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Synthesis and Evaluation of 15-(4-(2-[¹⁸F]Fluoroethoxy)phenyl)pentadecanoic Acid: a Potential PET Tracer for Studying Myocardial Fatty Acid Metabolism

Zhude Tu[†], Shihong Li[†], Terry L. Sharp[†], Pilar Herrero[†], Carmen S. Dence[†], Robert J. Gropler[†], and Robert H. Mach^{†,*}

[†]Division of Radiological Sciences, Washington University School of Medicine, 510 South Kingshighway Blvd. St. Louis, MO 63110, USA

Abstract

15-(4-(2-[¹⁸F]fluoroethoxy)phenyl)pentadecanoic acid ([¹⁸F]7) was synthesized as a PET probe for assessing myocardial fatty acid metabolism. The radiosynthesis of [¹⁸F]7 was accomplished using a two step reaction, starting with the corresponding tosylate ester, methyl 15-(4-(2-(tosyloxy)ethoxy)phenyl)pentadecanoate (**5**) and gave the radiolabeled fatty acid, [¹⁸F]7 in a radiolabeling yield of 55 – 60% and a specific activity of > 2,000 Ci/mmol (decay corrected to EOB). The biological evaluation of [¹⁸F]7 in rats displayed high uptake in heart (1.94%.ID/g at 5 min), which was higher than the uptake (%ID/g) in blood, lung, muscle, pancreas and brain. MicroPET studies of [¹⁸F]7 in Sprague-Dawley rats demonstrated excellent images of the myocardium when compared with [¹¹C]palmitate images in the same animal. Moreover, the tracer kinetics of [¹⁸F]7 paralleled those seen with [¹¹C]palmitate, with an early peak followed by biphasic washout. When compared to [¹¹C]palmitate, [¹⁸F]7 exhibited a slower early clearance (0.17 ± 0.01 vs. 0.30 ± 0.02, P < 0.0001) and a significantly higher late clearance (0.0030 ± 0.0005 vs. 0.0006 ± 0.00013, P < 0.01). These initial studies suggest that [¹⁸F]7 could be a potentially useful clinical PET tracer to assess abnormal myocardial fatty acid metabolism.

Keywords

Fatty acid; PET imaging; F-18

INTRODUCTION

Fatty acid metabolism is the major source of energy production in the normal myocardium contributing 90% of aerobic metabolism through the β-oxidation pathway (1–3). Abnormalities in myocardial fatty acid metabolism play a central role in a variety of cardiac diseases such as obesity, diabetes mellitus, dilated cardiomyopathy and myocardial ischemia (1,4). An overdependence on fatty acid metabolism typifies obesity and diabetes mellitus. This increase in fatty acid use can initiate a cascade of events such as worsening myocardial insulin resistance, impaired energetics, an increase in oxidative stress and lipid accumulation, all of which have detrimental effects on myocellular health and can lead to reduced cardiac function (5–7). In contrast, a reduction in fatty acid metabolism leading to an overdependence on glucose use occurs in left ventricular hypertrophy, dilated cardiomyopathy and myocardial ischemia. This metabolic pattern can impair cardiac

* Address correspondence to: Robert H. Mach, Ph.D. Division of Radiological Sciences Washington University School of Medicine Campus Box 8225 510 S. Kingshighway Blvd. St. Louis, MO 63110 Tel: 314-362-8538, Fax: 314-362-8555 rhmach@mir.wustl.edu.

function through a variety mechanisms including reduced energy production, increased oxidative stress and accelerated cell death (8,9). The importance of altered fatty acid metabolism in these various cardiac disease processes is highlighted by the robust development of new therapeutics designed to favorably manipulate myocardial fatty acid use such as the peroxisome proliferator-activated receptor alpha (PPAR α) agents and partial fatty acid oxidation inhibitors (10–12).

Detection of these different patterns in myocardial fatty acid metabolism may have important diagnostic, prognostic and therapeutic implications in the management of the cardiac patient. However, to achieve this goal requires the availability of an accurate and widely applicable noninvasive method to image myocardial fatty acid metabolism. To this end, investigators have focused on optimizing the structures of the fatty acids to identify radioligands that have extended retention time in heart and suitable pharmacokinetic properties for use as positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging probes (Figure 1) (13–16). However, because PET has higher sensitivity and higher spatial and temporal resolution compared to SPECT, developing a suitable PET probe which is capable of accurately quantifying myocardial fatty acid metabolism is highly desirable. Currently, [^{11}C]palmitate (Figure 1) is the most accurate PET radiotracer for measuring myocardial fatty acid metabolism. However, the short half-life of carbon-11 ($t_{1/2} = 20$ min) limits the use of [^{11}C]palmitate in the study of cardiovascular disease to imaging centers having the capability to produce the radiotracer onsite. Therefore, a number of groups have focused on developing fluorine-18 labeled PET tracers which are capable of imaging fatty acid uptake in the heart.

