min; wavelength: 254 nm) failed to separate *p*-[^{18}F]MPPF from *p*-MPPNO$_2$ in their laboratory. Their results may be due to using a large amount of *p*-MPPNO$_2$ (10 mg). Therefore, in the preparative HPLC separation of *p*-[^{18}F]MPPF from *p*-MPPNO$_2$, it may be useful to use a smaller amount of *p*-MPPNO$_2$.

Our result seems to be similar to the reported result of Le Bars *et al.*[9] that the retention times of *p*-[^{18}F]MPPF and *p*-MPPNO$_2$ were 15 and 24 min, respectively. On the other hand, they extracted the crude *p*-[^{18}F]MPPF in DMSO with a Sep-Pak tC18 cartridge (Waters, Milford, MA, USA) before the preparative HPLC separation and injected the eluate from the cartridge into an HPLC system. Koivula *et al.* reported that the preparative HPLC conditions described by Le Bars *et al.*[9] failed occasionally to separate *p*-[^{18}F]MPPF from the radioactive by-products with close retention time because the crude *p*-[^{18}F]MPPF in DMSO was diluted with the mobile phase and injected straight into an HPLC system.[20] In contrast, we diluted the crude *p*-[^{18}F]MPPF in DMSO with 20% MeCN(aq) and injected it straight into an HPLC system. In all batches of the final product, radioactive impurities with retention times of 7.6 and 10.8 min were not contaminating (Figure 1(b)). It might be suitable for the routine synthesis of *p*-[^{18}F]MPPF because our preparative HPLC method is simple, rapid, and reliable.

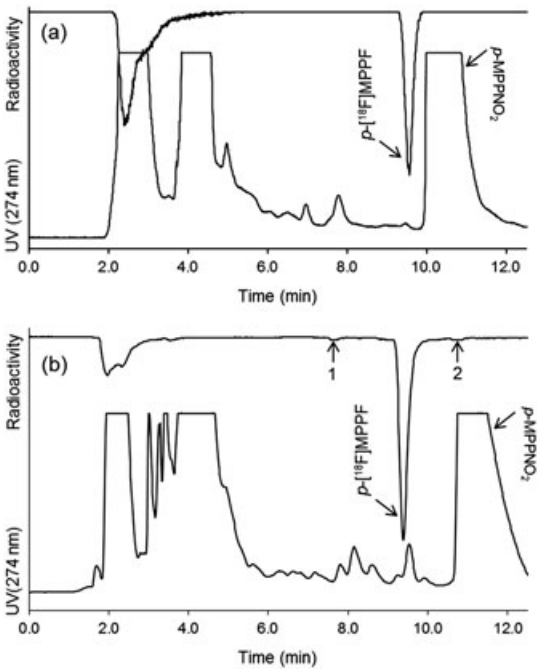

Figure 1. Preparative HPLC separation of *p*-[^{18}F]MPPF (mobile phase: MeCN/25 mM $AcONH_4$/AcOH = 200/300/0.15, flow rate: 6.0 mL/min, wavelength: 274 nm): (a) column: COSMOSIL MS-II, *p*-[^{18}F]MPPF: retention time = 9.6 min, *p*-MPPNO$_2$: retention time = 10.4 min; (b) column: COSMOSIL Cholester, *p*-[^{18}F]MPPF: retention time = 9.5 min, *p*-MPPNO$_2$: retention time = 11.1 min. Peak 1 (retention time = 7.6 min) and peak 2 (retention time = 10.8 min) are unknown radioactive impurities.

Quality control of *p*-[^{18}F]MPPF

The retention times of *p*-[^{18}F]MPPF and *p*-MPPNO$_2$ were 2.8 and 3.1 min, respectively. In all batches of the final product, the contamination of *p*-MPPNO$_2$ was below the detection limit.

