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Structure Dependence of Long-Chain [^{18}F]Fluorothia Fatty Acids as Myocardial Fatty Acid Oxidation Probes

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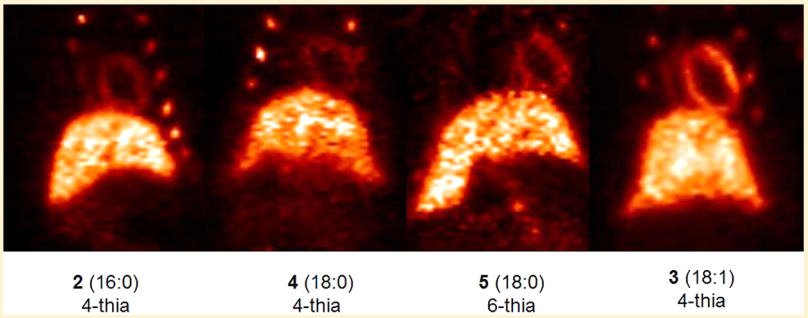
Supporting Information



ABSTRACT: In vivo imaging of regional fatty acid oxidation (FAO) rates would have considerable potential for evaluation of mammalian diseases. We have synthesized and evaluated ^{18}F-labeled thia fatty acid analogues as metabolically trapped FAO probes to understand the effect of chain length, degree of unsaturation, and placement of the thia substituent on myocardial uptake and retention. 18-[^{18}F]Fluoro-4-thia-(9*Z*)-octadec-9-enoic acid (**3**) showed excellent heart/background radioactivity concentration ratios along with highest retention in heart and liver. Pretreatment of rats with the CPT-1 inhibitor, POCA, caused >80% reduction in myocardial uptake of 16-[^{18}F]fluoro-4-thiahexadecanoic acid (**2**) and **3**, indicating high specificity for FAO. In contrast, 18-[^{18}F]fluoro-4-thiaoctadecanoic acid (**4**) showed dramatically reduced myocardial uptake and blunted response to POCA. 18-[^{18}F]Fluoro-6-thiaoctadecanoic acid (**5**) showed moderate myocardial uptake and no sensitivity of myocardial uptake to POCA. The results demonstrate relationships between structures of ^{18}F-labeled thia fatty acid and uptake and their utility as FAO probes in various tissues.

INTRODUCTION

Fatty acid oxidation (FAO) plays a pivotal role in energy production in many tissues and is a candidate biomarker for various disease states in cardiology, endocrinology, neurology, and oncology. Myocardial FAO is a highly regulated process, governed by fatty acid supply, competing energy substrates, energy demand, oxygen supply, and hormonal status among other factors.[1] The potential diagnostic utility of a noninvasive PET (positron emission tomography) approach to estimate regional FAO rates in the heart becomes apparent, as cardiac dysfunction often displays metabolic changes that alter FAO,[1] and could be applicable for evaluation of myocardial ischemia or heart failure. According to the metabolic trapping approach for radiotracer design, the ideal radiotracer for myocardial FAO imaging will mimic natural long chain fatty acids in their transport from the bloodstream into myocytes as well as their intracellular trafficking to the mitochondrion for oxidation.[1] FAO occurs in the mitochondrion matrix through the cyclical oxidative process of β-oxidation. The overall rate of β-oxidation is dependent on the flux control exerted by a multitude of processes, including fatty acid transport across the cell membrane (CD36/FATP), activation by fatty acyl CoA synthetase, transport into the mitochondrial matrix facilitated by carnitine palmitoyltransferases I and II (CPT-I, CTP-II), carnitine−acylcarnitine translocase (CAT), and the four enzymes responsible for β-oxidation.[1] CPT-I present at the outer mitochondrial membrane often represents the major step for flux control for FAO of fatty acids.[1] Therefore, CPT-I inhibitors such as POCA (sodium 2-[5-(4-chlorophenyl)-pentyl]oxirane-2-carboxylate) have been extensively employed to reduce FAO rates in experimental studies.[2]

The ideal FAO probe that is based on the metabolic trapping concept should follow the flux of fatty acids through at least the initial step(s) of β-oxidation, at which point the tracer becomes metabolically trapped. The probe should not undergo esterification into various complex lipids, as it renders the accumulation of radioactivity less specific to FAO.[1,3−5] Over the decades, our research has been focused on the potential of radiolabeled thia-substituted long-chain fatty acid analogues as such metabolically trapped probes of FAO in heart and other tissues.[2,3,6−9] The first molecule developed was [^{18}F]FTHA

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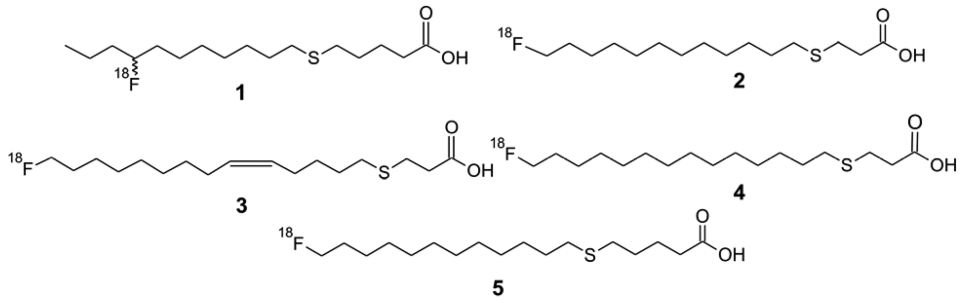
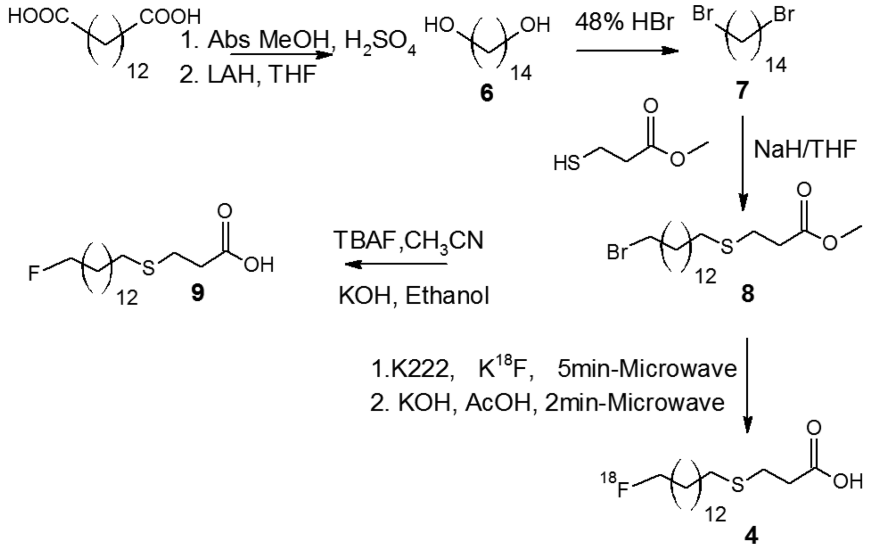
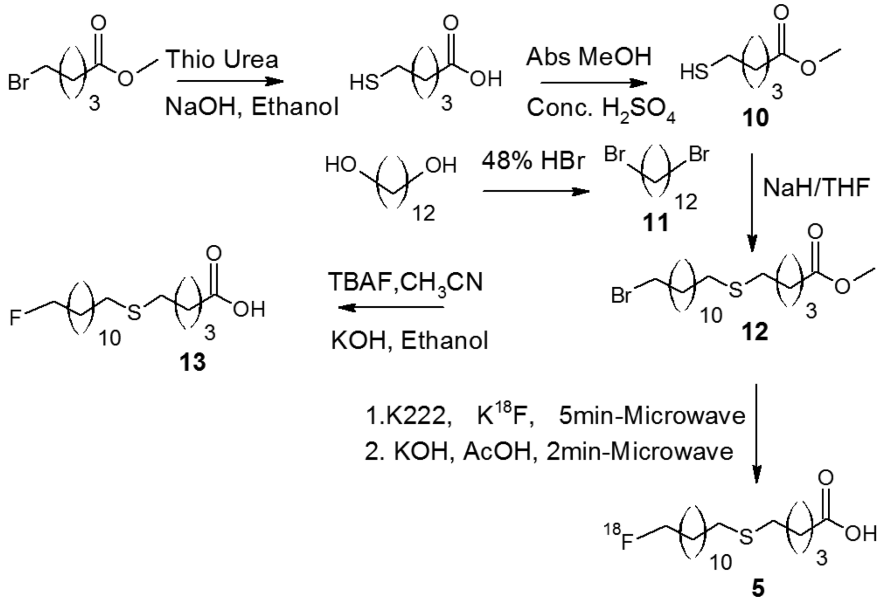


Figure 1. Structure of thia-substituted fatty acids synthesized and evaluated as metabolically trapped myocardial FAO probes.

