Synthesis and *In Vivo* Evaluation of a Kit-Type ^{99m}Tc-labeled *N*-(2-Aminoethyl)-3-(4-(2-hydroxy-3-(isopropylaminopropoxy)phenyl) propanamide as a Selective β_1-Adrenoceptor-binding SPECT Radiotracer

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To develop a novel β_1-adrenoceptor selective radiotracer, ^{99m}Tc-labeld *N*-(2-aminoethyl)-3-(4-(2-hydroxy-3-(isopropylaminopropoxy)phenyl)propanamide ([^{99m}Tc]**1**) was prepared by incorporating [$^{99m}TcO_4]^-$ into the precursor **3**. Radiotracer [^{99m}Tc]**1** was optimized in several reaction conditions for kit-type preparation of ^{99m}Tc-radiopharmaceuticals. The radiochemical yield was around 95% (non-decay corrected) within 30 s labeling time and radiochemical purity showed over 98%. In image studies, [^{99m}Tc]**1** provided a good heart accumulation, whereas liver and lung uptakes showed relatively low at initial time. Furthermore, [^{99m}Tc]**1** images at 25 min post-injection provided a clearly visible cardiac image with high contrast. To confirm the β_1-adrenoceptor-binding specificity of [^{99m}Tc]**1**, a blocking experiment was performed. *In vivo* blocking with excess esmolol (18 mg/kg) or atenolol (2 mg/kg) resulted in significantly reduced radioactivity uptake in the heart around 92 and 76%, respectively. The results suggested that [^{99m}Tc]**1** might be of worth applying to the assessment of β_1-adrenoceptor expression in the heart diseases.

Keywords: β_1-Adrenoceptor, Technetium-99m, Single photon emission computed tomography, Cardiac imaging

Introduction

It is generally accepted that β-adrenoceptors play a decisive role in various cardiac diseases such as heart failure.[1] β-Adrenoceptors belong to the G protein-coupled receptor (GPCR) super-family usually related to various cardiovascular functions which can be subdivided into three different subtypes including β_1-, β_2- and the atypical β_3-adrenoceptor.[2,3] In β-adrenoceptor family, β_1-adrenoceptor are found on cardiac muscle cells whereas β_2-adrenoceptor are found on arterial and bronchial smooth muscle at a ratio of about 7:3.[4] β_1-Adrenoceptor agonists stimulate the heart rate and cardiac contractility representing roughly 70% of the total β-adrenoceptor density,[1c] while selective β_2-adrenoceptor-binding agents mainly monitor bronchodilation and vasodepression. The atypical β_3-adrenoceptor participate in lipolysis and the assumed subtype β_4-adrenoceptor mediated by the β_1-adrenoceptor were also identified in the cardiac tissue.[5] β_1-Adrenoceptor stimulation is one of the most effective measures for maintaining cardiac output. Even though β_2-adrenoceptor is prominent, the density of β_1-adrenoceptor is decreased in patients with congestive heart failure.[6] Recently, most studies confirmed that the failing human hearts often perform a selective reduction in β_1-adrenoceptor without change in β_2-adrenoceptor density.[7] On account of its crucial effect to cardiac disease, β_1-adrenoceptor is an ideal target for specific cardiac imaging and also for the development of fixed point therapy.

As shown Figure 1, (*S*)-[^{11}C]CGP 12177 (4-(3-*tert*-butylamino-2-hydroxypropoxy)benzimidazol-2-one), the first reported radiotracer in 1991 by Delforege *et al.*, was developed for assessment of β-adrenoceptor density by positron emission tomography (PET), but the benzimidazol-2-one analog has not enough binding affinity for imaging of β_1-adrenoceptor as the radiotracer.[8–11] As another the benzimidazol-2-one analog, (*S*)-[^{11}C]CGP 12388, has been presented more easily accessible for the β_1-adrenoceptor but it also showed non-selective receptor targeting *in vivo*.[12] For measuring of β_1-adrenoceptor density usually abnormally changes in cardiac disease, a selective β_1-

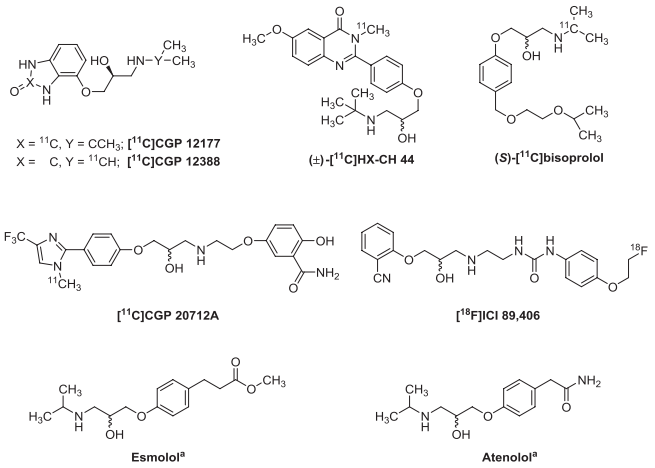


Figure 1. Chemical structure of current radiotracers and blockers[a] for β_1-adrenoreceptor.

adrenoceptor-binding radiotracer is imminently needed on cardiac image for cardiopathic diagnosis and therapy effective evaluation as well as prognosis judgment. However, specific and selective β_1-adrenoceptor radiopharmaceuticals for clinical use are still very rare so far.

