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Synthesis of [^{18}F]-Labeled (6-Fluorohexyl)triphenylphosphonium Cation as a Potential Agent for Myocardial Imaging using Positron Emission Tomography

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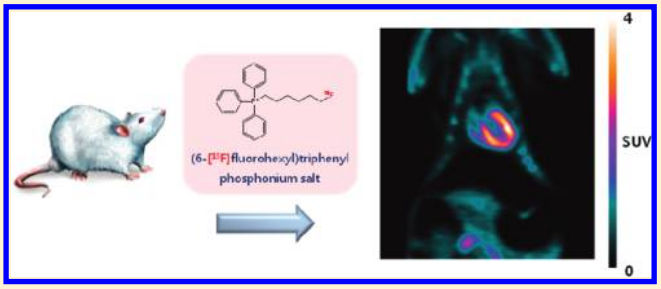
Supporting Information

ABSTRACT: Lipophilic cations such as phosphonium salts penetrate the hydrophobic barriers of the plasma and mitochondrial membranes and accumulate in mitochondria in response to the negative inner-transmembrane potentials. Thus, as newly developed noninvasive imaging agents, [^{18}F]-labeled phosphonium salts may serve as molecular "voltage sensor" probes to investigate the role of mitochondria, particularly in myocardial disease. The present study reports the radiosynthesis of (6-fluorohexyl)triphenylphosphonium salt (**3**) as a potential agent for myocardial imaging by using positron emission tomography (PET). The reference compound of (6-[^{18}F]fluorohexyl)triphenylphosphonium salt ([^{18}**F**]**3**) was synthesized with 74% yield via three-step nucleophilic substitution reactions. The reference compound was radiolabeled via two-step nucleophilic substitution reactions of no-carrier-added [^{18}F]fluoride with the precursor hexane-1,6-diyl bis(4-methylbenzenesulfonate) in the presence of Kryptofix 2.2.2 and K_2CO_3. The radiolabeled compound was synthesized with 15−20% yield. The radiochemical purity was >98% by analytical HPLC, and the specific activity was >6.10−6.47 TBq/μmol. The cellular uptake assay showed preferential uptake of [^{18}**F**]**3** in cardiomyocytes. The results of biodistribution and micro-PET imaging studies of [^{18}**F**]**3** in mice and rats showed preferential accumulation in the myocardium. The results suggest that this compound would be a promising candidate for myocardial imaging.



INTRODUCTION

Nuclear medicine imaging by single-photon emission tomography (SPECT) using [^{99m}Tc]-sestamibi, [^{99m}Tc]-tetrofosmin, or ^{201}thallium has been validated extensively. SPECT is a widely used tool for the assessment of myocardial infarction (MI) size[1,2] for the risk stratification of patients. It also is an attractive surrogate end point instead of mortality for determining the efficacy of therapeutic strategies in clinical trials.[1] However, the technical limitations of SPECT imaging, such as low spatial resolution and the assessment of only relative tracer inhomogeneities resulting from soft tissue attenuation or scatter, may compromise the delineation of small infarcts and the diagnostic accuracy of SPECT.[2]

Positron emission tomography (PET) has several technical advantages over SPECT, such as higher spatial resolution. Owing to accurate attenuation correction, PET can provide quantitative measures of myocardial tracer uptake.[3] However, the short half-life of currently used PET tracers for myocardial imaging, including [^{13}N]-ammonia, ^{82}rubidium, and [^{15}O]-water, limit the widespread clinical use of PET due to the need for a nearby cyclotron or generator. Because of their longer half-life, [^{18}F]-labeled tracers would avoid this limitation and facilitate clinical protocols.

To address this need, we developed a [^{18}F]-labeled phosphonium cation.[4] Similar to SPECT tracers such as [^{99m}Tc]-sestamibi and [^{99m}Tc]-tetrofosmin, phosphonium cations accumulate in cardiomyocytes to a higher degree than in normal cells.[5] This phenomenon is attributed to the highest density and higher electrochemical membrane potential of the mitochondria in cardiomyocytes.[5−7] Loss of the mitochondrial membrane potential is an early event in cell death caused by myocardial ischemia.[8] Cumulative evidence suggests that mitochondria-controlled apoptosis underlies cell loss in heart failure.[9] Previous publications have reported that [^{18}F]-labeled phosphonium cations such as [^{18}F]-fluorobenzyl triphenylphosphonium ([^{18}F]-FBnTP) are metabolically stable and demonstrate excellent characteristics as cardiac imaging agents in healthy and coronary artery disease models.[5,7,10]

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Herein, we report the synthesis and characterization of a novel [^{18}F]-labeled phosphonium cation, (6-[^{18}F]fluorohexyl)-triphenylphosphonium salt ([^{18}F]**3**) (Figure 1). Biological

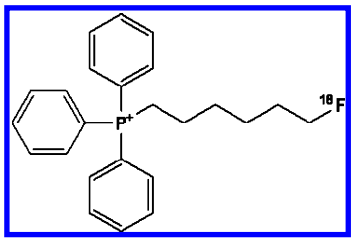

Figure 1. Schematic structure of (6-[^{18}F]fluorohexyl)-triphenylphosphonium salt ([^{18}F]**3**).

studies, such as a cellular uptake assay, biodistribution studies, and micro-PET imaging, were performed to test and optimize the kinetics for radiolabeled phosphonium cation.

