Full paper

Applied Organometallic Chemistry

Received: 24 October 2015 Revised: 28 January 2016 Accepted: 15 February 2016 Published online in Wiley Online Library: 26 April 2016

(wileyonlinelibrary.com) DOI 10.1002/aoc.3476

Synthesis and bio-evaluation of Tc-99m-labeled fatty acid derivatives for myocardial metabolism imaging

Jianping Liu, Qianqian Xue, Huan Wang, Hang Wang, Dawei Wang, Yu Fang and Huabei Zhang*

^{11}C, ^{18}F and ^{123}I fatty acids are used for myocardial imaging, and ^{99m}Tc-labeled fatty acids are more desirable substitutes than other radiolabeled fatty acids. In the work reported, [^{99m}Tc]-CpTT-10-oxo-FPA (1c), [^{99m}Tc]-CpTT-12-oxo-FPA (2c), [^{99m}Tc]-CpTT-14-oxo-FPA (3c) and [^{99m}Tc]-CpTT-16-oxo-FPA (4c) were prepared with 60.76–70.92% of radiochemical yield and purity of more than 95%. These radiotracers (1c, 2c, 3c, 4c) were chemically stable when incubated in Sprague Dawley rat serum for 3 h at 37 °C. Tissue distribution studies in female mice indicated that 2c had high initial heart uptake (8.84%ID g^{-1} at 1 min post-injection) and 4c had long retention in the heart (1.45%ID g^{-1} at 30 min post-injection). Metabolite analysis showed 4c could be metabolized to 5c via β-oxidation with loss of two –CH_2– in the myocardium, the radiometabolite being excreted via urine. However, low heart uptake suggested that 4c cannot be used as a diagnostic imaging agent. Copyright © 2016 John Wiley & Sons, Ltd.

Additional supporting information may be found in the online version of this article at the publisher's web site.

Keywords: long-chain fatty acid; myocardium metabolism; initial uptake

Introduction

Long-chain fatty acid analogs provide energy through β-oxidation in the normal myocardium.[1,2] Therefore, long-chain fatty acid analogs have important roles in detecting heart disease. A number of groups have reported radiolabeled tracers for positron emission tomography such as [^{11}C]-palmitate[3] and single-photon emission computed tomography (SPECT) such as [^{123}I]-IPPA ([^{123}I]-iodophenylpentadecanoic acid)[4] and [^{123}I]-BMIPP (15-(*p*-[^{123}I]-iodophenyl)-3-methylpentadecanoic acid).[5] Technetium-99m-labeled imaging agents are required because of their ideal nuclear properties and widespread SPECT-based imaging methods for diagnosis.

In the past two decades, many different ^{99m}Tc-labeled long-chain fatty acids have been reported,[6–13] but no compounds have been used as diagnostic imaging agents due to their poor uptake in and rapid clearance from the heart. Magata *et al.*[6] reported [^{99m}Tc]-MAMA-hexadecanoic acid which had higher liver uptake. Using [^{99m}Tc]-CpTT-PA, it was shown[7] that ^{99m}Tc-labeled fatty acid analogs are metabolized via β-oxidation with loss of two-carbon units in the heart. Lee *et al.*[8] reported ^{99m}Tc(CO)$_3$-15-[*N*-(acetyloxy)-2-picolylamino]pentadecanoic acid, which had an initial heart uptake of 12.67 ± 1.48%ID g^{-1} at 0.5 min and 6.38 ± 0.69%ID g^{-1} at 1 min, but the heart-to-blood ratio was low. Later Lee *et al.*[9] reported [^{99m}Tc]-CpTT-16-oxo-HDA which showed higher initial heart uptake (9.03 ± 0.17%ID g^{-1}). Chu *et al.*[10] reported ^{99m}Tc(CO)$_3$-IUA and ^{99m}Tc(CO)$_3$-BPIUA which showed higher heart uptake and lower blood background. [^{99m}Tc(N)(PNP)(L)]$^{0/+}$ fatty acids were reported by Emiliano *et al.*[11] who showed that monocationic complexes are more favorable for myocardial uptake. CpTT-16-oxo-HAUA[12] showed initial heart uptake of 4.37%ID g^{-1} at 1 min. [^{99m}Tc]-CpTT-15-oxo-PTA[13] had the highest heart uptake of 9.39%ID g^{-1} at 1 min and the highest heart-to-blood ratio of 5.7 at 5 min.

In the study reported here, four ^{99m}Tc-labeled fatty acid analogs were prepared with furan group involved in ^{99m}Tc-CpTT at the γ-position. And different lengths of carbon chains were studied to seek higher initial uptake and extend retention in the heart. We prepared ^{99m}Tc-CpTT-10-oxo-FPA (**1c**), ^{99m}Tc-CpTT-12-oxo-FPA (**2c**), ^{99m}Tc-CpTT-14-oxo-FPA (**3c**) and ^{99m}Tc-CpTT-16-oxo-FPA (**4c**) and conducted biological evaluations *in vitro* and *in vivo*.

Experimental

Materials and methods

Chemicals and solvents were obtained from commercial suppliers and used without further purification. Na^{99m}TcO$_4$ was obtained by elution with saline from a ^{99}Mo–^{99m}Tc generator (Beijing Atomic High-tech Co., Beijing). All reactions were monitored by TLC, using silica gel plates with fluorescence F254 and UV light visualization. Flash column chromatography employed silica gel 60 (230–400 mesh). HPLC was performed

* *Correspondence to: Huabei Zhang, Laboratory of Radiopharmaceuticals of Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, China. E-mail: hbzhang@bnu.edu.cn*

Key Laboratory of Radiopharmaceuticals of Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, China

using a Shimadzu SCL-10AVP system equipped with a semi-preparative column (Venusil MP C-18, Agela Technologies, 10 mm × 250 mm) with a UV detector (254 nm) and a NaI radioisotope detector. NMR spectra were obtained using a Bruker ARX-400 spectrometer with $CDCl_3$ as a solvent. Mass spectra (EI-MS) were obtained with a Trance MS-2000 mass spectrometer (Finnigan, USA). Radioactivity was measured with a dose calibrator (Beijing Nuclear Instrument Factory) and tissue radioactivity in a Bioscan-g-detector B-FC3200. All animal experiments were carried out using normal mice (Vital River Laboratory Animal Technology Co. Ltd, Beijing), and in compliance with the relevant national laws, as approved by the local committee on the conduct and ethics of animal experimentation.

Syntheses

*Synthesis of ethyl 3-(5-(10-ferrocene-10-oxodecanoyl)furan-2-yl)propanoate (**1a**)*

Sulfoxide chloride (45 ml, 618 mol) was added dropwise to decanedioic acid (6.01 g, 29.7 mmol) and heated at 85 °C for 12 h. Sulfoxide chloride was removed under vacuum, and then 1,10-decanedioyl dichloride was obtained without further purification. Ferrocene (5.53 g, 29.7 mmol) and ethyl 3-(furan-2-yl)propanoate (5 g, 29.7 mmol) were stirred well in dichloromethane (150 ml). The reaction flask was charged with 1,10-decanedioyl dichloride in dichloromethane (150 ml) and the stirred mixture was added slowly in an ice bath. Anhydrous aluminium chloride (7.92 g, 59.4 mmol) was added to this solution carefully over 30 min. After most of the solvent was removed in vacuum, the crude mixture was diluted with hydrochloric acid (30 ml, 1 mol l^{-1}) and extracted with dichloromethane (3 × 25 ml), then dried over anhydrous Na_2SO_4. When the solvent was evaporated, the residue was subjected to chromatography (10:90 ethyl acetate–petroleum ether) to afford **1a** (1.20 g, 2.3 mmol, 7.76%) as an orange solid.

Elemental analysis; found (%): C, 65.85; H, 6.97. Calculated for $C_{29}H_{36}FeO_5$ (%): C, 66.93; H, 6.97. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.08 (d, J = 3.4 Hz, 1H, –O–C_1=C_2–H), 6.19 (d, J = 3.4 Hz, 1H, –O–C_4&=C_3–H), 4.78 (s, 2H, –Fe–Cp–H(α)), 4.49 (s, 2H, –Fe–Cp–H(β)), 4.20 (s, 5H, Cp–H), 4.16 (q, J = 7.2 Hz, 2H, –O–CH_2–CH_3), 3.04 (t, J = 7.4 Hz, 2H, –C=O–CH_2–), 2.66–2.76 (m, 6H, –C=O–CH_2–), 1.69–1.70 (m, 4H, –C=O–CH_2–CH_2–), 1.35 (s, 8H, –CH_2–), 1.24 (t, J = 7.2 Hz, 3H, –CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 204.35 (–Fe–Cp–C=O), 188.94 (–furan–C=O), 171.76 (–COO–), 159.14 (–O–C_1=C_2–H), 151.65 (–O–C_4=C_3–H), 118.38 (–O–C_1=C_2–H), 108.44 (–O–C_4=C_3–H), 79.13 (–Fe–C–C=O), 72.02 (–Fe–Cp(α)), 69.64 (–Fe–Cp(β)), 69.23 (–Cp), 60.59 (–O–CH_2–), 39.57 (–C=O–CH_2–), 38.11 (–C=O–CH_2–), 32.02 (–CH_2–), 29.36 (–CH_2–), 29.25 (–CH_2–), 29.19 (–CH_2–), 24.45 (–furan–CH_2–CH_2–C=O–), 24.41 (–furan–CH_2–CH_2–C=O–), 23.65 (–C=O–CH_2–), 14.14 (–CH_3). MS (ESI) *m/z*: calcd for [$C_{29}H_{36}FeO_5$] 520.19; found $(M + H)^+$ 521.2, $(M + Na)^+$ 543.2. M.p. 55.2–56.5 °C.