Subsequently, only few radiotracers, for example, (±)-[^{11}C]HX-CH 44,[13] (*S*)-[^{11}C]bisoprolol,[14] [^{11}C]CGP 20712A,[15] and ICI 89406[16] were developed. Nevertheless, their applications are mostly restricted due to high non-specific binding, instability *in vivo* or a tissue uptake that does not reflect binding to β_1-adrenoceptor.[14,15,17] Furthermore, these radiotracers usually need to be prepared in harsh conditions, complicated procedures, and multiple subsequent purification processes which lead to low radiochemical yields.[18] Among all possible commercialized drugs reacting with β_1-adrenoceptor, we chose esmolol, an early example of a well-designed cardioselective and an ultra-short acting β_1-adrenergic blocking agent, which has been widely used in the sympathetic nervous system.[19] Esmolol can be easily available to modify its structure with no touching the functional group reacting with β_1-adrenoceptor. In addition, we focused on the radionuclide, single photon emitting ^{99m}Tc to develop a selective β_1-adrenoceptor-binding radiotracer because of its ideal physical properties (141 keV, $t_{1/2}$ = 6 h) and convenient preparation for routine clinical use. As a chelating functional group for ^{99m}Tc, ethylenediamine was embedded to esmolol instead of methoxy group, which can be easily labeling with ^{99m}Tc. It is a strategic ^{99m}Tc chelating moiety, which has been effectively applied in targeting receptors for minimizing its interruption to receptor-binding site. Therefore, the present study describes a new β_1-adrenoceptor-binding single photon emission computed tomography (SPECT) radiotracer, ^{99m}Tc-labeled *N*-(2-aminoethyl)-3-(4-(2-hydroxy-3-(isopropyl amino-propoxy)phenyl)propanamide, ^{99m}Tc-labeled esmolol analog, for diagnostic purpose in cardiac dysfunction related β_1-adrenoceptor expression. Also, this study includes a much more convenient and instantaneous radiolabeling procedure, a kit-type of radiolabeling procedure. Kit-type preparation for radiopharmaceuticals can date back to 1980s when Dougan *et al.* managed to label [^{123}I]*m*IBG by kit form, and gradually play a significant role in optimized labeling process with high reliability, especially for radiopharmaceuticals.[20] Not only dramatically shortens the reaction time, kit-type labeling also largely improves the safety for reaction as well as stability on storage. Herein, the preliminary biological evaluation of [^{99m}Tc]**1** for heart imaging based on β_1-adrenoceptor expression was carried by serial SPECT imaging experiments.

Experimental

All chemicals, reagents, and solvents for the synthesis used were analytical grade and purchased from Sigma-Aldrich (Darmstadt, Germany). Esmolol provided from Dongwoo Syntech Co., Ltd. (Seongnam, Korea). Flash column chromatography was performed with silica gel (230–400 mesh, ASTM) from Merck (Darmstadt, Germany). All reactions were controlled by thin-layer chromatography (TLC), which was performed on Merck silica gel glass plates ($60F_{254}$). Proton nuclear magnetic resonance (^{1}H NMR) spectra were analyzed by an INOVA-400 (400 MHz) at room temperature using $CDCl_3$ or D_2O as solvent. Chemical shifts were recorded in parts per million (ppm, δ units). Electrospray mass spectrometry (ESI-MS) was performed on a LC/MS spectrometer (Agilent 6130 Series). $Na^{99m}TcO_4$ was eluted on a daily basis from ^{99}Mo/^{99m}Tc generators (Samyoung Unitech, Seoul, Korea). HPLC was carried out on a Thermo Separation Products System (Fremont, CA, USA) equipped with an analytical column (YMC triart C18, 4.6 mm × 250 mm) and equipped with a UV detector (wavelength set at 280 nm) and a gamma-ray detector (Bioscan). HPLC-grade Solvents (J. T. Baker, Pennsylvania, USA) are used for HPLC purification through a 0.22 μm filtering (Whatman, Maidstone, UK). The column was eluted with a mixture of 15% acetonitrile–0.1 M phosphate buffered saline (pH = 6.0) at a flow rate of 0.5 mL/min. Radio-TLC was carried out by a Bioscan radio-TLC scanner (Washington, DC, USA). All radioactivitive counting were analyzed by a VDC-505 activity calibrator from Veenstra Instruments (Joure, The Netherlands). All of the animal experiments using Sprague-Dawley (SD) rats were approved by the Institutional Animal Care and Use Committee of the Seoul National University Bundang Hospital (SNUBH).

***N*-(2-Aminoethyl)-3-(4-(2-hydroxy-3-(isopropylamino)-propoxy)phenyl)propanamide dihydrochloride (3).** To compound **2** (600 mg, 2.03 mmol) in 10 mL of methanol, ethylenediamine (1.22 g, 20.3 mmol) was added and the solution was heated at reflux for 30 h. Upon completion, the reaction mixture was filtered and washed with methanol. Filtrate was concentrated under reduced pressure at 55°C. The residue was dissolved in toluene and