Scheme 1. Synthesis of 18-[^{18}F]Fluoro-4-thiaoctadecanoic Acid (4)



Scheme 2. Synthesis of 18-[^{18}F]Fluoro-6-thiaoctadecanoic Acid (5)



(**1**)[2] (Figure 1), a 6-thia analogue that showed excellent myocardial imaging characteristics . Surprisingly, **1** was found to be metabolized in heart to protein-bound species, presumably metabolic byproducts from β-oxidation. However, **1** lacked specificity to measure FAO in hypoxic conditions because of maintained myocardial retention of radiotracer.[3,8] Furthermore, metabolite analyses in liver and skeletal muscle tissues revealed a predominant accumulation of **1** in lipid pools, further limiting its application as a general mitochondrial FAO probe.[2] The second tracer developed was a 4-thia analogue of palmitic acid, **2** (Figure 1). The 4-thia analogue showed enhanced specificity to myocardial FAO, correlating with FAO rates of natural fatty

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Table 1. Uptake (% dose kg/g) of 16-[^{18}F]Fluoro-4-thiahexadecanoic Acid (2) in Fasted Sprague–Dawley Rats

organ/tissue (N = 6)	control 30 min[b]	poca treated 30 min[a]	control 120 min[b]	POCA treated 120 min[a]
blood	0.070 ± 0.059	0.0633 ± 0.0077	0.0105 ± 0.0028	0.0392 ± 0.0094[c]
heart	0.589 ± 0.257[d]	0.0792 ± 0.0174[e]	0.1069 ± 0.0649	0.1042 ± 0.0387
lung	0.099 ± 0.140	0.0676 ± 0.0126	0.0183 ± 0.0072	0.0472 ± 0.0098[c]
liver	0.899 ± 0.314	0.6197 ± 0.0949	0.4700 ± 0.1888	0.4433 ± 0.1201
kidney	0.209 ± 0.144	0.2477 ± 0.0310	0.1014 ± 0.0512	0.1637 ± 0.0659
brain	0.067 ± 0.049[d]	0.04879 ± 0.0142	0.0154 ± 0.0056	0.0578 ± 0.0114[c]
bone	0.235 ± 0.168	0.1790 ± 0.0758	0.3143 ± 0.1334	0.6549 ± 0.1112[c]
muscle	0.031 ± 0.013[d]	0.0333 ± 0.0080	0.0109 ± 0.0059	0.0298 ± 0.0069[c]

[a]Pretreated with POCA (30 mg/kg ip) 2 h prior. [b]Data from ref 10. [c]$p < 0.05$ for 120 min control vs 120 min POCA. [d]$p < 0.05$ for 30 min control vs 120 min control. [e]$p < 0.01$ for 30 min control vs 30 min POCA.

acids in hypoxic conditions.[6,7] However, uptake of **2** in liver was not sensitive to CPT-I inhibition, and there was poor retention of the "metabolically trapped" radioactivity in rat myocardium over a 2 h period.[3] Subsequently, a 4-thia substituted oleic acid analogue was developed as **3**[10] (Figure 1). Compound **3** showed enhanced uptake and retention in rat myocardium relative to both **1** and **2**.[10] Pharmacologic inhibition of CPT-1 with etomoxir evoked >80% decrease in myocardial uptake, demonstrating excellent sensitivity to change in myocardial FAO.[10] The differences in the biodistribution properties of compounds **1**–**3** raised the questions: What aspects of the structure of **3** were responsible for its favorable myocardial uptake properties, and could they be further enhanced? To begin to answer these questions, two additional molecules were designed and evaluated as stearic acid analogues: 18-[^{18}F]-fluoro-4-thiaoctadecanoic acid (18:0) (**4**) and 18-[^{18}F]fluoro-6-thiaoctadecanoic acid (18:0) (**5**) (Figure 1). The in vivo characteristics of **4** and **5** were examined in comparison with those of the previously developed **2** and **3**, since these two radiotracers fill the structural gap between the **2** and **3** to investigate the effects of chain length (**2** versus **4**), thia position (**4** versus **5**), and saturation (**3** versus **4**). Our present study provides considerable information on the structural dependence of thia-substituted fatty acid radiotracer uptake and retention in myocardium and provides a stronger basis for rational design of PET imaging probes for FAO.

RESULTS

Synthesis of 18-[^{18}F]Fluoro-4-thiaoctadecanoic Acid (4) and 18-[^{18}F]Fluoro-6-thiaoctadecanoic Acid (5). Schemes 1 and 2 describe the synthesis of **4** and **5**, respectively. To synthesize the labeling precursor for **4**, 1,14-tetradecadioic acid was converted to 1,14-tetradecane diol (**6**) in two steps: esterification using absolute methanol and catalytic amount of sulfuric acid followed by LAH (lithium aluminum hydride) reduction in THF (tetrahydrofuran).[11] The obtained diol was refluxed with 48% aqueous HBr to yield 1,14-dibromotetradecane (**7**), which on subsequent reaction with methyl 3-mercaptopropanoate in the presence of sodium hydride in THF yielded methyl 18-bromo-4-thiaoctadecanoate (**8**) as precursor for radiofluorination. The formation of compound **8** was characterized by the presence of two triplets peaks in the ^{1}H NMR spectrum, one at δ 3.41 ppm for two protons (CH_2Br) and another at δ 2.60 ppm for two protons (CH_2S). The product formation was further confirmed by carbon NMR, gradient HSQC, and HR(EI) mass spectrometry. The bromo ester **8** was treated with tetrabutylammonium fluoride in acetonitrile followed by ester hydrolysis to obtain non-radioactive standard (**9**) as a HPLC standard. The formation of **9** was characterized by the appearance of a doublet of a triplet (dt) at δ 4.46 ppm for two protons due to the formation of the new carbon–fluorine bond (CH_2F) and the absence of a triplet peak at δ 3.41 ppm for two protons (CH_2Br). The formation of cold standard **9** was also confirmed and supported by a doublet peak at δ 83.7–84.8 ppm due to fluorine–carbon coupling in its carbon NMR. HRMS confirmed the product formation with a molecular ion peak at 320.217 55 amu. The radiofluorination of the bromo ester **8** using cyclotron-produced K^{18}F was carried out under microwave heating (75 °C) as previously described.[12] Radiofluorination proceeded efficiently, with >90% incorporation of [^{18}F]fluoride into the corresponding ester. The [^{18}F]fluoro ester was quantitatively hydrolyzed by addition of 0.2 N KOH and microwave heating (75 °C) for 2 min. Overall radiochemical yields after semipreparative HPLC isolation (t_R = 9.4 min) were 30–33% decay-corrected. Radiochemical purity of the product **4** was >99% by radio-HPLC. The product was formulated in 0.5–1.0% BSA (bovine serum albumin) in saline solution and passed through a sterile filter before administration to rats.

The synthesis of the labeling precursor for **5** required a modified approach because of the unavailability of methyl 6-mercaptopentanoate. The methyl 6-mercaptopentanoate (**10**) was obtained on reaction between methyl 5-bromopentanoate and thiourea followed by treatment with ethanolic NaOH and subsequent esterification with absolute methanol.[13] Compound **10** was treated with 1,12-dibromododecane (**11**) in the presence of sodium hydride in THF, forming methyl 18-bromo-6-thiaoctadecanoate (**12**) as a precursor for radiofluorination. Compound **12** was characterized by the proton NMR presence of a triplet at δ 3.42 ppm for two protons (CH_2Br) and another multiplet at δ 2.52–2.50 ppm for four protons due to the formation of a thioether (CH_2-S-CH_2). The product formation was further supported by carbon NMR and HR(EI) mass spectrometry. The bromo ester **12** was also treated with tetrabutylammonium fluoride in acetonitrile followed by ester hydrolysis to obtain nonradioactive standard **13** as nonradioactive HPLC standard. Compound **13** was confirmed by proton and carbon NMR and HRMS. The radiofluorination, hydrolysis, and formulation were carried out in a similar manner as with **4**. The radiochemical yield of the labeled product **5** after semipreparative HPLC isolation (t_R = 8.7 min) was 30–33% decay-corrected. Radiochemical purity of the product (**5**) by r-HPLC was >99%.