After proton bombardment (18 MeV, 15 μA) of [^{18}O]H_2O for 20 min, the radioactivity and the specific activity of *p*-[^{18}F]MPPF were 1.6 ± 0.2 GBq/vial and 214.3 ± 21.1 GBq/μmol, respectively. The radiochemical purity determined with HPLC was >99%. The pH of *p*-[^{18}F]MPPF ranged from 5.4 to 5.8.

Materials and methods

The nitro-precursor, *p*-MPPNO$_2$, and the reference standard, *p*-MPPF, were purchased from ABX Advanced Biomedical Compounds (Radeberg, Germany). K.222 and K_2CO_3 were purchased from Merck (Darmstadt, Germany). Anhydrous MeCN and anhydrous DMSO were purchased from Wako Pure Chemical Industries (Osaka, Japan). Pharmaceutical grades of 25% ascorbic acid injection, polysorbate 80, and EtOH were used. All chemicals were obtained from commercial sources and used without further purification. Accell plus QMA anion exchange resin (bulk packing material) was purchased from Waters (Milford, MA, USA). The empty cartridge (Luer lock type cartridge, type mini, 0.1 mL) for anion exchange cartridge was purchased from Tomoe Works (Hyogo, Japan). We prepared a small QMA cartridge for eluting [^{18}F]F^- with a small amount of K_2CO_3. The empty cartridge was filled with about 25 mg of QMA resin (small QMA cartridge),[13] which was activated with 5 mL of 1 M K_2CO_3 solution and then washed with 15 mL of sterile water for injection prior to use.

Production of [^{18}F]fluoride

No-carrier-added [^{18}F]fluoride ([^{18}F]F^-) was obtained through the nuclear reaction of ^{18}O(*p*,*n*)^{18}F by irradiation of ^{18}O-enriched water ([^{18}O]H_2O) target (>98% isotopic enrichment; Taiyo Nippon Sanso, Tokyo, Japan) with an 18 MeV proton beam in a BC2010 cyclotron ([^{18}O]H_2O volume: ~1.0 mL; Japan Steel Works, Muroran, Japan) at the NIRS.

Synthesis module

The fully automated synthesis of *p*-[^{18}F]MPPF was carried out using a novel NIRS synthesis module (main synthesis unit: NIRS-SX.01; fluorination unit: NIRS-SF.02; Dainippon Seiki, Kyoto, Japan), which was connected through an injection unit (NIRS-SX.08) to an HPLC purification unit and a formulation unit (NIRS-SX.03). The HPLC purification unit consisted of a pump (PU-2080; Jasco, Tokyo, Japan), a UV detector (UV-2075; Jasco), a column (COSMOSIL Cholester, 10 mm i.d. × 250 mm, 5 μm), and a NaI(Tl) radioactivity detector (Oyokoken, Tokyo, Japan). A PEEK tube loop (0.125 mm i.d. × 600 mm) was inserted between the terminal end of the small QMA

cartridge and the three-way valve for reducing fluid velocity of solutions, that is, (1) the irradiated [^{18}O]H_2O containing [^{18}F]F^- and (2) 70% MeCN(aq) containing K.222 and K_2CO_3 (Figure 2). The sequence program of the whole system, including the main synthesis unit, the fluorination unit, the HPLC purification unit, and the formulation unit, was also modified to reflect the succeeding modification and reaction conditions optimized by automated experiments.

Automated synthesis of *p*-[^{18}F]MPPF

Before starting synthesis, K.222 (2.3 mg) and K_2CO_3 (0.7 mg) in 70% MeCN(aq) (0.4 mL), anhydrous MeCN (0.2 mL), *p*-MPPNO_2 (3 mg) in anhydrous DMSO (0.4 mL), 20% MeCN(aq) (1.0 mL), saline (10 mL), and 25% ascorbic acid injection (0.1 mL) + polysorbate 80/EtOH (1/4 (v/v); 375 μl) were injected into vials A to F in the synthesis module, respectively (Figure 2).

