Supporting Information

Comparison of the Cardiac MicroPET Images Obtained Using [^{18}F]FPTP and [^{13}N]NH_3 in Rat Myocardial Infarction Models

Dong-Yeon Kim,[†, §] Hyeon Sik Kim,[†, §] Hwa Youn Jang,[†] Ju Han Kim,[‡]
Hee-Seung Bom,[†] Jung-Joon Min*[, †]

[†]*Department of Nuclear Medicine, Chonnam National University Medical School, Gwangju, Korea*

[‡]*Department of Cardiology, Chonnam National University Medical School, Gwangju, Korea*

*Author to whom correspondence should be addressed: jjmin@jnu.ac.kr (J.J. Min).

Jung-Joon Min, M.D.,Ph.D.
Department of Nuclear Medicine, Chonnam National University Medical School,
160 Ilsimri, Hwasun, Jeonnam 519-763, Republic of Korea
Phone: 82-61-379-8476
Fax: 82-61-379-8455
E-mail: jjmin@jnu.ac.kr

Contents

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. 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 Inter-University 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). Normal rats were imaged and analyzed using microPET (Inveon, Siemens Medical Solutions, Malvern, PA, USA). No-carrier-added (n.c.a) [^{18}F]fluoride was produced on a GE PET trace cyclotron (16.4 MeV, General Electric healthcare, USA) by irradiation of a [^{18}O]H_2O water target (Chonnam National University Hwasun Hospital, Korea).

Chemistry

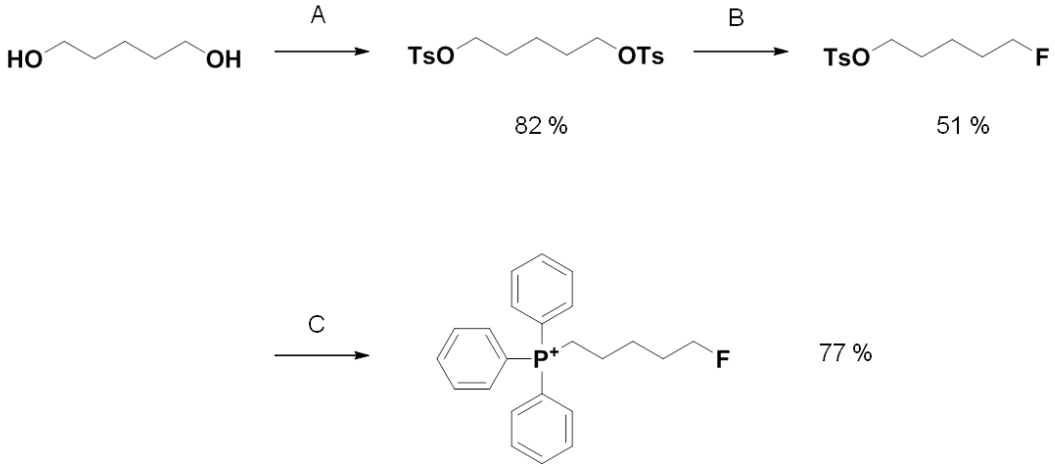
Synthesis of pentane-1,5-diyl bis(4-methylbenzenesulfonate) Pentane-1,5-diyl bis(4-methylbenzenesulfonate) was prepared by modification of a reported previously method.[1] Pentane-1,5-diol (1.56 g, 15.0 mmol) in 30.0 mL of anhydrous pyridine was added to 4-methylbenzene-1-

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 reaction mixture and the pyridine was extracted from 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) recrystallized from methylene chloride : *n*-hexane to yield 5.07 g (82 %) of pentane-1,5-diyl bis(4-methylbenzenesulfonate). mp 81-83 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.3 Hz, 4H), 7.34 (d, *J* = 8.0 Hz, 4H), 3.97 (t, *J* = 6.3 Hz, 4H), 2.44 (s, 6H), 1.59 (m, 4H), 1.35 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 144.96, 133.01, 130.00, 127.96. 70.08, 28.26, 21.75, 21.60; HRMS (ESI) *m/z* calculated for $C_{19}H_{24}O_6S_2Na$ $[M+Na]^+$ 435.0987, found 435.0982.

