16-Cyclopentadienyl Tricarbonyl ^{99m}Tc 16-Oxo-hexadecanoic Acid: Synthesis and Evaluation of Fatty Acid Metabolism in Mouse Myocardium†

Byung Chul Lee,‡,§,⊥,|| Dong Hyun Kim,‡,⊥ Iljung Lee,‡ Yearn Seong Choe,*,‡ Dae Yoon Chi,§ Kyung-Han Lee,‡ Yong Choi,‡ and Byung-Tae Kim‡

Department of Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-ku, Seoul 135-710, Korea, Department of Chemistry, Inha University, 253 Yonghyundong, Namgu, Inchon 402-751, Korea

Received January 18, 2008

We synthesized 16-cyclopentadienyl tricarbonyl ^{99m}Tc 16-oxo-hexadecanoic acid (^{99m}Tc-CpTT-16-oxo-HDA, **1**) and investigated its potential as a radiotracer for evaluating fatty acid metabolism in myocardium. Radiotracer **1** was synthesized in 22.6 ± 6.3% decay-corrected yield by a double ligand transfer reaction between the ferrocene adduct of methyl hexadecanoate (**2**) and $Na^{99m}TcO_4$ in the presence of $Cr(CO)_6$ and $CrCl_3$, followed by hydrolysis of the methyl ester group. Radiotracer **1** was found to be chemically stable (99% at 6 h) when incubated in human serum. A tissue distribution study in mice showed that high radioactivity accumulated in heart (9.03%ID/g at 1 min and 5.41%ID/g at 5 min postinjection) with rapid clearance and that heart to blood uptake ratios increased with time (2.13 at 5 min and 3.76 at 30 min postinjection). Metabolite analysis of the heart tissues using a simple extraction method showed that ^{99m}Tc-CpTT-4-oxo-butyric acid was detected as the major radioactive metabolite by HPLC, suggesting that **1** is metabolized to ^{99m}Tc-CpTT-4-oxo-butyric acid via β-oxidation in myocardium.

Introduction

Fatty acids are important myocardial metabolic substrates under aerobic conditions. In the fasting state, fatty acid oxidation is enhanced and anaerobic glycolysis is suppressed, whereas in the nonfasting state, fatty acid oxidation decreases and glucose is utilized as the primary energy source.[1] In the myocardium, long chain fatty acids are metabolized by β-oxidation, which degrades them by removing two-carbon units.[2–4] Moreover, fatty acid oxidation is impaired during myocardial ischemia.[1] Accordingly, long chain fatty acids have been radiolabeled with various radionuclides to evaluate myocardial fatty acid metabolism using single photon emission computed tomography (SPECT) or positron emission tomography (PET),[5–14] and some of these radiolabeled fatty acid analogues have been utilized for diagnosis of myocardial ischemia and other heart diseases.[5,6] Furthermore, it has been reported that a fatty acid chain length of 16 or 17 carbon atoms is optimal for myocardial imaging.[7] ^{99m}Tc is the most widely used radionuclide in nuclear medicine imaging due to its near ideal physical properties (141 keV, $t_{1/2}$ = 6 h) and ready availability.[15,16] However, although ^{99m}Tc-labeled long chain fatty acid analogues have been developed, most of them have poor myocardial uptakes. Among these analogues, ^{99m}Tc-[9,10-bis[*N*-(2′-methyl-2′-mercapto)propyl]-aminooctadecanoic acid and ^{99m}Tc-[7,10-bis(2-mercapto-2-methyl)propyl]-7,10-diazapentadecanoic acid showed higher heart uptakes than those of ^{99m}Tc-fatty acid analogues containing DTPA or EDTA, ^{99m}Tc-BAT-pentadecanoic acid, and ^{99m}Tc-BAT-hexadecanoic acid, probably due to branching effect of the fatty acid analogues.[9–12] Recently, metabolite analysis of fatty acid analogues was performed using rodent heart or urine samples to elucidate whether the fatty acid analogues are metabolized via β-oxidation. Magata et al. detected ^{99m}Tc-MAMA-butyric acid in the urine samples of rats injected with ^{99m}Tc-MAMA-heptadecanoic acid, which demonstrated that the fatty acid is metabolized by β-oxidation in the body.[13] Our group also found that ^{99m}Tc(CO)$_3$-[*N*-(acetyloxy)-2-picolylamino]pentadecanoic acid is primarily metabolized to ^{99m}Tc(CO)$_3$-[*N*-(acetyloxy)-2-picolylamino]pentanoic acid in mice.[14]

Small cyclopentadienyl tricarbonyl Re cores have been developed and applied to preparation of cyclopentadienyl tricarbonyl ^{99m}Tc (^{99m}Tc-CpTT) radiopharmaceuticals.[17–22] Furthermore, the ^{99m}Tc-CpTT core has been utilized for the preparation of various functionalized complexes because ferrocenes with different substituents can be prepared by Friedel−Crafts acylation and subsequent double ligand transfer is straightforward. The diminutive and lipophilic ^{99m}Tc-CpTT core is expected to only minimally perturb biological activity of fatty acid. Alberto and co-workers also synthesized ^{99m}Tc-CpTT complexes by coordinating $[^{99m}Tc(CO)_3]^+$ to carbonyl-Cp ligand in water.[23]

