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A novel gallium bisaminothiolate complex as a myocardial perfusion imaging agent

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Abstract

The development of new myocardial perfusion imaging agents for positron emission tomography (PET) may improve the resolution and quantitation of changes in regional myocardial perfusion measurement. It is known that a ⁶⁸Ge/⁶⁸Ga generator can provide a convenient source of PET tracers because of the long physical half-life of ⁶⁸Ge (271 days). A new ligand, 7,8-dithia-16,24-diaza-trispiro[5.2.5.2.5.3] pentacosa-15,24-diene, which consists of a N₂S₂-chelating core incorporated into three cyclohexyl rings, was prepared. To test feasibility and potential utility, the N₂S₂ ligand was successfully labeled and tested with ⁶⁷Ga (half-life=3.26 day; γ=93.3, 184.6 and 300.2 keV), which showed >92% radiochemical purity. The corresponding “cold” Ga complex was synthesized, and its structure containing a pyramidal N₂S₂ chloride core was elucidated with X-ray crystallography. In vivo biodistribution of this novel ⁶⁷Ga complex, evaluated in normal rats, exhibited excellent heart uptake and retention, with 2.1% and 0.9% initial dose/organ at 2 and 60 min, respectively, after an intravenous injection. Autoradiography was performed in normal rats and in rats that had the left anterior descending coronary artery permanently ligated surgically. Autoradiography showed an even uptake of activity in the normal heart, and there was a distinctively lower uptake in the damaged side of the surgically modified heart. In conclusion, the new N₂S₂ ligand was readily prepared and labeled with radioactive ⁶⁷Ga. Biodistribution in rats revealed high initial heart uptake and relatively high retention reflecting regional myocardial perfusion.

Keywords

PET imaging; Myocardial perfusion; ^{67/68}Ga labeling; In vivo biodistribution; Autoradiography

1. Introduction

Myocardial perfusion has been traditionally evaluated with planar imaging or single photon emission computed tomography (SPECT), in conjunction with radiopharmaceuticals such as ²⁰¹Tl, [^{99m}Tc]sestamibi or [^{99m}Tc]tetrofosmin [1,2]. Currently, these types of myocardial perfusion imaging studies are part of normal patient workup and are performed on a routine basis. Recent advances in positron emission tomography (PET)/computed tomography (CT), as well as in multichannel CT for cardiac applications, advocated the need to develop new PET radiopharmaceuticals to improve the resolution and quantification of regional myocardial perfusion studies [3]. The purpose of this article is to report a new gallium N₂S₂ complex,

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which may take advantage of a $^{68}\text{Ge}/^{68}\text{Ga}$ generator to provide a convenient myocardial perfusion imaging in PET.

In the past few decades, many types of $^{68}\text{Ge}/^{68}\text{Ga}$ generator systems used for producing positron-emitting isotopes without an on-site cyclotron have been reported [4–7]. The unique features of a radionuclide generator system are a relatively long parent half-life (^{68}Ge ; $t_{1/2}=270$ days) and a suitable daughter half-life (^{68}Ga ; $t_{1/2}=68$ min). Usually, separation of the desired daughter ^{68}Ga from the parent ^{68}Ge is achieved by using solid oxides (such as TiO_2 , ZrO_2 or SiO_2) supported with column chromatography. The column is eluted with a strong acid leading to Ga(III) complexes and avoids the formation of gallium hydroxide as a solid precipitate. A tin dioxide/1-N HCl generator also provides a sterile solution of Ga-68 in ionic form, which is ready for use in the preparation of many radiopharmaceuticals [6]. A similar column chromatography separation system using an organic polymer (phenolic ion exchanger), coupled in series with a small anion exchange column (AG-1), has been successfully employed for producing ^{68}Ga for labeling [8]. Alternatively, a new organic polymer (macroporous styrene–divinylbenzene copolymer) containing *N*-methylglucamine groups has been reported for a new $^{68}\text{Ge}/^{68}\text{Ga}$ generator system [9]. The criteria for an ideal $^{68}\text{Ge}/^{68}\text{Ga}$ generator system include the following: (a) a high efficient separation of ^{68}Ga from the column; (b) a minimum amount of “parent breakthrough” (a low level of ^{68}Ge in the eluent); and (c) stability of the column over time. All of the reported $^{68}\text{Ge}/^{68}\text{Ga}$ generator systems can meet the basic criteria listed above; however, one major unmet need in the field of nuclear medicine is the lack of Food and Drug Administration-approved commercial $^{68}\text{Ge}/^{68}\text{Ga}$ generator system(s) for human use, which limits the potential for developing ^{68}Ga -labeled radiopharmaceuticals for PET imaging [10].