*Synthesis of ethyl 3-(5-(12-ferrocene-12-oxododecanoyl)furan-2-yl)propanoate (**2a**)*

Sulfoxide chloride (45 ml, 618 mol) was added dropwise to dodecanedioic acid (6.84 g, 29.7 mmol) and heated at 85 °C for 12 h. Sulfoxide chloride was removed in vacuum, and then 1,12-decanoyl dichloride was obtained without further purification. Ferrocene (5.53 g, 29.7 mmol) and 3-(furan-2-yl) propanoate (5 g, 29.7 mmol) were stirred well in dichloromethane (150 ml). The reaction flask was charged with 1,12-decanoyl dichloride in dichloromethane (150 ml) and the stirred mixture was added slowly in an ice bath. Anhydrous aluminium chloride (7.92 g, 59.4 mmol) was added to this solution carefully over 30 min. After most of the solvent was removed under vacuum, the crude mixture was diluted with hydrochloric acid (30 ml, 1 mol l^{-1}) and extracted with dichloromethane (3 × 25 ml), then dried over anhydrous Na_2SO_4. When the solvent was evaporated, the residue was subjected to chromatography (10:90 ethyl acetate–petroleum ether) to afford **2a** (1.42 g, 2.6 mmol, 8.72%) as an orange solid.

Elemental analysis; found (%): C, 66.80; H, 7.42. Calculated for $C_{31}H_{40}FeO_5$ (%): C, 67.92; H, 7.35. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.09 (d, J = 3.4 Hz, 1H, –O–C_1=C_2–H), 6.20 (d, J = 3.4 Hz, 1H, –O–C_4=C_3–H), 4.78 (s, 2H, –Fe–Cp–H(α)), 4.49 (s, 2H, –Fe–Cp–H(β)), 4.20 (s, 5H, Cp–H), 4.16 (q, J = 7.2 Hz, 2H, –O–CH_2–CH_3), 3.04 (t, J = 7.5 Hz, 2H, –C=O–CH_2–), 2.67–2.76 (m, 6H, –C=O–CH_2–), 1.66–1.71 (m, 4H, –C=O–CH_2–CH_2–), 1.23–1.29 (m, 12H, –CH_2–), 1.18 (t, 3H, J = 7.2Hz, –CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 203.87 (–Fe–Cp–C=O), 188.32 (–furan–C=O), 171.02 (–COO–), 158.25 (–O–C_1=C_2–H), 150.75 (–O–C_4=C_3–H), 117.50 (–O–C_1=C_2–H), 107.50 (–O–C_4=C_3–H), 78.18 (–Fe–C–C=O), 71.12 (–Fe–Cp(α)), 68.73 (–Fe–Cp(β)), 68.35 (–Cp), 59.72 (–O–CH_2–), 38.77 (–C=O–CH_2–), 37.27 (–C=O–CH_2–), 31.12 (–CH_2–), 28.51 (–CH_2–), 28.46 (–CH_2–), 28.43 (–CH_2–), 28.41 (–CH_2–), 28.36, 28.31 (–CH_2–), 23.64 (–furan–CH_2–CH_2–C=O–), 23.57 (–furan–CH_2–CH_2–C=O–), 22.73 (–C=O–CH_2–), 13.18 (–CH_3). MS (ESI) *m/z*: calcd for [$C_{31}H_{40}FeO_5$] 548.22; found $(M + H)^+$ 549.1, $(M + Na)^+$ 571.1. M.p. 56.7–57.8 °C.

*Synthesis of ethyl 3-(5-(14-ferrocene-14-oxotetradecanoyl)furan-2-yl) propanoate (**3a**)*

Sulfoxide chloride (45 ml,618 mol) was added dropwise to tetradecanedioic acid (7.67 g, 29.7 mmol) and heated at 85 °C for 12 h. Sulfoxide chloride was removed in vacuum, then 1,14-tetradecanedioyl dichloride was obtained without further purification. Ferrocene (5.53 g, 29.7 mmol) and 3-(furan-2-yl)propanoate (5 g, 29.7 mmol) were stirred well in dichloromethane (150 ml). The reaction flask was charged with 1,14-tetradecanedioyl dichloride in dichloromethane (150 ml) and the stirred mixture was added slowly in an ice bath. Anhydrous aluminium chloride (7.92 g, 59.4 mmol) was added to this solution carefully over 30 min. After most of the solvent was removed under vacuum, the crude mixture was diluted with hydrochloric acid (30 ml, 1 mol l^{-1}) and extracted with dichloromethane (3 × 25 ml), then dried over anhydrous Na_2SO_4. When the solvent was evaporated, the residue was subjected to chromatography (10:90 ethyl acetate–petroleum ether) to afford **3a** (0.88 g, 1.5 mmol, 5.14%) as an orange solid.

Elemental analysis; found (%): C, 67.66; H, 7.66. Calculated for $C_{33}H_{44}FeO_5$ (%): C, 68.75; H, 7.69. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.08 (d, J = 3.4 Hz, 1H, –O–C_1=C_2–H), 6.19 (d, J = 3.4 Hz, 1H, –O–C_4=C_3–H), 4.78 (s, 2H, –Fe–Cp–H(α)), 4.48 (s, 2H, –Fe–Cp–H(β)), 4.19 (s, 5H, Cp–H), 4.14 (q, J = 7.2 Hz, 2H, –O–CH_2CH_3), 3.04 (t, J = 7.5 Hz, 2H, –C=O–CH_2–), 2.67–2.75 (m, 6H, –C=O–CH_2–), 1.67–1.69 (m, 4H, –C=O–CH_2–CH_2–), 1.23–1.26 (m, 19H, –CH_2–, –CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 203.40 (–Fe–Cp–C=O), 188.02 (–furan–C=O), 171.79 (–COO–), 158.15 (–O–C_1=C_2–H), 150.72 (–O–C_4=C_3–H), 117.36 (–O–C_1=C_2–H), 107.45 (–O–C_4=C_3–H), 78.21 (–Fe–C–C=O), 71.03 (–Fe–Cp(α)), 68.67 (–Fe–Cp(β)), 68.27 (–Cp), 59.60 (–O–CH_2–), 38.66 (–C=O–CH_2–), 37.20 (–C=O–CH_2–), 31.07 (–CH_2–), 28.53 (–CH_2–), 28.51 (–CH_2–), 28.48 (–CH_2–), 28.47 (–CH_2–), 28.40 (–CH_2–), 28.35 (–CH_2–), 28.28 (–furan–CH_2–CH_2–C=O–), 23.55 (–furan–CH_2–CH_2–C=O–), 22.70 (–C=O–CH_2–), 13.18

(–CH_3). MS (ESI) *m/z*: calcd for [$C_{33}H_{44}FeO_5$] 576.25; found $(M+H)^+$ 577.3, $(M+Na)^+$ 599.3. M.p. 58.7-61.9 °C

*Synthesis of ethyl 3-(5-(16-ferrocene-16-oxohexadenoyl)furan-2-yl)propanoate (**4a**)*

Sulfoxide chloride (45 ml, 618 mol) was added dropwise to hexadecanedioic acid (8.5 g, 29.7 mmol) and heated at 85 °C for 12 h. Sulfoxide chloride was removed in vacuum, and then 1,16-hexadecanedioyl dichloride was obtained without further purification. Ferrocene (5.53 g, 29.7 mmol) and 3-(furan-2-yl)propanoate (5 g, 29.7 mmol) were stirred well in dichloromethane (150 ml). The reaction flask was charged with 1,16-hexadecanedioyl dichloride in dichloromethane (150 ml) and the stirred mixture was added slowly in an ice bath. Anhydrous aluminium chloride (7.92 g, 59.4 mmol) was added to this solution carefully over 30 min. After most of the solvent was removed under vacuum, the crude mixture was diluted with hydrochloric acid (30 ml, 1 mol l^{-1}) and extracted with dichloromethane (3 × 25 ml), then dried over anhydrous Na_2SO_4. When the solvent was evaporated, the residue was subjected to chromatography (10:90 ethyl acetate–petroleum ether) to afford **4a** (0.92 g, 1.5 mmol, 5.12%) as an orange solid.