We previously showed that ^{99m}Tc-CpTT-8-oxo-octanoic acid is metabolized to ^{99m}Tc-CpTT-4-oxo-butyric acid via β-oxidation in the body and thus proved that it is recognized as a medium chain fatty acid.[24] Recently, Uehara et al. showed that ^{99m}Tc-CpTT-pentadecanoic acid is metabolized to ^{99m}Tc-CpTT-propionic acid via β-oxidation in the heart using the isolated Langendorff-perfused rat heart model.[25] Taken together, ^{99m}Tc-CpTT-fatty acid analogues are shown to be superior to other ^{99m}Tc-labeled fatty acid analogues.[9–14,25] However, synthesis of ^{99m}Tc-CpTT-pentadecanoic acid required an additional long reduction step of the carbonyl group next to the Cp ring prior to hydrolysis of the methyl ester group.[25]

In the present study, we synthesized 16-cyclopentadienyl tricarbonyl ^{99m}Tc 16-oxo-hexadecanoic acid (^{99m}Tc-CpTT-16-oxo-HDA, **1**) retaining the carbonyl group next to the Cp ring,

† Presented in part at the 48th Annual Meeting of the Society of Nuclear Medicine, Toronto, Canada, June 23−27, 2001.

* To whom correspondence should be addressed. Phone: +82-2-3410-2623. Fax: +82-2-3410-2639. E-mail: ysnm.choe@samsung.com.

‡ Department of Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine.

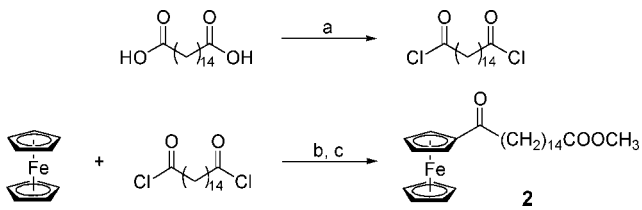
§ Department of Chemistry, Inha University.

|| Present address: Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, Korea.

⊥ These authors contributed equally to this work.

10.1021/jm800049h CCC: $40.75
Published on Web 05/27/2008

Scheme 1[a]



[a] Reaction conditions: (a) $SOCl_2$, 90 °C, overnight; (b) $AlCl_3$, CH_2Cl_2, 0 °C, 1 h; (c) CH_3OH, Et_3N, reflux, 1 h.

and evaluated its potential as a β-oxidation substrate in mouse myocardium using a simple extraction method.

Results

Chemistry. Radiotracer **1** was synthesized by the incorporation of $^{99m}Tc(CO)_3$ into the Cp-fatty acid analogue by double ligand transfer, followed by hydrolysis of the methyl ester group (Schemes 1 and 2). Subsequent HPLC purification furnished **1** in overall radiochemical yield of 22.6 ± 6.3% (decay-corrected) and with a radiochemical purity >99% as determined by HPLC and radio-TLC. Total synthesis time including HPLC purification was 150−170 min. The Re complex **3** was synthesized using the procedure described for **1** (Scheme 2).

In Vitro Stability. Radiotracer **1** was incubated in human serum for 6 h at 37 °C and analyzed by radio-TLC. The results obtained showed that 99% of **1** remained intact even at 6 h, which amply demonstrated its high chemical stability.

Tissue Distribution. Because fatty acids are predominantly metabolized in the fasting state, mice were fasted for 12 h prior to experiments. High radioactivity levels accumulated in blood, heart, lung, liver, and kidneys from 1 min postinjection, but washed out rapidly with time from all tissues except liver and kidneys (Table 1). Heart uptake by **1** was eliminated with a half-life of ca. 10 min (9.03%ID/g at 1 min and 4.12%ID/g at 10 min postinjection) and the heart to blood uptake ratios increased with time (1.64 at 1 min, 2.13 at 5 min, 3.27 at 10 min, and 3.76 at 30 min).

Analysis of Metabolites. Radioactive metabolites of **1** were analyzed in heart tissue samples and in urine. Although metabolites can be detected in urine samples, heart samples were further analyzed for metabolites because fatty acids are also metabolized in liver. When heart samples were homogenized in $CHCl_3-CH_3OH-0.001$ N NaOH, most of the radioactivity partitioned into the aqueous fraction and tissue pellet (50.7:8.2:39.9 at 10 min and 54.1:6.7:38.6 at 30 min; aqueous fraction:organic fraction:tissue pellet) (Table 2). When the aqueous fractions were analyzed, the radioactive metabolite, ^{99m}Tc-CpTT-4-oxo-butyric acid was detected by HPLC (Figure 1A,B), suggesting that **1** was metabolized to [^{99m}Tc]CpTT-4-oxo-butyric acid after six cycles of β-oxidation, which is entirely consistent with the same metabolite formation from [^{99m}Tc-]CpTT-8-oxo-octanoic acid.[24] [^{99m}Tc]CpTT-4-oxo-butyric acid was also detected in urine samples (Figure 1C,D). This result is comparable to that of ^{99m}Tc-CpTT-pentadecanoic acid, which was found to be metabolized to ^{99m}Tc-CpTT-propionic acid.[25] Most radioactivity in residual tissue pellets after dissolution in hot NaOH solution was precipitated by adding trichloroacetic acid (68−70%), which suggested that **1** was covalently bound to protein involved in β-oxidation in myocardium.