concentrated under reduced pressure at 65°C. Ethyl acetate (10 mL) was added to the residue and the mixture was stirred at room temperature for 12 h. The precipitate was filtered, washed with ethyl acetate, and dried under reduced pressure at 55°C, to give *N*-(2-aminoethyl)-3-(4-(2-hydroxy-3-(isopropyl-amino)propoxy)phenyl)propanamide (513 mg, 78.2%) as a white solid: MS (ESI) *m/z* 324.02 $[M+H]^+$. Subsequently, the obtained compound (500 mg, 1.55 mmol) in 10 mL of methanol, isopropyl alcohol (0.888 mL) were added. The solution was filtered, washed with 10 mL of methanol, and cooled to 0°C. The hydrogen chloride solution (230 mg of hydrogen chloride in 713 mg of isopropyl alcohol) was added slowly to cooled solution and then stirred for 1 h at room temperature. Isopropyl alcohol (10 mL) was added additionally and the solution was stirred for 3 h at room temperature. The resulting precipitate was filtered, washed with isopropyl alcohol (5 mL), and dried under reduced pressure at 55°C to give crude mixture (301 mg). The crude mixture in 5 mL of isopropyl alcohol was heated at 75°C for 30 min. The solution was filtered and washed with isopropyl alcohol. Filtrate was cooled slowly to 20°C for 3 h. The precipitate was filtered and washed with isopropyl alcohol again. The product was dried under reduced pressure at 55°C to give white crystalline solid product **3** (271 mg, 48.4%): m.p. 124.5–127.3°C; ^{1}H NMR (D_2O, 400 MHz) δ 7.24 (d, *J* = 11.6 Hz, 2H), 7.01 (d, *J* = 11.6 Hz, 2H), 4.33–4.29 (m, 1H), 4.16–4.07 (m, 2H), 3.55–3.49 (m, 1H), 3.41 (t, *J* = 6.2 Hz, 2H), 3.36–3.22 (m, 2H), 3.04 (t, *J* = 6.2 Hz, 2H), 2.89 (t, *J* = 7.4 Hz, 2H), 2.58 (t, *J* = 7.4 Hz, 2H), 1.38–1.36 (m, 6H); ^{13}C NMR (D_2O, 100 MHz) δ 176.8, 156.4, 133.5, 129.6, 114.8, 69.6, 65.6, 51.0, 46.6, 38.9, 37.3, 36.5, 30.2, 18.2, 17.8. MS (ESI) *m/z* 324.24 $[M+H-2HCl]^+$.

Re-coordinated *N*-(2-Aminoethyl)-3-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)propanamide ([Re]1). To $ReOCl_3[P(Ph)_3]_2$ (trichlorooxobis(triphenyl phosphine) rhenium(V), 333 mg, 0.4 mmol) in 4.0 mL of methanol, aqueous solution of sodium hydroxide (48 mg, 1.2 mmol) and compound **3** (158 mg, 0.4 mmol) in 2.0 mL of water were added (water:methanol:0.3 N NaOH = (v/v/v) 1:2:2). The mixture solution was heated at 90°C for 30 min. Upon completion, reaction mixture was cooled to 0°C, then filtered, and washed with methanol (2.0 mL × 2). The solid was dissolved in 10 mL of H_2O and filtered again. Filtrate was frozen and dried to give white solid (178 mg, 85.2%). ^{1}H NMR (D_2O, 400 MHz) δ 7.14 (d, *J* = 10.4 Hz, 4H), 6.89 (d, *J* = 10.4 Hz, 4H), 4.02-3.99 (m, 4H), 3.93–3.89 (m, 2H), 3.02 (t, *J* = 7.8 Hz, 4H), 2.87–2.71 (m, 8H), 2.64–2.58 (m, 2H), 2.47–2.37 (m, 8H), 0.98–0.96 (m, 12H). MS (MALDI) *m/z* 866.05, 868.06 $[M+H]^+$.

Three Different Stabilizers Contained Kit Formulation and General Method for ^{99m}Tc-labeled *N*-(2-Aminoethyl)-3-(4-(2-hydroxy-3-(isopropylamino)propoxy) phenyl)propanamide ([^{99m}Tc]1). Reagents solution A includes compound **3** (100 mg), ascorbic acid (100 mg), and mannitol (500 mg) in 100 mL of distilled water. Reagents solution B includes SnF_2 (25 mg) in 25 mL of 0.1 N HCl. In aseptic room, the mixed solution (solutions A and B) was filtered by PVDF (polyvinylidene difluoride) membrane filter (0.22 μm) and divided by micropipette for 1.25 mL per vial. In result, every kit-vials contained 1 mg of compound **3**, 1 mg of ascorbic acid, 5 mg of mannitol, 0.25 mg of SnF_2, and 0.02 N HCl and dried over vacuum freeze-drying at −50°C. Pure N_2 (99.9999%) was used to protect ingredients from O_2 and all vials were sealed completely with rubber cap. In preparation of kit-vials for NaCl (5 mg) or cyclodextrin (5 mg) as other stabilizers, all processes were performed as same described above. For synthesis of [^{99m}Tc]**1**, $Na^{99m}TcO_4$ (approximately 740 MBq, 1 mL of saline) and 0.1 N HCl (63 μL) were added in an empty vial. After voltexing for few seconds, the mixture was injected into kit-vial and voltexed at room temperature for 30 s. The solution was filtered by PVDF membrane filter (0.22 μm, 13 mm). The radiochemical yield of [^{99m}Tc]**1** was more than 95% and there was no byproduct peak except for negligible free $^{99m}TcO_4^-$ which had checked by radio-TLC and HPLC. The identity was confirmed by coinjection with authentic [Re]**1** in the analytical HPLC system (YMC triart C18, 4.6 mm × 250 mm, 15% acetonitrile–0.1 M phosphate buffered saline (pH 6.0), flow rate = 0.5 mL/min). For biological evaluation by SPECT in rat, [^{99m}Tc]**1** was injected as below one-tenth of total volume (v/v) per rat.