Synthesis of 5-fluoropentyl 4-methylbenzenesulfonate 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. pentane-1,5-diyl bis(4-methylbenzenesulfonate) (1.87 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 (51 %) of 5-fluoropentyl 4-methylbenzenesulfonate as a yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 4.39 (dt, *J* = 47.2, 6.05 Hz, 2H), 4.03 (t, *J* = 6.3 Hz, 2H), 2.44 (s, 3H), 1.64 (m, 4H), 1.44 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 144.87, 133.15, 129.94, 127.97, 83.75, 70.32, 29.85, 28.53, 21.73, 21.46; MS (FAB) *m/z* 261 $[M+H]^+$, 173 (100); HRMS (FAB) *m/z* calculated for $C_{12}H_{18}FO_3S$ $[M+H]^+$ 261.0961, found 261.0958.

Synthesis of (5-fluoropentyl)triphenylphosphonium salt Triphenylphosphine (1.0 g, 3.81 mmol) dissolved in 10.0 mL anhydrous acetonitrile was added to 5-fluoropentyl 4-methylbenzenesulfonate (0.99 g, 3.81 mmol). The solution was refluxed 19 h. The solvent was evaporated under reduced pressure, the solution was purified by column chromatography (methylene chloride : methanol : ethyl acetate = 8 : 1 :1) provided 1.03 g (77 %) of (5-fluoropentyl)triphenylphosphonium salt as a powder.

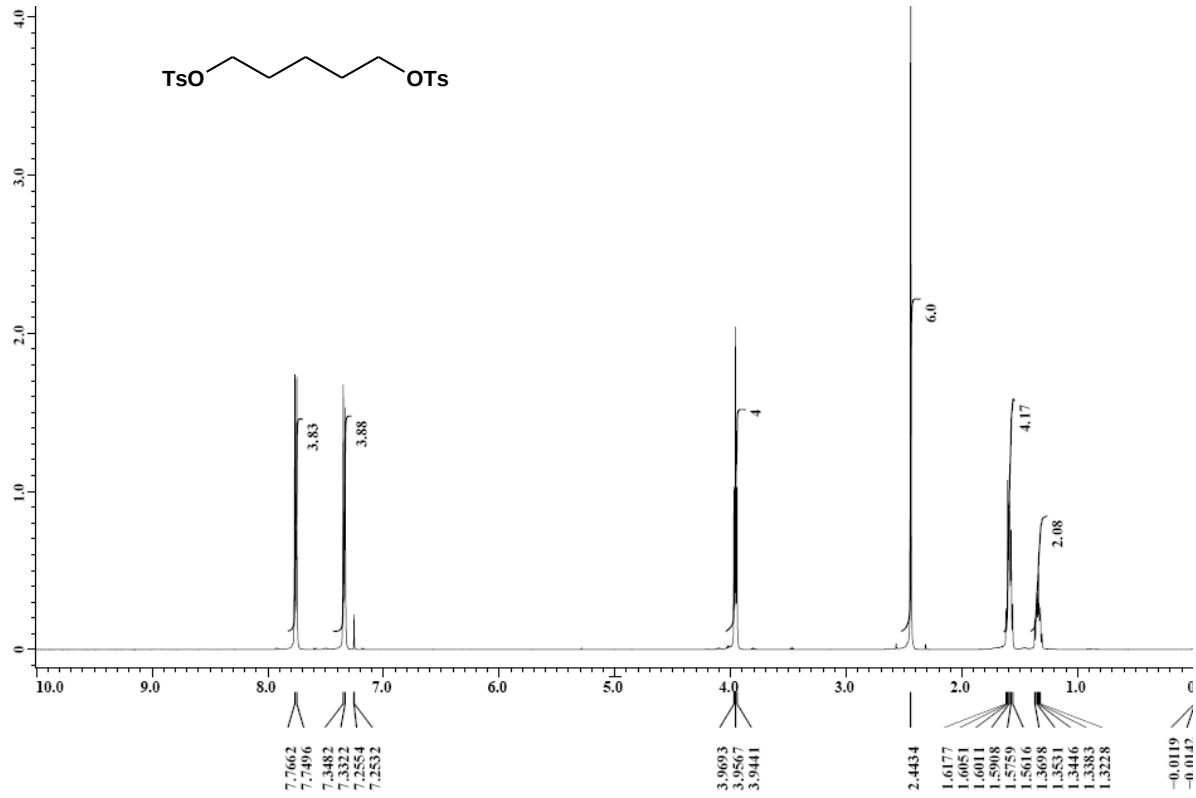
mp 218-220 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.72 (m, 17H), 7.06 (d, *J* = 8.0 Hz, 2H), 4.33 (dt, *J* = 47.6, 5.7 Hz, 2H), 3.63 (m, 2H), 2.29 (s, 3H), 1.66 (m, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 144.58, 138.58, 135.01, 133.71, 130.49, 128.37, 126.22, 118.82, 118.14, 84.05, 29.54, 26.25, 22.27, 21.49; MS (FAB) *m/z* 351 $[M]^+$, 351 (100); HRMS (FAB) *m/z* calculated for $C_{23}H_{25}FP$ $[M]^+$ 351.1678, found 351.1685.