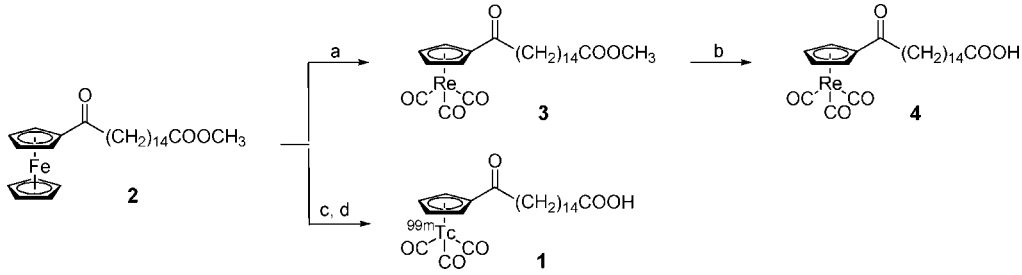
Discussion

Radiotracer **1** was prepared by a double ligand transfer reaction between the precursor **2** and $Na^{99m}TcO_4$ in the presence of $Cr(CO)_6$ and $CrCl_3$ and subsequent methyl ester hydrolysis. Moreover, incorporation of ^{99m}Tc into the Cp-fatty acid analogue was quantitative. However, during the hydrolysis of the obtained methyl ester, an unknown polar peak was observed on TLC, which was probably derived from decomposition of the ^{99m}Tc core and, therefore, mild conditions were employed to reduce side product formation. In contrast, hydrolysis of Re complex **3** was carried out in high yield (79%), indicating that Re core is more stable than the corresponding ^{99m}Tc core. ^{99m}Tc-labeling after deprotecting the methyl ester group of **2** was not carried out because our previous studies showed that double ligand transfer between 8-ferrocenyl-8-oxo-octanoic acid and $Na^{99m}TcO_4$ in the presence of a CO source in methanol produced the methyl ester form of the product.[24] This is not surprising because some ferrocenyl side chains were shown to be modified during double ligand transfer in methanol.[17] Although HPLC retention times of **1** and Re complex **4** were not identical, **1** was identified based on the similar chemical properties between Tc and Re complexes as was done for other ^{99m}Tc-labeled complexes.[14,19,24]

Tissue distribution studies on **1** (Table 1) showed that ^{99m}Tc-CpTT-oxo-fatty acid analogues are superior to other ^{99m}Tc-labeled fatty acid analogues because they have higher heart uptakes and heart to blood uptake ratios. Heart uptake by **1** (9.03%ID/g at 1 min, 5.41%ID/g at 5 min, 4.12%ID/g at 10 min, and 2.16%ID/g at 30 min postinjection) was higher than those of other ^{99m}Tc-labled fatty acid analogues; ^{99m}Tc-CpTT-pentadecanoic acid (3.85%ID/g at 1 min, 2.71%ID/g at 5 min, 1.87%ID/g at 10 min, and 1.27%ID/g at 30 min postinjection),[25] $^{99m}Tc(CO)_3$-15-[*N*-(acetyloxy)-2-picolylamino]pentadecanoic acid (6.38%ID/g at 1 min and 0.73%ID/g at 5 min postinjection),[14] ^{99m}Tc-MAMA-pentadecanoic acid (5.46%ID/g at 2 min and 2.40%ID/g at 5 min postinjection),[13] ^{99m}Tc-BAT-pentadecanoic acid and hexadecanoic acid (0.31−0.46%ID/organ at 5 min postinjection),[10] and ^{99m}Tc-[9,10-bis[*N*-(2′-methyl-2′-mercapto)propyl]aminooctadecanoic acid and ^{99m}Tc-[7,10-bis(2-mercapto-2-methyl)propyl]-7,10-diazapentadecanoic acid (1.30%ID/g at 5 min and 0.56%ID/g at 15 min postinjection, respectively).[11,12] Heart to blood uptake ratios of **1** were similar to those of the ^{99m}Tc-CpTT-pentadecanoic acid (1.64 vs 0.84 at 1 min, 2.13 vs 2.93 at 5 min, 3.27 vs 4.60 at 10 min, and 3.76 vs 3.44 at 30 min)[25] but higher than those of other ^{99m}Tc-labeled fatty acid analogues (^{99m}Tc-MAMA-hexadecanoic acid: 1.51 at 2 min; $^{99m}Tc(CO)_3$-[*N*-(acetyloxy)-2-picolylamino]-pentadecanoic acid: 1.87 at 5 min; ^{99m}Tc-BAT-pentadecanoic acid: 1.39 at 5 min and 0.98 at 15 min; ^{99m}Tc-[9,10-bis[*N*-(2′-methyl-2′-mercapto)propyl]aminooctadecanoic acid: 1.78 at 5 min and 1.67 at 10 min).[10−14]