In this regard, two different strategies have been pursued: 1) developing radiotracers such as [^{11}C]palmitate whose kinetics closely track unlabeled fatty acids and thus reflect all aspects of myocardial fatty acid oxidation and storage (7,16–19); and, 2) developing radiolabeled tracers that will be trapped within the myocardium and can thus reflect a specific aspect of fatty acid metabolism such as cellular uptake (16,20–28). For the first strategy, the advantages are the assessment of various aspects of myocardial fatty acid metabolism, such as uptake, oxidation and storage, with appropriate mathematical modeling techniques capable of quantifying fatty acid metabolism in each pathway. In contrast, trapped fatty acid tracers offer the potential of higher image quality, but are not capable of discriminating between the oxidation and storage pathways. A prime example of the latter approach is the radiotracer, 14-(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA) (Figure 1) (20,21). Imaging studies of [^{18}F]FTHA showed high uptake and retention in the myocardium in various diseases (23–25), but this radiotracer lacked sensitivity to measure the inhibition of β -oxidation by myocardium under conditions of hypoxia (14). Subsequently, 16-[^{18}F]fluoro-4-thia-palmitate ([^{18}F]FTP) (Figure 1) was developed and its metabolic trapping function was proportional to β -oxidation under normal oxygenation and hypoxic conditions (22). However, it is not clear if [^{18}F]FTP can accurately differentiate between fatty acid oxidation and esterification (27).

Recently, trans-9-(RS)-[^{18}F]fluoro-3,4-(RS,RS) methyleneheptadecanoic acid ([^{18}F]FCPHA) (Figure 1), which incorporates a cyclopropyl group at C3–C4 to block β -oxidation was reported as a radiolabeled fatty acid analogue (26). The uptake of [^{18}F]FCPHA in rat myocardium reached approximately 1.5 %ID/gram tissue at 5 min and displayed little change over a period of 60 min. However, the impact of altered plasma substrate levels, cardiac workload and myocardial blood flow on the pharmacokinetics of [^{18}F]FCPHA is not known. It is also not clear if the kinetics of [^{18}F]FCPHA will be able to differentiate between fatty acid uptake, oxidation and esterification. Currently, both [^{18}F]FTP and [^{18}F]FCPHA are being evaluated in phase-1 clinical trials (4). In 2009, 17-[4-(2-[^{18}F]fluoroethyl)-1H-1,2,3-triazol-1-yl]-6-thia-heptadecanoic acid, a “click-labeling”

analogue of [^{18}F]FTHA, was reported as a potential PET agent for visualizing fatty acid metabolism in myocardium (28). However, the same issues apply to this radiotracer regarding the ability to differentiate between fatty acid oxidation and storage mechanisms in the heart.

The goal of our research was to identify an ^{18}F -labeled fatty acid analogue which is capable of mimicking [^{11}C]palmitate with regard to its ability to measure fatty acid uptake, β -oxidation, and storage as triglycerides. The lead compound for our studies was 15-(para-iodophenyl)-pentadecanoic acid (IPPA) (Figure 1), which was developed over twenty years ago as a SPECT myocardial imaging agent. In this case, the *p*-iodine atom of IPPA was replaced with a 2-fluoroethoxy group to make 15-(4-(2-fluoroethoxy)phenyl)pentadecanoic acid (**7**) (Figure 1). IPPA was chosen as the lead compound for developing a PET radiotracer because its kinetics closely match those of [^{11}C]palmitate but are too rapid to be imaged with clinical SPECT scanners.

In this article, we report the synthesis and radiosynthesis of [^{18}F]**7**, and our initial results measuring myocardial uptake in rats. MicroPET imaging studies of [^{18}F]**7** were also performed in Sprague-Dawley rats and its pharmacokinetics were compared with that of [^{11}C]palmitate.

EXPERIMENTAL PROCEDURES

General Chemistry

All analytical grade chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI) and were used without further purification unless otherwise specified. Flash column chromatography was conducted using Scientific Adsorbents, Inc. silica gel, 60Å, "40 Micron Flash" (32–63 μ). Melting points were determined using MEL-TEMP 3.0 apparatus and uncorrected. ^1H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer with CDCl_3 as solvent and tetramethylsilane (TMS) as the internal standard. All chemical shift values are reported in ppm (δ). Elemental analyses (C, H) were determined by Atlantic Microlab, Inc. (Norcross, GA).