Recently, there has been renewed interest in the use of ^{68}Ga for PET imaging [10–13]. Of particular interest is the recent development of ^{68}Ga -labeled peptides targeting endocrine tumor receptors [14–19]. Through the use of 1,4,7,10-tetraazacyclododecane=1,4,7,10-tetraacetic (DOTA) or diethylenetriaminepentaacetic acid (DTPA) as the chelating group, various peptides, including analogs of somatostatin [11,14,20], epidermal growth factor receptor [15], substance P [21], bombesin [16], gastrin and cholecystokinin-B [22], have been successfully labeled with ^{68}Ga for PET imaging. These complexes, when labeled with other radionuclides, can also be useful as radiotherapeutic agents, thus providing a “see-and-treat” approach in tumor diagnosis and treatment [16,20,23–25].

Gallium complexes of N_2S_2 (bisaminoethanethiolate (**1**)) [26–28], as well as NS_3 [29], have been previously reported (Fig. 1). One unique feature of $[\text{Ga}]\mathbf{1}$ complexes is that they have cationic character—+1-charged cation in aqueous solution. When Compound **1** is dissolved in water, the chloride ion would most likely be dissociated to form a Ga bisaminothiolate complex containing a positive charge. It was believed that the highly lipophilic cationic complex was trapped in myocardial tissues similar to those in $^{99\text{m}}\text{Tc}$ -labeled myocardial imaging agents [$^{99\text{m}}\text{Tc}$ methoxyisobutylisonitrile (MIBI) and tetrafosamine]. To improve myocardial uptake and retention, we have prepared a new gallium complex consisting of Compound **1** with three “cyclohexyl” rings as a potential imaging agent for myocardial perfusion studies by PET. The extra “cyclohexyl” rings were added to improve stability and to enhance the lipophilicity of the gallium complex. The improved lipophilicity may enhance the first-pass extraction and the retention of the +1-charged cation in the myocardium. Reported herein are the preparation and in vivo testing of this novel Ga complex.

2. Materials and methods

2.1. Chemistry

2.1.1. General procedures— ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined with a Bruker DPX 200 spectrometer using tetramethylsilane or residual solvent peak as an internal standard. High-resolution mass spectra (HRMS) were recorded at the McMaster Regional Center for Mass Spectrometry using a Micromass/Waters GCT instrument (GC-EI/CI Time of Flight Mass Spectrometer), or at the University of Pennsylvania, Department of Radiology, Radiopharmaceutical Chemistry Section, using Agilent Technologies LC/MSD Time of Flight Mass Spectrometer. Microwave reactions were performed on a Biotage Initiator microwave reactor. X-ray crystallography was performed at the University of Pennsylvania, Department of Chemistry, on a Rigaku Mercury area detector at -130°C . Solvents were dried through a molecular sieve system (Pure Solve Solvent Purification System; Innovative Technology, Inc.). All other chemicals were purchased from Aldrich Chemical Co. and used without further purification. ^{67}Ga for labeling was purchased from Mallinckrodt, Inc. (Folcroft, PA) as gallium citrate.

2.1.2. Syntheses

2.1.2.1. Cyano-cyclohexylamine (4): A solution of cyclohexanone (5 g, 51 mmol) in 20 ml of anhydrous methanol was cooled to 0°C . Through that cold solution, anhydrous ammonia gas was bubbled for 1 h, after which the solution was poured into a mixture of potassium cyanide (4.40 g, 68 mmol) and ammonium chloride (6.2 g, 117 mmol) in 50 ml of aqueous ammonia (28%). The mixture was stirred at room temperature for 24 h. It was then filtered to remove insolubles, and the filtrate was extracted with dichloromethane (DCM) (4×25 ml). The DCM extracts were combined, dried (Na_2SO_4) and concentrated to afford Compound **4** as a pale yellow liquid. It was purified as its HCl salt by bubbling anhydrous HCl gas through its solution in anhydrous ether (6.39 g, 78%). ^1H NMR [200 MHz, CDCl_3 , δ (ppm)]: 1.94–2.00 (m, 2H), 1.37–1.78 (m, 9H), 1.10–1.28 (m, 1H); ^{13}C NMR [50 MHz, CDCl_3 , δ (ppm)]: 123.84, 37.58, 24.24, 22.19. HRMS [electrospray ionization (ESI)] calculated for $\text{C}_7\text{H}_{13}\text{N}_2$ (MH^+), 125.1079; found, 125.1065.