Indirect metabolite analysis using urine samples was carried out for ^{99m}Tc-MAMA-hexadecanoic acid and $^{99m}Tc(CO)_3$(*N*-acetyloxy-2-picolylamino)-pentadecanoic acid.[13,14] However, because β-oxidation may also occur in tissues other than the heart, direct metabolite analysis using myocardial tissues is preferred for evaluation of the radiolabeled fatty acid analogues as substrates for β-oxidation. Recently, the radioactive metabolite of ^{99m}Tc-CpTT-pentadecanoic acid was analyzed using an isolated Langendorff-perfused rat heart model. In this study, most radioactivity in perfused heart homogenates extracted into the organic fraction, and most of this was found to be more nonpolar than ^{99m}Tc-CpTT-pentadecanoic acid by TLC and its subsequent hydrolysis resulted in the formation of ^{99m}Tc-CpTT-pentadecanoic acid and a small fraction of ^{99m}Tc-CpTT-propionic acid by HPLC, which suggested that ^{99m}Tc-CpTT-pentadecanoic acid was metabolized to ^{99m}Tc-CpTT-propionic acid

Scheme 2[a]



[a] Reaction conditions: (a) NH_4ReO_4, $Cr(CO)_6$, $CrCl_3$, CH_3OH, 180 °C, 1 h; (b) 0.4 N NaOH-CH_3OH (1:3), 80 °C, 40 min; (c) $Na^{99m}TcO_4$, $Cr(CO)_6$, $CrCl_3$, CH_3OH, 180 °C, 1 h; (d) 0.3 N NaOH-CH_3OH (1:4), 70 °C, 10 min.

Table 1. Tissue Distribution of **1** in Mice[a]

	%ID/g			
tissue	1 min	5 min	10 min	30 min
blood	5.51 ± 0.32	2.54 ± 0.30	1.26 ± 0.06	0.58 ± 0.05
heart	9.03 ± 0.17	5.41 ± 0.15	4.12 ± 0.32	2.16 ± 0.19
lung	7.17 ± 0.39	2.41 ± 0.14	1.38 ± 0.04	0.61 ± 0.09
liver	25.6 ± 1.60	33.4 ± 1.97	36.4 ± 1.20	37.4 ± 1.33
kidney	8.48 ± 1.00	15.7 ± 1.65	19.1 ± 1.90	24.6 ± 0.09
stomach	1.86 ± 0.12	1.06 ± 0.15	0.66 ± 0.08	0.71 ± 0.17

[a] Values are given as means ± SD of groups of 4 mice.

Table 2. Distribution of Radioactivity in Mouse Heart Homogenates after Injection of **1**[a]

	% aqueous fraction	% organic fraction	% tissue pellet
10 min	50.7 ± 4.6	8.2 ± 1.1	39.9 ± 5.0
30 min	54.1 ± 4.0	6.7 ± 1.2	38.6 ± 4.9

[a] Values are given as means ± SD of 4 experiments.

via β-oxidation in myocardium.[25] In the present study, we used a simple extraction method based on a 2:1:1 mixture of $CHCl_3$−CH_3OH−0.001 N NaOH to analyze the metabolite of **1** directly in heart tissue samples,[26] and subsequent HPLC analysis of the aqueous fraction suggested that **1** was metabolized to ^{99m}Tc-CpTT-4-oxo-butyric acid in myocardium (Figure 1). Taken together, the presence of a carbonyl group next to the Cp ring of **1** does not appear to noticeably affect the biological properties of ^{99m}Tc-CpTT-fatty acid analogues.[25]

Conclusion

Radiotracer **1** was prepared in high yield (22.6 ± 6.3%) from a double ligand transfer reaction between the ferrocene adduct of methyl hexadecanoate and $Na^{99m}TcO_4$ in the presence of a CO source and subsequent hydrolysis of the methyl ester group. Tissue distribution of **1** in mice showed high radioactivity accumulation in heart with high heart to blood uptake ratios, and radioactive metabolite analysis of heart samples using a simple extraction method suggested that **1** was metabolized to ^{99m}Tc-CpTT-4-oxo-butyric acid via β-oxidation in myocardium. These results suggest that **1** can be used as a substrate for fatty acid metabolism.

Experimental Section

Materials and Methods. Solvents and reagents were purchased from Sigma-Aldrich (St. Louis, MO). ^{1}H NMR spectra were obtained on a Varian Gemini 400 (Palo Alto, CA) and ^{13}C NMR spectra at 100 MHz. Chemical shifts are reported in parts per million (ppm, δ units) downfield from tetramethylsilane. Fast atom bombardment (FAB) mass spectra were obtained on a JMS-700 Mstation (Jeol Ltd., Tokyo, Japan). HPLC was carried out using a Thermo Separation Products System (Fremont, CA) equipped with a semipreparative column (YMC silica gel, 5 μ, 10 mm × 250 mm) or an analytical column (YMC C18, 5 μ, 4.6 mm × 250 mm). Eluants were simultaneously monitored using a UV detector (254 nm) and a NaI(Tl) radioactivity detector. TLC was performed on Merck F_{254} silica plates and analyzed using a Bioscan radio-TLC scanner (Washington, DC). $Na^{99m}TcO_4$ was eluted on a daily basis from a ^{99}Mo/^{99m}Tc generator (Samyoung Unitech, Co., Ltd., Seoul, Korea; DuPont Pharmaceuticals Co., Delaware; and Daiichi Radioisotope Laboratories, Chiba, Japan). Radioactivity was measured using a dose calibrator (Biodex Medical Systems, Shirley, NY) and tissue radioactivity using a Wallac automated gamma counter (efficiency 89%) (Boston, MA). All animal experiments were performed in compliance with the rules of the Samsung Medical Center Laboratory Animal Care, which are in accord with NIH guidelines.