EXPERIMENTAL PROCEDURES

General. All commercial reagents and solvents were purchased from Sigma-Aldrich or Merck, were of analytical grade, and were used without further purification. [^{3}H]-tetraphenylphosphonium ([^{3}H]-TPP) was purchased from Moravek Biochemicals, Inc. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECA-500 FT-NMR spectrometer (Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute). All chemical shifts were reported on the ppm scale with tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL JMS-AX505WA spectrometer. Compounds were measured by electrospray ionization (ESI) and fast atom bombardment (FAB) methods at the National Center for Interuniversity Research Facilities (NCIRF). Gravity column chromatography was performed on Merck silica gel 60 (70−230 mesh ASTM). Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} glass plates and was visualized by UV light. Purification was achieved by HPLC, with a SP930D pump, UV730D UV detector (Young-Lin Inc., Korea), and FC-3200 high energy gamma detector (Bioscan, USA) to measure the radioactive flow. The UV detection wavelength was 254 nm for all experiments. Both semipreparative (Phenomenex Luna, C18, 10 mm × 250 mm) and analytical (Waters Atlantis C18, 4.6 mm × 250 mm) reverse-phase HPLC columns were used. A CRC-712MH radioisotope calibrator (Capintec Instruments, USA) was used for radioactivity measurements. [^{18}F] analysis was performed with a 1480 WIZARD 3 gamma counter (Perkin-Elmer, USA), and [^{3}H] was measured with an LS 6500 liquid scintillation counter (Beckman, USA). Normal rats were imaged and analyzed by micro-PET (Inveon, Siemens). No-carrier-added (n.c.a) [^{18}F]fluoride was produced on a PETtrace cyclotron (16.4 MeV, General Electric Company, USA) by irradiation of a [^{18}O]H_2O water target.

Synthesis of Hexane-1,6-diyl Bis(4-methylbenzenesulfonate) (1). Compound **1** was prepared by the modification of a previously reported method.[11] Hexane-1,6-diol (1.77 g, 15.0 mmol) in 30.0 mL of anhydrous pyridine was added to 4-methylbenzene-1-sulfonyl chloride (8.58 g, 45.0 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h, quenched with 3.0 mL of water, and stirred for a further 30 min. Methylene chloride and 1.0 M HCl were added to the reaction mixture, and the pyridine was extracted from the organic phase. The organic phase was washed twice with water and brine, dried over sodium sulfate, and filtered. After evaporation of the solvent, the solution was purified by column chromatography (methylene chloride:*n*-hexane:acetone = 48:50:2) and recrystallized from methylene chloride:*n*-hexane to yield 5.12 g (80%) of compound **1**. mp 77−79 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25−1.27 (m, 4H), 1.57−1.60 (m, 4H), 2.45 (s, 6H), 3.97 (t, J = 6.3 Hz, 4H), 7.34 (d, J = 8.4 Hz, 4H), 7.77 (d, J = 8.4 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.60, 21.75, 28.26, 70.08, 127.96, 130.00, 133.01, 144.96; HRMS (ESI) m/z calculated for $C_{20}H_{26}O_6S_2Na$ $[M+Na]^+$ 449.1064; found 449.1068.

Synthesis of 6-Fluorohexyl 4-Methylbenzenesulfonate (2). Anhydrous acetonitrile (3.0 mL) was added to tetrabutylammonium fluoride trihydrate (TBAF, 1.43 g, 4.54 mmol). The mixture was evaporated under reduced pressure to remove the water. This procedure was repeated twice. Compound **1** (1.94 g, 4.54 mmol) in 10.0 mL of anhydrous acetonitrile was added to the reaction flask. The mixture was stirred for 4 h at 85 °C in a closed tube. The solvent was evaporated under reduced pressure. Column chromatography (methylene chloride:*n*-hexane:acetone = 49:50:1) provided 0.60 g (48% yield) of compound **2** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34−1.35 (m, 4H), 1.58−1.65 (m, 4H), 2.44 (s, 3H), 4.02 (t, J = 6.3 Hz, 2H), 4.39 (dt, J = 47.3, 6.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.73, 24.74, 28.82, 30.16, 70.49, 83.95, 127.98, 129.92, 133.21, 144.81; MS (FAB) m/z 275 $[M+H]^+$, 173 (100); HRMS (FAB) m/z calculated for $C_{13}H_{20}FO_3S$ $[M+H]^+$ 275.1117; found 275.1120.