SPECT/CT Imaging. Animal SPECT imaging study was performed using low-energy and high-resolution pyramid collimator (4 × 9, 1.4 mm pinhole) in SPECT/CT (Single photon emission computed tomography/ Computed tomography) scanner system (NanoSPECT/CT, Bioscan Inc., Washington, DC, USA). [^{99m}Tc]**1** (approximately 74 MBq, *n* = 3) was administrated to normal SD rat (8–9 weeks) through the intravenous injection under anesthesia with 2% isoflurane. Whole body images were obtained in 24 projections over 10 min periods. For measurement of heart, lung, and liver uptake of [^{99m}Tc]**1**, HiSPECT (version 1.0, Bioscan Inc., Washington, DC, USA) and InVivoScope (version 1.43, Bioscan Inc., Washington, DC, USA) were used for image reconstruction and quantification, respectively. The reconstructed SPECT was described with an image size of 176 × 176 × 136 and a voxel size of 0.2 mm × 0.2 mm × 0.2 mm (*x*, *y*, *z*). The accumulated radioactivity of each organs was determined by drawing regions of interest (ROI) using the reference source (^{99m}Tc-pertechnate, 37 MBq). The percentage of the injected dose per gram of weight (% ID/g) was calculated from the radioactivity contained in the ROI divided by the dose administered to the rat.

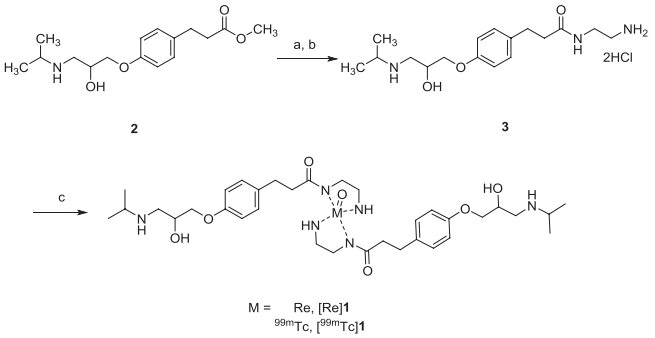
Blocking Study. Normal SD rats (8–9 weeks) were administered with esmolol (18 mg/kg) or atenolol (2 mg/kg) into the tail vein before 10 min the injection of [^{99m}Tc]**1**. After that, the rats were anesthetized with 2% isoflurane in a 7:3 mixture of N_2/O_2 and the intravenous injection of [^{99m}Tc]**1** (approximately 74 MBq, *n* = 3) was performed.

Whole body SPECT images were acquired at a mid-scan time of 25 min for scan duration of 10 min, followed by a CT scan of body for attenuation correction.

Results and Discussion

The precursor **3** was simply prepared by the amide condensation from esmolol (**2**) and diethylamine as shown in Scheme 1. The obtained *N*-(2-aminoethyl)-3-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)propanamide was treated with hydrogen chloride, resulted in the chlorine salt form **3**. The "cold" rhenium coordination reaction with the precursor was performed in a water–methanol–sodium hydroxide mixture at 90°C using $ReOCl_3(PPh_3)_2$. The rhenium complex [Re]**1** was characterized by ^{1}H NMR and mass spectrum. The coordination of the ^{99m}Tc radioisotope to the precursor **3** was performed in aqueous media from ^{99m}Tc-pertechnetate in the presence of SnF_2, ascorbic acid, and stabilizers.

As the ingredient in a kit formulation, SnF_2 was served as an alternative reducing agent to $SnCl_2$ to avoid ^{99m}Tc-colloidal phenomenon. Though $SnCl_2$ is a very common used reducing agent in various ^{99m}Tc-labeled procedures, the use of $SnCl_2$ induced a mass of ^{99m}Tc-colloidal resulted in high liver uptake of [^{99m}Tc]**1** in SPECT/CT images. An anti-oxidant ascorbic acid was also employed to enhance the *in vivo* stability of [^{99m}Tc]**1** for this reaction. In addition, various stabilizers were investigated to improve target to non-target ratio, *i.e.*, heart-to-liver and heart-to-lung. Figure 2 summarize the *in vivo* imaging results of the [^{99m}Tc]**1** without or with different stabilizer. Among four images, [^{99m}Tc]**1** exhibited significantly the high liver accumulation at non-use of stabilizer. Comparison of the serial SPECT/CT images of [^{99m}Tc]**1** with stabilizer-dependent was performed in normal rats (n = 3), respectively. Through comparative experiments, an optimal stabilizer was chosen from the most excellent performance focusing on cardiac SPECT images. Among three SPECT/CT images by different stabilizers contained kit-vials, mannitol induced the highest uptake of [^{99m}Tc]**1** in the heart about



Scheme 1 (a) ethylenediamine, MeOH, reflux, 30 h; (b) 0.6 N HCl, *iso*-propyl alcohol, MeOH, rt, 4 h; (c) $ReOCl_3(PPh_3)_2$, NaOH, 90°C, 30 min for [Re]**1**; Na$^{99m}TcO_4$, ascorbic acid, stabilizer, SnF_2, 0.02 N HCl, rt, 30 s for [^{99m}Tc]**1**.

1.95 ± 0.3% ID/g and the lowest uptake in the liver (1.10 ± 0.2% ID/g) at 5 min post-injection compared to those of other stabilizers as shown Figure 2.