The automated synthesis of *p*-[^{18}F]MPPF was performed according to the synthesis scheme (Figure 3). We modified the ^{18}F-labeling conditions described by Shiue *et al.*[8] In fact, we reduced the amount of precursor from 4 mg (8.7 μmol) to 3 mg (6.5 μmol) and increased ^{18}F-labeling temperature to 190 °C from 150 °C.

No-carrier-added [^{18}F]F^- (3.4–5.3 GBq) from the cyclotron was isolated from [^{18}O]H_2O by trapping on a small QMA cartridge and eluted slowly with a mixture of K.222 (2.3 mg) and K_2CO_3 (0.7 mg) in 70% MeCN(aq) (0.4 mL) in a reaction vessel pushed by He pressure (3 mL/min). The [^{18}F]F^- solution was evaporated under a He gas stream (250 mL/min) at 120 °C. The residue was dried by azeotropic evaporation with anhydrous MeCN (0.2 mL) to ensure anhydrous reaction conditions for fluorine labeling. The precursor, *p*-MPPNO_2 (3 mg, 6.5 μmol), dissolved in anhydrous DMSO (0.4 mL), was added to the reaction vessel; the mixture was heated at 190 °C for 20 min. After the reaction mixture had been cooled to 90 °C, 20% MeCN(aq) (1.0 mL) was added to the reaction vessel. The reaction mixture was passed through a 0.20 μm filter (Millex-LG, 13 mm) by He pressure and then was introduced into a 3 mL sample loop of an injector valve (NIRS-SX.08). The injector valve was turned on when the He gas flow was stopped by the air-lock phenomenon on the filter, to inject the reaction mixture onto the HPLC column (mobile phase: MeCN/25 mM $AcONH_4$/AcOH = 200/300/0.15; flow rate: 6.0 mL/min; wavelength: 274 nm). The peak corresponding to *p*-[^{18}F]MPPF (retention time = 9.4 min) was collected into a rotary evaporator flask containing 25% ascorbic acid injection (0.1 mL) and polysorbate 80/EtOH (1/4 (v/v); 375 μl). The solvent was removed *in vacuo* at 150 °C, and the residue was dissolved in saline (10 mL) and then passed through a 0.22 μm sterile filter (vented Millex-GS; Millipore) to give the final product. All the aforementioned procedures were performed using a remote processing unit (NIRS-PC.001; Noie, Sapporo, Japan).

Quality control of *p*-[^{18}F]MPPF

The radioactivity of the final product was measured with a dose calibrator (Curiemeter IGC-7; Aloka, Tokyo, Japan). The chemical