Biodistribution Studies in Rats. Tables 1–4 show the biodistribution data for ^{18}F-radioactivity in rats after intravenous administration of **2**–**5** at 30 and 120 min postinjection (pi). To indicate the sensitivity of the biodisposition of the fatty acid analogues to FAO impairment, data were obtained with

Table 2. Uptake (% dose kg/g) of 18-[^{18}F]Fluoro-4-thiaoctadecanoic Acid (4) in Fasted Sprague−Dawley Rats

organ/tissue (N = 6)	control 30 min	POCA treated 30 min[a]	control 120 min	POCA treated 120 min[a]
blood	0.0392 ± 0.0063	0.0510 ± 0.0026[b]	0.0098 ± 0.0006[c]	0.0125 ± 0.0033
heart	0.3140 ± 0.0738	0.1814 ± 0.0276[b]	0.2144 ± 0.1017[c]	0.1025 ± 0.0260
lung	0.0755 ± 0.0168	0.0998 ± 0.0245	0.0339 ± 0.0122[c]	0.0244 ± 0.0092
liver	0.6292 ± 0.1114	0.4064 ± 0.0787[b]	0.3759 ± 0.0655[c]	0.1341 ± 0.0686[d]
kidney	0.1872 ± 0.0248	0.2350 ± 0.0199[b]	0.0726 ± 0.0166[c]	0.0947 ± 0.0350
brain	0.0200 ± 0.0037	0.0385 ± 0.0033[b]	0.0143 ± 0.0015	0.0310 ± 0.0111[d]
bone	0.2309 ± 0.0588	0.2289 ± 0.0332	0.2934 ± 0.0826	0.4042 ± 0.0612[d]
muscle	0.0303 ± 0.0061	0.0443 ± 0.0047[b]	0.0181 ± 0.0070[c]	0.0197 ± 0.0060

[a]Pretreated with POCA (30 mg/kg i.p.) 2 h prior. [b]$p < 0.05$ for 30 m control vs 30 m POCA. [c]$p < 0.05$ for 30 m control vs 120 m control. [d]$p < 0.05$ for 120 m control vs 120 m POCA.

Table 3. Uptake (% dose kg/g) of 18-[^{18}F]Fluoro-6-thiaoctadecanoic Acid (5) in Fasted Sprague−Dawley Rats

organ/tissue (N = 6)	control 30 min	POCA treated 30 min[a]	control 120 min	POCA treated 120 min[a]
blood	0.0290 ± 0.0120	0.0510 ± 0.0190[b]	0.0241 ± 0.0137	0.0271 ± 0.0038
heart	0.4413 ± 0.1749	0.3331 ± 0.1210	0.2815 ± 0.0895	0.2288 ± 0.1051
lung	0.0621 ± 0.0180	0.1163 ± 0.0421	0.0702 ± 0.0118	0.0536 ± 0.0090
liver	1.1260 ± 0.5112	0.8492 ± 0.3636	0.7548 ± 0.1403	0.4190 ± 0.0941[d]
kidney	0.2095 ± 0.0734	0.3441 ± 0.1484	0.1629 ± 0.0587	0.1554 ± 0.0249
brain	0.0330 ± 0.0223	0.0879 ± 0.0479	0.0220 ± 0.0041	0.0519 ± 0.0124[d]
bone	0.1171 ± 0.0493	0.1037 ± 0.0297	0.2467 ± 0.0766[c]	0.2104 ± 0.0494
muscle	0.0390 ± 0.0268	0.0639 ± 0.0242	0.0263 ± 0.0063	0.0599 ± 0.0127

[a]Pretreated with POCA (30 mg/kg ip) 2 h prior. [b]$p < 0.05$ for 30 min control vs 30 min POCA. [c]$p < 0.05$ for 30 min control vs 120 min control. [d]$p < 0.05$ for 120 min control vs 120 min POCA.

Table 4. Uptake (% dose kg/g) of 18-[^{18}F]Fluoro-4-thia-(9Z)-octadec-9-enoic Acid (3) in Fasted Sprague−Dawley Rats

organ/tissue (N = 5)	control 30 min[a,b]	POCA treated 30 min	control 120 min	POCA treated 120 min[a,b]
blood	0.0441 ± 0.0121	0.0606 ± 0.0096	0.0185 ± 0.005[d]	0.0141 ± 0.0098
heart	0.7028 ± 0.3019	0.1155 ± 0.0211[c]	0.8164 ± 0.2249	0.0760 ± 0.0360
lung	0.0496 ± 0.0096	0.0458 ± 0.0061	0.0228 ± 0.005[d]	0.0179 ± 0.0109
liver	1.1847 ± 0.1550	0.8162 ± 0.1420[c]	0.8488 ± 0.2232[d]	0.1666 ± 0.0706[e]
kidney	0.2035 ± 0.0510	0.1932 ± 0.0173	0.1244 ± 0.0310	0.0611 ± 0.0222[e]
brain	0.0206 ± 0.0057	0.0368 ± 0.0044[c]	0.0215 ± 0.0051	0.0206 ± 0.009
bone	0.1531 ± 0.0796	0.1475 ± 0.0355	0.4610 ± 0.4140	0.2990 ± 0.1645
muscle	0.0201 ± 0.007	0.0297 ± 0.0039[c]	0.0171 ± 0.0024	0.0216 ± 0.0137

[a]Pretreated with POCA (30 mg/kg ip) 2 h prior. [b]Reference 10. [c]$p < 0.05$ for 30 min control vs 30 min POCA. [d]$p < 0.05$ for 30 min control vs 120 min control. [e]$p < 0.05$ for 120 min control vs 120 min POCA.

and without pretreatment of rats with the CPT-1 inhibitor POCA (sodium 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate).[2,14]

The palmitate analogue **2** showed high myocardial uptake at 30 min (0.589 ± 0.257% ID kg/g, Table 1). However, there was >80% washout of radioactivity from control hearts from 30 to 120 min. The pretreatment of rats with POCA markedly decreased (>90%) myocardial uptake at 30 min, indicating very high sensitivity of **2** toward FAO. The effect of POCA was not observed at 120 min because of clearance of **2** from control hearts. The metabolic fate of **2** studied by Folch type extraction of heart tissues (Table 5) showed 58.8 ± 19.2% of activity bound to the protein pellet at 30 min control compared to 32.8 ± 6.1% of activity with POCA treated rats at 30 min. Radioactivity in the protein pellet was interpreted as the fraction of radiotracer that had undergone β-oxidation.[2,3,5,10] Inhibition of β-oxidation by POCA results in reduced radioactivity bound to protein and increased radioactivity in organic soluble metabolites (nonmetabolized fatty acid or complex lipids) or to a lesser extent aqueous metabolites.[5] Therefore, these data further supported the sensitivity of **2** to FAO. This effect was more pronounced at 120 min, showing 76.9 ± 10.8% of radioactivity bound to protein pellet in control hearts compared to 24.5 ± 5.4% in POCA hearts (Table 5).

Table 2 summarizes the biodistribution data for **4** (18:0). It showed 1.7 fold higher ($p < 0.05$) heart uptake of tracer at 30 min control rats than 30 min POCA treated rat, indicating considerable sensitivity of **4** toward FAO. More importantly, **4** showed longer retention in heart over time as indicated by 120 min control uptake studies. The Folch type extraction of heart tissues treated with **4** showed 78.7 ± 3.5% radioactivity bound with protein pellet at 30 min control, supporting sensitivity of the tracer toward FAO. On comparison with **2**, **4** had 1.8-fold lower myocardial uptake at 30 min control but longer retention in the heart. The longer retention was also supported by metabolic studies where it showed 71.5 ± 5.5% of radioactivity bound to protein pellet at 120 min control.