Conversely, lung uptake of [^{99m}Tc]**1** was 0.38 ± 0.1% ID/g and slowly decreased with elapsed time by urinary excretion. In case of NaCl, the uptake of [^{99m}Tc]**1** in the heart was decreased (40% reduction vs. the mannitol treated group) and the uptake in the liver reached up to 4.7% ID/g which was fourfold higher than that of mannitol. Even though the cyclodextrin treated group showed the similar heart uptake, the liver uptake showed 3.9% ID/g. Consequently, we established that mannitol was the best stabilizer for preparing a kit-type [^{99m}Tc]**1** superior to NaCl and cyclodextrin by measuring of heart-to-lung and heart-to-liver ratios.

In terms of purity, [^{99m}Tc]**1** could be prepared in high radiochemical yield over 95% with more than 98% of radiochemical purity only through filtration of PVDF filter (0.22 μm) after incorporation of $^{99m}TcO_4^-$ and the precursor (**3**) in the presence of SnF_2, ascorbic acid, and mannitol at room temperature for 30 s. Radio-TLC was used to identify [^{99m}Tc]**1**. The R_f value of [^{99m}Tc]**1** and unreacted ^{99m}Tc was 0.0 with 99.1% and 1.0 with 0.9%, respectively, using an absolute acetone as developing solvent. In HPLC analysis, [^{99m}Tc]**1** was eluted at 4.9 min while the retention of $^{99m}TcO_4^-$ was 10.9 min. Without additional purification process such as HPLC or the C_{18} Sep-Pak cartridge, [^{99m}Tc]**1** was readily prepare to use directly for biological experiment or *in vivo* images by simple filtering (Figure 3).

After determining the optimal kit formulation, we obtained high-resolution scans (12 frames) of each rat from 0 to 120 min after the intravenous injection of [^{99m}Tc]**1** (approximately 74 MBq). In SPECT images, [^{99m}Tc]**1** showed high accumulation in the heart and kidneys, while the liver and lung uptakes were relatively low as shown Figure 4. Timed organ distributions (% ID/g) was addressed in the Table 1. The desirable scan time, 25 min post-injection was derived from serial SPECT imaging study by considering of the ratios of heart-to-lung and heart-to-liver.

To confirm the β_1-adrenoceptor-binding specificity of [^{99m}Tc]**1**, the blocking experiment was performed with esmolol and atenolol, respectively, which was well known as β_1-adrenoceptor blockers.[19,21] Using excess esmolol (18 mg/kg) or atenolol (2 mg/kg),[22] both images resulted in significantly reduced radioactivity uptake in the heart (92 and 76% reduction in heart uptake vs. control, respectively). Our results indicated that the heart images of [^{99m}Tc]**1** was mediated selectively by β_1-adrenoceptor as shown in Figure 5.

In summary, To better prepare a kit-type ^{99m}Tc-labeled esmolol derivative ([^{99m}Tc]**1**), several attempts were investigated with different reagents and stabilizers. [^{99m}Tc]**1** can be prepared extremely simple and instantaneous by reacting $^{99m}TcO_4^-$ with the precursor in the presence of SnF_2, ascorbic acid and mannitol at room temperature. Total preparation time was around 5 min including radiolabeling and