Methyl 15-bromopentadecanoate (**2**)

A 2.0 M solution of trimethylsilyl diazomethane in hexane (3.4 mL, 6.8 mmol) was added to the solution of 15-bromopentadecanoic acid **1** (1 g, 3 mmol) in THF/MeOH (v/v, 7/2) (18 mL) and the reaction mixture was stirred at room temperature for 2 hr. The solvent was removed under reduced pressure. Chloroform (80 mL) was used to dissolve the residue and the solution was washed with water (2 $\times$ 30 mL) and brine (2 $\times$ 30 mL). The organic solution was dried over sodium sulfate. After the solvent was removed under reduced pressure, it afforded **2** as colorless syrup (0.99 g, 99%). ^1H NMR (300 MHz, CDCl_3): δ 1.2 – 1.36 (m, 20H); 1.36 – 1.48 (2H, m); 1.58–1.78 (m, 2H); 1.79 – 1.92 (m, 2H); 2.26 – 2.34 (t, 2H); 3.36 – 3.48 (t, 2H); 3.67 (s, 3H).

Methyl 15-(4-(benzyloxy)phenyl)pentadecanoate (**3**)

A solution of compound **2** (400 mg, 1.2 mmol) in 2-methyl-2-butanol (5 mL) was added to a round bottom flask (25 mL) that contained 4-benzyloxyphenylboronic acid methyl ester (408 mg, 1.79 mmol), $\text{Pd}(\text{OAc})_2$ (26.8 mg, 12 mmol), $[\text{HP}(\text{t-Bu})_2\text{Me}]\text{BF}_4$ (62.7 mg, 0.25 mmol), and potassium *tert*-butoxide (KO-t-Bu) (53.5 mg, 0.48 mmol), and the reaction mixture was stirred overnight at ambient temperature. The reaction was quenched with ethyl acetate (60 mL), water (13 mL) and glacial acetic acid (2 mL). The organic layer was separated and washed with brine (3 $\times$ 10 mL). The organic solution was dried over sodium sulfate and the solvent was removed under reduced pressure. The product was purified by

silica gel column chromatography using ethyl acetate/hexane (1/10, v/v) as the eluent to afford the intermediate compound, 15-(4-(benzyloxy)phenyl)pentadecanoic acid, as a white solid (240 mg, 45%), mp: 95–96°C. ¹H NMR (300MHz, CDCl₃): δ 1.38 – 1.18 (m, 20H), 1.70 – 1.50 (m, 2H), 2.34 (t, 2H), 5.04 (s, 2H), 2.54 (t, 2H), 6.93 – 6.87 (m, 2H), 7.12 – 7.05 (m, 2H), δ 7.50 – 7.30 (m, 5H).

0.5 mL of concentrated sulfuric acid was added into a solution of 15-(4-(benzyloxy)phenyl)pentadecanoic acid (230 mg, 0.54 mmol) in 30 mL methanol. The reaction mixture was stirred at reflux overnight and quenched with a saturated aqueous solution of sodium bicarbonate. After removing the methanol under reduced pressure, the residue was partitioned between dichloromethane and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using ethyl acetate/hexane (v/v, 1/10) as the eluent to afford **3** (185 mg, 78%) as a white solid: mp: 56 – 57°C. ¹H NMR (300 MHz, CDCl₃): δ 1.18 – 1.40 (m, 20H); 1.50 – 1.68 (4H, m); 2.26–2.36 (t, 2H); 2.48 – 2.58 (t, 2H); 3.66 (s, 3H); 5.04 (s, 2H); 6.86 – 6.94 (m, 2H); 7.06 – 7.13 (m, 2H); 7.28 – 7.46 (m, 5H), elemental analysis: C₂₉H₄₂O₃ Calcd: C: 79.41, H: 9.65; found: C: 79.14, H: 9.81.

Methyl 15-(4-hydroxyphenyl)pentadecanoate (**4**)

Palladium (10 wt.% on activated carbon; 50 mg) was added under a nitrogen atmosphere to a solution of compound **3** (185 mg, 0.42 mmol) in ethyl acetate (10 mL). Hydrogen was added into the system to reach 6 pounds per square inch (psi) pressure and the mixture was stirred for over 5 hr. The reaction mixture was filtered through Celite and washed with ethyl acetate (5 mL). After removing excess solvent, the product was purified by silica gel column chromatography using ethyl acetate/hexane (v/v, 1/5) as the eluent to afford **4** as a white solid (111 mg, 75%): mp: 75 – 76°C. ¹H NMR (300 MHz, CDCl₃): δ 1.30 – 1.47 (m, 20H); 1.50 – 1.68 (m, 4H); 2.26 – 2.36 (t, 2H); 2.49 – 2.58 (t, 2H); 3.67 (s, 3H); 6.72 – 6.78 (m, 2H); 7.04–7.09 (m, 2H). Elemental analysis: C₂₂H₃₆O₃ Calcd: C: 75.82, H: 10.41; found: C: 75.71, H: 10.46