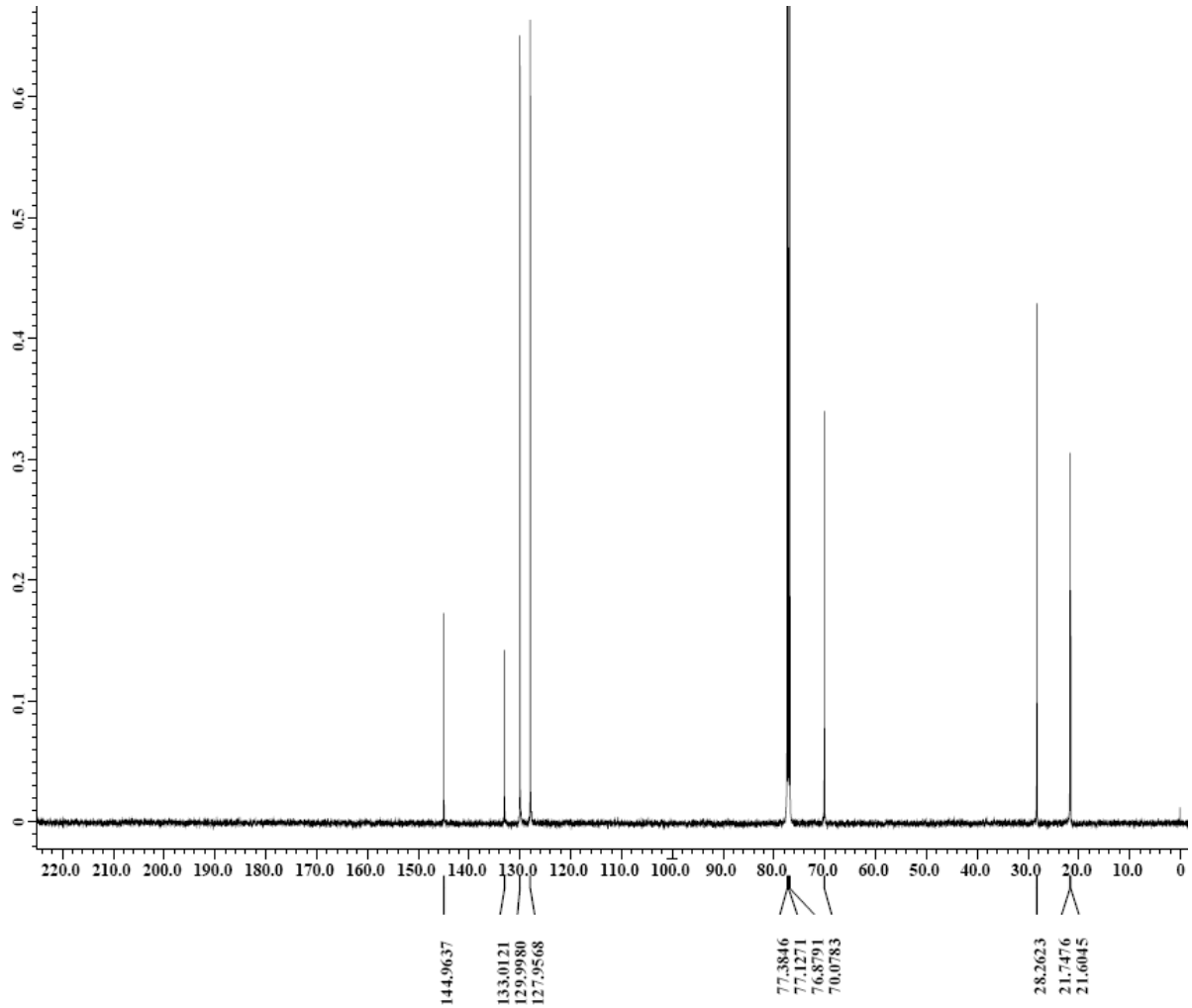
S Figure 1. Synthetic scheme of (5-fluoropentyl)triphenylphosphonium salt