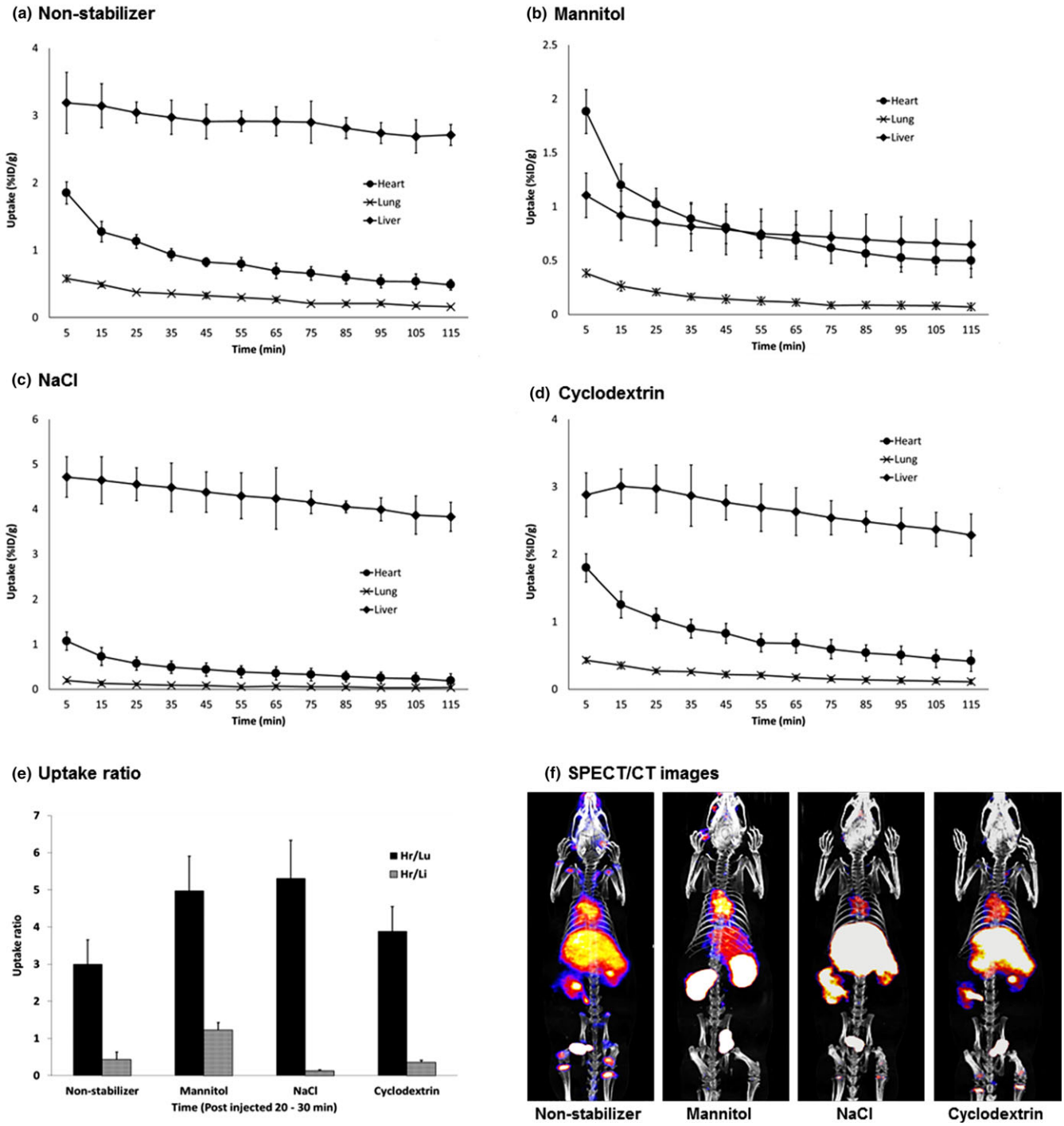


Figure 2. Time-activity curves (a–d), ratios of heart-to-lung and heart-to-liver (e), and representative SPECT/CT images (f) of [^{99m}Tc]**1** without or with different stabilizers.

filtration. Compared to the reported β_1-adrenoceptor-binding radiotracers, ^{99m}Tc-labeled esmolol derivative is much more attractive in terms of its longer half-life ($t_{1/2}$ = 6 h), convenient to acquire from generator and simple radiolabeling procedure. In this work, ^{99m}Tc-labeled esmolol derivative was prepared by a kit-type radiotracer, which was able to perform as the final *in vivo* injection through filtration of PVDF filter (0.22 μm) without HPLC further separation or any other further purification processes. The kit-type radiolabeling method takes great potentials for commercialization and development of clinical radiopharmaceuticals. It can be also readily automated using a commercial radiosynthetic module fleetly and largely without burdensome operation or sophisticated diversiform instruments. In SPECT imaging studies, [^{99m}Tc]**1** showed relatively high radiotracer uptake in the heart in comparison

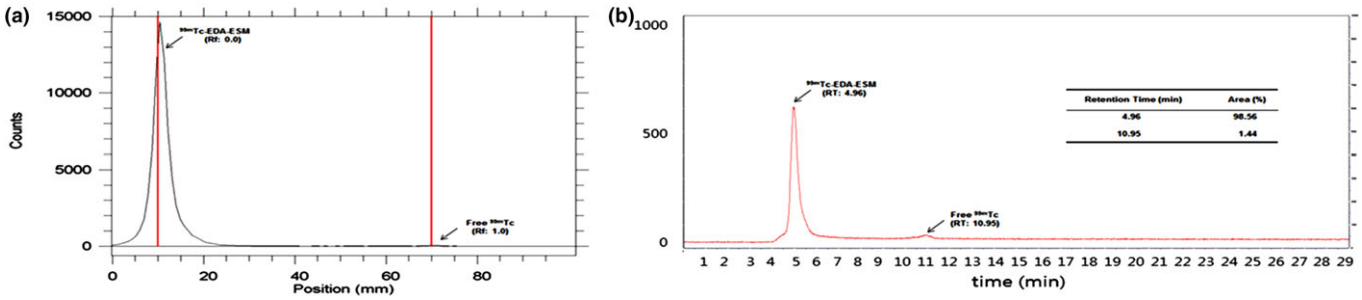


Figure 3. Identification of [^{99m}Tc]**1** using radio-TLC scanner and HPLC.

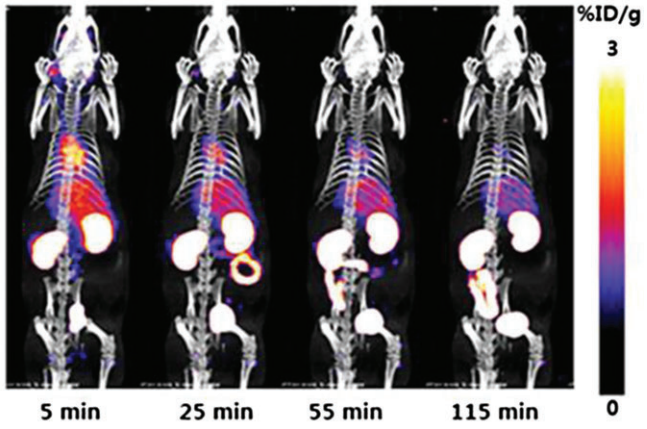


Figure 4. Serial SPECT/CT images of [^{99m}Tc]**1** in the presence of mannitol.