Synthesis of (6-Fluorohexyl)triphenylphosphonium Salt (3). Compound **3** was prepared by the modification of a previously reported method.[12] Triphenylphosphine (1.0 g, 3.81 mmol) dissolved in 10.0 mL anhydrous acetonitrile was added to compound **2** (1.05 g, 3.81 mmol). The solution was refluxed for 19 h. The solvent was evaporated under reduced pressure. Column chromatography (methylene chloride:methanol:ethyl acetate = 8:1:1) provided 1.03 g (74%) of compound **3** as a powder. Retention factor (R_f): 0.45−0.50 (methylene chloride:methanol = 9:2); mp 220−223 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31−1.36 (m, 2H), 1.50−1.61 (m, 6H), 2.28 (s, 3H), 3.56−3.61 (m, 2H), 4.33 (dt, J = 47.2, 6.1 Hz, 2H), 7.04 (d, J = 7.8 Hz, 2H), 7.63−7.79 (m, 17H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.60, 22.01, 22.56, 24.75, 29.90, 84.03, 118.08, 118.75, 126.22, 128.32, 130.55, 133.61, 135.03. 138.50, 144.67; MS (FAB) m/z 365 $[M]^+$, 365 (100); HRMS (FAB) m/z calculated for $C_{24}H_{27}FP$ $[M]^+$ 365.1834; found 365.1834.

Radiolabeling of (6-Fluorohexyl)-triphenylphosphonium Salt ([^{18}F]3). [^{18}F]Fluoride was produced by an ^{18}O(p, n)^{18}F reaction on a PETtrace cyclotron. The activity was extracted from $H_2{}^{18}O$ by an anion exchanger and was eluted by aqueous potassium carbonate (25.0 mmol) into the reaction vessel. The radioactive solution was dried together with 4.0 mg of Kryptofix 2.2.2 in 1.0 mL of acetonitrile under nitrogen at 100 °C. The solution was evaporated at 100 °C by bubbling nitrogen gas. The residue was dried by azeotropic distillation with acetonitrile (1 mL, 3 times). Then, 4.0 mg of compound **1** dissolved in 1.0 mL of anhydrous acetonitrile were added. The mixture was heated for 5 min at 90 °C in the closed state. Radio-TLC showed a >80% yield of compound [^{18}F]**2**. The solution was passed through a small silica Sep-Pak cartridge. Six milligrams of triphenylphosphine dissolved in 1.0 mL of toluene were added to the reaction

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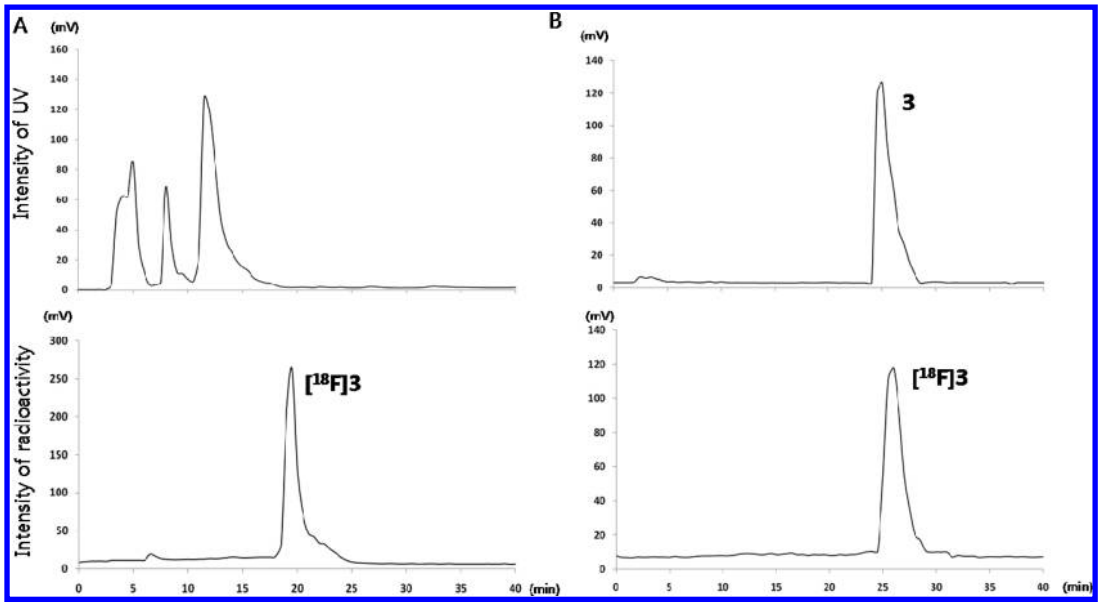

Figure 2. (A) Purification of [^{18}F]**3** on semipreparative HPLC. The reaction mixture was injected on a C18 column and was eluted with acetonitrile: phosphate-buffered saline = 50:50 at a flow rate of 3.0 mL/min. UV impurities were not observed. (B) Analytical HPLC chromatogram of [^{18}F]**3** coinjected with the nonradioactive compound **3**.

vessel, and the solution was heated to 220 °C for 3 min with no separation step (Scheme 2).