2.1.2.2. Aminomethyl-cyclohexylamine (5): To a solution of Compound **4** (HCl salt, 338 mg, 2.11 mmol) in absolute ethanol was added PtO_2 (53 mg, 20 mol%), followed by concentrated HCl (1.1 ml). The resulting solution was hydrogenated (48 psi) at room temperature for 24 h. The catalyst was removed by passing the mixture through a short pad of Celite. The Celite was repeatedly washed with methanol. The combined alcohol fractions were concentrated to afford Compound **5** (424 mg, quantitative), which was pure enough for subsequent use. ^1H NMR [200 MHz, D_2O , δ (ppm)]: 3.26 (s, 2H), 1.40–1.81 (m, 10H). HRMS (ESI) calculated for $\text{C}_7\text{H}_{17}\text{N}_2$ (MH^+), 129.1392; found, 129.1398.

2.1.2.3. 1-(((1-((1-Mercaptocyclohexyl)methylamino)cyclohexyl)methylamino)methyl)cyclohexanethiol (7): To a solution of Compound **5** (424 mg, 2.11 mmol) in 8 ml of anhydrous methanol was added sodium methoxide (1 M in MeOH, 4.22 ml, 4.22 mmol), and the resulting mixture was stirred under argon for 5 min. It was then filtered to remove insolubles and concentrated. The residue was redissolved in 5 ml of anhydrous methanol, and a solution of dialdehyde **6** (603 mg, 2.11 mmol) [30] in 2 ml of toluene was added. The resulting cloudy solution was refluxed for 2 h. Volatiles were then removed, and the residue was taken in 10 anhydrous DCM. The insolubles were then filtered off, and the filtrate was concentrated to yield a viscous liquid that solidified under vacuum. It was then recrystallized from hexane (575 mg, 72%). ^1H NMR [200 MHz, CDCl_3 , δ (ppm)]: 6.85 (s, 1H), 6.79 (s, 1H), 3.81–3.94 (br, 1H), 2.86 (br, 1H), 1.14–2.14 (m, 30H).

2.1.2.4. 7,8-Dithia-16,24-diaza-trispiro[5.2.5.2.5.3]pentacosa-15,24-diene (9): To a solution of Red-Al (70% in toluene, 1.3 ml, 4.56 mmol) in toluene (10 ml) was added dropwise, at room temperature, a suspension of Compound **7** (230 mg, 0.61 mmol) in 1 ml of toluene. The resulting solution was refluxed for 2 h. The solution was then cooled to 0°C, and excess hydride was carefully destroyed using 1.6 ml of concentrated HCl. The pH of the solution was adjusted to 10 using a concentrated NaOH solution. The mixture was then filtered, and the filtrate was concentrated to a foul-smelling oil that was dissolved in absolute ethanol (4 ml). Anhydrous HCl gas was passed through the ethanolic solution to precipitate Compound **8** as its hydrochloride salt **9** (162 mg, 58%). The hydrochloride salt **9** was dissolved in water, and the pH was adjusted to 10 by adding a 50% NaOH solution. The compound was then extracted with 10% MeOH in DCM to afford pure free base **8**. ¹H NMR [200 MHz, CDCl₃, δ (ppm)]: 2.63 (s, 2H), 2.49 (s, 4H), 1.18–1.70 (m, 34H); ¹³C NMR [50 MHz, CDCl₃, δ (ppm)]: 63.15, 55.88, 53.30, 50.36, 37.99, 33.87, 25.95, 25.79, 25.75, 22.07, 21.36. HRMS (CI) calculated for C₂₁H₃₉N₂S₂ (M-H⁺), 383.2555; found, 383.2541.