Table 1. Uptake (% ID/g) of [^{99m}Tc]**1** in major organs of normal rats.[a]

	% ID/g			
Organs	5 min	25 min	55 min	115 min
Lung	0.38 ± 0.1	0.21 ± 0.1	0.13 ± 0.1	0.07 ± 0.1
Liver	1.10 ± 0.2	0.85 ± 0.2	0.75 ± 0.2	0.65 ± 0.2
Heart	1.88 ± 0.2	1.02 ± 0.2	0.73 ± 0.1	0.50 ± 0.2
Hr/Lu[b]	4.94 ± 0.7	4.98 ± 0.9	5.98 ± 1.3	7.61 ± 2.2
Hr/Li[c]	1.73 ± 0.2	1.22 ± 0.2	0.99 ± 0.2	0.77 ± 0.1

[a] The data expressed the percentage of injected dose per gram (% ID/g, n = 3).
[b] The ratio of heart to liver.
[c] The ratio of heart to lung.

with liver and lung. Compared to the initial scan time, the 20–30 min summed (25 min post-injection) image was shown a clearly visible image of the heart with high contrast. The radioactivity of heart at 55 min post-injection was decreased to half (0.92 ± 0.15%ID/g) of that uptake in 5 min post-injection and then rapidly washout via mainly urinary excretion. Appropriate clearance rate greatly eases the risk of the negative influence due to a long time of radiotracer left in the heart and other vital organs. Simultaneously, *in vivo* blocking experiments with excess esmolol displayed remarkable reduction of radioactivity uptake in the heart. From the other side, it demonstrated that [^{99m}Tc]**1** inherits a series of advantages from esmolol. It illustrates that ethylenediamide modification does not impact the original cardioselective β_1-adrenoceptor blocker function of esmolol, *i.e.*, short duration of action and no significant intrinsic sympathomimetic performance.[23] The short duration of action of esmolol is based on rapid hydrolysis by plasma esterase on the ester-methyl chain. This metabolism produces a free acid and methanol. In a similar, [^{99m}Tc]**1** exhibits relatively little slower but still rapid clearance rate as the peptide bond would be broken by enzymes first, then releases ethylenediamide moiety and ^{99m}Tc, radioactive marker, immediately. These results indicate that [^{99m}Tc]**1** is a highly sensitive radiotracer for β_1-adrenoceptor imaging in the heart. Based on the success modification using ethylenediamide, we will focus on the optimized formula linked with esmolol and radioactive markers, *e.g.*, Ga-68, Cu-64 and In-111 to further enhance the quality of cardiac images. Furthermore, animal models for cardiovascular disease will be selected to evaluate the application of [^{99m}Tc]**1**.

Conclusion

In conclusion, ^{99m}Tc-labeld esmolol derivative, [^{99m}Tc]**1**, was successfully synthesized in excellent yield by "kit"-type formulation. Also, the presented preclinical biological study suggests that [^{99m}Tc]**1** may have potential as a useful SPECT radiotracer for assessment of β_1-adrenoceptor in heart diseases.

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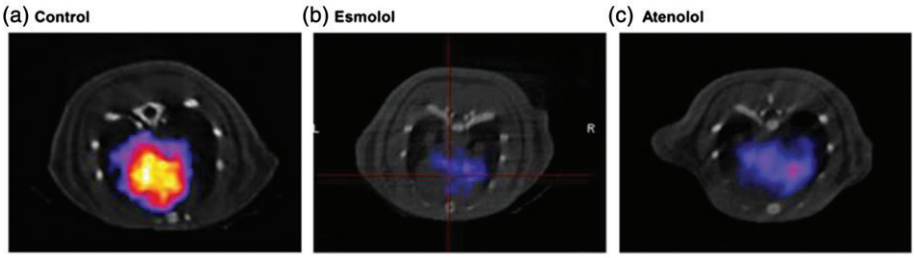

Figure 5. Axial heart SPECT/CT images of [^{99m}Tc]**1** without or with esmolol or atenolol.