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

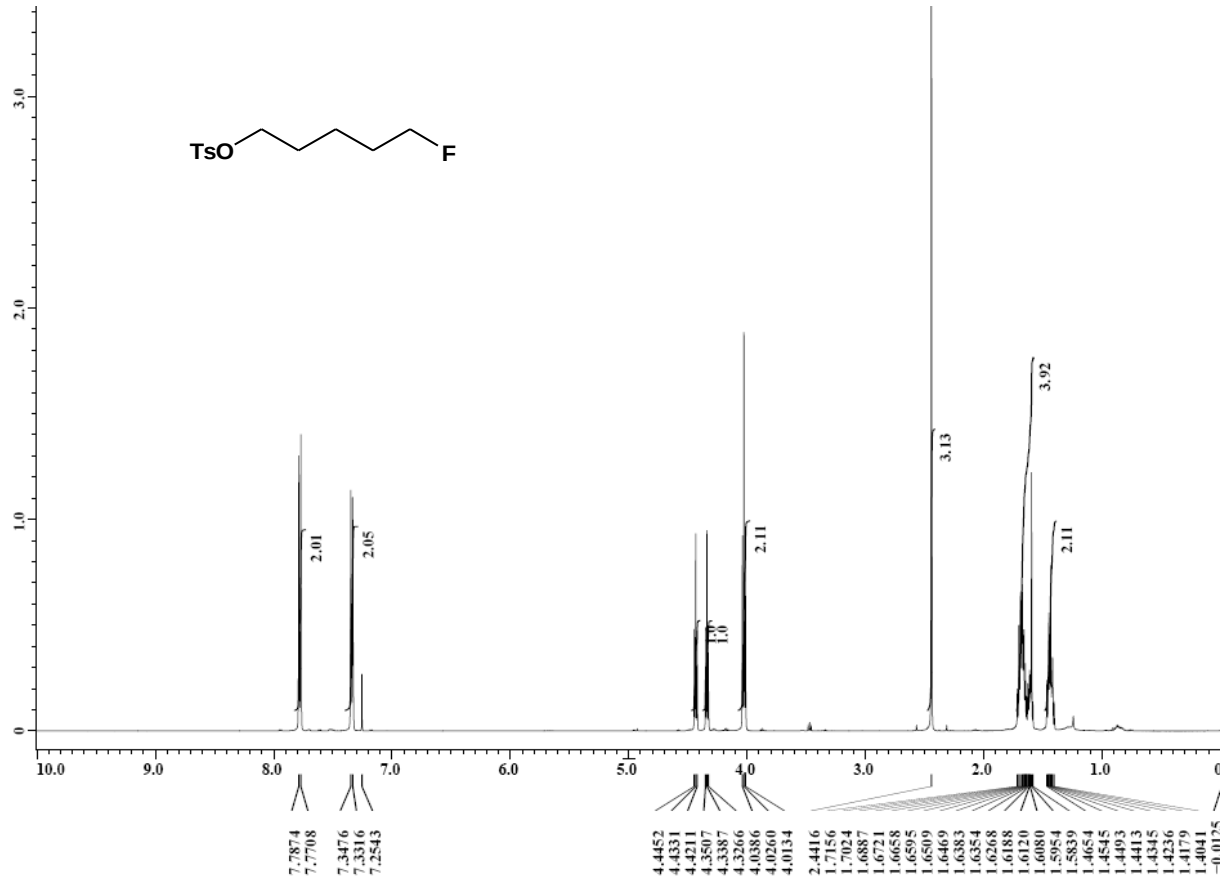
^{1}H and ^{13}C NMR spectra of all compounds