Methyl 15-(4-(2-(tosyloxy)ethoxy)phenyl)pentadecanoate (**5**)

Compound **4** (106 mg, 0.30 mmol) and ethyleneglycol-1,2-bistosylate (338 mg, 0.91 mmol) were dissolved into anhydrous acetonitrile (20 mL), and then potassium carbonate (42 mg, 0.30 mmol) was added into the reaction mixture. The reaction mixture was stirred at reflux under a nitrogen atmosphere for 18 hr until thin layer chromatography (TLC) indicated the reaction was complete. After removing the solvent, dichloromethane (10 mL) was used to dissolve the residue. The organic layer was washed with brine (10 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using ether/hexane (v/v, 1/2) as the eluent to afford compound **5** as a white solid (145 mg, 87%): mp: 44 – 45°C. ¹H NMR (300 MHz, CDCl₃): δ 1.18 – 1.40 (m, 20H); 1.46 – 1.68 (4H, m); 2.26 – 2.36 (t, 2H); 2.45 (s, 3H); 2.48 – 2.56 (t, 2H); 3.66 (s, 3H); 4.09 – 4.15 (2H); 4.32 – 4.38; 6.66 – 6.74 (m, 2H); 7.01 – 7.08 (m 2H); 7.30 – 7.37 (m, 2H); 7.78 – 7.853 (m, 2H); Elemental analysis: C₃₁H₄₆O₆·4H₂O Calcd: C: 60.17, H: 8.80; found: C: 60.03, H: 8.44

Methyl 15-(4-(2-fluoroethoxy)phenyl)pentadecanoate (**6**)

Compound **4** (104 mg, 0.3 mmol), 1-bromo-2-fluoroethane (227 mg, 1.8 mmol) and potassium carbonate (246 mg, 1.8 mmol) were added into a flask containing acetone (40 mL). The reaction mixture was stirred at reflux under a nitrogen atmosphere for 18 hr. Volatiles were removed under reduced pressure, and the residue was partitioned between ethyl acetate (60 mL) and water (20 mL). The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was

purified by silica gel column chromatography using ethyl acetate/hexane (v/v, 1/10) as the eluent to afford compound **6** as a white solid (115 mg, 98%): mp: 66 – 67°C. ¹H NMR (300 MHz, CDCl₃): δ 1.18 – 1.40 (m, 20H); 1.50 – 1.68 (m, 4H); 2.26 – 2.37 (t, 2H); 2.49 – 2.58 (t, 2H); 3.67 (s, 3H); 4.12 – 4.18 (t, 1H), 4.22 – 4.27 (t, 3H); 4.64 – 4.70 (t, 1H); 4.80 – 4.85 (t, 1H); 6.82 – 6.88 (m, 2H); 7.06 – 7.13 (m, 2H). Elemental analysis: C₂₄H₃₉FO₃ Calcd: C: 73.06, H: 9.96; found: C: 73.30, H: 10.14

5-(4-(2-Fluoroethoxy)phenyl)pentadecanoic acid (**7**)

Compound **6** (110 mg, 0.28 mmol) and NaOH (180 mg, 4.5 mmol) were dissolved into 11 mL of dichloromethane/methanol/water (5/5/1) and the reaction mixture was stirred at ambient temperature for 18 hr. Solvents were removed under reduced pressure, the residue was acidified to pH 2.0 with 1N aqueous HCl and extracted into ethyl acetate (3 × 20 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to afford compound **7** as a white solid (95 mg, 89%): mp: 84 – 85°C. ¹H NMR (300 MHz, CDCl₃): δ 1.18 – 1.40 (m, 20H); 1.50 – 1.708 (m, 4H); 2.30 – 2.39 (t, 2H); 2.49 – 2.58 (t, 2H); 4.12 – 4.17 (t, 1H), 4.21 – 4.37 (t, 3H); 4.64 – 4.70 (t, 1H); 4.80 – 4.85 (t, 1H); 6.81 – 6.88 (m, 2H); 7.06 – 7.13 (m, 2H). Elemental analysis: C₂₃H₃₇FO₃ Calcd: C: 75.29, H: 9.80; found: C: 72.77, H: 9.82.

Radiochemistry

Production of [¹⁸F]Fluoride—H₂ ¹⁸O was purchased from Rotem Industries (Israel). [¹⁸F]Fluoride was produced in our institution by proton irradiation of enriched ¹⁸O water (95%) [reaction: ¹⁸O(p, n)¹⁸F] using a Siemens Eclipse Cyclotron (Siemens Medical Systems, Inc, Knoxville, TN). ¹⁸F-enriched H₂ ¹⁸O (up to 1,500 mCi) was delivered from the Eclipse Cyclotron and transferred to the reaction vial through an anion-exchange column, Sep-Pak® Light QMA. [¹⁸F]fluoride was eluted from the Sep-Pak by addition of an aqueous solution of potassium carbonate (2 mg of K₂CO₃ in 0.5 mL of water) into the reactor vessel.