Elemental analysis; found (%): C, 68.48; H, 7.95. Calculated for $C_{35}H_{48}FeO_5$ (%): C, 69.53; H, 8.00. ^{1}H NMR (400 MHz,$CDCl_3$, δ, ppm): 7.08 (d, J = 3.4 Hz, 1H, –O–C_1=C_2–H), 6.20 (d, J = 3.4 Hz, 1H, –O–C_4=C_3–H), 4.79 (s, 2H, –Fe–Cp–H(α)), 4.50 (s, 2H, –Fe–Cp–H(β)), 4.21 (s, 5H, Cp–H), 4.16 (q, J = 7.1 Hz, 2H, –O–CH_2–CH_3), 3.04 (t, J = 7.4 Hz, 2H, –C=O–CH_2–), 2.67–2.76 (m, 6H, –C=O–CH_2–), 1.66–1.71 (m, 4H, –C=O–CH_2–CH_2–), 1.23–1.34 (m, 23H, –CH_2–, –CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 204.78 (–Fe–Cp–*C*=O), 189.27 (–furan–*C*=O), 171.95 (–COO–), 159.21 (–O–C_1=C_2–H), 151.75 (–O–C_4=C_3–H), 118.44 (–O–C_1=C_2–H), 108.48 (–O–C_4=C_3–H), 79.29 (–Fe–*C*–C=O), 72.07 (–Fe–Cp(α)), 69.71 (–Fe–Cp(β)), 69.32 (–Cp), 60.72 (–O–CH_2–), 39.80 (–C=O–CH_2–), 38.29, (–C=O–CH_2–) 32.13 (–CH_2–), 29.62 (–CH_2–), 29.59 (–CH_2–), 29.56 (–CH_2–), 29.52 (–CH_2–), 29.47 (–CH_2–), 29.40 (–CH_2–), 29.35 (–CH_2–), 24.63 (–furan–CH_2–CH_2–C=O–), 24.60 (–furan–CH_2–CH_2–C=O–), 23.73 (–C=O–CH_2–), 14.19 (–CH_3). MS (ESI) *m/z*: calcd for [$C_{35}H_{48}FeO_5$] 604.29; found M^+ 605.1, $(M+H)^+$ 606.2. M.p. 62.7–66.3 °C.

*Synthesis of methyl 5-(16-ferrocene-16-oxohexadenoyl)furan-2-carboxylate (**5a**)*

A stirred mixture of 2-furaldehyde (5.71 g, 59.2 mmol), ethylene glycol (3.68 g, 59.4 mmol) and *p*-TsOH (1 mol%) in toluene (350 ml) was refluxed for 5 h with azeotropic removal of water; the solvent was removed under vacuum. Sulfoxide chloride (45 ml, 618 mol) was added dropwise to hexadecanedioic acid (8.5 g, 29.7 mmol) and heated at 85 °C for 12 h. Sulfoxide chloride was removed in vacuum, and then 1,16-hexadecanedioyl dichloride was obtained without further purification. Ferrocene (5.53 g, 29.7 mmol) was added to the mixture and stirred well in dichloromethane (150 ml). The reaction flask was charged with 1,16-hexadecanedioyl dichloride in dichloromethane (150 ml) and the stirred mixture was added slowly in an ice bath. Anhydrous aluminium chloride (7.92 g, 59.4 mmol) was added to this solution carefully over 30 min. After most of the solvent was removed under vacuum, the crude mixture was diluted with hydrochloric acid (30 ml, 1 mol l^{-1}) and extracted with dichloromethane (3 × 25 ml), then dried over anhydrous Na_2SO_4. When the solvent was evaporated, the residue was subjected to chromatography (10:90 ethyl acetate–petroleum ether) to afford **5**. Compound **5** (0.56 g, 1 mmol) was dissolved in methanol (5 ml) to which was added V_2O_5 (0.01 mmol) dissolved in 30% H_2O_2 (1 mmol) in an ice bath for 10 h. This was then extracted with dichloromethane and dried. After removal of solvent, the residue was subjected to chromatography (10:90 ethyl acetate–petroleum ether) to afford **5a** (0.12 g, 0.21 mmol, 21%) as an orange solid.

Elemental analysis; found (%): C, 68.36; H, 7.97. Calculated for $C_{32}H_{42}FeO_5$ (%): C, 68.05; H, 7.51. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.98 (d, J = 3.4 Hz, 1H, –O–C_1=C_2–H), 6.90 (d, J = 3.4 Hz, 1H, –O–C_4=C_3–H), 4.79 (s, 2H, –Fe–Cp–H(α)), 4.50 (s, 2H, –Fe–Cp–H(β)), 4.20 (s, 5H, Cp–H), 3.88 (s, 3H, –$COOCH_3$), 2.69 (t, J = 7.3 Hz, 2H, –C=O–CH_2–), 1.79 (t, J = 6.7 Hz, 2H, –C=O–CH_2–), 1.68–1.70 (m, 2H, –C=O–CH_2–CH_2–), 1.44–1.46 (m, 2H, –C=O–CH_2–CH_2–), 1.26–1.33 (m, 20H, –CH_2–). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 203.64 (–Fe–Cp–C=O), 192.23 (–furan–C=O), 172.32 (–COO–), 153.48 (–O–C_1=C_2–H), 141.39 (–O–C_4=C_3–H), 131.05 (–O–C_1=C_2–H), 124.95 (–O–C_4=C_3–H), 78.23 (–Fe–C–C=O), 71.08 (–Fe–Cp(α)), 68.72 (–Fe–Cp(β)), 68.33 (–Cp), 50.61 (–COO–CH_3), 38.73 (–C=O–CH_2–), 37.97 (–C=O–CH_2–) 31.94 (–CH_2–), 28.77 (–CH_2–), 28.50 (–CH_2–), 28.42 (–CH_2–), 28.35 (–CH_2–), 28.31 (–CH_2–), 25.33 (–CH_2–), 23.90 (–CH_2–), 23.60 (–CH_2–). MS (ESI) *m/z*: calcd for [$C_{32}H_{42}FeO_5$] 564.76; found $(M+H)^+$ 565.87.

*Synthesis of tricarbonyl (3-(5-(10-cyclopentadienyl-10-oxodecanoyl)furan-2-yl) propanoic acid) rhenium (CpTRe-10-oxo-FPA, **1b**)*

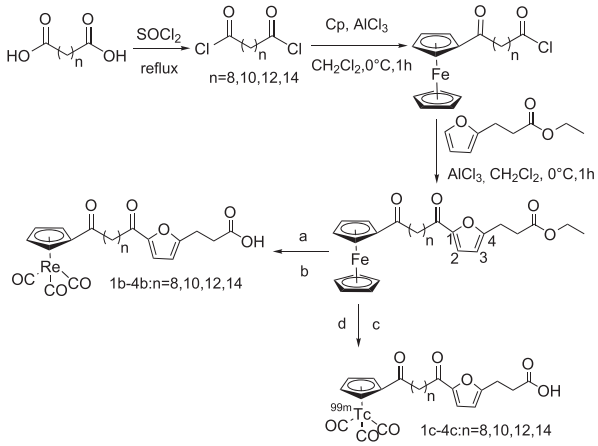
Ester **1a** (100 mg), NH_4ReO_4 (30 mg, 0.11 mmol), $Cr(CO)_6$ (128 mg, 0.58 mmol) and $CrCl_3$ (30 mg, 0.19 mmol) with dry methanol (0.8 ml) were placed in a high-pressure tank of polytetrafluoroethylene, then heated at 180 °C. After 3 h the mixture was cooled for 30 min in an ice bath. The residue was dissolved in CH_2Cl_2, passed through a 0.2 μm Millipore filter, and the solvent was removed under vacuum. The residue was added to 3 ml of 0.3 M NaOH and methanol (10 ml) and heated at 80 °C for 40 min. Methanol was removed under reduced pressure at 40 °C, and the solution was acidified to pH = 7 with 0.1 M HCl. The reaction mixture was extracted with dichloromethane, and the organic layer was dried over anhydrous Na_2SO_4 then concentrated. The crude products were purified by flash column chromatography (10:20 ethyl acetate–petroleum ether with 1% acetic acid) to afford **1b** (10.0 mg, 15.6 nmol, 8.2%) as a gray solid.