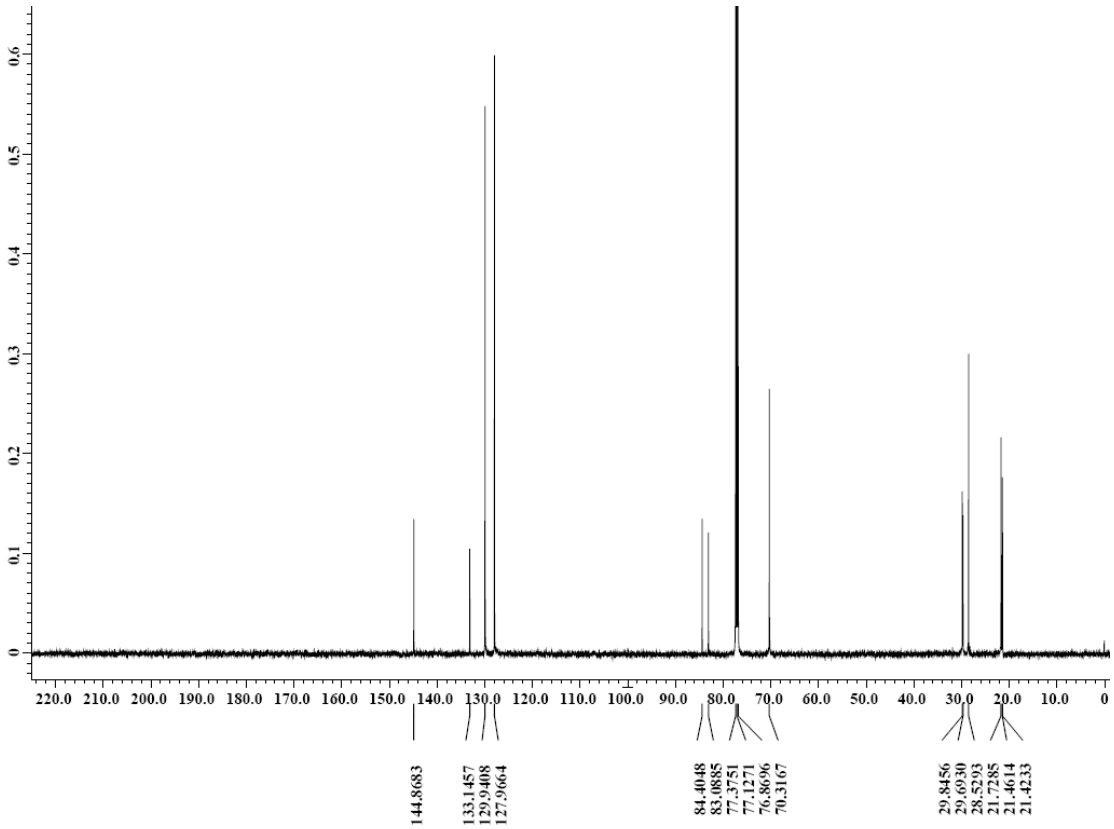
S Figure 2A. ^{1}H spectra of pentane-1,5-diyl bis(4-methylbenzenesulfonate)



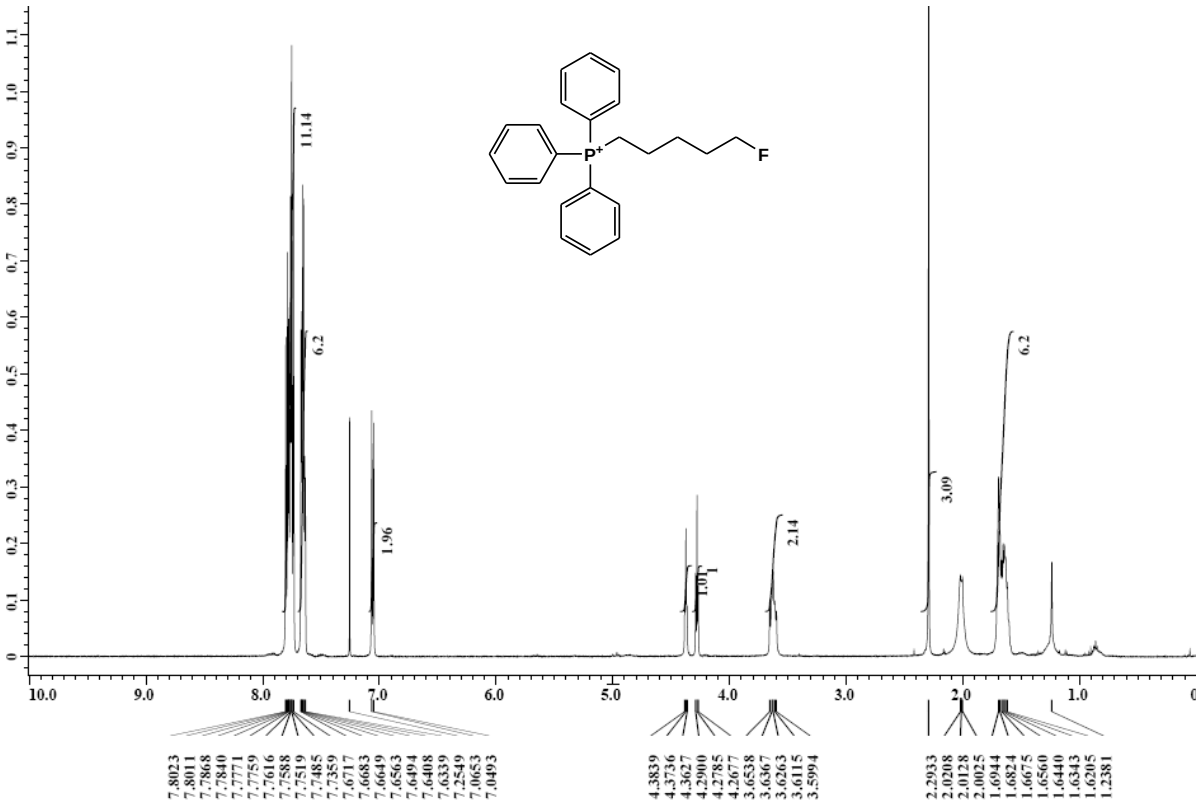
S Figure 2B. ^{13}C spectra of pentane-1,5-diyl bis(4-methylbenzenesulfonate)