References

1. (a) J. W. Calvert, D. J. Lefer, *Physiology* **2013**, *28*, 216; (b) G. Vaniotis, D. Del Duca, P. Trieu, C. V. Rohlicek, T. E. Hébert, B. G. Allen, *Cell. Signal.* **2011**, *23*, 89; (c) H. T. Tevaearai, W. J. Koch, *Trends Cardiovasc. Med.* **2004**, *14*, 252.
2. K. Makaritsis, F. Triposkiadis, In *Introduction to Translational Cardiovascular Research*, D. V. Cokkinos Ed., Springer; Academic Press, Athens, **2015**, p. 73.
3. (a) J. R. S. Arch, A. T. Ainsworth, M. A. Cawthorne, V. Piercy, M. V. Sennitt, V. E. Thody, C. Wilson, S. Wilson, *Nature* **1984**, *309*, 163; (b) R. A. Bond, D. E. Clarke, *Br. J. Pharmacol.* **1988**, *95*, 723.
4. J. G. Baker, *Br. J. Pharmacol.* **2005**, *144*, 317.
5. D. Sarsero, P. Molenaar, A. J. Kaumann, N. Freestone, *Br. J. Pharmacol.* **1999**, *128*, 1445.
6. (a) M. Ungerer, M. Böhm, J. S. Elce, E. Erdmann, M. J. Lohse, *Circulation* **1993**, *87*, 454; (b) M. R. Bristow, F. L. Anderson, D. P. Port, L. Skerl, R. S. Hershberger, P. Larabee, J. B. O'Conell, D. G. Renlund, K. Volkman, J. Murray, A. M. Feldman, *Circulation* **1991**, *84*, 1024; (c) O. E. Brodde, *Pharmacol. Rev.* **1991**, *43*, 203; (d) M. Steinfath, B. Geertz, W. Schmitz, H. Scholz, A. Haverich, I. Breil, P. Hanrath, C. Reupcke, M. Sigmund, H.-B. Lo, *Naunyn-Schmiedebergs Arch. Pharmacol.* **1991**, *343*, 217.
7. (a) O. E. Brodde, M. C. Michel, *Pharmacol. Rev.* **1999**, *51*, 651; (b) K. Kopka, S. Wagner, B. Riemann, M. P. Law, C. Puke, S. K. Luthra, V. W. Pike, T. Wichter, W. Schmitz, O. Schlber, M. Schäfers, *Bioorg. Med. Chem.* **2003**, *11*, 3513.
8. J. Delforge, A. Syrota, J. P. Lançon, K. Nakjima, C. Loc'h, M. Janier, J. M. Vallois, J. Cayla, C. Crouzel, *J. Nucl. Med.* **1991**, *32*, 739.
9. (a) K. Gherbi, L. T. May, J. G. Baker, S. J. Briddon, S. J. Hill, *FASEB J.* **2015**, *29*, 2859; (b) R. H. J. A. Slart, P. van der Meer, R. A. Tio, D. J. van Veldhuisen, P. H. Elsinga, In *Autonomic Innervation of the Heart*, R. H. J. A. Slart, R. A. Tio, P. H. Elsinga, M. Schwaiger Eds., Springer; Academic Press, Berlin and Heidelberg; Groningen, **2015**, p. 235.
10. (a) P. Molenaar, L. Chen, A. B. Semmler, W. A. Parsonage, A. J. Kaumann, *Clin. Exp. Pharmacol. Physiol.* **2007**, *34*, 1020; (b) J. G. Granneman, *Am. J. Physiol. Endocrinol. Metab.* **2001**, *280*, E199; (c) P. Molenaar, *Br. J. Pharmacol.* **2003**, *140*, 1; (d) J. R. Arch, *Br. J. Pharmacol.* **2004**, *143*, 517; (e) A. J. Kaumann, P. Molenaar, *Pharmacol. Ther.* **2008**, *118*, 303.
11. S. S. Joseph, J. A. Lynham, A. A. Grace, W. H. Colledge, A. J. Kaumann, *Br. J. Pharmacol.* **2004**, *142*, 51.
12. P. H. Elsinga, A. van Waarde, K. A. Jaeggi, G. Schreiber, M. Heldoorn, W. Vaalburg, *J. Med. Chem.* **1997**, *40*, 3829.
13. H. Valette, F. Dollé, I. Guenther, S. Demphel, C. Rasetti, F. Hinnen, C. Fuseau, C. Crouzel, *Nucl. Med. Biol.* **1999**, *26*, 105.
14. D. V. Soloviev, M. Matarrese, R. M. Moresco, S. Todde, T. A. Bonasera, F. Sudati, P. Simonelli, F. Magni, D. Colombo, A. Carpinelli, M. G. Kienle, F. Fazio, *Neurochem. Int.* **2001**, *38*, 169.
15. P. H. Elsinga, A. van Waarde, G. M. Visser, W. Vaalburg, *Nucl. Med. Biol.* **1994**, *21*, 211.
16. (a) S. Wagner, K. Kopka, M. P. Law, B. Riemann, V. W. Pike, O. Schober, M. Schäfers, *Bioorg. Med. Chem.* **2004**, *12*, 4117; (b) V. W. Pike, M. P. Law, S. Osman, R. J. Davenport, O. Rimoldi, D. Giardina, P. G. Camici, *Pharm. Acta Helv.* **2000**, *74*, 191.
17. A. van Waarde, J. G. Meeder, P. K. Blanksma, J. Bouwer, G. M. Visser, P. H. Elsinga, A. M. J. Paans, W. Vaalburg, K. I. Lie, *Int. J. Rad. Appl. Instrum. B* **1992**, *19*, 711.
18. K. Kopka, M. P. Law, H. J. Breyholz, A. Faust, C. Holtke, B. Riemann, O. Schober, M. Schafers, S. Wagner, *Curr. Med. Chem.* **2005**, *12*, 2057.
19. C. Y. Deng, S. G. Lin, W. C. Zhang, S. J. Kuang, W. M. Qian, S. L. Wu, Z. X. Shan, M. Yang, X. Y. Yu, *Methods Find. Exp. Clin. Pharmacol.* **2006**, *28*, 697.
20. (a) H. Dougan, D. M. Lyster, J. S. Vincent, T. Rihela, *J. Labelled Comp. Radiopharm.* **1988**, *25*, 531; (b) P. Kumar, B. Singh, A. Ghai, P. P. Hazari, B. R. Mittal, A. K. Mishra, *J. Labelled Comp. Radiopharm.* **2015**, *58*, 242; (c) D. İlem-Özdemir, M. Aşıkoğlu, H. Özkılıç, *J. Radioanal. Nucl. Chem.* **2013**, *298*, 1635.
21. P. Agon, P. Goethals, D. V. Haver, J. M. Kaufman, *J. Pharm. Pharmacol.* **1991**, *43*, 597.
22. (a) S. H. Haidar, J. E. Moreton, Z. Liang, J. F. Hoke, K. T. Muir, N. D. Eddington, *J. Pharm. Sci.* **1997**, *86*, 1278; (b) K. A. Kane, J. R. Parratt, F. M. Williams, *Br. J. Pharmacol.* **1984**, *82*, 349.
23. P. Jaillon, M. Drici, *Am. J. Cardiol.* **1989**, *64*, J65.