Elemental analysis; found (%): C, 47.68; H, 4.47. Calculated for $C_{25}H_{27}^{185}ReO_8$ (%): C, 46.79; H, 4.24. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.08 (d, J = 3.4Hz, 1H, –O–C_1=C_2–H), 6.02 (d, J = 3.4 Hz, 1H, –O–C_4=C_3–H), 5.99 (t, J = 2.3 Hz, 2H, –Re–Cp–H(α)), 5.39 (t, J = 2.3 Hz, 2H, –Re–Cp–H(β)), 3.06 (t, J = 7.4 Hz, 2H, –C=O–CH_2–), 2.73–2.79 (m, 4H), 2.58 (t, J = 7.2 Hz, 2H, –C=O–CH_2–), 1.67–1.69 (m, 4H, –C=O–CH_2–CH_2–), 1.33 (s, 8H, –CH_2–); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 194.44 (–Re–C=O), 190.84 (–Re–C=O), 188.39 (–furan–C=O), 176.16 (–COO–), 157.81 (–O–C_1=C_2–H), 150.79 (–O–C_4=C_3–H), 117.56 (–O–C_1=C_2–H), 107.65 (–O–C_4=C_3–H), 95.15 (–Re–C–C=O), 86.92 (–Re–Cp(α)), 84.16 (–Re–Cp(β)), 37.78 (–C=O–CH_2–), 37.22 (–C=O–CH_2–), 30.76 (–CH_2–), 28.17 (–CH_2–), 28.10 (–CH_2–), 27.94 (–CH_2–), 23.50 (–CH_2–), 22.44 (–furan–CH_2–CH_2–C=O–), 23.34 (–furan–CH_2–CH_2–C=O–). MS (ESI) *m/z*: calcd for [$C_{25}H_{27}^{185}ReO_8$] 640.52; found ($M+H^+$, 185 Re) 640.8, ($M+H^+$, 187 Re) 642.8.

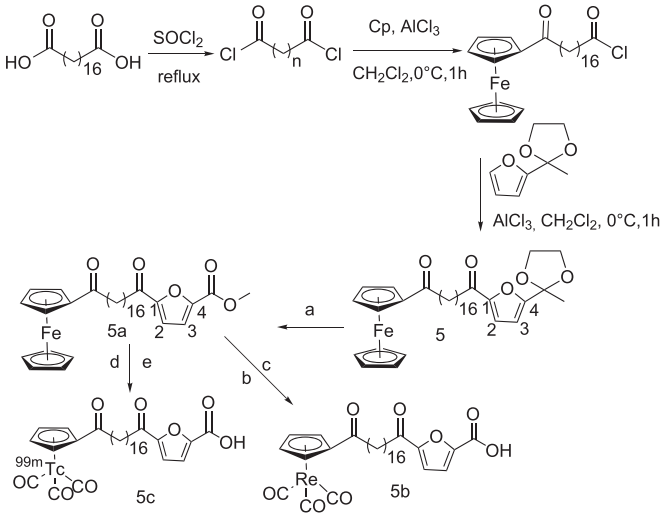
*Synthesis of tricarbonyl (3-(5-(12-cyclopentadineyl-12-oxododecanoyl)furan-2-yl)propanoic acid) rhenium (CpTRe-12-oxo-FPA, **2b**)*

Prepared analogously as described above, compound **2b** as a gray solid was obtained from **1b** (13 mg, 19.4 nmol, 10.22%).

Elemental analysis; found (%): C, 47.78; H, 4.62. Calculated for $C_{27}H_{31}^{185}ReO_8$ (%): C, 48.42; H, 4.67. ^{1}H NMR (400 MHz,$CDCl_3$, δ, ppm): 7.08 (d, J = 3.4 Hz, 1H, –O–C_1=C_2–H), 6.02 (d, J = 3.4 Hz, 1H,



Scheme 1. Synthetic route. Reaction conditions: (a) NH_4ReO_4, $Cr(CO)_6$, $CrCl_3$, CH_3OH, 180 °C, 1 h; (b) 0.3 N NaOH–CH_3OH (1:3), 80 °C, 40 min; (c) $Na^{99m}TcO_4$, $Mn(CO)_5Br$, DMF, 150 °C, 1 h; (d) 0.3 N NaOH–CH_3OH (1:3), 80 °C, 10 min.



Scheme 2. Synthetic route to **5b** and **5c**. Reaction conditions: (a) V_2O_5, H_2O_2 (30%), CH_3OH, reflux, 9 h; (b) NH_4ReO_4, $Cr(CO)_6$, $CrCl_3$, CH_3OH, 180 °C, 1 h; (c) 0.3 N NaOH–CH_3OH (1:3), 80 °C, 40 min; (d) $Na^{99m}TcO_4$, $Mn(CO)_5Br$, DMF, 150 °C, 1 h; (e) 0.3 N NaOH–CH_3OH (1:3), 80 °C, 10 min.

–O–C_4=C_3–H), 5.99 (t, J = 1.9 Hz, 2H, –Re–Cp–H(α)), 5.39 (t, J = 1.9 Hz, 2H, –Re–Cp–H(β)), 3.05 (t, J = 7.4 Hz, 2H, –C=O–CH_2–), 2.73–2.80 (m,4H, –C=O–CH_2–), 2.58 (t, J = 7.2 Hz, 2H, –C=O–CH_2–), 1.66–1.71 (m, 4H, –C=O–CH_2–CH_2–), 1.29 (s,12H, –CH_2–). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 194.49 (–Re–C=O), 190.85 (–Re–C=O), 188.44 (furan–C=O), 176.17 (–COO–), 157.81 (–O–C_1=C_2–H), 150.77 (–O–C_4=C_3–H), 117.58 (–O–C_1=C_2–H), 107.64 (–O–C_4=C_3–H), 95.13 (–Re–C–C=O), 86.93 (–Re–Cp(α)), 84.15 (–Re–Cp(β)), 37.81 (–C=O–CH_2–), 37.27 (–C=O–CH_2–), 30.75 (–CH_2–), 28.31 (–CH_2–), 28.27 (–CH_2–), 28.02 (–CH_2–), 23.58 (–CH_2–), 23.39 (–furan–CH_2–CH_2–C=O–), 22.42 (–furan–CH_2–CH_2–C=O–). MS (ESI) *m/z*: calcd for [$C_{27}H_{31}^{185}ReO_8$] 668.58; found (M + H^+, 185 Re) 669.2, (M + H^+, 187 Re) 671.2.

*Synthesis of tricarbonyl (3-(5-(14-cyclopentadineyl-14-oxotetradecanoyl)furan-2-yl)propanoic acid) rhenium (CpTRe-14-oxo-FPA, **3b**)*

Prepared analogously as described above, compound **3b** as a gray solid was obtained from **1b** (18 mg, 25.8 nmol, 13.58%).

Elemental analysis; found (%): C, 49.94; H, 5.36. Calculated for $C_{29}H_{35}^{185}ReO_8$ (%): C, 49.92; H, 5.06. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.03 (d, J = 3.4 Hz, 1H, -O–C_1=C_2–H), 6.15 (d, J = 3.4 Hz, 1H, –O–C_4=C_3–H), 5.92 (t, J = 2.2 Hz, 2H, –Re–Cp–H(α)), 5.32 (t, J = 2.2 Hz, 2H, –Re–Cp–H(β)), 2.98 (t, J = 7.4 Hz, 2H, –C=O–CH_2–), 2.66–2.73 (m, 4H, –C=O–CH_2–), 2.51 (t, J = 7.2 Hz, 2H, –C=O–CH_2–), 1.59–1.64 (m, 4H, –C=O–CH_2–CH_2–), 1.19–1.22 (d,16H, –CH_2–). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 194.48 (–Re–C=O), 190.84 (–Re–C=O), 188.42 (furan–C=O), 175.77 (–COO–), 157.77 (–O–C_1=C_2–H), 150.80 (–O–C_4=C_3–H), 117.52 (–O–C_1=C_2–H), 107.62 (–O–C_4=C_3–H), 95.16 (–Re–C–C=O), 86.91 (–Re–Cp(α)), 84.12 (–Re–Cp(β)), 37.83 (–C=O–CH_2–), 37.29 (–C=O–CH_2–), 30.69 (–CH_2–), 28.51 (–CH_2–), 28.49 (–CH_2–), 28.40 (–CH_2–), 28.34 (–CH_2–), 28.31 (–CH_2–), 28.06 (–CH_2–), 23.61 (–CH_2–), 23.42 (–furan–CH_2–CH_2–C=O–), 22.43 (–furan–CH_2–CH_2–C=O–). MS (ESI) *m/z*: calcd for [$C_{29}H_{35}^{185}ReO_8$] 696.64; found (M + H^+, 185 Re) 697.2, (M + H^+, 187 Re) 699.2.