Synthesis of Methyl 16-Ferrocenyl-16-oxo-hexadecanoate (2). Thionyl chloride (550 μL, 7.55 mmol) was added dropwise to hexadecanedioic acid (432 mg, 1.51 mmol), and heated at 90 °C overnight. After thionyl chloride had been removed in vacuo, the resulting 1,16-hexadecanedioyl dichloride was used without further purification. The reaction flask was charged with ferrocene (281 mg, 1.51 mmol) and 1,16-hexadecanedioyl dichloride in dichloromethane (30 mL) and chilled in an ice bath. To this solution was added anhydrous aluminum chloride (221 mg, 1.66 mmol) carefully over 20 min using a spatula under N_2. The reaction mixture, which turned a deep-violet color, was stirred for 1 h in an ice bath and then allowed to stand for 10 min at room temperature. At the end of the reaction, the flask was placed again in an ice bath, and methanol (6 mL) and triethylamine (1.2 mL) were slowly added. The mixture was refluxed vigorously for 1 h, and after solvent had been removed under reduced pressure, the crude mixture was diluted with water and extracted with dichloromethane (3 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4, and solvent was removed in vacuo. The residue obtained was purified by flash column chromatography (10:90 ethyl acetate−hexane) to gived **2** (240 mg, 34%) as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.77 (t, J = 2.0 Hz, 2H, −Fe-Cp-H (α)), 4.48 (t, J = 2.0 Hz, 2H, −Fe-Cp-H (β)), 4.19 (s, 5H, Cp-H), 3.66 (s, 3H, OCH_3), 2.68 (t, J = 7.4 Hz, 2H, CH_2), 2.29 (t, J = 7.6 Hz, 2H, CH_2), 1.70−1.58 (m, 4H), 1.32−1.26 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 202.037, 171.762, 76.868, 69.548, 67.241, 66.870, 48.876, 37.314, 31.648, 27.149, 27.104, 27.051, 26.960, 26.770, 26.679, 22.499, 22.188. MS (FAB) m/z 468 (M^+). HRMS calcd for $C_{27}H_{40}O_3Fe$ 468.2327, found 468.2328. Anal. ($C_{27}H_{40}O_3Fe$) C, H.

Synthesis of Methyl 16-Cyclopentadienyl Tricarbonyl Rhenium 16-Oxo-hexadecanoate (3). Compound **3** was synthesized as described in the literature with some modification.[17] A mixture of the ester **2** (20 mg, 0.043 mmol), NH_4ReO_4 (4 mg, 0.014 mmol), $Cr(CO)_6$ (17 mg, 0.077 mmol), and $CrCl_3$ (4 mg, 0.026 mmol) was placed in a pressure tube containing a magnetic stir bar. After methanol (300 μL) was added, the pressure tube was sealed with a Teflon cap/O-ring and heated at 180 °C (oil bath) for 1 h. At the end of the reaction, the mixture was cooled in an ice bath for 15 min and solvent was removed in vacuo. Flash column chromatography (1:4 ethyl acetate−hexane) of the crude mixture gave **3** (5.2 mg, 20%) as a pale-yellow solid: mp 79.5−80.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.98 (t, J = 2.2 Hz, 2H, −Re-Cp-H (α)), 5.39 (t, J = 2.4 Hz, 2H, −Re-Cp-H (β)), 3.66 (s, 3H,