2.1.2.5. Synthesis of [Ga]2: Ligand **9** (223.93 mg, 0.49 mmol) was dissolved in 50 ml of degassed and nitrogen-saturated water. An excess amount of gallium chloride (180 mg 1.02 mmol) was added and stirred. The pH was adjusted with a 0.1-N ammonium hydroxide solution to a final pH of 4.5 just before cloudiness occurred. The solution was refluxed overnight, filtered from some small precipitates that had formed and concentrated under vacuum. The residue was extracted from the aqueous phase with chloroform (2×50 ml). The organic phase was dried over sodium sulfate and concentrated. Yield: 78 mg of [Ga]2 (0.16 mmol; 33%). ¹H NMR [200 MHz, MeOH-d₄, δ (ppm)]: 2.2–3.0 (m, 8H), 1.2–1.9 [m (broad), 30H]. HRMS (ESI) calculated for C₂₁H₃₈GaN₂S₂ [(M-HCl)+H⁺], 451.1732; found, 451.1744.

Crystals for X-ray structure determination were obtained through recrystallization from methylene chloride/hexane.

2.1.3. Radiolabeling—About 0.7 mg of ligand **9** was dissolved in 100 μl of water (pH 6), and 500 μl of a no-carrier-added Ga-67–citrate solution (540 μCi) was added (pH 6). The sample was heated in a heating block at 75°C for 30 min. The reaction mixture was cooled to room temperature, and a small sample was used for radiochemical purity (RCP) determination by thin-layer chromatography (TLC; TLC aluminum sheet, silica gel 60, Merck; mobile phase: acetone/acetic acid, 3/1, vol/vol). The RCP, as determined by TLC, was 92% [*R_f*(⁶⁷Ga)2=0.8, *R_f*(⁶⁷Ga)citrate=0.1]. The complex was stable over 24 h at room temperature. The *R_f* value for the cold compound was the same as that for [⁶⁷Ga]2.

2.2. Biological studies

2.2.1. General—Three rats per group were used for each biodistribution study. While under isoflurane anesthesia, 0.2 ml of a saline solution containing 10–100 μCi of radioactive tracer was injected into the femoral vein. The rats were sacrificed at the time indicated by cardiac excision while under anesthesia. Organs of interest were removed and weighed, and radioactivity was counted. The percent dose per organ was calculated by comparing the tissue counts to counts of 1% of the initial dose (aliquots of the injected material diluted 100 times) measured at the same time. Partition coefficient was measured by mixing [⁶⁷Ga]2 complex with 3 g each of 1-octanol and buffer (pH 7.4, 0.1 M phosphate) in a test tube. The test tube was vortexed for 3 min at room temperature, then centrifuged for 5 min. Two weighed samples (1.5 g each) from the 1-octanol and buffer layers were counted in a well counter. The partition coefficient was determined by calculating the ratio of 1-octanol to buffer (cpm/g). Samples from the 1-octanol layer were repartitioned until consistent partitions of coefficient values were obtained. The measurement was repeated three times.

2.2.2. Rat model of myocardial infarction—Male Sprague–Dawley rats (6–8 weeks old, 220–250 g) were purchased from Charles River Laboratories. Anesthesia was induced in rats with 3% isoflurane mixed with oxygen. The rat was then intubated and ventilated through a small-animal ventilator (model 680; Harvard Apparatus). Tidal volume (1.5–2.5 ml) was determined for each animal according to its respiratory rate and body weight. One percent isoflurane mixed with oxygen was used to maintain the anesthesia during surgery. Body temperature was maintained by a heating lamp. Electrocardiogram was monitored throughout the surgery. After the thoracic cavity and pericardium had been opened, the left anterior descending coronary artery was permanently ligated by passing a piece of 6-0 silk suture underneath the vessel and the surrounding myocardium without damaging it. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

2.2.2.1. Autoradiography in normal and infarct rat heart models: About 100 μCi of [^{67}Ga] **2** was injected intravenously into a normal Sprague–Dawley rat or a Sprague–Dawley rat that underwent a procedure to have the anterior branch of the left main coronary artery permanently ligated. The rat was sacrificed at 30 min postinjection (~ 180 min after ligation for the infarct model), and the heart was removed and placed within an embedding medium. Upon freezing, the heart was sliced into 20- μm sections on a cryostat microtome. The heart slices were mounted upon microscope glass slides and exposed to X-ray film for 24 h.