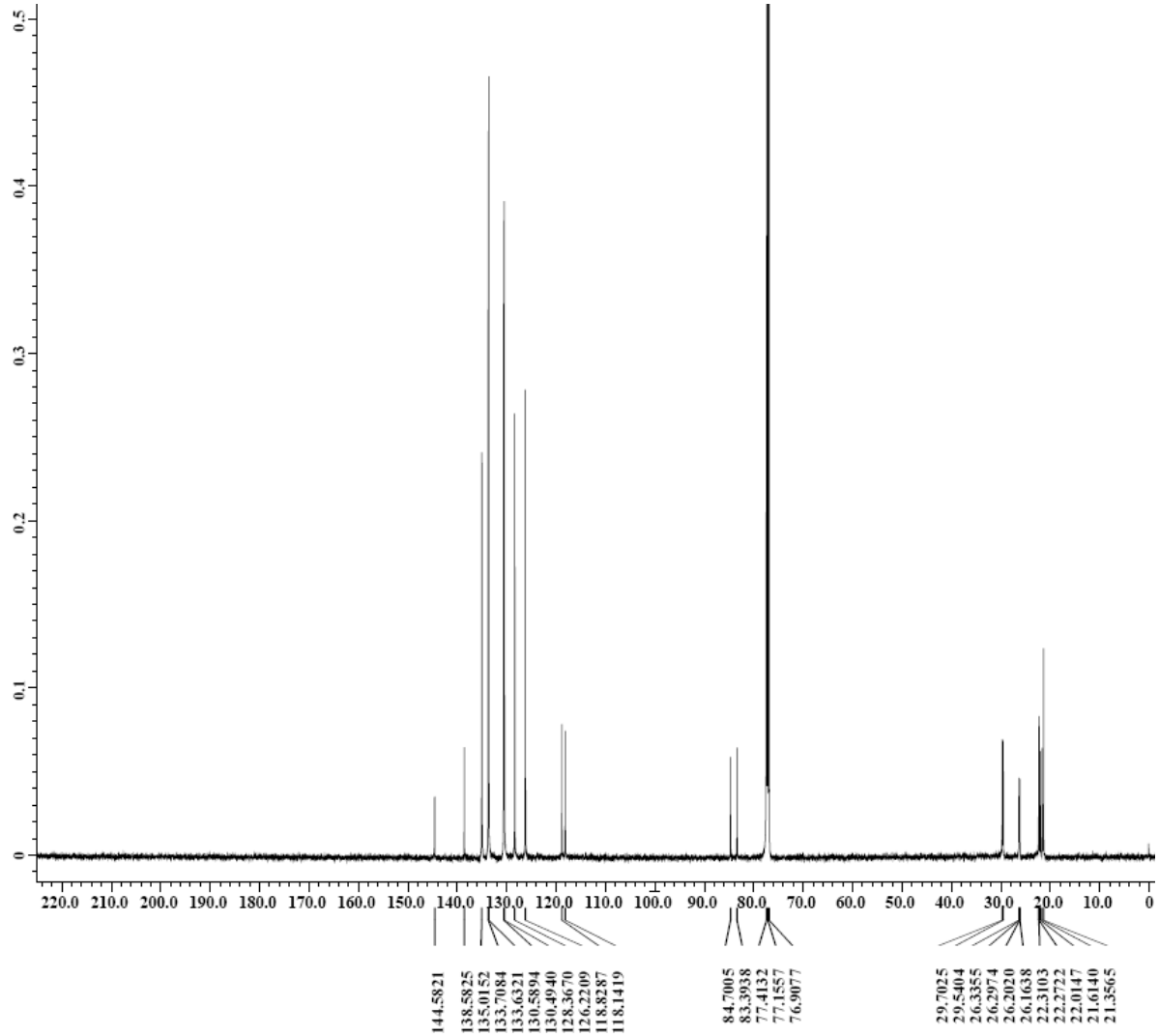
S Figure 3A. ^{1}H spectra of 5-fluoropentyl 4-methylbenzenesulfonate