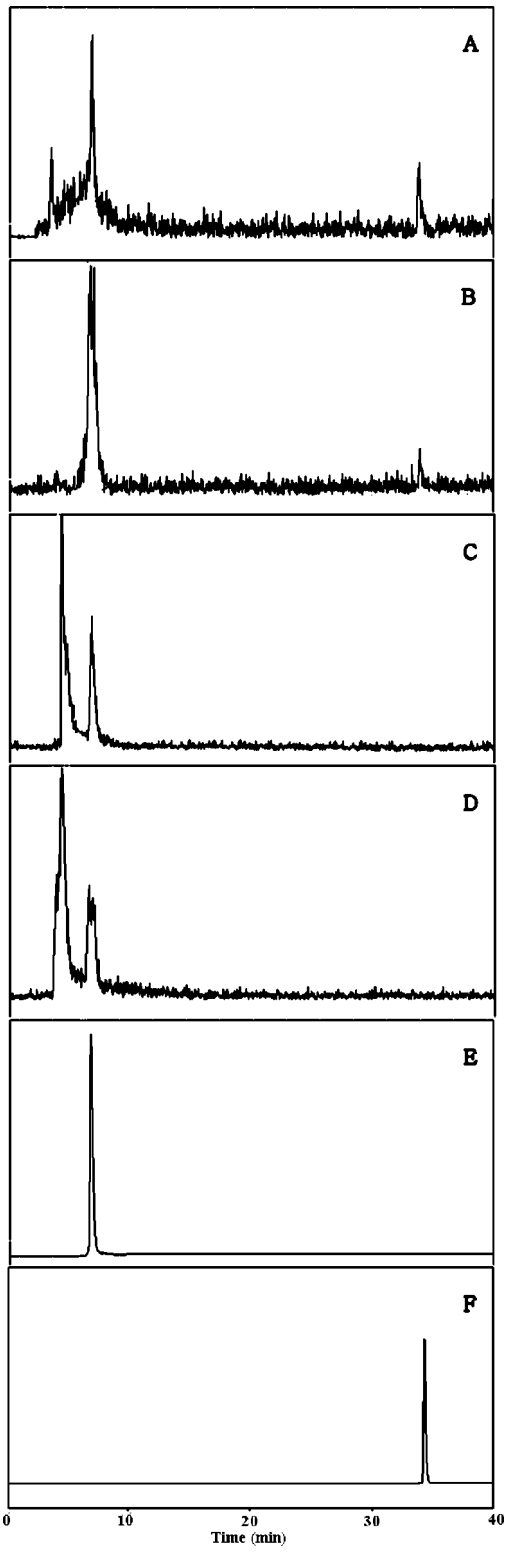


Figure 1. HPLC analyses of 10 min (A) and 30 min (B) heart tissue samples, and of 10 min (C) and 30 min (D) urine samples of mice injected with **1**. HPLC profiles of the radioactive metabolite standard, [^{99m}Tc]CpTT-4-oxo-butyric acid (t_R = 7.1 min) (E) and the radiotracer **1** (t_R = 33.5 min) (F). HPLC analysis was conducted using an analytical column (YMC C18, 5 μ, 4.6 mm × 250 mm) and a NaI(T1) radioactivity detector.

OCH_3), 2.57 (t, J = 7.1 Hz, 2H, CH_2), 2.30 (t, J = 7.5 Hz, 2H, CH_2), 1.71−1.61 (m, 4H), 1.38−1.25 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 195.348, 191.846, 174.365, 96.179, 87.886, 85.096, 51.431, 38.832, 34.101, 29.591, 29.553, 29.424, 29.348, 29.235, 29.128, 29.075, 24.936, 24.406. MS (FAB) m/z 618 (M^+). HRMS calcd for $C_{25}H_{35}O_6{}^{187}Re$ (M^+), 618.1992, found 618.2009.

Synthesis of 16-Cyclopentadienyl Tricarbonyl Rhenium 16-Oxo-hexadecanoic Acid (4). A solution of **3** (20 mg, 0.032 mmol) in 3 mL of 0.4 N NaOH and methanol (1:3) was heated at 80 °C for 40 min. After removing methanol under reduced pressure, the solution was cooled and acidified with 0.1 N HCl in an ice bath. The reaction mixture was then extracted with dichloromethane, and the organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product so obtained was purified by flash column chromatography (70:30 ethyl acetate−hexane) to give **4** (14 mg, 79%) as a white solid: mp 73.0−73.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.98 (t, J = 2.0 Hz, 2H, −Re-Cp-H (α)), 5.38 (t, J = 1.8 Hz, 2H, −Re-Cp-H (β)), 2.57 (t, J = 7.3 Hz, 2H, CH_2), 2.34 (t, J = 7.5 Hz, 2H, CH_2), 1.66−1.63 (m, 4H), 1.25−1.17 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 195.351, 191.777, 179.525, 96.323, 87.841, 85.072, 38.870, 33.825, 29.523, 29.409, 29.197, 29.098, 29.038, 24.683, 24.463. MS (FAB) m/z 604 (M^+). HRMS calcd for $C_{24}H_{33}O_6{}^{187}Re$ 604.1835, found 604.1824.

Radiochemical Synthesis of 1. Radiotracer **1** was synthesized, as described in the literature with some modification.[17,18] A solution of $Na^{99m}TcO_4$ (740 MBq, 0.2 mL) eluted from a generator was placed in a pressure tube and dried under a stream of N_2 at 70 °C. Ester **2** (2.5 mg, 5.34 μmol), $Cr(CO)_6$ (2.1 mg, 9.64 μmol), $CrCl_3$ (0.5 mg, 3.21 μmol), and methanol (300 μL) were then added, and the reaction mixture was sealed with a Teflon cap/O-ring and heated at 180 °C (oil bath) for 1 h. At the end of the reaction, the mixture was cooled in an ice bath and methanol was then removed under a stream of N_2 at 50 °C (water bath). The residue so obtained was dissolved in dichloromethane, passed through a short silica plug, and the solvent was removed under a stream of N_2. The residue was then redissolved in methanol (200 μL) and treated with 0.3 N NaOH (50 μL) at 70 °C for 10 min. The solution was then cooled, acidified with 0.1 N HCl (150 μL), and extracted with dichloromethane. The reaction mixture was purified by HPLC (YMC silica gel, 5 μ, 10 mm × 250 mm) at a flow 4 mL/min using a 90:10:0.1 mixture of hexane−2-propanol−acetic acid, and **1** was eluted between 16.3 and 17.0 min. Radiotracer **1** obtained by HPLC (106−127 MBq) was dried under a gentle stream of N_2 at 50 °C (water bath), redissolved in ethanol, and diluted in saline containing 1% bovine serum albumin (BSA) at 40 °C to give a final solution of 10% ethanol in saline containing 1% BSA. ^{99m}Tc-CpTT-4-oxo-butyric acid was synthesized as previously reported.[24]

In Vitro Stability. An aliquot (7.4 MBq) of **1** in 10% ethanol−saline was added to human serum (1 mL) and incubated at 37 °C. The solution was analyzed at 1, 3, and 6 h by radio-TLC using dichloromethane−methanol (15:1) as the developing solvent.