15-(4-(2-[¹⁸F]fluoroethoxy)phenyl)pentadecanoic acid ([¹⁸F]**7**)

[¹⁸F]Fluoride (100 – 150 mCi) was added to a 10-mL Pyrex screw cap tube containing 5–6 mg of Kryptofix 2.2.2. and 1 – 2 mg of K₂CO₃. HPLC grade acetonitrile (3 × 1.0 mL) was used to remove the water by azeotropic evaporation from this reaction vessel at 110°C under a stream of nitrogen. After all the water was removed, a solution of precursor **11** (1 – 2 mg) in 0.25 – 0.30 mL acetonitrile was added. The tube was capped and heated at 110 – 115°C for 5 ~ 10 min. This solution was diluted with 1.0 mL acetonitrile and passed through a silica gel Sep-Pak (Alltech Associates, Inc, Deerfield, Ill) into a collection vial. An additional 2 mL of acetonitrile was used to rinse out the remaining radioactive product from the Sep-Pak into the above collection vial. Radio-thin-layer chromatography (radio-TLC) showed the radiochemical purity was > 95% on a Bioscan System 2000 system (Bioscan, Inc., Washington, DC). The acetonitrile was removed by a stream of nitrogen gas at 80 – 85°C. [¹⁸F]**6** was used directly in the next step, without further purification. 0.5 mL of ethanol and 0.2 mL of a 2N NaOH aqueous solution were added into the above collection vessel containing dried [¹⁸F]**6**. The vessel was heated at 80°C for 2 min, and then 0.4 mL of 1N aqueous HCl was added into the reaction mixture to neutralize the mixture to pH = 7.0. Acetonitrile (2 mL) was added and the reaction mixture was loaded through a 0.45 μm filter into a high-performance liquid chromatography (HPLC) system equipped with an Alltech Econosil C18 reverse phase column (10 × 250 mm) and eluted with acetonitrile/0.1 M ammonium formate buffer (v/v, 85/15) at a flow rate of 4.0 ml/min. A vial was used to collect the radioactive product from 24 – 25.5 min. The HPLC mobile phase solution was concentrated under reduced pressure and the residue was azeotroped with ethanol (2 × 1

mL) to remove the residual mobile phase. The labeled fatty acid [^{18}F]7 was formulated in 6 mL of 3% human serum albumin (HSA) pre-incubated at 45°C for 3 min and passed through 0.22 μm Millex-GP sterile syringe filter (cat#: SLMP025SS, Millipore, Billerica, MA) into a final dose vial. The dose sample was authenticated by co-injection with the corresponding cold standard onto an analytical HPLC system which included an Alltech Econosil C18 column (10 μm , 250 mm $\times$ 4.6 mm), using acetonitrile: 0.1 M ammonium formate buffer (v/v, 90/10) as mobile phase, 1.5 mL/min flow rate, and 274 nm UV wavelength. Under these conditions, the retention time of the product [^{18}F]7 was 7.4 min. The total radiosynthesis took ~2 hr, the labeling yield ranged from 50 – 60% (decay corrected to EOB), radiochemical purity >95%, and specific activity > 2,000 Ci/mmol (decay correct to EOB).

Biodistribution Studies

All animal experiments were conducted in compliance with the NIH Guidelines for the Care and Use of Research Animals under IACUC approved protocols reviewed by the Washington University School of Medicine Animal Studies Committee. The biodistribution of [^{18}F]7 was performed in adult male Sprague-Dawley rats (170–250 g). Rats were anesthetized with 2–3 % isoflurane/O₂ and [^{18}F]7 (approximately 20 – 25 $\mu\text{Ci}/100\text{ }\mu\text{L}$) was injected via the lateral tail vein. Rats were euthanized under anesthesia at 5, 30, 60 and 120 min post injection, 4–8 rats for each time point. Samples of blood, lung, liver, kidney, spleen, muscle, heart, brain, and pancreas were removed from each rat, weighed, and counted in a Beckman Gamma 8000 counter. Bone and fat were taken only for the 5 and 60 min time points from 4 rats. A standard dilution of the injectate was counted with the samples from each animal. The biodistribution results were calculated as percent injected dose per gram of tissue (% ID/g) and are presented in Table 1.