Table 3 summarizes the biodistribution data for **5** where the sulfur atom replaced the sixth carbon in the stearate analogue. CPT-I inhibition by POCA had no effect on myocardial uptake at either time points, indicating poor sensitivity to indicate mitochondrial FAO. Myocardial washout was slow over the 30−120 min period. The retained radioactivity was primarily in the form of protein-bound metabolites in control hearts but in

the organic phase of Folch extractions in POCA hearts (Table 5). Similar results were also obtained at the 120 min time point. On comparison to **4**, **5** had similar uptake and retention in heart tissue at 30 and 120 min controls but uptake of **5** by the heart was found to be less sensitive to POCA treatment.

Table 4 provides the biodistribution data for the oleate analogue **3**. Compound **3** showed the highest heart uptake of the four tracers. Myocardial uptake of **3** in control animals was statistically unchanged from 30 to 120 min, demonstrating effective trapping in heart tissue. On treatment with POCA, the heart uptake dramatically decreased at both 30 min (−84%) and 120 min (−90%) time points, indicating very high specificity and sensitivity of **3** for FAO (Figure 2). The

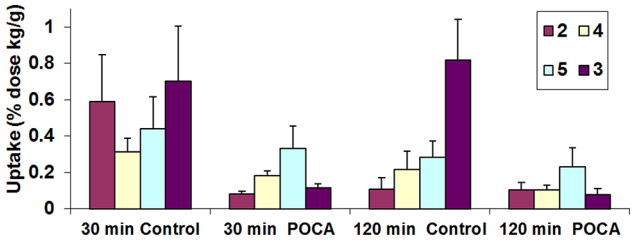

Figure 2. Comparison of heart uptake of **2**−**5** in fasted rats. Compound **3** uptake at 120 min control rats is statistically higher than for **2**, **4**, and **5** ($p < 0.05$). Compound **3** showed the highest degree of blockade of heart uptake at both 30 and 120 min time points. The data are derived from Tables 2−4.

Folch-type extraction of heart tissues after administration of **3** demonstrated the highest levels of protein binding for all tracers studied (>88%), suggesting the highest metabolic conversion to protein-bound metabolites. In control animals, myocardial uptake of **3** was 1.2-, 2.2-, and 1.6-fold higher ($p < 0.05$) than that of **2**, **4**, and **5** at 30 min pi (Figure 2). At the 120 min time point, the heart uptake of **3** was 2.6- to 8.0-fold higher ($p < 0.05$) than the other tracers (Figure 2).

With regard to the whole-body distribution of the tracers, **2** and **4** showed high uptake in liver, followed by heart, bone, kidney, and other organs at 30 min in fasted control rats. Compounds **3** and **5** showed highest uptake in liver, followed by heart, kidney, bone, and other organs at 30 min control fasted rats. Furthermore, all four tracers (**2**−**5**) showed the highest uptake in liver at 120 min. However, this trend differed slightly in the cases of **5** and **3**, where the heart showed the second highest uptake after liver, indicating longer myocardial retention. Hepatic clearance was greater for **2**, **4**, and **5** in comparison with **3** (Figure 3) between 30 and 120 min in control animals. Bone uptake for the all the four tracers was comparable at 120 min, suggesting their equivalent defluorination rates in rat.

Heart/organ ratios for **2**−**5** at 120 min after administration of tracers are shown in Figure 4. Heart/blood ratio for **3** was

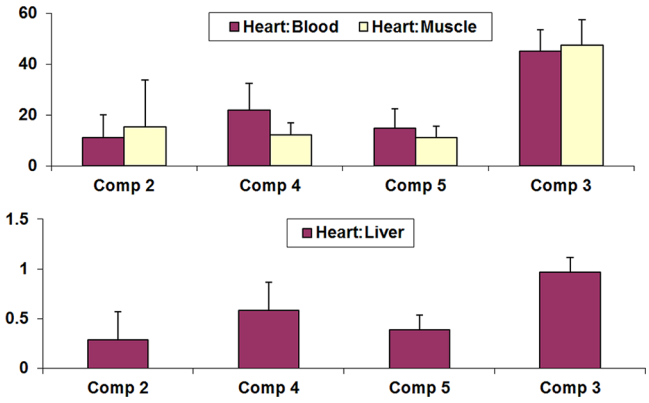

Figure 4. Comparison of heart/blood, heart/muscle, and heart/liver ratios for compounds **2**−**5** in fasted rats at 120 min pi. Heart/blood and heart/muscle are statistically higher for **3** than for **2**, **4**, and **5** ($p < 0.05$). Heart/liver values for **3** are also statistically higher than for **2** and **5** ($p < 0.05$). The data are derived from Tables 2−4.

found to be 4.0-fold higher than **2**, 1.8-fold higher than **4**, and 3.0-fold higher than **5** ($p < 0.005$). The heart/muscle ratio for **3** was 3.0-, 3.5-, and 4.2-fold higher than **2**, **4**, and **5**, respectively ($p < 0.005$). Furthermore, heart/liver ratios for **3** were 3.3- and 2.5-fold higher than **2** and **5**, respectively ($p < 0.005$).

Folch-type extraction analysis of tissues showed that **3** is metabolized predominantly to protein-bound species in control hearts (Figure 5). At 120 min, the radioactivity of protein-bound fraction in heart was significantly higher for **3** (89%) relative to **2** (77%), **4** (71%), and **5** (67%) ($p < 0.05$). At 30 min, protein-bound, aqueous, and organic fractions in liver and skeletal muscle were similar for **2**, **4**, and **5** (Figure 5). However, **3** showed up to 71% ($p < 0.01$) and 59% protein-bound fractions in liver and skeletal muscle, respectively (Table 5). The protein-bound fractions of **2**, **4**, and **5** were lower than those for **3** in liver and skeletal muscle, indicating lower FAO-dependent metabolic trapping relative to **3** in these organs.

It was also observed that POCA-treated rats had relatively higher uptake in blood, brain, and muscle. For example, POCA-treated rats with **2** and **5** showed 2.7-fold ($p < 0.005$) and 2.2-fold ($p < 0.005$) higher muscle uptake at 120 min than control

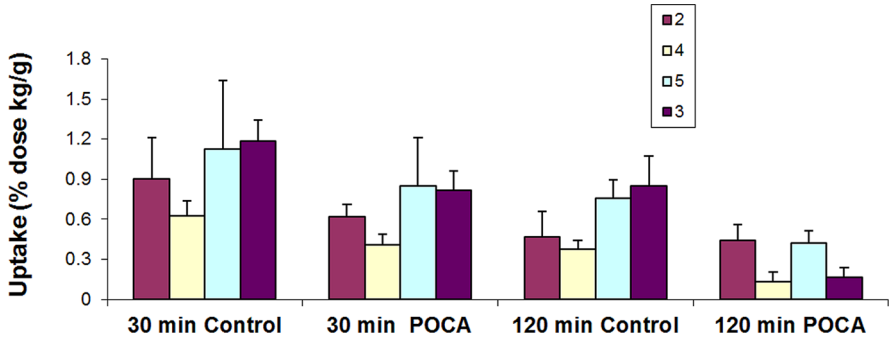

Figure 3. Comparison of liver uptake of **2**−**5** in fasted rats. Compounds **3** and **4** showed the highest degree of blockade of liver uptake at 120 m time point ($p < 0.05$). The data are derived from Tables 2−4.

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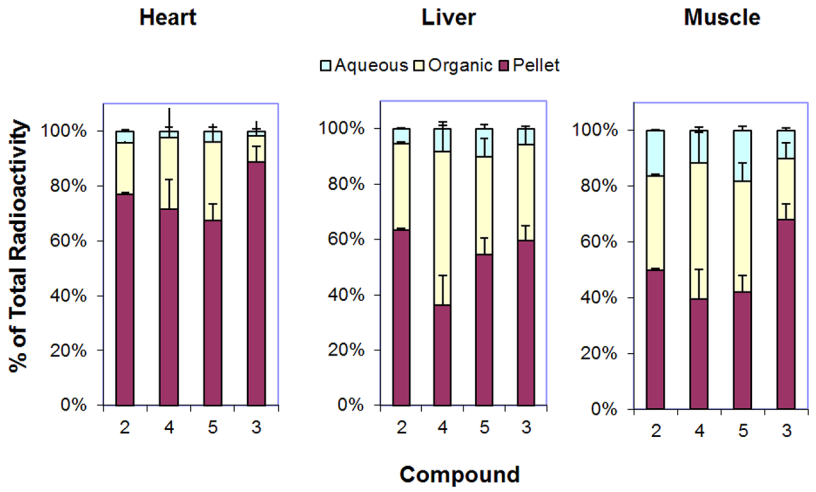

Figure 5. Folch-type extraction analysis of metabolites at 120 min (pi) in control Sprague–Dawley rats. The data are derived from Table 5.

rats, respectively. In contrast, such enhancement with POCA treatment was not observed for **3** and **4**. POCA-treated rats showed ~3.0-fold higher fatty acid concentration and reduced glucose concentration in plasma with respect to control rats (Table 6), whereas triglyceride and lactate concentrations were independent of CPT-I inhibition. As a CPT-I inhibitor, POCA was developed to be a potential antidiabetic drug. It increases plasma fatty acid levels by lowering the rate of fatty acid clearance from the blood because of decreased β-oxidation in liver, heart, and skeletal muscle. Conversely, POCA lowers blood glucose. The decrease in FAO stimulates glucose oxidation in liver, heart, and skeletal muscle to maintain energy status. The higher glucose metabolic rates in these tissues results in a decrease in plasma glucose levels.