Tissue Distribution. ICR mice (male, 25−28 g, 4 mice per time point) were fasted for 12 h before injection of **1** (1.1 MBq/mouse) in 0.2 mL of 10% ethanol−saline (1% BSA) via a tail vein. Mice were sacrificed by cervical dislocation at 1, 5, 10, and 30 min postinjection. Blood, heart, lung, liver, kidney, and stomach were removed, weighed, and counted. Data are expressed as percentages of injected dose per gram of tissue (%ID/g). The percent injected dose was calculated by comparing sample counts to standard solution counts measured in the gamma counter.

Analysis of Metabolites. ICR mice were injected with **1** (34 MBq) via a tail vein, and heart tissue and urine samples were collected at 10 and 30 min postinjection. Heart samples were homogenized in a 2:1:1 mixture of $CHCl_3$−CH_3OH−0.001 N NaOH and centrifuged[26] and the organic and aqueous fractions and residual tissue pellets were counted. Aqueous fractions were then analyzed by HPLC at a flow rate of 1 mL/min using a 30:70 mixture of 0.1% acetic acid (aq) and methanol for 20 min, followed by a linear gradient from 30:70 to 0:100 mixture of the same solvents over 10 min and then 100% methanol for 10 min. ^{99m}Tc-CpTT-4-oxo-butyric acid was also analyzed by HPLC as the radioactive metabolite standard (t_R = 7.1 min). Radiotracer **1** and Re complex **4** were coinjected into a reverse phase HPLC column (YMC C18, 5 μ, 4.6 mm × 250 mm) and had retention times of 33.5 and 32.9 min, respectively.

Tissue pellets were dissolved in 1 N NaOH at 90 °C for 1 h, cooled, and then treated with 50% trichloroacetic acid,[27] and resulting supernatant and precipitate fractions were counted. Urine samples were filtered through a membrane filter (Millipore, 0.22 μm) and analyzed by HPLC using the HPLC conditions mentioned above.

Acknowledgment. We thank Eun Sun Cha for her technical assistance. This research was supported by a grant (CBM31-B3002-01-01-00) from the Center for Biological Modulators of the 21st Century Frontier R&D Program, the Ministry of Science and Technology, Korea.