MicroPET Studies

MicroPET imaging studies were performed in fasted male Sprague-Dawley rats using a microPET Focus 220 scanner. The rats were secured using a custom-designed acrylic restraining device, and placed inside the field of the window of the microPET Focus 220 scanner (29,30). After a bolus injection of radiotracer via the right jugular catheter, microPET imaging studies of each rat were performed using a 1 hr dynamic data acquisition. The microPET studies were first conducted with [^{11}C]palmitate (0.30 – 0.40 mCi/150 μL). After a 1 hr period for biological clearance and radioactive decay, [^{18}F]7 (0.30 – 0.40 mCi/150 μL) was administered into the same rat and a second microPET dynamic imaging scan was acquired. [^{11}C]palmitate and [^{18}F]7 blood time–activity curves were generated from dynamic PET images by placing one 3 $\times$ 3 voxel region of interest (ROI) on the left ventricle. Myocardial ROIs encompassing the antero-lateral myocardium were drawn on three consecutive PET slices as shown in Figure 2C and 2D.

RESULTS AND DISCUSSION

Chemistry

The synthesis of compound 7 is shown in Scheme 1. The ester 2 was made via O-alkylation of 15-bromopentadecanoic acid, 1 with trimethylsilyl diazomethane in tetrahydrofuran as described in the literature (31). The Suzuki cross-coupling of 2 with 4-benzyloxyphenylboronic acid gave 15-(4-(benzyloxy)phenyl)pentadecanoic acid in modest yield (45%). The methyl group was removed from the ester 3 because potassium *tert*-butoxide (KO-*t*-Bu) is a strong Lewis base that led to the hydrolysis of intermediate ester, methyl 15-(4-(benzyloxy)phenyl)pentadecanoate. It is of interest to note that when the Suzuki cross-coupling reaction was directly performed between 15-bromopentadecanoic acid and 4-benzyloxyphenylboronic acid, no product was obtained. Esterification of 15-(4-(benzyloxy)phenyl)pentadecanoic acid in methanol with concentrated sulfuric acid as a

catalyst afforded compound **3** in 78% yield. Removal of the benzyl group of **3** was accomplished by hydrogenolysis over 10% palladium-activated carbon to afford **4** in 75% yield. O-alkylation of the phenol group of **4** with 1-bromo-2-fluoroethane in acetone using potassium carbonate as the base afforded the fluoroethoxy ester **6** in high yield (98%). Hydrolysis of the ester group afforded the target compound **7** in 89% yield. Compound **5**, the precursor for making [^{18}F]**7**, was prepared via O-alkylation of **4** with ethyleneglycol-1,2-bistosylate in acetonitrile using potassium carbonate as the base (87% yield) as shown in Scheme 1.

Radiochemistry

The radiosynthesis of the target compound [^{18}F]**7** was made using the two step reaction sequence shown in Scheme 2. First, nucleophilic displacement of the tosylate group of ester **5** with [^{18}F]KF/Kryptofix 2.2.2. in acetonitrile afforded [^{18}F]**6**. Hydrolysis of the ester with aqueous sodium hydroxide followed by neutralization with aqueous hydrochloric acid and semi-preparative HPLC gave the target fatty acid analogue, [^{18}F]**7**. The identity of the target compound was confirmed by co-injection with the cold standard **7** using an analytical HPLC system. Radiosynthesis and formulation of [^{18}F]**7** took approximately 2 hr, with the yield ranging from 50 – 60%, the specific activity was $>2,000 \text{ mCi}/\mu\text{mol}$ (decay corrected to the end of synthesis), and the radiochemical and chemical purities were $>95\%$ ($n = 10$). The radiosynthesis is straightforward and can be easily adapted to an automated synthesis module. Radiosynthesis of [^{11}C]palmitate was performed as previously described (32).