MicroPET Studies in Rats. A preliminary microPET imaging study was performed with **2**–**5** in fasted rats (Figure 6). Initial dynamic images showed rapid blood clearance of all four tracers to low radioactivity concentrations within 2 min pi (postinjection). Similarly, lung clearance was also very rapid and gave low background to the heart by 2 min pi. The hepatic uptake of all four thia fatty acid tracers (**2**–**5**) was extremely high at 5 min pi, followed by slow clearance over time. Myocardial uptakes of all four PET probes plateaued at about 10 min, but faster clearance was observed with **2**, whereas myocardial clearance was nominal for **4** and **5** but remained stable for **3**. Whole-body images acquired 55–115 min pi demonstrated superior myocardial imaging abilities of **3** among the thia fatty acid tracers (Figure 6). The biodistribution data for all four tracers (Tables 1–4) were consistent with microPET images (Figure 6). Bone uptake in ribs were seen with all four radiotracers, indicating in vivo defluorination in the rat.

DISCUSSION

Fatty acid oxidation (FAO) is the key energy producer in heart, liver, and skeletal muscle. Furthermore, it has recently been investigated that the brown adipose tissue plays a vital role in thermogenesis during cold exposure via oxidative metabolism of fat in human adults.[15] A FAO-specific PET imaging technique has a considerable potential to improve existing medical care by determining disease state and progression and also for evaluation of potential drug therapies targeted to alter FAO rates.[16–18] Several non-thia fatty acid analogues of palmitic acid labeled with ^{18}F and ^{11}C were studied and documented in the literature.[4,19–23] However, the fate of the radiolabel involves multiple catabolic and anabolic processes and requires complex kinetic models to derive an estimate for FAO.[4,19–24] All the radiotracers in the current study were radiolabeled with ^{18}F at the terminal (ω) position, a site known to undergo metabolic defluorination in liver.[25] Although we have previously shown that the label can be stabilized against defluorination if placed at the (ω-3) position,[25] the synthesis burden is greatly increased to produce the labeling precursors. Furthermore, subsequent to our early work with (ω-3) labeled tracers, we found that ω-labeling provided satisfactorily stability against defluorination in pigs and humans.[3] Defluorination of ω-labeled F-18 fatty acids in rodents is significant but does not render the present biodistribution data useless, since bone uptake is quantitatively a minor contribution of the overall biodistribution.

The present study extends our work on the structure uptake relationship of thia-substituted fatty acid analogues as metabolically trapped probes for PET imaging of FAO.[2,3,10] Thia-substituted FA analogues are designed to undergo metabolic trapping in myocardium after commitment of the FA toward mitochondrial FAO.[5] β-Oxidation of ω-[^{18}F]fluoro and 4- and 6-thia-fatty acid analogues give rise to protein-bound metabolites in the myocardium, putatively explained by β-oxidation dependent decomposition of the thia fatty acid analogues to ω-[^{18}F]fluoro long-chain thiols that covalently react with mitochondrial proteins.[2,3] The sulfur heteroatom mimics the methylene group of normal fatty acids, although it subtly increases the local polarity. The sulfur atom was initially inserted at the sixth position in the first thia fatty acid radiotracer **1**, showing sensitivity toward CPT-I inhibition in the myocardium in a mouse model.[2] However, further studies indicated FAO-independent mechanisms for retention of radiotracer in hypoxic myocardium.[2] Later, other positions of thia substitutions were investigated and it was found that sulfur substitution at the fourth position of the palmitate analogue **2** gave the best correlation with FAO rates in isolated rat hearts in both normoxic and hypoxic conditions.[3] Although **2** showed higher specificity for FAO, it showed suboptimal retention in rat myocardium.

Therefore, we further explored the structure of the natural FAO substrate oleic acid to form the 4-thia oleate analogue **3**.[10] The monounsaturated **3** (18:1) showed high myocardial uptake and longer myocardial retention measured at 120 min in rat[10]

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Table 5. Distribution of ^{18}F-Radioactivity (% of Total) in Aqueous, Organic, and Pellet Fractions after Folch-Type Extraction of Tissues

		30 min control	30 min POCA[a]	120 min control	120 min POCA[a]
		Compound **2**			
heart (n = 6)	aqueous	2.63 ± 1.00	15.28 ± 3.96[b]	4.17 ± 1.31	7.53 ± 2.25[b]
	organic	38.56 ± 19.03	51.90 ± 3.86	18.88 ± 10.79	67.98 ± 6.82[b]
	pellet	58.80 ± 19.21	32.81 ± 6.12[b]	76.93 ± 10.77	24.47 ± 5.35[b]
liver (n = 6)	aqueous	13.52 ± 8.30	20.23 ± 4.88	5.32 ± 0.82	11.52 ± 1.13[b]
	organic	48.25 ± 8.35	41.68 ± 6.53	31.39 ± 9.80	50.00 ± 8.31[b]
	pellet	38.21 ± 13.23	38.07 ± 4.09	63.27 ± 9.25	38.47 ± 8.58[b]
muscle (n = 6)	aqueous	23.24 ± 8.37	25.13 ± 2.90	16.17 ± 4.64	19.16 ± 4.09
	organic	33.56 ± 13.11	42.23 ± 6.23	34.00 ± 12.3	48.14 ± 7.73
	pellet	43.18 ± 16.90	32.63 ± 5.99	49.81 ± 16.94	32.69 ± 5.13
		compound **3**			
heart (n = 5)	aqueous	2.86 ± 1.55	10.15 ± 2.55[b]	1.86 ± 0.37	4.19 ± 1.04[b]
	organic	8.46 ± 0.86	62.03 ± 7.43[b]	9.34 ± 0.42	66.66 ± 11.65[b]
	pellet	88.67 ± 1.63	23.80 ± 5.76[b]	88.78 ± 0.71	29.14 ± 10.80[b]
liver (n = 5)	aqueous	8.43 ± 1.10	16.30 ± 3.66[b]	5.68 ± 0.19	8.10 ± 2.07
	organic	19.87 ± 3.32	58.80 ± 4.90[b]	34.75 ± 12.14	68.06 ± 3.39[b]
	pellet	71.69 ± 4.34	24.88 ± 3.54[b]	59.56 ± 12.02	23.82 ± 3.75[b]
muscle (n = 5)	aqueous	18.79 ± 3.83	24.06 ± 5.81	9.91 ± 2.52	11.37 ± 1.57
	organic	21.75 ± 4.86	43.19 ± 4.95[b]	21.98 ± 4.18	53.25 ± 9.73[b]
	pellet	59.12 ± 8.66	32.74 ± 6.02[b]	67.95 ± 1.75	35.36 ± 11.08[b]
		compound **4**			
heart (n = 6)	aqueous	3.97 ± 1.01	7.73 ± 0.62[b]	2.39 ± 0.95	3.39 ± 3.17
	organic	17.29 ± 2.61	67.58 ± 3.94[b]	26.14 ± 5.50	74.91 ± 6.52[b]
	pellet	78.72 ± 3.49	24.67 ± 3.92[b]	71.45 ± 5.49	21.68 ± 3.63[b]
liver (n = 6)	aqueous	15.36 ± 1.68	31.69 ± 5.95[b]	8.18 ± 4.73	10.23 ± 3.80
	organic	51.66 ± 5.80	51.76 ± 7.03	55.69 ± 8.48	67.69 ± 13.75
	pellet	32.97 ± 6.71	16.53 ± 1.87[b]	36.13 ± 11.75	22.07 ± 13.63
muscle (n = 6)	aqueous	28.99 ± 3.73	25.80 ± 5.01	11.58 ± 3.65	9.12 ± 2.18
	organic	38.77 ± 5.36	47.15 ± 2.67[b]	48.90 ± 7.54	58.25 ± 8.75
	pellet	32.22 ± 5.24	27.04 ± 3.35	39.51 ± 4.831	32.62 ± 8.20
		compound **5**			
heart (n = 6)	aqueous	4.02 ± 1.24	3.84 ± 1.31	4.03 ± 1.42	3.30 ± 1.07
	organic	25.81 ± 10.12	63.99 ± 9.67[b]	28.56 ± 6.62	74.20 ± 7.78[b]
	pellet	70.16 ± 9.05	32.16 ± 10.47[b]	67.39 ± 6.01	22.48 ± 6.91[b]
liver (n = 6)	aqueous	9.65 ± 3.27	10.75 ± 2.01	10.15 ± 4.50	7.45 ± 2.00
	organic	45.61 ± 5.10	54.88 ± 6.96	35.26 ± 4.02	64.78 ± 10.98[b]
	pellet	44.72 ± 4.54	34.35 ± 7.76	54.58 ± 5.08	27.76 ± 9.07[b]
muscle (n = 6)	aqueous	16.49 ± 2.26	7.70 ± 2.81[b]	18.18 ± 5.46	9.30 ± 1.56[b]
	organic	44.55 ± 8.06	43.43 ± 11.19	39.74 ± 10.78	59.27 ± 6.95[b]
	pellet	38.95 ± 7.41	48.85 ± 12.63	42.06 ± 9.84	31.41 ± 7.23