S Figure 3B. ^{13}C spectra of 5-fluoropentyl 4-methylbenzenesulfonate



S Figure 4A. ^{1}H spectra of (5-fluoropentyl)triphenylphosphonium salt

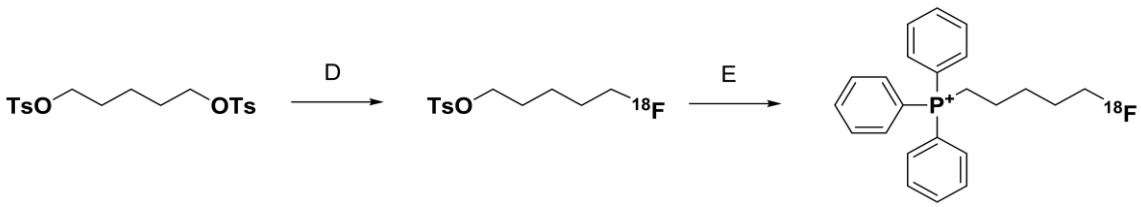


S Figure 4B. ^{13}C spectra of (5-fluoropentyl)triphenylphosphonium salt

Radiochemistry

Radiosynthesis of [^{18}F]FPTP: ^{18}F fluoride was produced by an ^{18}O(p, n)^{18}F reaction on a GE PETtrace cyclotron. Activity was extracted from $H_2{}^{18}O$ by an anion exchanger and then 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, and the residue was dried by azeotropic distillation with acetonitrile (1 mL, 3 times). Next, 4.0 mg of pentane-1,5-diyl bis(4-methylbenzenesulfonate) dissolved in 1.0 mL of anhydrous acetonitrile was added. The mixture was heated for 5 min at 90 °C in the closed state. Radio-TLC showed a yield of > 80% of compound ^{18}F-fluoropentyl 4-methylbenzenesulfonate. The solution was passed through a small silica Sep-Pak cartridge. Triphenylphosphine (6.0 mg) was dissolved in 1.0 mL of toluene, added to the reaction vessel, and heated to 220 °C for 3 min with no separation step.[2]

The solution was cooled and injected onto a semipreparative HPLC column system for purification (acetonitrile : phosphate-buffered saline = 45 : 55; flow rate: 3 mL/min; UV: 254 nm; t_R: 21.8 min). For identification of the radioproduct, the collected HPLC fraction was co-injected with its nonradioactive compound. ^{18}F-FPTP was dried, made isotonic with sodium chloride, and passed through a 0.20 µm membrane filter into a sterile multi-dose vial for in vitro and in vivo experiments.



S Figure 5. Radiosynthetic scheme of (5-[^{18}F]fluoropentyl)triphenylphosphonium salt
Reagents and conditions (D) [^{18}F]KF, acetonitrile, Kryptofix 2.2.2, 90°C, 5 min; (E) triphenylphosphine, toluene, 220°C, 3 min.

Radiosynthesis of [^{13}N]NH_3: [^{13}N]NH_3 was synthesized by GE [^{13}N]NH_3 synthesis module. [^{13}N]NO_x was produced by an ^{16}O(p, α)^{13}N reaction on a GE PETtrace cyclotron. [^{13}N]NO_x reacted with devardas alloy and sodium hydroxide in a disposable unit and then radioproduct dissolved in 0.9 % sodium chloride. Finally that passed through a 0.20-μm membrane filter into a sterile multidose vial for in vivo.

Animal Model

Eight-week old, male Sprague-Dawley rats (250–260 g, Orient, Kyunggido, Korea) underwent left coronary artery (LCA) ligation, as previously described.[3] 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). Animals underwent imaging studies at 24 h after LCA occlusion.

Small Animal PET and Quantitative Analysis

A dedicated microPET/CT scanner was used for in vivo imaging of [^{18}F]FPTP and [^{13}N]NH_3. Normal or MI rats were anesthetized with isoflurane, placed in a cradle, and equipped with masks for anesthesia gas supply and warm water pads at the tail veins for injection. Dynamic microPET images were acquired for 30 min after injection of [^{18}F]FPTP and [^{13}N]NH_3 of 37MBq and reconstructed by using the 3D-ordered subset expectation maximization (3D-OSEM) algorithm with four iterations. Potential artifacts by respiration and/or cardiac motion were not corrected during microPET imaging studies.