*Synthesis of tricarbonyl (3-(5-(16-cyclopentadineyl-16-oxohexadenoyl)furan-2-yl)propanoic acid) rhenium (CpTRe-16-oxo-FPA, **4b**)*

Prepared analogously as described above, compound **4b** as a gray solid was obtained from **1b** (17 mg, 23.4 nmol, 12.35%).

Elemental analysis; found (%): C, 51.80; H, 5.64. Calculated for $C_{31}H_{39}^{185}ReO_8$ (%): C, 51.30; H, 5.42.^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.03 (d, J = 3.4 Hz, 1H, –O–C_1=C_2–H), 6.15 (d, J = 3.4 Hz, 1H, –O–C_4=C_3–H), 5.91 (t, J = 2.3 Hz, 2H, –Re–Cp–H(α)), 5.32 (t, J = 2.3 Hz, 2H, –Re–Cp–H(β)), 2.98 (t, J = 7.4 Hz, 2H, –C=O–CH_2–), 2.66–2.73 (m, 4H, –C=O–CH_2–), 2.51 (t, J = 7.2 Hz, 2H, –C=O–CH_2–), 1.59–1.64 (m, 4H, –C=O–CH_2–CH_2–), 1.19–1.22 (d, 20H, –CH_2–). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 194.47 (–Re–C=O), 190.84 (–Re–C=O), 188.43 (furan–C=O), 176.07 (–COO–), 157.80 (–O–C_1=C_2–H), 150.80 (–O–C_4=C_3–H), 117.53 (–O–C_1=C_2–H), 107.62 (–O–C_4=C_3–H), 95.19 (–Re–C–C=O), 86.89 (–Re–Cp(α)), 84.13 (–Re–Cp(β)), 37.84 (–C=O–CH_2–), 37.30 (–C=O–CH_2–), 30.75 (–CH_2–), 28.58 (–CH_2–), 28.53 (–CH_2–), 28.44 (–CH_2–), 28.37 (–CH_2–), 28.41 (–CH_2–), 28.33 (–CH_2–), 28.07 (–CH_2–), 23.60 (–CH_2–), 23.44 (–furan–CH_2–CH_2–C=O–), 22.44 (–furan–CH_2–CH_2–C=O–). MS (ESI) *m/z*: calcd for [$C_{31}H_{39}^{185}ReO_8$] 724.7; found (M + H^+, 185 Re) 725.7, (M + H^+, 187 Re) 727.7.

Table 1. HPLC retention time of Re/^{99m}Tc-labeled fatty acids and partition coefficient of ^{99m}Tc-labeled fatty acids

Compound	Retention time (min)	Compound	Retention time (min)	Log *P*
CpTRe-10-oxo-FPA (**1b**)	10.32	[^{99m}Tc]-CpTT-10-oxo-FPA (**1c**)	11.20	0.51 ± 0.01
CpTRe-12-oxo-FPA (**2b**)	12.93	[^{99m}Tc]-CpTT-12-oxo-FPA (**2c**)	14.12	0.62 ± 0.0.01
CpTRe-14-oxo-FPA (**3b**)	16.32	[^{99m}Tc]-CpTT-14-oxo-FPA (**3c**)	18.02	0.73 ± 0.01
CpTRe-16-oxo-FPA (**4b**)	22.27	[^{99m}Tc]-CpTT-16-oxo-FPA (**4c**)	24.36	1.05 ± 0.0001
CpTRe-16-oxo-FA (**5b**)	18.26	[^{99m}Tc]-CpTT-16-oxo-FA (**5c**)	19.87	

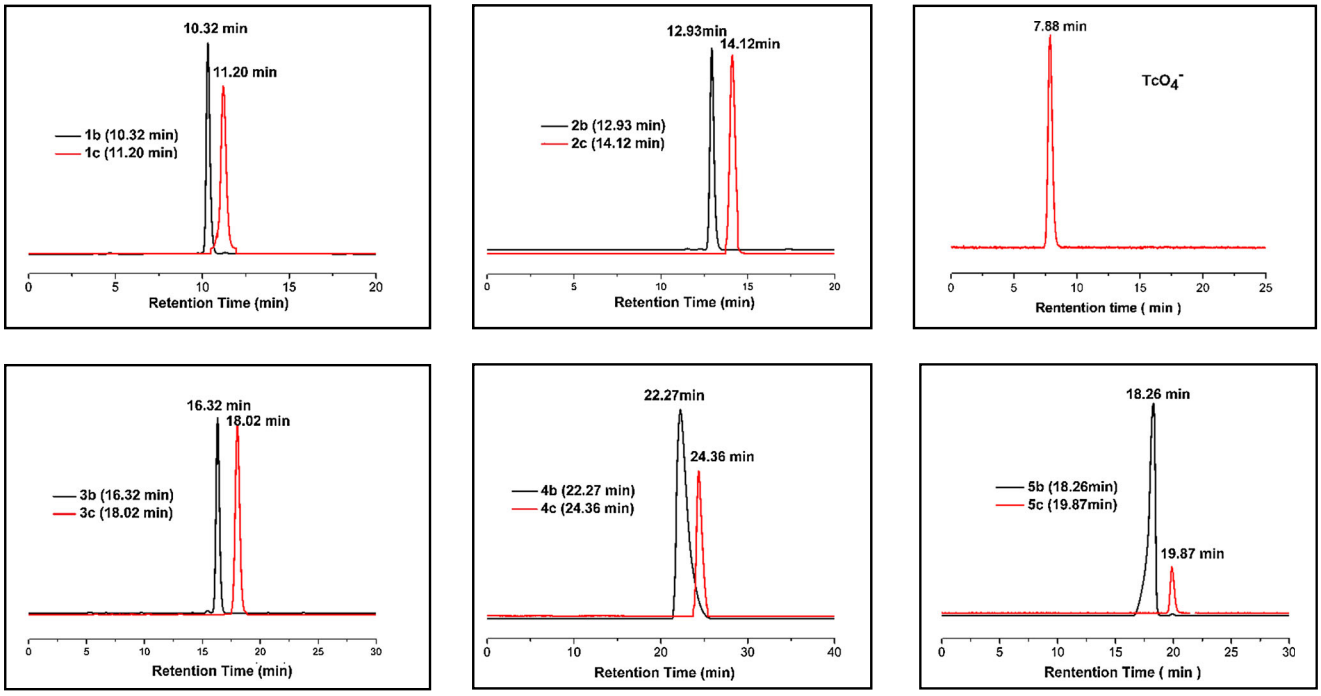


Figure 1. HPLC profiles of ^{99m}Tc-labeled complexes (**1c**, **2c**, **3c**, **4c**, **5c** and $^{99m}TcO_4^-$) and corresponding nonradioactive rhenium fatty acids.

Table 2. Electrophoresis pattern of ^{99m}Tc-labeled fatty acids

Compound	Position of radioactive counts (%)		
	Cathode	Origin	Anode
1c	0.8	98.1	1.1
2c	1.4	96.8	1.8
3c	1.0	97.5	1.5
4c	2.1	95.4	2.5

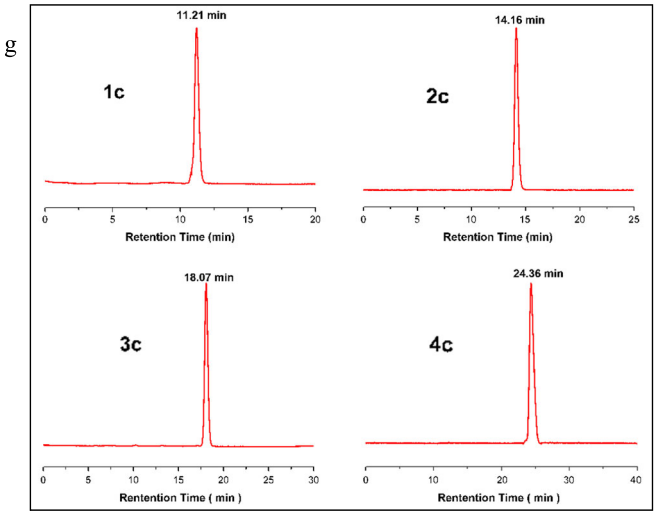


Figure 2. *In vitro* stability of ^{99m}Tc-labeled complexes (**1c**, **2c**, **3c**, **4c**) in rat serum after incubating at 37 °C for 3 h.

*Synthesis of tricarbonyl (5-(16-cyclopentadineyl-16-oxohexadenoyl)furan-2-carboxylic acid) rhenium (CpTRe-16-oxo-FA, **5b**)*

Prepared analogously as described above, compound **5b** as a gray solid was obtained from **1b** (10 mg, 14.3 nmol, 7.55%).