Biodistribution Studies

The tissue distribution of [^{18}F]**7** in adult male Sprague-Dawley rats is shown in Table 1. At 5 min post-i.v. injection, the uptake of [^{18}F]**7** in the heart ($2.00 \pm 0.44 \text{ \%ID/g}$) and liver ($2.71 \pm 0.37 \text{ \%ID/g}$) was higher than any of the other tissues sampled. The ratio of activity in heart: non-target tissues was 2.55:1 for blood, and 8.3:1 for skeletal muscle. The brain had the lowest initial uptake of [^{18}F]**7**, $0.11 \pm 0.01 \text{ \%ID/g}$; tracer uptake in fat activity was also very low, $0.14 \pm 0.02 \text{ \%ID/g}$. [^{18}F]**7** has a calculated log P value of 5.56 (ACD, Log D/suite 7.0). Consequently, this high lipophilicity will make it difficult to passively cross the blood-brain-barrier, which explains the low uptake of [^{18}F]**7** in the brain. The 5 min uptake of [^{18}F]**7** in liver was $2.71 \pm 0.37 \text{ \%ID/g}$, the only organ with higher uptake than that seen in heart. By 30 min post injection, the uptake of [^{18}F]**7** in heart had decreased to 35% of the 5 min activity and remained stable between 30 and 60 min (0.72 ± 0.10 and $0.86 \pm 0.28 \text{ \%ID/g}$, respectively). At 120 min, the uptake of [^{18}F]**7** in the heart remained $0.34 \pm 0.04 \text{ \%ID/g}$, which was approximately 1.4 fold higher than that in blood ($0.26 \pm 0.06 \text{ \%ID/g}$) at 120 min. Between 30 min and 60 min, activity in blood, liver and kidney continued to decrease, while activity levels in lung, heart, spleen muscle pancreas and brain remained fairly stable. The increase in mean uptake of [^{18}F]**7** in lung and heart from 30 to 60 min was within experimental error and was not significant (P values comparing the 30 and 60 min time points using the Students t-test were 0.56 for lung and 0.66 for heart). Bone and fat were taken only at the 5 and 60 min time points. Although the activity in fat increased 20% (from 0.14 ± 0.02 to $0.17 \pm 0.01 \text{ \%ID/g}$, Students t-test P value = 0.04) there was no significant accumulation of [^{18}F]**7** activity in bone between 5 and 60 min (from 0.28 ± 0.01 to $0.33 \pm 0.06 \text{ \%ID/g}$, Students t-test P value = 0.14). These data indicate that there was no significant metabolic defluorination during the period of time needed for PET data acquisition.

MicroPET Studies

MicroPET imaging studies in adult male Sprague-Dawley rats ($N = 3$) were also conducted in order to compare the myocardial uptake of [^{18}F]**7** with that of [^{11}C]palmitate. Representative myocardial microPET images obtained from same rat are displayed in Figure 2A when using [^{11}C]palmitate and Figure 2B when using [^{18}F]**7**. Images are displayed on

the transaxial axis and represent data acquired 20 – 30 min post-injection. [^{18}F]7 images displayed excellent quality and higher tracer activity than the corresponding [^{11}C]palmitate images. PET images were consistent with the biodistribution data and showed no evidence of accumulation in bone. Because the biological half-life of [^{11}C]palmitate in human heart is short, a tissue time-activity curve for 0 – 20 min provides sufficient information for measuring the kinetics of fatty acid metabolism (33). To be consistent, Figure 2C and 2D display 0 – 20 min tissue time-activity curves for both radiotracers. [^{18}F]7 exhibited significant tracer uptake in the heart and a rapid biphasic washout. However, some differences were noted when the data were compared with the time-active curves of [^{11}C]palmitate. For example, [^{18}F]7 exhibited an early plateau followed by a slower early tracer clearance (0.17 ± 0.01 vs. 0.30 ± 0.02 , $P < 0.0001$) and a significantly higher late clearance (0.0030 ± 0.0005 vs. 0.0006 ± 0.00013 , $P < 0.01$). Studies to determine if the differences in washout kinetics of [^{18}F]7 versus [^{11}C]palmitate reflect a difference in fatty acid metabolism or are due to the position of the radiolabel (C-1 position in [^{11}C]palmitate vs. *p*-position of the benzene ring in [^{18}F]7) are currently ongoing in our group.

CONCLUSION

We have successfully synthesized the fatty acid analogue [^{18}F]7 with good yield, high radiochemical purity, and high specific activity. The initial biodistribution and microPET imaging studies of [^{18}F]7 in rats indicates that [^{18}F]7 displays a high uptake in heart and good contrast vs. blood and other tissues. The initial microPET studies of [^{18}F]7 in male Sprague-Dawley rats indicated that the tissue time-activity of [^{18}F]7 displays rapid biphasic washout kinetics similar to that of [^{11}C]palmitate.

There is a compelling need for widely applicable radiotracer method that provides accurate and comprehensive measurements of myocardial fatty acid metabolism in humans. Current radiotracers are limited either by their lack of quantitative capability (e.g., [^{123}I]BMIPP), inability to track various aspects of fatty acid metabolism (e.g., [^{18}F]FTHA or [^{18}F]FTP) or wide spread applicability (e.g., [^{11}C]palmitate). The availability of [^{18}F]7 combined with appropriate image analysis tools should overcome these limits. The availability of these methods will facilitate the identification of relevant fatty acid metabolic biomarkers predictive of cardiac disease presence and/or progression and to evaluate specific therapeutic strategies. Once these data are obtained, the usefulness of this radiotracer technique in the clinical management of patients could be determined. Moreover, PET studies with [^{18}F]7 could be used to measure fatty acid metabolism in other organs such as skeletal muscle and liver. Thus, the impact on human health of an ^{18}F -labeled radiotracer for measuring fatty acid metabolism could be further expanded by enabling the study of other common diseases where alterations in cellular fatty acid metabolism are central to disease pathophysiology, such as insulin resistance syndrome and non-alcoholic fatty liver disease.