To determine pharmacokinetics, a region of interest was drawn around the heart. Time-activity curves of [^{18}F]FPTP and [^{13}N]NH_3 were generated to obtain the counts per pixel. Reconstructed pixel sizes were approximately 0.78 mm in the transverse and axial directions. The dimensions of the reconstructed images were 128 × 128 in each of the 159 transverse slices. Data were normalized and corrected for randoms, dead time, and decay.

The 2,3,5-triphenyltetrazolium chloride (TTC) staining was performed to compare the defect size of MI models, as previously described.[4-6] In the presence of dehydrogenase enzyme that exists in viable myocardium, TTC is reduced and forms a formazan precipitate that makes viable tissue turn brick-red, whereas nonviable infarcted tissue without dehydrogenase activity remains pale. Finally, we compared the defect size on small-animal PET images with TTC stained images to measure correlation.

MicroPET Image Analysis

Analysis of the microPET images was performed with PMOD software package (PMOD Technologies Ltd.).[7-9] To measure the average defect size, reconstructed PET data were reoriented into 17 segments of polar map images.

Statistical Analysis

Correlation between infarct size measured using small-animal PET and TTC staining was calculated with a Pearson test (2-tailed, 95% confidence interval) using SPSS software (version 18.0; SPSS Inc.) *P* values of less than 0.05 were considered statistically significant.

References

(1) Yu, K. H.; Kim, Y. S.; Kim, S. W.; Park, J. H.; Yang, S. D.; Herdering, W.; Knoechel, A. Synthesis of [^{18}F]Fluoroclofilium as a potential cardiac imaging agent for PET studies. *J. Label. Compd. Radiopharm.* **2003**, *46*, 1151-1160.

(2) Ravert, H. T.; Madar, I.; Dannals, R. F. Radiosynthesis of 3-[^{18}F]fluoropropyl and 4-[^{18}F]fluorobenzyl triarylphosphonium ions. *J. Label. Compd. Radiopharm.* **2004**, *47*, 469-476.

(3) Samsamshariat, S. A.; Samsamshariat, Z. A.; Movahed, M. R. A novel method for safe and accurate left anterior descending coronary artery ligation for research in rats. *Cardiovasc. Revasc. Med.* **2005**, *6*, 121-123.

(4) Acton, P. D.; Thomas, D.; Zhou, R. Quantitative imaging of myocardial infarct in rats with high resolution pinhole SPECT. *Int. J. Cardiovasc. Imaging* **2006**, *22*, 429-434.

(5) Gibbons, R. J.; Valeti, U. S.; Araoz, P. A.; Jaffe, A. S. The quantification of infarct size. *J. Am. Coll. Cardiol.* **2004**, *44*, 1533-1542.

(6) Kim, D. Y.; Kim, H. S.; Le, U. N.; Jiang, S. N.; Kim, H. J.; Lee, K. C.; Woo, S. K.; Chung, J.; Bom, H. S.; Yu, K. H.; Min, J. J. Evaluation of a Mitochondrial Voltage Sensor, ([^{18}F]fluoropentyl)triphenylphosphonium cation, in a rat myocardial infarction model. *J. Nucl. Med.* **2012**, *53*, 1779-1785.

(7) Siegrist, P. T.; Husmann, L.; Knabenhans, M.; Gaemperli, O.; Valenta, I.; Hoefflinghaus, T.; Scheffel, H.; Stolzmann, P.; Alkadhi, H.; Kaufmann, P. A. ^{13}N-ammonia myocardial perfusion imaging with a PET/CT scanner: impact on clinical decision making and cost-effectiveness. *Eur. J. Nucl. Med. Mol. Imaging* **2008**, *35*, 889-895.

(8) Schepis, T.; Gaemperli, O.; Treyer, V.; Valenta, I.; Burger, C.; Koepfli, P.; Namdar, M.; Adachi, I.; Alkadhi, H.; Kaufmann, P. A. Absolute quantification of myocardial blood flow with ^{13}N-ammonia and 3-dimensional PET. *J. Nucl. Med.* **2007**, *48*, 1783-1789.

(9) Koepfli, P.; Hany, T. F.; Wyss, C. A.; Namdar, M.; Burger, C.; Konstantinidis, A. V.; Berthold, T.; Von Schulthess, G. K.; Kaufmann, P. A. CT attenuation correction for myocardial

perfusion quantification using a PET/CT hybrid scanner. *J. Nucl. Med.* **2004**, *45*, 537-542.