2.2.2.2. Dual-isotope study of [$^{99\text{m}}\text{Tc}$]sestamibi and [^{67}Ga] **2 in normal rats:** [$^{99\text{m}}\text{Tc}$]sestamibi (obtained from Mallinckrodt, Inc.) was diluted in 0.1% bovine serum albumin. The ligand **9** was labeled as described above (Section 2.1.3) to yield [^{67}Ga] **2** with a RCP of $>90\%$. The following procedure was used for the preparation of the dose used in dual-isotope biodistribution studies: Two equal activity solutions of [^{67}Ga] **2** and [$^{99\text{m}}\text{Tc}$]sestamibi were made up (~ 100 $\mu\text{Ci}/1$ ml). Equal volumes of [^{67}Ga] **2** and [$^{99\text{m}}\text{Tc}$]sestamibi complex solutions were mixed. [^{67}Ga] **2**/[$^{99\text{m}}\text{Tc}$]sestamibi complex solution (0.2 ml) was injected into animals and prepared for initial dose (~ 5 – 10 $\mu\text{Ci}/\text{dose}$). Tissue samples were counted on a Perkin-Elmer Packard Cobra II Auto Gamma counter at an energy window setting of 80–230 keV (sum of ^{67}Ga and $^{99\text{m}}\text{Tc}$ γ photons). After 70 h [~ 12 half-lives for $^{99\text{m}}\text{Tc}$ ($t_{1/2}=6.02$ h); ~ 1 half-life for ^{67}Ga ($t_{1/2}=78.24$ h)], the tissue samples were counted again for “pure” ^{67}Ga γ photons. The counts were decay corrected for ^{67}Ga , yielding the contribution of ^{67}Ga ; the numbers were subtracted from the original counts. The difference was the count for the contribution of $^{99\text{m}}\text{Tc}$ from [$^{99\text{m}}\text{Tc}$]sestamibi.

3. Results and discussion

3.1. Synthesis

The desired N_2S_2 ligand **9** containing three tricyclohexyl groups was prepared from two simple fragments, Compounds **5** and **6**. The strategy involves the formation of diimine linkage between diamine **5** and dialdehyde **6**. Subsequent one-pot reduction of imine and disulfide bonds affords the target ligand **9**. The preparation of synthon **5** is depicted in Scheme 1. The amino-cyanation (Strecker) reaction of commercially available cyclohexanone **3** afforded the aminonitrile **4** in good yield (78%). The subsequent hydrogenation of the HCl salt of Compound **4** over PtO_2 yielded the diamine **5** as its dihydrochloride salt (Scheme 1). Synthesis of Compound **6** was achieved by treating commercially available 1-cyclohexanecarboxaldehyde with sulfurmonochloride (Scheme 2) [30]. The preparation of ligand **9** from Compounds **5** and **6** is depicted in Scheme 3. Treatment of dialdehyde **6** with the free base of Compound **5** in refluxing methanol afforded the diimine **7**. Red-Al reduction of Compound **7** in refluxing toluene brought about cleavage of disulfide bond, along with the reduction of diimine moiety to afford Compound **8**, which was then purified as its dihydrochloride salt **9** in 58% yield. The reaction

schemes provided for the synthesis of the desired N₂S₂ ligand **9** are sufficiently flexible and could be adapted for the preparation of new N₂S₂ ligands containing other substitution groups to adjust the properties of the Ga N₂S₂ complexes.

To confirm the formation of the desired radioactive gallium complex, the “cold” gallium complex [Ga]**2** was prepared. The ligand, Compound **9**, was reacted with gallium chloride under a refluxing condition for 18 h. The resulting mixture was condensed under vacuum, and the residue was redissolved in a small amount of water and extracted with chloroform. Removing the chloroform under vacuum gave the final product [Ga]**2** in 33% yield. Alternatively, the compound was purified by preparative TLC on a silica gel plate with acetone/acetic acid (3/1, vol/vol) as mobile phase. The expected gallium complex [Ga]**2** has no UV/Vis peaks; therefore, it is very difficult to determine the formation of the complex. To monitor the product on the TLC, the mixture was first spiked with [⁶⁷Ga]**2**. The formation of the product was then monitored with a Geiger counter, which led to the identification of the desired gallium product band. After extracting the material from silica gel with 10% methanolic methylene chloride and removing the solvent under vacuum, the compound was redissolved in acetone and precipitated with ether to yield the desired complex.