The solution was cooled and injected into a semipreparative HPLC column system for purification (mobile phase, acetonitrile:phosphate-buffered saline = 50:50; flow rate, 3 mL/min; 254 nm; t_R: 19.5 min) (Figure 2A). For identification of the radioproduct, the collected HPLC fraction was coinjected with cold compound **3**. [^{18}F]**3** was dried, made isotonic with sodium chloride, and passed through a 0.20 μm membrane filter into a sterile multidose vial for in vitro and in vivo experiments. The total reaction time of [^{18}F]**3** was within 60 min, and the overall decay-corrected radiochemical yield was approximately 15−20%. Radiochemical purity was >98% by the analytical HPLC system (with the same isocratic as used for the semipreparative HPLC system). The specific activity was >6.10−6.47 TBq/μmol (Figure 2B).

In Vitro Stability Study. For the labeling stability test, [^{18}F]**3** (0.37 MBq/100 μL) was incubated with human serum (1.0 mL) in a 37 °C water bath for 4 h and then was analyzed by chromatography on ITLC-sg strips developed with methylene chloride:methanol = 9:2. After they were developed, the chromatographic strips were scanned on an automatic TLC scanner, and TLC was performed at various time points. All experiments were performed in triplicate.

Partition Coefficient. After the complete removal of volatiles of [^{18}F]**3**, the residue was dissolved in a mixture of 3 mL of saline and 3 mL of *n*-octanol in a round-bottom flask. The mixture was vigorously stirred for 20 min at room temperature and then was transferred to a 15 mL Falcon conical tube. The tube was centrifuged at 3000 rpm for 5 min. Samples in triplets from *n*-octanol and aqueous layers were obtained and were counted on a gamma counter. The log *P* value was reported as an average of the data obtained in three independent measurements.[13]

Cell Lines, Culture Conditions, and Cell Uptake Studies. [^{18}F]**3** accumulation was measured in cell culture with rat embryonic cardiomyoblast (H9c2) and mouse normal fibroblast (NIH/3T3) cell lines. All cells were grown in Deficient DMEM (Dulbecco's modified Eagle medium) High Glucose (containing 5.3 mmol/L KCl and 110.34 mmol/L NaCl; Life Technologies) plus 5% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine. Cells were incubated at 37 °C for 0.5, 1, or 2 h with radioactive culture medium and [^{18}F]**3** (0.74 MBq/200 μL). The radioactive medium was removed, and the radioactivity was measured. The wells were washed three times with cold phosphate-buffered saline. The cells were harvested, and the cell-associated radioactivity was determined. The mitochondrial membrane potential-dependent cellular uptake of [^{18}F]**3** was further assessed with H9c2 cells treated with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), which is a protonphore that selectively abolishes the mitochondrial membrane potential.[14] The compound was dissolved in dimethyl sulfoxide and diluted to the desired concentration with low K^+ HEPES buffer. The final concentration of dimethyl sulfoxide was <0.1%. CCCP solution (0.50 μM) was added to 5.0×10^4 cells at 30 min before the start of the experiment. After incubation with [^{18}F]**3**, radioactivity was determined by a gamma counter.[15] The mitochondrial membrane potentials of both cell lines were assessed by a cell uptake study with [^{3}H]-TPP (37 kBq/200 μL). The [^{3}H]-TPP uptake assay is a standard method for measuring mitochondrial membrane potential in vitro.[7] After incubation with [^{3}H]-TPP, radioactivity was determined with a liquid scintillation counter. Triplicate samples were obtained for all uptake studies. Data are expressed as the accumulation ratio (%) ± SD calculated by dividing the radioactivity in the pellet by the total radioactivity (supernatant + pellet).