Elemental analysis; found (%): C, 49.86; H, 5.28. Calculated for $C_{28}H_{33}^{185}ReO_8$ (%): C, 49.26; H, 4.88. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.04 (d, J = 3.4 Hz, 1H, –O–C_1=C_2–H), 6.94 (d, J = 3.4 Hz, 1H, –O–C_4=C_3–H), 5.98 (t, J = 2.2 Hz, 2H, –Re–Cp–H(α)), 5.39 (t, J = 2.2 Hz, 2H, –Re–Cp–H(β)), 2.98 (t, J = 7.4 Hz, 2H, –C=O–CH_2–), 2.58 (t, J = 6.3 Hz, 2H, –C=O–CH_2–), 1.98–2.03 (m, 2H, –C=O–CH_2–*CH_2*–), 1.76–1.82 (m, 2H, –C=O–CH_2–*CH_2*–), 1.65–1.67 (m, 2H, –C=O–CH_2–CH_2–*CH_2*–), 1.44–1.46 (m, 2H, –C=O–CH_2–CH_2–*CH_2*–), 1.26–1.31 (d, 1614H, –CH_2–). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 194.39 (–Re–C=O), 192.36 (–Re–C=O), 190.86 (furan–C=O), 176.40 (–COO–), 152.30 (–O–C_1=C_2–H), 141.46 (–O–C_4=C_3–H), 131.09 (–O–C_1=C_2–H), 125.03 (–O–C_4=C_3–H), 95.25 (–Re–C–C=O), 86.93 (–Re–Cp(α)), 84.15 (–Re–Cp(β)), 52.43 (–$COOCH_3$) 37.99 (–C=O–CH_2–), 37.81 (–C=O–CH_2–), 31.81 (–CH_2–), 28.76 (–CH_2–), 28.67 (–CH_2–), 28.26 (–CH_2–), 28.20 (–CH_2–), 28.01 (–CH_2–), 25.08 (–CH_2–), 23.90 (–CH_2–), 23.39 (–CH_2–). MS (ESI) *m/z*: calcd for [$C_{28}H_{33}^{185}ReO_8$] 682.61; found (M + H^+, 185 Re) 683.82, (M + H^+, 187 Re) 685.82.

Radiochemical synthesis and biological experiments

Radiochemical synthesis

[^{99m}Tc]-Tricarbonyl (3-(5-(10-cyclopentadineyl-10-oxodecanoyl)furan-2-yl)propanoic acid) technetium (^{99m}Tc-CpTT-10-oxo-FPA, **1c**), [^{99m}Tc]-tricarbonyl (3-(5-(12-cyclopentadineyl-12-oxododecanoyl)furan-2-yl)propanoic acid) technetium (^{99m}Tc-CpTT-12-oxo-FPA, **2c**), [^{99m}Tc]-tricarbonyl (3-(5-(14-cyclopentadineyl-14-oxotetradecanoyl)

Table 3. Biodistribution of [^{99m}Tc]-CpTT-10-oxo-FPA (**1c**) in normal mice (%ID g^{-1}, $n = 5$, mean ± SD)

Organ	Time after injection				
	1 min	5 min	15 min	30 min	60 min
Blood	9.62 ± 0.52	1.51 ± 0.02	0.57 ± 0.01	0.42 ± 0.06	0.27 ± 0.01
Brain	0.64 ± 0.03	0.24 ± 0.01	0.12 ± 0.01	0.13 ± 0.004	0.09 ± 0.01
Heart	5.98 ± 0.06	1.31 ± 0.05	0.69 ± 0.13	0.41 ± 0.01	0.21 ± 0.01
Liver	22.81 ± 0.73	29.81 ± 0.87	34.57 ± 0.67	35.31 ± 0.89	24.84 ± 1.03
Spleen	2.38 ± 0.26	1.40 ± 0.11	1.13 ± 0.02	1.20 ± 0.08	0.74 ± 0.05
Lung	8.63 ± 0.26	1.70 ± 0.12	0.97 ± 0.08	0.77 ± 0.03	0.50 ± 0.07
Kidney	13.02 ± 0.02	11.87 ± 0.38	12.50 ± 0.85	19.51 ± 0.71	13.57 ± 0.67
Muscle	1.53 ± 0.27	0.80 ± 0.05	0.40 ± 0.02	0.31 ± 0.16	0.26 ± 0.01
Bone	2.17 ± 0.008	0.56 ± 0.04	0.50 ± 0.10	0.59 ± 0.03	0.45 ± 0.11

Table 4. Biodistribution of [^{99m}Tc]-CpTT-12-oxo-FPA (**2c**) in normal mice (%ID g^{-1}, $n = 5$, mean ± SD)

Organ	Time after injection				
	1 min	5 min	15 min	30 min	60 min
Blood	8.43 ± 0.85	1.53 ± 0.003	0.72 ± 0.05	0.53 ± 0.04	0.46 ± 0.04
Brain	0.49 ± 0.07	0.26 ± 0.01	0.18 ± 0.01	0.19 ± 0.11	0.10 ± 0.05
Heart	8.84 ± 0.81	1.53 ± 0.13	0.99 ± 0.62	0.50 ± 0.01	0.26 ± 0.08
Liver	19.54 ± 0.09	33.26 ± 0.72	31.49 ± 0.04	30.53 ± 2.23	15.70 ± 2.30
Spleen	2.74 ± 0.13	1.69 ± 0.08	1.36 ± 0.15	1.37 ± 0.32	0.85 ± 0.14
Lung	14.78 ± 0.45	2.68 ± 0.29	5.22 ± 0.95	2.03 ± 0.34	3.42 ± 1.06
Kidney	7.04 ± 0.51	7.25 ± 0.05	7.73 ± 0.31	8.71 ± 0.69	5.79 ± 0.28
Muscle	1.87 ± 0.10	1.07 ± 0.01	0.54 ± 0.21	0.36 ± 0.18	0.27 ± 0.01
Bone	1.06 ± 0.09	0.85 ± 0.16	0.67 ± 0.40	0.25 ± 0.13	0.26 ± 0.05

Table 5. Biodistribution of [^{99m}Tc]-CpTT-14-oxo-FPA (**3c**) in normal mice (%ID g^{-1}, $n = 5$, mean ± SD)

Organ	Time after injection				
	1 min	5 min	15 min	30 min	60 min
Blood	13.54 ± 0.34	2.59 ± 0.14	0.57 ± 0.08	0.35 ± 0.03	0.30 ± 0.02
Brain	0.50 ± 0.08	0.20 ± 0.01	0.16 ± 0.01	0.11 ± 0.002	0.13 ± 0.01
Heart	7.94 ± 0.40	3.67 ± 0.001	1.09 ± 0.003	0.42 ± 0.04	0.51 ± 0.07
Liver	19.54 ± 0.10	44.96 ± 1.95	37.47 ± 1.37	33.97 ± 0.18	26.22 ± 0.27
Spleen	3.88 ± 0.18	4.36 ± 0.48	2.18 ± 0.08	2.45 ± 0.39	1.20 ± 0.10
Lung	11.84 ± 0.81	4.90 ± 0.02	2.71 ± 0.08	2.59 ± 0.20	1.85 ± 0.34
Kidney	6.45 ± 0.02	5.91 ± 0.09	6.74 ± 0.17	6.42 ± 0.41	6.17 ± 0.12
Muscle	1.87 ± 0.11	1.66 ± 0.13	0.75 ± 0.09	0.36 ± 0.01	0.46 ± 0.17
Bone	1.96 ± 0.11	1.76 ± 0.30	0.90 ± 0.18	0.72 ± 0.06	0.56 ± 0.13

Table 6. Biodistribution of [^{99m}Tc]-CpTT-16-oxo-FPA (**4c**) in normal mice (%ID g^{-1}, $n = 5$, mean ± SD)