[a]Pretreated with POCA (30 mg/kg ip) 2 h prior. [b]$p < 0.05$ for control vs POCA.

Table 6. Plasma Concentration of Metabolic Substrates in Sprague−Dawley Rats

	fatty acid (mM)	glucose (mg/dL)	triglyceride (mg/dL)	lactate (mmol)
control (n = 12)	0.242 ± 0.161	111.660 ± 14.768	46.484 ± 36.701	1.797 ± 0.577
POCA (n = 6)	0.905 ± 0.085	66.748 ± 15.945	47.971 ± 18.730	1.850 ± 0.523
p	4.84×10^{-09}	0.000226	0.9107	0.8470

(Tables 4 and 5). To increase our understanding of the different biochemical behavior of **2** and **3**, two additional radiotracers **4** and **5** were synthesized and allowed for the study of the effects of chain length (**2** (16:0) versus **4** (18:0)), monounsaturation (**4** (18:0) versus **3** (18:1)), and position of the thia substituent (4-thia **4** versus 6-thia **5**). It was observed that **2** (16:0), **4** (18:0), and **5** (18:0) have almost similar myocardial uptake at 30 min. However, the stearate analogues (18:0) showed better retention in the myocardium than **2** at 120 min. Similar to the stearate (18:0) analogues, the monounsaturated (18:1) **3** also showed excellent myocardial retention. On comparison of the myocardial kinetics of the unsaturated oleate analogue **3** to the saturated stearate analogue **4**, the presence of monounsaturation resulted in more than double the myocardial uptake at 30 min with excellent retention of both radiotracers at 120 min. Furthermore, **3** showed the highest protein-bound fractions of ^{18}F-radioactivity in heart homogenates and markedly higher sensitivity to POCA induced inhibition of myocardial uptake and formation of protein-bound metabolites in the heart. Thus, our findings

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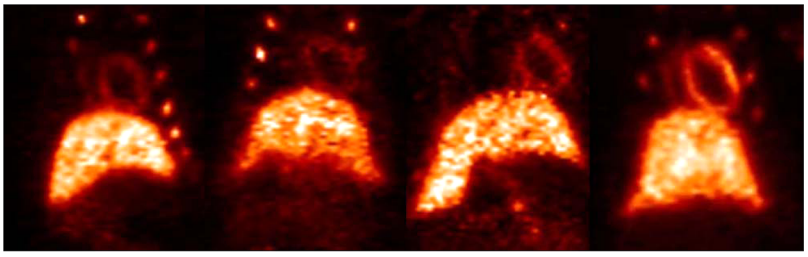

Figure 6. Representative microPET images of **2**–**5** at 55–115 min pi. Images are presented as maximum intensity projections (MIPs) of rats in coronal orientation with snout up. Accumulation of the radiotracers is visualized in heart, liver, and ribs (indicating defluorination).

suggest that monounsaturation enhances FAO-dependent transport and intracellular trafficking of **3** to the mitochondrion for oxidative metabolic trapping. The inhibition of myocardial uptake and formation of protein-bound metabolites of FTO (and to a lesser extent the other FA analogues) with CPT-I inhibition (POCA effect) is a strong indication of mitochondrial trafficking. The significance of this fact is that FTO may serve as a noninvasive biomarker of the flux of fatty acids to the mitochondrion for β-oxidation. We had confirmed in an earlier work with FTP that the tracer had localized to the mitochondrial fraction by ultracentrifugation.[3] Extending the chain length of the palmitate analogue (**2**) by two carbons to form **4** results in similar uptake and metabolic trapping properties but lengthened myocardial retention, suggesting longer retention of the putative radiolabeled long-chain thiol metabolite for **4**.

Thia substition at the fourth carbon appears to optimize specificity of metabolic trapping to FAO. FAO-dependent metabolism of the 4-thia analogues to protein-bound metabolites in heart was strongly indicated from the finding that pretreatment of rats with the CPT-I inhibitor POCA resulted in dramatic reductions of protein-bound fractions from 70–89% to 20–25%. To further clarify the nature of the protein binding binding of metabolites, a gel electrophoresis experiment was performed with heart and liver homogenates using **2** and **3**. ^{18}F-Radioactivity was found to be distributed among at least five different molecular weight proteins across the gels and not specific to one particular protein (data not shown). This finding is consistent with the previously reported mechanism of metabolism of thia fatty acids to long-chain thiol fragments which continues to bear the ^{18}F-radiolabel and nonspecifically bind to mitochondrial protein by formation of disulfide bonds through various cysteine residues.[5] Interestingly, the 6-thia fatty acid analogue **5** showed no significant change in myocardial uptake in response to CPT-1 inhibition (Table 3), although protein-bound fractions of ^{18}F radioactivity in heart homogenates were decreased in a manner similar to the 4-thia fatty acid analogues. It appears that **5** was taken up by POCA treated hearts by an amount near control levels despite the inhibition of FAO at the CPT-1 step. This behavior differs from that previously seen for **1** in POCA-treated mouse hearts where myocardial uptake was reduced by 87% ($p < 5 \times 10^{-4}$) at 60 min[2]. It is not clear whether this discrepancy is caused by the difference in fatty acid length (C-18 versus C-17), placement of the ^{18}F (ω versus ω-3), or species differences (rat versus mouse). Profound differences in metabolism have been observed for fatty acids differing by as little as one or two methylene groups.[2,3,5,10]

CONCLUSIONS

Several long-chain, thia-substituted fatty acid analogues were synthesized and preliminarily evaluated in rats as PET probes for myocardial fatty acid oxidation (FAO). The radiosyntheses of **4** and **5** were developed in analogy to those previously developed for **2** and **3**. Biodistribution and PET imaging studies of the tracers were performed to establish a plausible structure–uptake relationship. The enhanced myocardial uptake and metabolic trapping of **3** are primarily due to the presence of monounsaturation, whereas improved retention in myocardium can be attributed to the longer chain length. Myocardial uptake and metabolism of 4-thia stearate analogues appear to be more specific to mitochondrial CPT-1 mediated trafficking to the mitochondrion than the 6-thia analogue **5**. Preliminary images of the accumulation of compound **3** in the rat myocardium were clearly superior to those of **2**, **4**, and **5**. In summary, **3** remains the most specific and sensitive FAO probe of the 4-thia fatty acid analogues evaluated and warrants further preclinical evaluation.