3.2. X-ray structure analysis of [Ga]**2**

Crystals suitable for X-ray structure analyses were obtained through recrystallization of the complex [Ga]**2** from a mixture of methylene chloride/hexane and by slow evaporation of the solution at room temperature. The colorless crystals were analyzed by X-ray crystallography. An ORTEP drawing of complex [Ga]**2** is shown in Fig. 2. The coordination geometry around the gallium is a distorted square pyramidal structure, with N(1), N(2), S(2) and Cl forming the base plane (dihedral angle, −2.9°) and with S(2) occupying the apical position. Gallium sits at about 0.6 Å above the base. Cyclohexyl moieties have a chair conformation. Bond distances of Ga–S [2.246 Å, Ga–S(3) (apical); 2.267 Å, Ga–S(4)], Ga–N [2.112 Å, Ga–N(5); 2.182 Å, Ga–N(7)] and Ga–Cl (2.391 Å) are very similar to the reported distances of the comparable chemical bonds in [Ga]**1** [27], and they all fall within the expected range of bond distances of known compounds [31–38].

3.3. Radiolabeling

[⁶⁷Ga]**2** was prepared by the labeling of N₂S₂ ligand **9** with [⁶⁷Ga]citrate (Scheme 4). The labeling was rapid (in 30 min) and efficient, with a radiochemical labeling yield (RCP) of >90%. The RCP was easily determined by normal-phase TLC with a mobile phase acetone/acetic acid (3/1, vol/vol). The retention factors *R_f* for [⁶⁷Ga]citrate and [⁶⁷Ga]**2** were 0.1 and 0.8, respectively, under these conditions (Fig. 3). Partition coefficient was determined to be 70 (PC=70I21). The ease of labeling may potentially be useful for developing a kit formulation, which is useful for routine clinical application.

3.4. Biodistribution in rats

Table 1 shows the biodistribution of [⁶⁷Ga]**2** and [^{99m}Tc] sestamibi in normal rats in a dual-isotope study. [^{99m}Tc] sestamibi showed the expected behavior, with high initial heart uptake (2.65% dose/g after 2 min) and retention of this value over the next couple of hours (2.43% dose/g after 1 h). Blood uptake and retention values dropped over this time interval from 0.17% dose/g after 2 min to 0.02 dose/g after 1 h, resulting in the very high heart/blood value of 122 after 1 h. [⁶⁷Ga]**2** also showed good initial heart uptake with 3.00% dose/g after 2 min and good retention after 1 h (1.25% dose/g). The blood uptake after 2 min was 0.58% dose/g after 2 min; after 1 h, the retention value was 0.07% dose/g, resulting in a respectable heart/blood value of 18.

Two types of myocardial perfusion agents have been reported. The most commonly used SPECT agents [^{99m}Tc] MIBI and [^{99m}Tc]tetrafosmin are trapped inside the myocardium by binding to the intracellular protein or mitochondria. One of the critical factors to consider is the first-pass extraction (the higher, the better) because the higher first-pass extraction could produce images that quantitatively reflect regional perfusion. The PET myocardial imaging agents [^{13}N]ammonia and ^{82}Rb (an analog of potassium) are more likely to be trapped in myocardial cells. They are, in general, termed “chemical microspheres.” The other type of the myocardial imaging agents are freely diffusible tracers. It has been reported that [^{99m}Tc]teboroxime [39,40] and [^{15}O] water [41] are the two best-characterized diffusible tracers for SPECT and PET studies of regional myocardial perfusion, respectively. The [Ga]2 complex reported in this article showed excellent initial myocardial uptake (3.00% dose/g at 2 min), reflecting a good first-pass extraction. However, the retention as measured by heart uptake at a later time point is 1.69% dose/g at 30 min. The kinetics of this tracer in the heart is neither trapped nor freely diffusible. In the future, advances in developing new Ga tracers for myocardial imaging may lead to agents that are either trapped permanently or freely diffusible. These distinct kinetic differences may be important for measuring regional myocardial perfusion using PET. The new tracer [^{67}Ga]2 may have another problem for use as a myocardial perfusion imaging agent. Liver uptake was very high throughout the time period. The high liver uptake may interfere with the imaging of the heart. Further studies of other gallium complexes with less liver uptake may improve the feasibility of measuring regional myocardial perfusion using PET.