Supporting Information Available: Elemental analysis, HPLC chromatograms, and ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Corbett, J. R. (1999) Fatty acids for myocardial imaging. *Semin. Nucl. Med.* **1999**, *29*, 237–258.
(2) Bergmann, S. R.; Weinheimer, C. J.; Markham, J.; Herrero, P. Quantitation of myocardial fatty acid metabolism using PET. *J. Nucl. Med.* **1996**, *37*, 1723–1730.
(3) Wisneski, J. A.; Gertz, E. W.; Neese, R. A.; Mayr, M. Myocardial metabolism of free fatty acids. Studies with ^{14}C-labeled substrates in humans. *J. Clin. Invest.* **1987**, *79*, 359–366.
(4) Nellis, S. H.; Liedtke, A. J.; Renstrom, B. Fatty acid kinetics in aerobic myocardium: characteristics of tracer carbon entry and washout and influence of metabolic demand. *J. Nucl. Med.* **1992**, *33*, 1864–1874.
(5) Goodman, M. M.; Kirsch, G.; Knapp, F. F., Jr. Synthesis of radioiodinated ω-(*p*-iodophenyl)-substituted methyl-branched long-chain fatty acids. *J. Med. Chem.* **1984**, *27*, 390–397.
(6) Knapp, F. F., Jr.; Franken, P.; Kropp, J. Cardiac SPECT with iodine-123-labeled fatty acids: evaluation of myocardial viability with BMIPP. *J. Nucl. Med.* **1995**, *36*, 1022–1030.
(7) Otto, C. A.; Brown, L. E.; Wieland, D. M.; Beierwaltes, W. H. Radioiodinated fatty acids for myocardial imaging: effects of chain length. *J. Nucl. Med.* **1981**, *22*, 613–618.
(8) Eckelman, W. C.; Karesh, S. M.; Reba, R. C. New compounds: fatty acid and long chain hydrocarbon derivatives containing a strong chelating agent. *J. Pharm. Sci.* **1975**, *64*, 704–706.
(9) Karesh, S. M.; Eckelman, W. C.; Reba, R. C. Biological distribution of chemical analogs of fatty acids and long chain hydrocarbons containing a strong chelating agent. *J. Pharm. Sci.* **1977**, *86*, 225–228.
(10) Mach, R. H.; Kung, H. F.; Jungwiwattanaporn, P.; Guo, Y. Z. Synthesis and biodistribution of a new class of ^{99m}Tc-labeled fatty acid analogs for myocardial imaging. *Nucl. Med. Biol.* **1991**, *18*, 215–226.
(11) Jones, G. S.; Elmaleh, D. R.; Strauss, H. W.; Fischman, A. J. Synthesis and biodistribution of new ^{99m}technetium fatty acid. *Nucl. Med. Biol.* **1994**, *21*, 117–123.
(12) Jones, G. S.; Elmaleh, D. R.; Strauss, H. W.; Fischman, A. J. 7,10-Bis(2-mercapto-2-methyl)propyl-7,10-diazapalmitic acid: a novel, N_2S_2 ligand for technetium-99m. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2399–2404.
(13) Magata, Y.; Kawaguchi, T.; Ukon, M.; Yamamura, N.; Uehara, T.; Ogawa, K.; Arano, Y.; Temma, T.; Mukai, T.; Tadamura, E.; Saji, H. A Tc-99m-labeled long chain fatty acid derivative for myocardial imaging. *Bioconjugate Chem.* **2004**, *15*, 389–393.
(14) Lee, B. C.; Kim, D. H.; Lee, J. H.; Sung, H. J.; Choe, Y. S.; Chi, D. Y.; Lee, K. H.; Choi, Y.; Kim, B. T. ^{99m}Tc(CO)$_3$-15-[*N*-(Acetyloxy)-2-picolylamino]pentadecanoic acid: a potential radiotracer for evaluation of fatty acid metabolism. *Bioconjugate Chem.* **2007**, *18*, 1332–1337.
(15) Schwochau, K. Technetium radiopharmaceuticals. Fundamentals, synthesis, structure, and development. *Angew. Chem., Int. Ed.* **1994**, *33*, 2258–2267.
(16) Jurisson, S.; Berning, D.; Jia, W.; Dangsche, M. Coordination compounds in nuclear medicine. *Chem. Rev.* **1993**, *93*, 1137–1156.
(17) Spradau, T. W.; Katzenellenbogen, J. A. Preparation of cyclopentadienyltricarbonylrhenium complexes using a double ligand transfer reaction. *Organometallics* **1998**, *17*, 2009–2017.
(18) Cesati, R. R., III; Tamagnan, G.; Baldwin, R. H.; Zoghbi, S. S.; Innis, R. B.; Katzenellenbogen, J. A. Synthesis of cyclopentadienyl tricarbonyl technetium phenyl-tropane derivatives by direct double ligand transfer with ferrocene precursors. *J. Labelled Compd. Radiopharm.* **1999**, *42*, S150−152.
(19) Skaddan, M. B.; Wust, F. R.; Jonson, S.; Syhe, R.; Welch, M. J.; Spies, H.; Katzenellenbogen, J. A. Radiochemical synthesis and tissue distribution of Tc-99m-labeled 7α-substituted estradiol complex. *Nucl. Med. Biol.* **2000**, *27*, 269–278.
(20) Cesati, R. R., III; Katzenellenbogen, J. A. One-pot formation of substituted cyclopentadienyl and indenyltricarbonyl rhenium complexes through in situ generation of cyclopentadienyl- and indenyltributylstannanes. *J. Am. Chem. Soc.* **2001**, *123*, 4093–4094.
(21) Mull, E. S.; Sattigeri, V. J.; Rodriguez, A. L.; Katzenellenbogen, J. A. Aryl cyclopentadienyl tricarbonyl rhenium complexes: novel ligands for the estrogen receptor with potential use as estrogen radiopharmaceuticals. *Bioorg. Med. Chem.* **2002**, *10*, 1381–1398.
(22) Bigott, H. M.; Parent, E.; Luyt, L. G.; Katzenellenbogen, J. A.; Welch, M. J. Design and synthesis of functionalized cyclopentadienyl tricarbonylmetal complexes for technetium-94m PET imaging of estrogen receptors. *Bioconjugate Chem.* **2005**, *16*, 255–264.
(23) Bernard, J.; Ortner, K.; Spingler, B.; Pietzsch, H. J.; Alberto, R. Aqueous synthesis of derivatized cyclopentadienyl complexes of technetium and rhenium directed toward radiopharmaceutical appication. *Inorg. Chem.* **2003**, *42*, 1014–1022.
(24) Lee, B. C.; Choe, Y. S.; Chi, D. Y.; Lee, K. H.; Choi, Y.; Kim, S. E.; Kim, B.-T. Cyclopentadieny tricarbonyl ^{99m}Tc octanoic acid: a novel radiotracer for evaluation of fatty acid metabolism in the liver. *Bioconjugate Chem.* **2004**, *15*, 121–127.
(25) Uehara, T.; Uemura, T.; Hirabayashi, S.; Adachi, S.; Odaka, K.; Akizawa, H.; Magata, Y.; Irie, T.; Arano, Y. Technetium-99m-labeled long chain fatty acid analogues metabolized by β-oxidation in the heart. *J. Med. Chem.* **2007**, *50*, 543–549.
(26) Forch, J.; Less, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509.
(27) DeGrado, T. R.; Coenen, H. H.; Stöcklin, G. 14(*R,S*)-[^{18}F]Fluoro-6-thia-heptadecanoic acid (FTHA): evaluation in mouse of a new probe of myocardial utilization of long chain fatty acids. *J. Nucl. Med.* **1991**, *32*, 1888–1896.

JM800049H