Organ	Time after injection				
	1 min	5 min	15 min	30 min	60 min
Blood	15.62 ± 0.73	2.51 ± 0.15	1.05 ± 0.11	0.65 ± 0.01	0.46 ± 0.02
Brain	0.36 ± 0.02	0.31 ± 0.09	0.40 ± 0.10	0.19 ± 0.02	0.20 ± 0.01
Heart	5.66 ± 0.33	3.56 ± 0.13	2.41 ± 0.31	1.45 ± 0.15	0.91 ± 0.03
Liver	20.28 ± 0.80	38.71 ± 0.60	34.01 ± 1.43	35.90 ± 2.05	25.58 ± 1.20
Spleen	2.92 ± 0.03	4.77 ± 0.22	2.62 ± 0.13	1.53 ± 0.12	1.46 ± 0.05
Lung	14.08 ± 1.16	5.33 ± 0.24	2.41 ± 0.13	1.52 ± 0.11	1.29 ± 0.07
Kidney	4.27 ± 0.60	4.84 ± 0.19	3.31 ± 0.06	3.55 ± 0.34	2.93 ± 0.12
Muscle	1.69 ± 0.15	1.87 ± 0.18	1.43 ± 0.12	1.14 ± 0.03	1.77 ± 0.28
Bone	2.62 ± 0.07	2.34 ± 0.24	2.22 ± 0.37	1.79 ± 0.06	3.30 ± 0.23

furan-2-yl)propanoic acid) technetium (^{99m}Tc-CpTT-14-oxo-FPA, **3c**), [^{99m}Tc]-tricarbonyl (3-(5-(16-cyclopentadineyl-16-oxohexadecanoyl) furan-2-yl)propanoic acid) technetium (^{99m}Tc-CpTT-16-oxo-FPA, **4c**) and [^{99m}Tc]-tricarbonyl (5-(16-cyclopentadineyl-16-oxohexadecanoyl)furan-2-carboxylic acid) technetium (^{99m}Tc-CpTT-16-oxo-FA, **5c**) were synthesized and the corresponding rhenium compounds as shown in Schemes 1 and 2. An amount of 1.2 mg of precursors and 3 mg of $Mn(CO)_5Br$ were dissolved in 0.6 ml of dimethylformamide (DMF) in a 10 ml sealed vial then TcO_4^- was added. This reaction was conducted at 150 °C for 1 h. The synthesis method has been reported in previous literature.[14–16] The residue was dissolved in CH_2Cl_2, passed through a 0.2 μm Millipore filter, and 3 ml of 0.3 M NaOH and methanol (10 ml) was added followed by heating at 80 °C for 10 min. The solution was acidified to pH = 7 with 0.1 M HCl (150 ml), and extracted with CH_2Cl_2. ^{99m}Tc-CpTT-10-oxo-FPA, ^{99m}Tc-CpTT-12-oxo-FPA, ^{99m}Tc-CpTT-14-oxo-FPA, ^{99m}Tc-CpTT-16-oxo-FPA and ^{99m}Tc-CpTT-16-oxo-FA were purified using HPLC (semi-preparative column, Venusil MP C-18, Agela Technologies, 10 mm × 250 mm) at a flow rate of 2 ml min^{-1} using CH_3CN–H_2O(90:10) and UV (254 nm).

In vitro stability

Rat serum (1 ml) was centrifuged at 3500 rpm for 30 min; the supernatant was rat serum albumin. The four ^{99m}Tc-labeled complexes (100 μl, 100 μCi) were each added to 100 μl of rat serum albumin in a centrifuge tube. After incubating at 37 °C for 3 h, the serum proteins were precipitated by adding 200 μl of acetonitrile, and the supernatant and precipitate were separated by centrifugation at 3500 rpm for 10 min. The supernatant passed through a 0.2 μm Millipore filter, and then the radiochemical purity of the four resulting mixtures was analyzed using radio-HPLC.

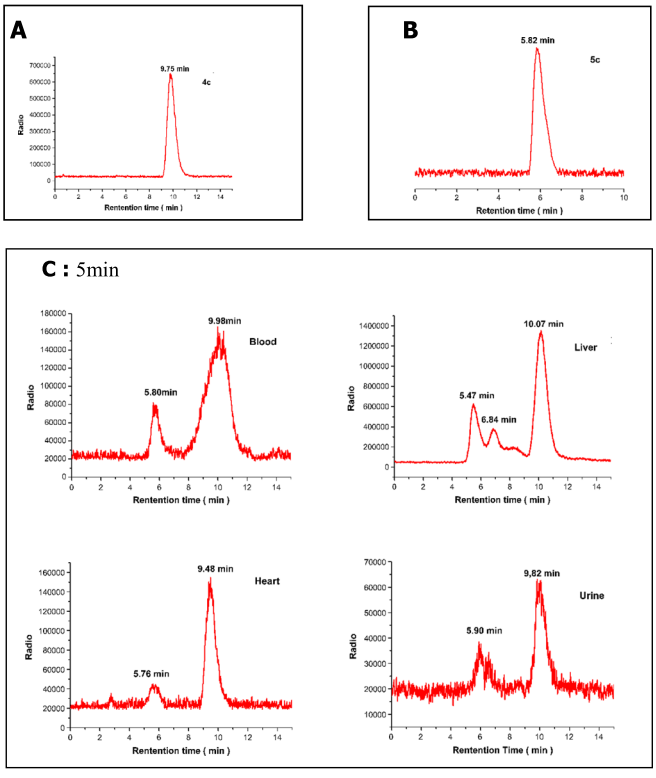
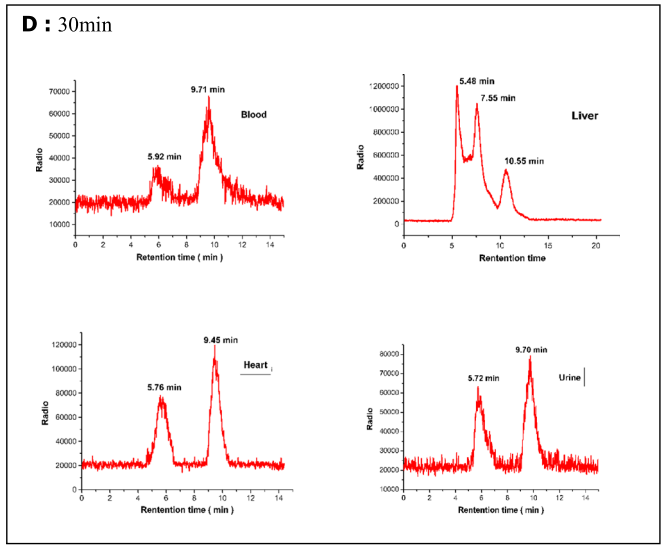


Figure 3. Metabolite analysis of heart, urine, blood and liver samples after administration of **4c**. HPLC analyses of **4c** (A), **5c** (B), 5 min (C), 30 min (D) and 60 min (E) tissue samples. (Agela Venusil MP C18, 150 mm × 4.6 mm, 5 μm, using a flow rate of 0.6 ml min^{-1} and acetonitrile eluent).



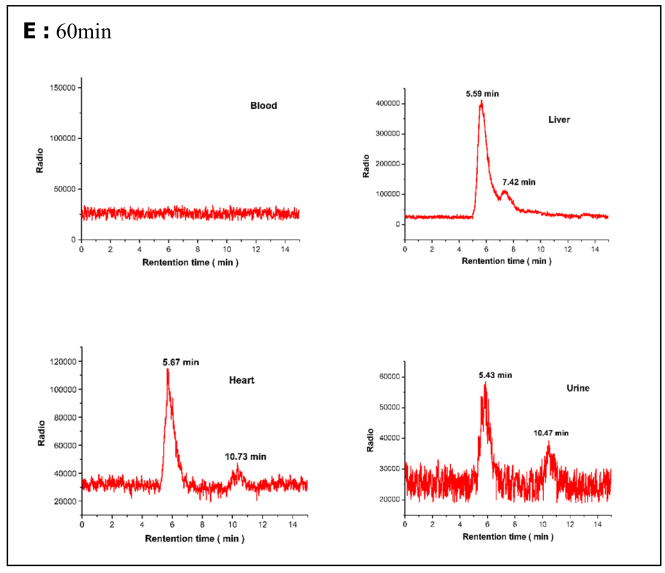


Figure 3. (Continued)

Partition coefficient determination

Previous literature has reported the method of determination of partition coefficients of the four ^{99m}Tc-labeled complexes. ^{99m}Tc-labeled complex (100 μl) dissolved in saline (0.37 MBq) was mixed with 1 ml of *n*-octanol and 900 μl of phosphate-buffered saline (PBS; 0.1 M, pH = 7.4) in a test tube. After vortexing for 3 min at room temperature, the test tube was centrifuged at 3500 rpm for 5 min. Samples from the *n*-octanol (100 μl) and PBS (100 μl) layers were collected and weighted. The partition coefficient was determined by the logarithm of the ratio of counts per gram of *n*-octanol to PBS. The measurement was carried out in triplicate and repeated three times.

Paper electrophoresis

Paper electrophoresis was used to determine the charge of radiotracers. Studies were performed using paper chromatography

(Xinhua No.1 chromatographic paper) in 20 ml of phosphate buffer (pH = 7.4). Each 0.1 ml sample was spotted and developed in electrophoresis containers with a constant potential of 150 V for 2 h. After drying the strips, three separate parts cut from the center of the paper were counted.