Murine Biodistribution Studies. To confirm the activity of [^{18}F]**3** in vivo, biodistribution studies were performed with a murine model. Biodistribution in different organs was assessed in BALB/c mice (18−20 g, Orient, Kyunggido, Korea) at 10, 30, 60, and 120 min after i.v. injection of 7.4 MBq radiotracer. Blood, heart, lung, liver, spleen, stomach, intestine, kidney, pancreas, bone, and muscle were sampled from mice, and the radioactivity of each organ was measured with a gamma counter. Radioactivity determinations were normalized by the

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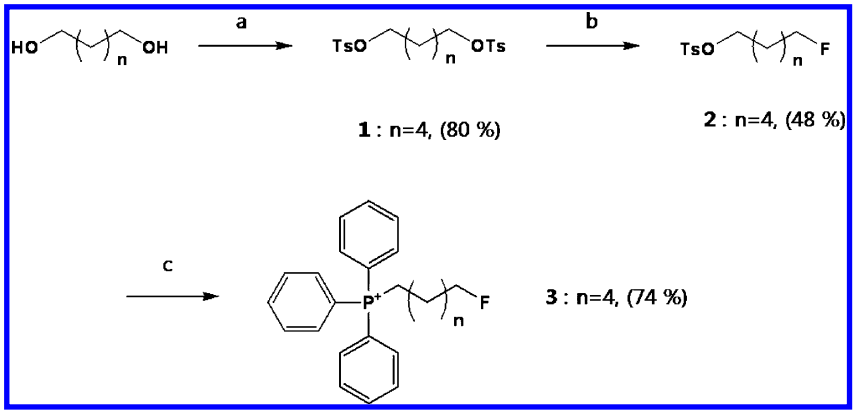
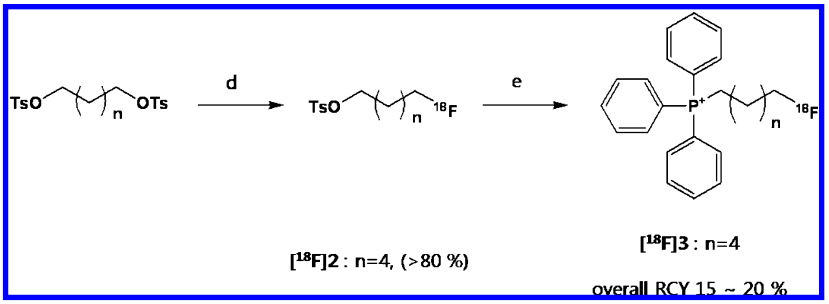
Scheme 1. Synthesis of (6-Fluorohexyl)triphenylphosphonium Salt[a]



[a]Reagents and conditions: (a) 4-methylbenzene-1-sulfonyl chloride, pyridine, room temp, 3 h; (b) tetra butyl ammonium fluoride, acetonitrile, 85 °C, 4 h; (c) triphenylphosphine, acetonitrile, reflux, 19 h.

Scheme 2. Radiosynthesis of (6-Fluorohexyl)triphenylphosphonium Salt[a]



[a]Reagents and conditions: (d) [^{18}F]KF, Kryptofix 2.2.2, acetonitrile, 90 °C, 5 min; (e) triphenylphosphine, toluene, 220 °C, 3 min.

weight of the tissue and the amount of radioactivity injected to obtain the % ID/g.

Micro-PET Imaging Studies. Normal rats were imaged with micro-PET at 0.5 and 1.0 h after i.v. injection of 37 MBq of [^{18}F]**3**. Eight-week-old male Sprague–Dawley rats (250–260 g, Orient, Kyunggido, Korea) were used for this study. Animal care, all experiments, and euthanasia were performed in accordance with protocols approved by the Chonnam National University Animal Research Committee and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 85–23, revised 1985). Rats were anesthetized by ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (2.5 mg/kg).

RESULTS AND DISCUSSION

Synthesis and Radiochemistry. The total synthesis of compounds **3** and [^{18}F]**3** is shown in Schemes 1 and 2. The reference compound was synthesized *via* three-step procedures and was analyzed by ^{1}H, ^{13}C NMR, and FAB high-resolution mass spectroscopy to confirm the identity.

For synthesis of the reference compound, compound **1** was first prepared by commercially available hexane-1,6-diol reacted with an excess of 4-methylbenzene-1-sulfonyl chloride in pyridine. Compound **1** was synthesized with an 80% yield and was treated thereafter with TBAF in acetonitrile, to generate the fluoro compound **2**. Finally, a nucleophilic substitution reaction was performed with triphenylphosphine, in which the *p*-tosyloxy residue was chosen as the leaving group. Methylene groups in the ω-position of the alkyl side chain were split into a doublet of triplets by fluorine in NMR ($^{2}J_{HF}$: 47.2 Hz). We also confirmed 4-methylbenzenesulfonate as counterions by NMR.