EXPERIMENTAL SECTION

Materials and Methods. All chemicals and solvents were purchased from Sigma-Aldrich and used as received. Anhydrous solvents were also purchased from Sigma-Aldrich in "Sure/Seal" bottles. TLC analysis of reaction mixtures and products was performed on Merck silica gel 60 F254 TLC plates. Liquid chromatography was carried out on Merck 60 silica gel (32–63 μm). ^{1}H and ^{13}C NMR results were recorded on a Varian 600 MHz spectrometer and referenced to $CDCl_3$ (^{1}H NMR $CDCl_3$ at 7.26 ppm and ^{13}C NMR $CDCl_3$ at 77.0 ppm). Mass spectral data were obtained from the Mass Spectral Lab of School of Chemical Sciences, University of Illinois, Urbana, IL. Analytical HPLC was performed on a Phenomenex Luna C-18 column (5 μm, 4.6 mm × 250 mm) with a flow rate of 0.8 mL/min. Semipreparative HPLC was performed on the final ^{18}F labeled product using a Phenomenex Luna C-18 column (5 μm, 10 mm × 250 mm) with a flow rate of 5.0 mL/min. POCA (sodium 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate) was obtained as a generous gift from Dr. S. John Gatley, Department of Pharmaceutical Sciences, Northeastern University, Boston, MA. The "5P30 DK 36836" specialized assay core facility of Joslin DERC, Boston, MA, was used to estimate the plasma concentration of metabolic substrates like glucose, triglyceride, free fatty acid, and lactate. All these substrate were measured by standard enzymatic laboratory assay.

Synthesis of Methyl 18-Bromo-4-thiaoctadecanoate (8). Synthesis of methyl 18-bromo-4-thiaoctadecanoate (**8**) was achieved by coupling methyl 3-mercaptopropionate with 1,14-dibromotetradecane (**7**) using sodium hydride. To a stirred solution of methyl 3-mercaptopropanoate (500 mg, 4.16 mmol) in dry THF (100 mL) under nitrogen at 0 °C was added NaH (100 mg, 4.16 mmol). The resulting solution was stirred for 15 min at 0 °C. A solution of **7** (1.32 g, 3.70 mmol) in THF (10 mL) was then gradually added, and the

resulting mixture was maintained at 0 °C for 30 min. The mixture was stirred for 3 h at room temperature and quenched with water. After extraction with the dichloromethane, the organic layer was dried over Na_2SO_4 and solvent was evaporated under vacuum. The residue was subjected to column chromatography (5:95 ethyl acetate/hexane) to yield **8** (1.30 g, 79% yield, mp 37 ± 1 °C) as a white solid. ^{1}H NMR (23 °C, 599.77 MHz, $CDCl_3$) δ ppm: 3.70 (s, 3H), 3.41 (t, 2H, $J_{1,2}$ = 12.0 Hz), 2.78 (t, 2H, $J_{1,2}$ = 12.0 Hz), 2.60 (t, 2H, $J_{1,2}$ = 12.0 Hz), 2.52 (t, 2H, $J_{1,2}$ = 12.0 Hz), 1.88−1.82 (m, 2H), 1.58−1.53 (m, 2H), 1.44−1.40 (m, 2H), 1.38−1.34 (m, 2H), 1.32−1.22 (brs, 16H). ^{13}C NMR (23 °C, 150.81 MHz, $CDCl_3$) δ ppm: 172.6 ($COOCH_3$), 51.9 (OCH_3), 34.7, 34.1, 32.9, 32.2, 29.6−29.4 (CH_2x6), 29.3, 29.2, 28.9, 28.7, 28.1, 26.9. HRMS (ES) calcd for $C_{18}H_{35}O_2SBr(M^+)$ 394.154 11, found 394.152 27 and (M + 2) at 396.0 (due to isotopic abundance).

Synthesis of 18-Fluoro-4-thiaoctadecanoic Acid (9). An anhydrous acetonitrile solution (3 mL) of bromo ester **8** (80 mg, 0.20 mmol) and 1 M THF solution of TBAF (3 mL, 3.0 mmol) was stirred at room temperature for 72 h under nitrogen (additional 0.5 mL (0.5 mmol) of 1 M THF solution was further added at 24 and 48 h). Solvent was evaporated under vacuum. The resulting solid was dissolved in 100 mL of dichloromethane, washed with water, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated under vacuum to dryness. The obtained fluoro ester residue was further subjected to hydrolysis using 0.5 mL of aqueous solution of KOH (10 mg, 0.18 mmol) and stirred at room temperature for 2 h. The solvent was evaporated under vacuum. The obtained residue was dissolved in 25 mL of dichloromethane, washed with 1 N HCl, dried over Na_2SO_4, and filtered. The filtrate was evaporated under vacuum and purified by column chromatography using 4:96 methanol/chloroform as solvent and silica gel as an adsorbent to obtain compound **9** as a white solid (40 mg, 61.6% overall yield, mp 57 ± 1 °C). ^{1}H NMR (23 °C, 599.77 MHz, $CDCl_3$) δ ppm: 4.46 (dt, 2H, J_1 = 47.3 Hz, J_2 = 6.2 Hz), 2.82 (t, 2H, $J_{1,2}$ = 7.3 Hz), 2.70 (t, 2H, $J_{1,2}$ = 7.3 Hz), 2.57 (t, 2H, $J_{1,2}$ = 7.3 Hz), 1.74−1.68 (m, 2H), 1.63−1.58 (m, 2H), 1.42−1.38 (m, 4H), 1.32−1.22 (brs, 16H). ^{13}C NMR (23 °C, 150.81 MHz, $CDCl_3$) δ ppm: 176.2, 84.8, and 83.7 (d, J = 163.8 Hz, F−CH_2 due to carbon−fluorine coupling), 34.3, 32.2, 30.4−30.3 (d, J = 19.6 Hz long-range fluorine carbon coupling), 29.6−29.2 (CH_2 × 7), 29.2, 28.8, 26.6, 25.2, 25.1. HRMS (ES) calcd for $C_{17}H_{33}O_2SF(M^+)$ 320.218 53, found 320.217 55.

Synthesis of Methyl 18-Bromo-6-thiaoctadecanoate (12). Synthesis of methy18-fluoro-6-thiaoctadecanoate (**12**) was achieved by coupling of methyl 6-mercaptopentanoate with 1,12-dibromododecane (**11**) using sodium hydride. To a stirred solution of methyl 6-mercaptopentanoate (500 mg, 3.37 mmol) in anhydrous THF (100 mL) under nitrogen at 0 °C was added NaH (81 mg, 3.37 mmol). The resulting solution was stirred for an additional 15 min at 0 °C. A solution of **11** (990 mg, 3.09 mmol) in THF (10 mL) was slowly added, and the resulting mixture was maintained at 0 °C for 30 min. The mixture was then stirred for 3 h at room temperature and quenched with water. The dichloromethane extract was dried over Na_2SO_4, filtered, and the filtrate was evaporated under vacuum. The residue was subjected to column chromatography (5:95 ethyl acetate/hexane) to yield compound **12** (1.09 g, 2.76 mmol, 82% yield) as a colorless oil. ^{1}H NMR (23 °C, 599.77 MHz, $CDCl_3$) δ ppm: 3.68 (s, 3H), 3.42 (t, 2H, $J_{1,2}$ = 12.0 Hz), 2.52−2.50 (m, 4H), 2.34 (t, 2H, $J_{1,2}$ = 12.0 Hz), 1.86−1.82 (m, 2H), 1.76−1.71 (m, 2H), 1.61−1.64 (m, 2H), 1.59−1.54 (m, 4H), 1.44−1.39 (m, 2H), 1.38−1.34 (m, 2H), 1.32−1.22 (brs, 10H). ^{13}C NMR (23 °C, 150.81 MHz, $CDCl_3$) δ ppm: 174.1 ($COOCH_3$), 51.8 (OCH_3), 34.0, 33.6, 32.8, 32.1, 31.6, 29.6−28.7 (CH_2 × 9), 28.1, 24.1. HRMS (ES) calcd for $C_{18}H_{35}O_2SBr$-(M^+) 394.154 11, found 394.152 27 and (M + 2) at 395.9 (due to isotopic abundance)