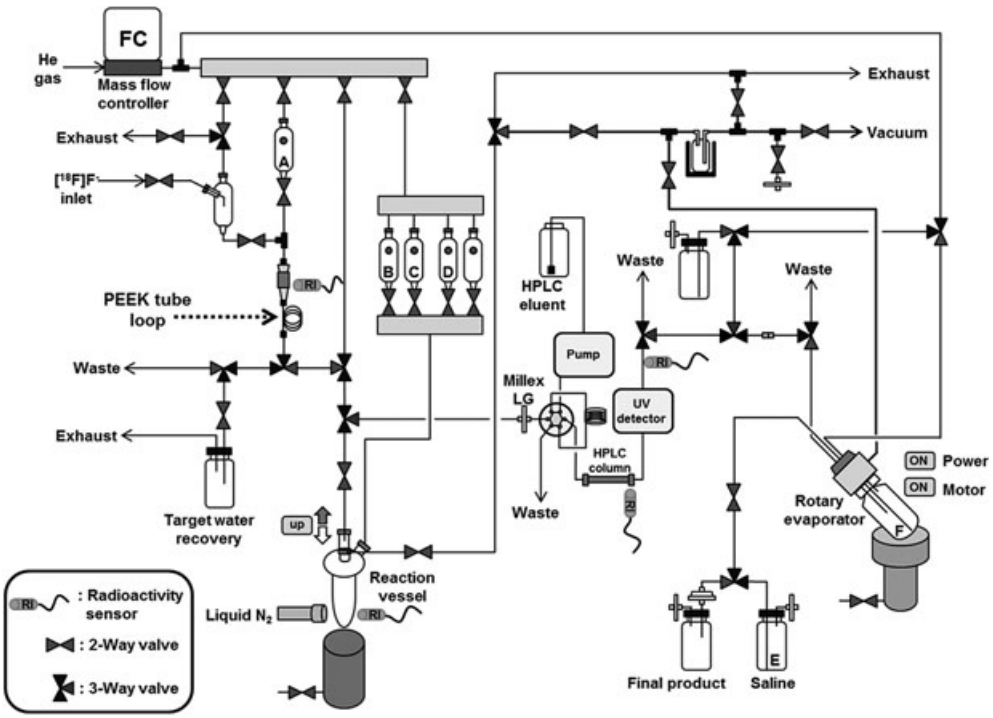

Figure 2. Schematic diagram of the automated synthesis of *p*-[^{18}F]MPPF.

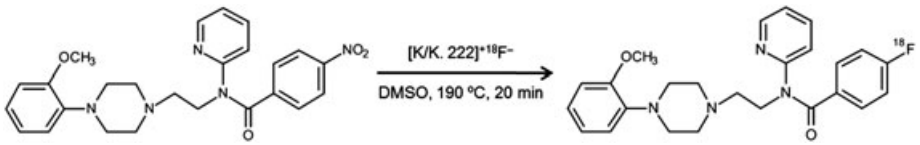

Figure 3. Reaction scheme for the synthesis of *p*-[^{18}F]MPPF.

purity, the radiochemical purity, and the specific activity were determined using an analytical HPLC system that consisted of a pump (515; Waters), a UV detector (2487; Waters), a sample injector valve (VICI AG International, Schenkon, Switzerland) with a 5 μl loop, and a NaI(Tl) radioactivity detector (ORTEC ACE Mate 925-SCINT; EG&G, München, Germany).[14] *p*-[^{18}F]MPPF was analyzed using an analytical reversed-phase column (XBridge RP_{18}, 3.0 mm i.d. × 50 mm, 2.5 μm; Waters) with 90(v/v)% MeCN/50 mM ammonium phosphate buffer (pH 9.3) = 2/3 (v/v) at a flow rate of 1.0 mL/min. The effluent from the column was monitored by UV absorption (274 nm) and radioactivity. The pH of the final product was measured by a pH meter (Orion 720Aplus; Thermo Fisher Scientific, Waltham, MA, USA) coupled with a micro-pH electrode (Orion 9863BN; Thermo Fisher Scientific).

Conclusion

The automated synthesis of *p*-[^{18}F]MPPF with a small amount of precursor was successfully accomplished with the optimized reaction condition and the improved HPLC separation. With the use of the NIRS synthesis system, *p*-[^{18}F]MPPF was synthesized with a radiochemical yield of 38.6 ± 5.0% (decay corrected, $n = 5$), a specific activity of 214.3 ± 21.1 GBq/μmol at EOS, a radiochemical purity of >99%, and a total synthesis time of 54.6 ± 1.3 min. This synthesis method was rapid, simple and reliable, and gave a consistently high radiochemical yield. With the use of the Cholester column instead of the traditional C18 column, *p*-[^{18}F]MPPF could be separated rapidly from the closely related *p*-$MPPNO_2$ and the radioactive by-products with a close retention time.

The preparative HPLC separation by using this column is promising for the routine production of other ^{18}F-labeled radiopharmaceuticals, such as [^{18}F]altanserin and [^{18}F]flumazenil, starting from the corresponding nitro-precursor.

Conflict of Interest

The authors did not report any conflict of interest.

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