For synthesis of the [^{18}F]-labeled compound, **1** was reacted with nca [^{18}F]fluoride in the presence of potassium carbonate (K_2CO_3) and the phase-transfer agent Kryptofix 2.2.2. Then, [^{18}F]**2** was reacted with triphenylphosphine to yield the target compound with no separation step. After purification of the [^{18}F]-labeled target compound by semiprep HPLC, [^{18}F]**3** was compared with the reference compound by analytical HPLC to determine the identity (Figure 2A and B). Figure 2B revealed a single peak, suggesting the formation of one product ([^{18}F]**3**) identical to the reference compound. Radiochemical yield ranged 15–20% in the labeled compound (EOS; corrected for decay). Radiochemical purity was >98% by analytical HPLC. The specific activity was >6.10–6.47 TBq/μmol.

When the radiotracer (0.37 MBq [^{18}F]**3**) was incubated in human serum at 37 °C for 4 h, the percentage of the remaining [^{18}F]**3** was >95%, indicating the relatively high in vitro stability of the radiotracer (Supporting Information).

A previous publication has reported the synthesis of the (3-[^{18}F]fluoropropyl)triphenylphosphonium cation, with 12% radiochemical yield.[12] In this previous study, the whole radiosynthesis was completed in 56 min, with an average specific radioactivity of 15.5 GBq/μmol. The radiochemical characteristics of [^{18}F]**3** and (3-[^{18}F]fluoropropyl)-triphenylphosphonium cation were quite similar, except that [^{18}F]**3** had a significantly higher specific activity. In the current study, we further used the fluoroalkylphosphonium cation derivative ([^{18}F]**3**) in biological studies, including a cellular uptake assay, biodistribution studies, and micro-PET imaging.

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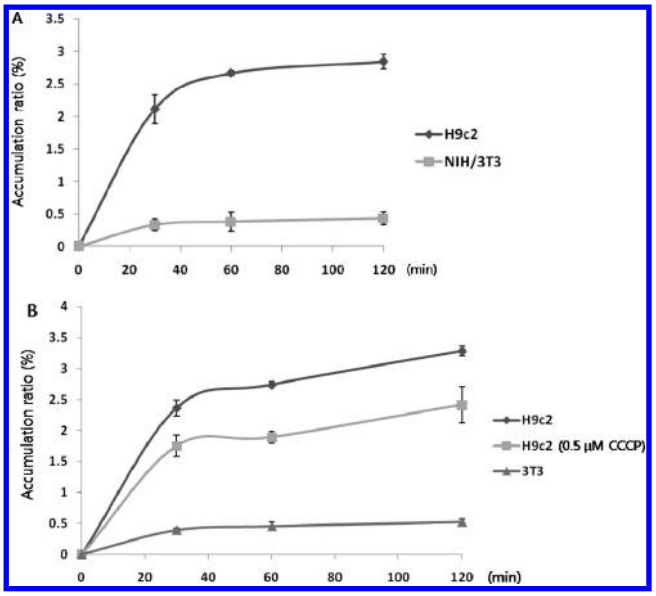

Figure 3. (A) Cell uptake of [^{3}H]-TPP in H9c2 and NIH/3T3 cells at 0.5, 1.0, or 2.0 h in a 37 °C incubator. Uptake by H9c2 cells was higher than uptake by NIH/3T3 cells for the entire time. This finding means that the mitochondria membrane potential of H9c2 cells was higher than that of NIH/3T3 cells. (B) Cell uptake of [^{18}F]**3** in H9c2 cells, H9c2 cells treated with 0.5 μM protonphore CCCP, and NIH/3T3 cells at 0.5, 1.0, or 2.0 h in a 37 °C incubator. Uptake by H9c2 cells was about 6-fold higher than uptake by NIH/3T3 cells. When incubated with 0.5 μM CCCP, the cellular uptake of [^{18}F]**3** was significantly inhibited ($P < 0.05$). All results are expressed as a percentage of applied radioactivity and are the means ± SD of three measurements.

Lipophilicity of Radiolabeled Compound. To assess the lipophilicity of [^{18}F]**3**, we measured the log P value of [^{18}F]**3**, which was 1.78 ± 0.05. Although there is little information available with regard to the optimal lipophilicity needed for the high myocardial selectivity of a radiotracer, a predictive model has been reported for the selective accumulation of phosphonium cations in myocardial cells.[16] For lipophilic cationic radiotracers (log P = 0.5−1.5) such as ^{99m}Tc-sestamibi

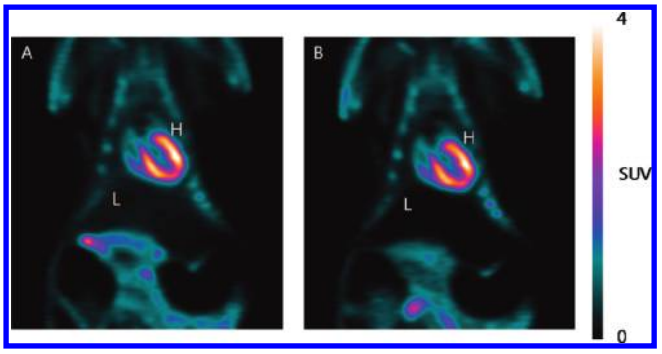

Figure 4. Micro-PET imaging (corona) of rats after i.v. injection of [^{18}F]**3**. Shown are images at 0.5 h (A) and 1.0 h (B) after injection of [^{18}F]**3**. H, heart; L, liver. The heart was visible with excellent heart-to-liver and heart-to-lung contrast at each time point after i.v. injection.