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ABBREVIATIONS

BMIPP	[^{123}I]β-methyl iodophenylpentadecanoic acid
[^{11}C]palmitate	[^{11}C]palmitic acid
[^{18}F]FCPHA	trans-9(RS)-[^{18}F]fluoro-3,4(RS,RS) methyleneheptadecanoic acid

[¹⁸F]FTHA	14(R,S)-[¹⁸ F]fluoro-6-thia-heptadecanoic acid
[¹⁸F]FTP	16-[¹⁸ F]fluoro-4-thia-palmitate
HPLC	high-performance liquid chromatography
%ID/g	percent injected dose per gram
IPPA	15-(para-iodophenyl)-pentadecanoic acid
MP	Melting Point
ND	Not determined
PPARα	peroxisome proliferator-activated receptor alpha
PET	positron emission tomography
SPECT	single photon emission computed tomography
TLC	thin layer chromatography
TMS	tetramethylsilane

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Figure 1.
Radiolabeled fatty acid analogs used in PET and SPECT imaging studies.

Figure 2.

MicroPET imaging studies of [^{11}C]palmitate (Panel A) and [^{18}F]7 (Panel B) in male Sprague-Dawley rats. Images are displayed on the transaxial axis and represent data acquired 20–30 min post-injection of the radiotracer. [^{18}F]7 images display excellent quality and higher tracer accumulation than the [^{11}C]palmitate images. Tissue-time-activity curve are shown in Panel C for [^{11}C]palmitate and Panel D for [^{18}F]7. Both radiotracers display biphasic washout kinetics but some differences are noted. For example, when compared to [^{11}C]palmitate, [^{18}F]7 exhibited an early plateau followed by a slower early tracer clearance (0.17 ± 0.01 vs. 0.30 ± 0.02 , $P < 0.0001$); and a significantly higher late clearance (0.0030 ± 0.0005 vs. 0.0006 ± 0.00013 , $P < 0.01$).

Scheme 1.

Synthesis of the precursor for radiolabeling and the HPLC standard.

Reagents and conditions: (a) trimethylsilyl diazomethane, THF, hexane, 2hr; (b) 4-benzyloxyphenylboronic acid, Pd(OAc)₂, [HP(t-Bu)₂Me]BF₄, KO-t-Bu, t-amyl alcohol, argon, RT; (c) 98% sulfuric acid, methanol; (d) Pd/c, EtOAc, H₂; (e) BrCH₂CH₂F, K₂CO₃, acetone; (f) TsOCH₂CH₂OTs, K₂CO₃, CH₃CN, reflux; g), 2N NaOH, aqueous MeOH.

Scheme 2.

Radiosynthesis of [^{18}F]7.

Reagents and conditions (a) [$^8\text{F}:\text{F}$ - Kryptof $\times$ 222 K_2CO_3 CH_3CN 80°C (b) 2N NaOH/
MeOH H_2O (c) 1N HC

Table 1Distribution of [^{18}F]**7** in male Sprague-Dawley rats (%ID/gram)^a

	5 min	30 min	60 min	120 min
blood	0.76 ± 0.05	0.75 ± 0.06	0.56 ± 0.06	0.26 ± 0.06
lung	1.26 ± 0.32	0.87 ± 0.06	0.95 ± 0.27	0.52 ± 0.06
liver	2.71 ± 0.37	1.39 ± 0.09	0.81 ± 0.10	0.49 ± 0.07
spleen	0.94 ± 0.05	0.63 ± 0.07	0.57 ± 0.11	0.40 ± 0.06
kidney	1.30 ± 0.09	1.26 ± 0.08	1.02 ± 0.15	0.52 ± 0.07
muscle	0.23 ± 0.03	0.24 ± 0.04	0.21 ± 0.02	0.12 ± 0.01
fat ^b	0.14 ± 0.02	ND	0.17 ± 0.01	ND
heart	2.00 ± 0.44	0.72 ± 0.10	0.86 ± 0.28	0.34 ± 0.04
pancreas	0.73 ± 0.07	0.50 ± 0.03	0.42 ± 0.04	0.28 ± 0.02
brain	0.11 ± 0.01	0.13 ± 0.01	0.10 ± 0.01	0.06 ± 0.01
bone ^b	0.28 ± 0.01	ND	0.33 ± 0.06	ND

^aData is shown as Mean ± Std Dev.^bResults not determined (ND) for fat and bone at 30 and 60 min.