Synthesis of 18-Fluoro-6-thiaoctadecanoic Acid (13). An anhydrous acetonitrile solution (3 mL) of bromo ester **12** (76 mg, 0.19 mmol) and 1 M THF solution of TBAF (3 mL, 3.0 mmol) was stirred at room temperature for 72 h under nitrogen (additional 0.5 mL (0.5 mmol) of 1 M THF solution was further added at 24 and 48 h). Solvent was evaporated under vacuum. The resulting solid was dissolved in 100 mL of dichloromethane, washed with water, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated under vacuum to dryness. The obtained fluoro ester residue was further subjected to hydrolysis using 0.5 mL of aqueous solution of KOH (10 mg, 0.18 mmol) and stirred at room temperature for 2 h. The solvent was evaporated under vacuum. The obtained residue was dissolved in 25 mL of dichloromethane, washed with 1 N HCl, dried over Na_2SO_4, and filtered. The filtrate was evaporated under vacuum and purified by column chromatography using 4:96 methanol/chloroform as solvent and silica gel as an adsorbent to obtain compound **13** as a white solid (38 mg, 61.7% over all yield, mp 51 ± 1 °C). ^{1}H NMR (23 °C, 599.77 MHz, $CDCl_3$) δ ppm: 4.46 (dt, 2H, J = 47.3 Hz, 6.2 Hz), 2.56−2.51 (m, 4H,), 2.41 (t, 2H, $J_{1,2}$ = 7.3 Hz), 1.79−1.66 (m, 6H), 1.61−1.58 (m, 2H), 1.40−1.38 (m, 4H), 1.33−1.29 (brs, 12H). ^{13}C NMR (23 ° C, 150.81 MHz, $CDCl_3$) δ ppm: 179.2, 84.8, and 83.7 (d, J = 163.5 Hz, F−CH_2 due to carbon−fluorine coupling), 33.7, 33.2, 32.1, 31.6, 30.4−30.3 (d, J = 19.1 Hz long-range fluorine carbon coupling), 29.6−28.9 (CH_2 × 8), 25.1, 23.9. HRMS (ES) calcd for $C_{17}H_{33}O_2SF$ (M^+), 320.2151, found 320.2194.

General Procedure for ^{18}F-Labeling. The labeling precursors for all the four tracers **2**,[3,6] **3**,[10] **4**, and **5** were the methyl esters of their corresponding bromides (Schemes 1 and 2). Nucleophilic [^{18}F]-fluorination and subsequent hydrolysis of the ester were performed as previously described.[19] Briefly, cyclotron-produced [^{18}F]fluoride was dried under nitrogen at 95 °C in a 2 mL glass vial containing Kryptofix 2.2.2 (10 mg), acetonitrile (0.8 mL), and K_2CO_3 (4 mg) solution in water (0.15 mL). The residue was further dried by azeotropic distillation with acetonitrile (2 × 0.5 mL). A solution of the precursor (approximately 2−3 mg) in acetonitrile (0.5 mL) was added, and the vial was sealed (screw tight cap) and subjected to microwave irradiation at 75 °C for 5 min. Subsequent hydrolysis of the resulting [^{18}F]fluoro ester was performed in the same vessel by the addition of 0.15 mL of 0.2 N KOH followed by microwave irradiation at 75 °C for 2 min. The mixture was then cooled, acidified with acetic acid (0.2 N), filtered, and injected onto the semipreparative HPLC column (Phenomenex Luna C-18, 5 μm, 10 mm × 250 mm). The mobile phase was acetonitrile/water/TFA (90:10:0.005 v/v), and flow rate was 5 mL/min (t_R = 9.4 min for **4** and t_R = 8.7 min for **5**). An in-line ultraviolet detector (210 nm) was used to monitor the elution of unlabeled materials. The [^{18}F]fluoro fatty acid fraction was diluted in 50 mL water and trapped on a C-18 Sep-Pak cartridge (Accel C-18 Plus, Waters, Milford, MA), followed by washing of the Sep-Pak with 10 mL of water. The product was eluted from the Sep-Pak using 0.1 mL of ethanol (multiple fractions). The fraction having the highest activity was used for formulation in 0.5−1% BSA in isotonic NaCl solution and filtered through a 0.22 μm filter (Millex-GS, Millipore, Bedford, MA, U.S.). Radiochemical purity (>99%) was analyzed by radio-HPLC in the system described above.

Biodistribution Studies in Rats. Female Sprague−Dawley rats were fasted overnight. The rats were anesthetized with isoflurane (3.5% induction, 1.5% maintenance). The ^{18}F-labeled radiotracer (0.7−1.4 MBq) was injected into the tail vein. The biodistributions were performed at either 30 or 120 min before procurement of heart, liver, lung, blood, kidney, bone (femur), brain, and skeletal muscle. The tissues were counted for ^{18}F-radioactivity and weighed. All ^{18}F-radioactivity measurements were corrected for radioactivity decay. Radiotracer uptake was calculated as

$$\text{uptake (\% dose kg/g)} = \frac{\text{CPM(tissue)} \times \text{body wt (kg)}}{\text{tissue wt (g)} \times \text{CPM(dose)}} \times 100 \quad (1)$$

In one group of fasted rats, the CPT-I inhibitor POCA (30 mg/kg) was administered intraperitoneally 120 min prior to **3** injection. Crude analysis of the nature of the metabolites in the heart, liver, and muscle was performed by a Folch-type extraction procedure. Approximately 0.5 g of tissue was excised and thoroughly homogenized and sonicated (20 s) in 7 mL of chloroform/methanol (2:1) at 0 °C. Urea (40%, 1.75 mL) and 5% sulfuric acid (1.75 mL) were added, and the mixture was sonicated for an additional 20 s. After centrifugation for 10 min at 1800g, aqueous, organic, and protein interphase (pellet) fractions were

separated and counted for ^{18}F-radioactivity. Furthermore, the three fractions are obtained by this extraction method, an organic phase (chloroform/methanol), aqueous phase, and protein-pellet. The percentage of ^{18}F-radioactivity distributed among the three phases indicated the following: (1) organic phase contained nonmetabolized fatty acid analogue and complex lipid metabolites such as triglycerides and phospholipids; (2) aqueous phase contained F-18 fluoride or other aqueous soluble metabolites; and (3) the remaining protein-pellet contained protein-bound ^{18}F-labeled β-oxidation metabolites.

MicroPET/CT Imaging Studies in Rats. Female Sprague–Dawley rats were fasted overnight. The rats were anesthetized with isoflurane (3.5% induction, 1.5% maintenance). PET/CT scanning was performed using an eXplore Vista PET/CT (General Electric Health Care, Milwaukee, WI). The animal was positioned in the prone position with the snout positioned into the scanner. Before the radiotracer imaging study, a whole-body CT scan was performed. This scan was used for anatomical visualization and for attenuation correction of the emission data. The radiotracers were studied in different rats to allow the biodistribution to be done following the PET scan. The ^{18}F-labeled radiotracer (9–11 MBq) was injected into a tail vein, and the emission data were collected in dynamic acquisition for 50 min over the upper thorax, followed by a static whole-body scan from 55 to 115 min postinjection. For **3**–**5**, two to five rats were imaged with each tracer. In this preliminary study, representative images are presented for each ^{18}F-labeled fatty acid analogue. The images were displayed as maximum intensity projections (MIPs) with coronal orientation. The pixel intensities were normalized to the highest pixel value within each image. The images were interpreted strictly qualitatively to show the imaging feasibility of the radiotracers as myocardial imaging agents and confirmation with the biodistribution data.

Statistical Analyses. All results were expressed as the mean ± SD. Comparison of the mean values was made using a two-tailed Student's *t* test. The acceptable level of significance was set at $p < 0.05$. Microsoft Excel was used to perform the statistical analysis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR spectra of nonradioactive standards and radio-HPLC chromatograms of compounds used in the present study. 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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■ ABBREVIATIONS USED

FAO, fatty acid oxidation; CPT-I, carnitine palmitoyltransferase I; POCA, sodium 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate; PET, positron emission tomography; CD36, cluster of differentiation 36, which is an integral membrane protein; FATP, fatty acid transport protein; CAT, carnitine–acylcarnitine translocase; **1**, 14(*R*,*S*)-[^{18}F]fluoro-6-thiaheptadecanoic acid; (16:0), 16 carbon chain with no double bond; (18:0), 18 carbon chain with no double bond; (18:1), 18 carbon chain with one double bond; LAH, lithium aluminum hydride; THF, tetrahydrofuran; BSA, bovine serum albumin; TBAF, tetrabutylammonium fluoride; pi, postinjection; amu, atomic mass unit

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