(log P = 1.1), their membrane diffusion kinetics are so fast they tend to localize in mitochondria-rich organs such as the heart.[16] Furthermore, because the alkyl group increases the hydrophobicity, the hydrophobic interaction between the triphenylphosphonium cation and the lipid core is attractive due to the hydrophobicity of the lipophilic phosphonium cation and increased entropy.[17−19]

In Vitro Cell-Uptake Assays. The mitochondrial membrane potential of H9c2 cells, which was assessed by a cellular [^{3}H]-TPP uptake assay, was significantly higher than that of NIH/3T3 cells (Figure 3A). Cell uptake values of [^{18}F]**3** in H9c2 and NIH/3T3 cells over incubation periods of 0.5, 1, and 2 h are shown in Figure 3B. The uptake increased from 2.37 ± 0.13% at 0.5 h to 3.28 ± 0.08% at 2 h. The uptake was about 6-fold higher than that of NIH/3T3 cells. When incubated with 0.5 μM of CCCP, cellular uptake of [^{18}F]**3** was significantly inhibited ($P < 0.05$) at all incubation time points (Figure 3B). These results clearly demonstrate that [^{18}F]**3** accumulates in the cells through the mitochondrial membrane potential.

Biodistribution Studies and Micro-PET Imaging. The in vivo biodistribution of [^{18}F]**3** was examined in BALB/c mice (Table 1). High levels of radioactivity accumulated in the heart. The myocardial uptake of [^{18}F]**3** was >20% ID/g at 10 min after radiotracer injection. The heart-to-blood ratio of [^{18}F]**3**

Table 1. Normal Biodistribution Studies in BALB/c Mice at 10, 30, 60, and 120 min after i.v. Injection of [^{18}F]3[a]

	10 min	30 min	60 min	120 min
Blood	0.72 ± 0.14	0.29 ± 0.02	0.19 ± 0.03	0.14 ± 0.01
Heart	21.84 ± 2.26	20.47 ± 4.35	23.20 ± 2.70	20.09 ± 3.12
Lung	6.57 ± 1.25	4.52 ± 0.57	3.70 ± 0.12	2.84 ± 0.17
Liver	5.13 ± 1.73	1.74 ± 0.11	1.30 ± 0.12	0.81 ± 0.20
Spleen	6.11 ± 2.22	3.29 ± 0.08	2.94 ± 0.19	2.37 ± 0.57
Stomach	6.48 ± 0.48	5.58 ± 1.11	5.58 ± 1.69	5.07 ± 2.13
Intestine	15.24 ± 2.90	11.06 ± 1.66	11.29 ± 2.10	3.47 ± 0.88
Kidney	56.63 ± 12.20	20.72 ± 1.20	12.68 ± 3.13	11.47 ± 1.84
Pancreas	12.32 ± 2.84	9.73 ± 0.32	10.09 ± 1.13	9.91 ± 1.68
Normal Muscle	4.24 ± 1.15	4.16 ± 0.49	4.53 ± 0.54	4.14 ± 0.28
Bone	5.22 ± 0.86	5.55 ± 1.15	7.30 ± 1.36	7.45 ± 1.86
Heart/blood	30.95 ± 4.80	71.87 ± 21.63	126.76 ± 22.51	138.61 ± 8.10
Heart/liver	4.58 ± 1.61	11.90 ± 3.37	17.83 ± 1.18	25.53 ± 5.88
Heart/lung	3.36 ± 0.30	4.50 ± 0.44	6.27 ± 0.65	7.07 ± 0.98
Heart/muscle	5.43 ± 1.76	4.91 ± 0.67	5.13 ± 0.35	4.83 ± 0.44

[a]Data are expressed as the percentage administered activity (injected dose) per gram of tissue (% ID/g, n = 12). The myocardial uptake of [^{18}F]**3** was >20% ID/g for the whole time after radiotracer injection. The heart-to-blood ratio of [^{18}F]**3** was >138, whereas the heart-to-lung, heart-to-liver, and heart-to-normal muscle ratios were >7, >25, and >4, respectively, at 120 min.