Tissue distribution studies

Female mice (Chinese Kunming, 22–25 g, $n = 5$) were fasted before experiments for 12 h. A saline solution with purified ^{99m}Tc-labeled complex (100 μl, 10 μCi) was injected via the tail vein. The mice were sacrificed at 1, 5, 15, 30 and 60 min. Samples of blood and of other organs of interest were obtained, weighed and counted. Data of blood and organs were expressed in the form of percentage of injected dose per gram of tissue (%ID g^{-1}).

In vivo metabolic stability

Female mice (Sprague Dawley, 200–250 g) were injected with **4c** (100 μl, 100 μCi) and sacrificed at 5, 30 and 60 min post-injection. The method has been reported in previously literature.[7,8,17,18] Heart, liver and blood were collected and washed with saline. The samples were placed separately in 500 μl of ice-cooled PBS (0.01 M, pH = 7.4) and homogenized for 2 min. Ice-cold CH_3CN (500 μl) was added, the mixtures were vortexed first and then centrifuged at 3500 rpm for 5 min. The supernatant was directly injected into HPLC column after passing through a 0.22 μm organic Millipore filter. Blood was collected and centrifuged, then precipitated with 200 μl of CH_3CN. After centrifugation, the supernatant was analyzed using HPLC.

Results and discussion

Four fatty acids with different chain lengths were prepared by Friedel–Crafts acylation in three steps (Scheme 1). ^{99m}Tc-labeled complexes (**1c**, **2c**, **3c**, **4c**) were obtained in two steps (Scheme 1). In the first step, ReO_4^-/$^{99m}TcO_4^-$ was reduced and carbonylated; at the same time, cyclopentadienyl (Cp) ligand of ferrocenyl precursor underwent a transfer reaction. The second step was hydrolysis of the ethyl ester at 80 °C for 10 min. The final products were purified using HPLC. The retention time of the ^{99m}Tc-labeled complexes (**1c**, **2c**, **3c**, **4c**) was determined by the corresponding nonradioactive rhenium fatty acid using HPLC (Table 1 and Fig. 1). Log *P* of shorter chain fatty acid is slightly lower than that of longer chain acid which indicates that the shorter chain fatty acid is more hydrophilic[19,20] (Table 1). Paper electrophoresis indicates that the ^{99m}Tc-labeled complexes are neutral (Table 2). *In vitro* stability results indicate that the ^{99m}Tc-labeled complexes (**1c**, **2c**, **3c**, **4c**) are stable in Kunming mice serum at 37 °C (Fig. 2).

Biodistribution studies

Radioactivity accumulated in organs (Tables 3–6) shows that there is high radioactivity in blood, heart, liver and lung along with rapid washout from blood and lung, and low radioactivity in brain, spleen, muscle, bone and kidney. The biodistribution shows **2c** has the highest initial heart uptake (8.84%ID g^{-1} at 1 min post-injection) while **4c** has the best myocardial retention time (1.45%ID g^{-1} at 30 min, 0.91%ID g^{-1} at 60 min post-injection). Also, **4c** is superior to **2c** in terms of heart-to-liver ratios (%ID g^{-1} ratio of **4c**: 0.28 at 1 min, 0.09 at 5 min, 0.07 at 15 min, 0.04 at 30 min, 0.04 at 60 min post-injection) and heart-to-blood ratios (%ID g^{-1} ratio of **4c**: 0.36 at 1 min, 1.42 at 5 min, 2.29 at 15 min, 2.23 at 30 min, 1.98 at 60 min post-injection). Compared with **1c**, **3c** has the higher heart uptake, heart-to-liver ratios (%ID g^{-1} ratio of **3c** versus **1c**: 0.41 versus 0.26 at 1 min, 0.08 versus 0.04 at 5 min, 0.03 versus 0.03 at 15 min, 0.01 versus 0.01 at 30 min, 0.02 versus 0.008 at 60 min post-injection) and heart-to-blood ratios (%ID g^{-1} ratio of **3c** versus **1c**: 0.59 versus 0.62 at 1 min, 1.42 versus 0.86 at 5 min, 1.91 versus 1.21 at 15 min, 1.20 versus 0.98 at 30 min, 1.70 versus 0.78 at 60 min post-injection). Among the four ^{99m}Tc-labeled complexes, along with an increase of the number of carbon atoms, kidney uptake shows a downward trend. In contrast, liver uptake exhibits an increasing trend. Moreover, the longer chain fatty acids reveal longer retention time compared with the shorter chain fatty acids.

In vivo metabolic stability

Radio-HPLC analysis of fractions is shown in Fig. 3. More than 75% of the radioactivity in the myocardium and liver homogenate is extracted with acetonitrile, 35.25% of the radioactivity in the blood is extracted. There are two radioactivity peaks in the myocardium and blood which represent the radioactive metabolite and **4c**, respectively. However, three radioactivity peaks are detected in the liver sample. More than 80% of ^{99m}Tc-CpTT-16-oxo-FPA is present in the myocardium and liver at 5 min post-injection. Over 50% of ^{99m}Tc-CpTT-16-oxo-FPA is present in the myocardium at 30 min post-injection. There is no radioactivity detected in blood at 60 min post-injection. Compound **4c** is metabolized to metabolite product **5c** via β-oxidation, and **4c** and metabolite product are excreted from tissue into urine. The radioactivity in blood is low, which is in accord with the rapid washout from the blood found in the study of biodistribution.

Conclusions

^{99m}Tc-labeled fatty acids were obtained in yields of 60.76–70.92% using a double ligand transfer reaction. Evaluation of **1c**, **2c**, **3c** and **4c** *in vivo* and *in vitro* demonstrated that **4c** was superior to the others because of its longest retention time and highest heart-to-liver and heart-to-blood uptake ratios. But **4c** cannot be used for myocardial evaluation imaging due to the poor myocardial uptake. Metabolite analysis of tissue samples using extraction method suggested that **4c** could be recognized and metabolized in myocardium and liver. The radiometabolite was eliminated from tissue into the urine.

Acknowledgments/Acknowledgements according to UK/US spelling of paper

We gratefully acknowledge the Significant New Drugs Development (grant no. 2014ZX09507007001), Natural Science Foundation of China (no. 21371026) and Science and Technology Support Program (no. 2014BAA03B03) for financial support.

References

[1] W. Kunau, V. Dommes, H. Schulzt, *Prog. Lipid Res.* **1995**, *34*, 267–342.
[2] K. Yoshinaga, N. Tamaki, *Curr. Opin. Biotechnol.* **2007**, *18*, 52–59.
[3] N. Tamaki, K. Morita, Y. Kuge, E. Tsukamoto, *J. Nucl. Med.* **2000**, *41*, 1525–1534.
[4] J. Kropp, J. Likungu, P. G. Kirchhoff, *Eur. J. Nucl. Med.* **1991**, *18*, 467–474.
[5] F. F. Knapp, Jr., P. Franken, J. Kropp, *J. Nucl. Med.* **1995**, *36*, 1022–1030.

[6] Y. Magata, T. Kawaguchi, M. Ukon, *J. Med. Chem.* **2004**, *15*, 389–393.
[7] T. Uehara, T. Uemura, S. Hirabayashi, *J. Med. Chem.* **2007**, *50*, 543–549.
[8] B. C. Lee, D. H. Kim, J. H. Lee, *Bioconj. Chem.* **2007**, *18*, 1332–1337.
[9] B. C. Lee, D. H. Kim, I. Lee, *J. Med. Chem.* **2008**, *51*, 3630–3634.
[10] T. W. Chu, Y. Zhang, X. Q. Liu, *Appl. Radiat. Isotopes* **2004**, *60*, 845–850.
[11] C. Emiliano, B. Elisa, P. Micol, *Bioconj. Chem.* **2008**, *19*, 450–460.
[12] H. H. Zeng, H. B. Zhang, X. X. Wu, *J. Label. Compd. Radiopharm.* **2013**, *56*, 1–5.
[13] H. H. Zeng, H. B. Zhang, *Eur. J. Med. Chem.* **2014**, *72*, 10–17.
[14] M. Wenzel, *J. Label. Compd. Radiopharm.* **1992**, *31*, 641–650.
[15] M. Saidi, K. Kothari, M. R. A. Pillai, *J. Label. Compd. Radiopharm.* **2001**, *44*, 603–618.
[16] X. Chen, M. C. Cui, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6352–6357.
[17] N. Yamamura, Y. Magata, Y. Arano, *Bioconj. Chem.* **1999**, *10*, 489–495.
[18] T. Uehara, M. Koike, H. Nakata, *Nucl. Med. Biol.* **2003**, *30*, 327–334.
[19] M. Orozco, F. J. Luque, *Chem. Rev.* **2000**, *100*, 4187–4226.
[20] H. Zhang, Y. Zhang, *J. Med. Chem.* **2006**, *49*, 5815–5829.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web site.