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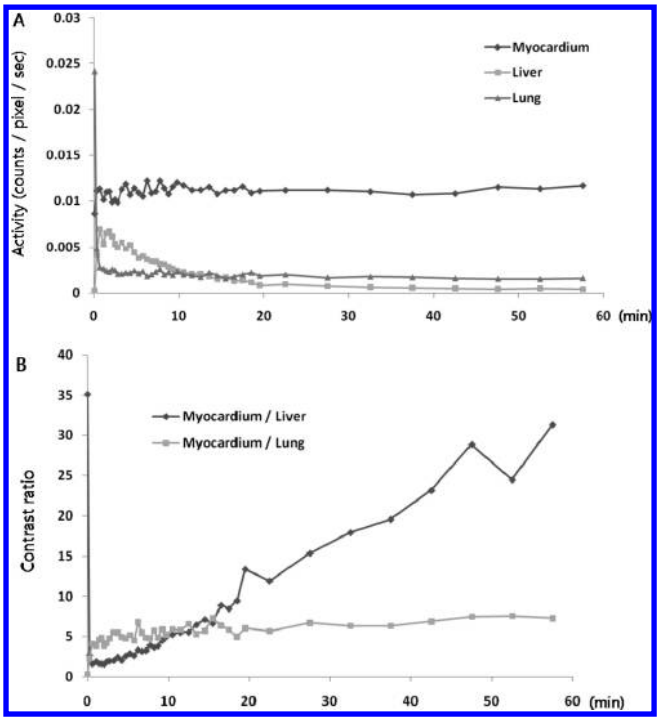

Figure 5. (A) Time−activity curves generated from dynamic PET images. [^{18}F]**3** accumulated specifically in the heart. The [^{18}F]**3** in liver was washed out rapidly but was retained in the myocardium for the whole time. (B) Contrast ratio of myocardium-to-liver and myocardium-to-lung for 1.0 h after i.v. injection of [^{18}F]**3**.

was >30 at 10 min, indicating rapid clearance of the compound from the blood. The heart-to-lung, heart-to-liver, and the heart-to-normal muscle ratios were >3, >4, and >5, respectively, at 10 min after radiotracer injection. Notably, the heart-to-blood and heart-to-liver ratios increased to >138 and >25 at 120 min after radiotracer injection, indicating that [^{18}F]**3** is optimal as a cardiac imaging agent.

Coronal micro-PET imaging was performed in rats at 0.5 h (Figure 4A) and 1 h (Figure 4B) after i.v. injection of [^{18}F]**3**. The results demonstrated good visualization of the heart, with excellent heart-to-background contrast at each time point. Time−activity curves for the myocardium, liver, and lung revealed rapid washout from the liver and lung after i.v. injection of [^{18}F]**3**. However, [^{18}F]**3** was retained at a constant level in the myocardium for up to 1.0 h after injection (Figure 5A). The myocardium-to-liver and myocardium-to-lung uptake ratios quickly reached 5.0 after [^{18}F]**3** injection (Figure 5B).

The rationale for preparing the 6-carbon spacer group was to decrease lipophilicity. Previous publications have reported that [^{18}F]-FBnTP is metabolically stable and demonstrates excellent characteristics as a cardiac imaging agent in healthy mice.[7] However, the myocardium-to-liver uptake ratio in the time-activity curve reached 1.0 at approximately 25 min after i.v. injection of [^{18}F]-FBnTP, indicating delayed washout from the liver. Clearance of the radiotracer from the liver would be dependent on the lipophilicity of the compound. Thus, in the current study, several kinds of radiolabeled phosphonium salts were synthesized to assess the appropriate lipophilicity by different carbon chain lengths; however, highly lipophilic structures (such as benzene rings) were not adopted for the radiolabeling of phosphonium salts. Compared to [^{18}F]**3**, the (3-[^{18}F]fluoropropyl)triphenylphosphonium cation[12] (a shorter-chain analog) showed lower myocardial uptake, whereas the (8-[^{18}F]fluorooctyl)triphenylphosphonium cation (a longer-chain analogue) showed higher liver uptake and delayed clearance (data not shown).

CONCLUSION

In the current study, we suggested the feasibility of a [^{18}F]-labeled phosphonium cation as a PET myocardial agent. Alkyltriphenylphosphonium cations are better candidates for the general delivery of compounds to mitochondria because they accumulate to a greater extent within the mitochondria in cells.[20] The [^{18}F]**3** compound was synthesized very easily from the reaction of triphenylphosphine with appropriate precursors, which have the proper cationic activity and lipophilicity to penetrate the mitochondrial membrane and accumulate inside. [^{18}F]**3** revealed good myocardial uptake, high myocardial selectivity and fast clearance from other organs. Future studies will be directed toward functional studies with diverse in vitro and small animal models.

ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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