3.5. Autoradiography in normal and infarct rat heart models

To demonstrate the feasibility of using [^{67}Ga]2 as a myocardial perfusion imaging agent, we performed autoradiography studies of the complex in normal and infarct heart models. [^{67}Ga]2 was injected intravenously into a normal rat or a rat that had the anterior branch of the left main coronary artery permanently ligated. In the healthy rat heart, [^{67}Ga]2 was evenly retained after 30 min, as shown in the upper row of Fig. 4. In the case where the anterior branch of the left main coronary artery was permanently ligated, there was nonexistent uptake compared to the other side of the heart, clearly demonstrating the potential of [^{67}Ga]2 as a myocardial imaging agent.

It is well known that the $^{82}\text{Sr}/^{82}\text{Rb}$ ($t_{1/2}=25$ days and 75 s, respectively) generator has been approved for studying myocardial perfusion with PET. Validation of the use of ^{82}Rb /PET myocardial perfusion imaging to study cardiovascular function related to blockage of the coronary artery has been previously reported [42,43]. In spite of the relatively short physical half-life of ^{82}Rb , the PET imaging studies were successful and showed comparable or improved detection of myocardial perfusion in patients with coronary artery diseases [44–46]. The convenience of using a generator system for routine imaging has fueled the expansion of PET imaging application in nuclear cardiology. The novel gallium complex reported in this article may provide an approach to developing a generator-based PET imaging tracer for studying myocardial function.

In conclusion, a novel Ga complex, based on a new bisamino–bisthiolate ligand, Compound 9, has been reported. The structure analyzed by X-ray crystallography showed a pyramidal core with one Ga–S bond pointing at the apex, while a Ga–Cl bond may be ionizable, leading to a +1-charged cation in solution. Biodistribution of [^{67}Ga]2 in rats showed a high heart uptake, albeit a high liver uptake. When labeled with ^{68}Ga , it may be potentially useful as an imaging agent for studying myocardial perfusion with PET.

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Fig. 1.
Chemical structures of two Ga bisaminobisthiolates **1** and **2**.

Fig. 2.

ORTEP diagram of the complex [Ga]2. Selected interatomic distances (Å) and angles (°) are as follows: Ga–Cl, 2.391; Ga–N1, 2.113; Ga–N2, 2.183; Ga–S1, 2.246; Ga–S2, 2.266; Cl–Ga–N1, 87.70; Cl–Ga–N2, 145.88; Cl–Ga–S1, 111.00; Cl–Ga–S2, 90.33; S1–Ga–S2, 119.86; N1–Ga–S1, 90.68; N1–Ga–S2, 147.73; N2–Ga–N1, 78.57; N2–Ga–S1, 100.35; N2–Ga–S2, 85.34. †The crystal system is rhombohedral; space group *R* (no. 148), with $a=32.567(3)$ Å, $c=13.6244(12)$ Å, $V=12514(2)$ Å³ and $Z=18$. The structure was solved by direct methods and was refined by full-matrix least squares methods using 4893 reflections, with $I>4\sigma(I)$ up to the final $R=0.0664$ and $R_w=0.1798$. Hydrogen atoms were calculated and refined according to a riding model. Data were collected on a Rigaku Mercury area detector at -130°C . Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center.

Fig. 3.
TLC profile of $[^{67}\text{Ga}]\mathbf{2}$ on silica, with acetone/acetic acid (3/1, vol/vol) as the developing solvent.

Fig. 4. Autoradiography of $[^{67}\text{Ga}]\mathbf{2}$ in a healthy Sprague–Dawley rat heart (upper row) and in a Sprague–Dawley rat that underwent a procedure to have the anterior branch of the left main coronary artery permanently ligated (lower row).

Scheme 1.
Synthesis of Compound **5**.

Scheme 2.
Synthesis of Compound 6.

Scheme 3.
Synthesis of Compound **8**.

Scheme 4.
Radiolabeling of Compound 9.

Table 1Dual-isotope study of [^{67}Ga]2/[$^{99\text{m}}\text{Tc}$]sestamibi in rats (% dose/g; average of three rats $\pm$ S.D.)

	2 min	30 min	60 min	120 min
[^{67}Ga]2				
Blood	0.58 $\pm$ 0.10	0.14 $\pm$ 0.01	0.07 $\pm$ 0.01	0.06 $\pm$ 0.00
Heart	3.00 $\pm$ 0.41	1.69 $\pm$ 0.14	1.25 $\pm$ 0.16	0.80 $\pm$ 0.09
Muscle	0.15 $\pm$ 0.02	0.14 $\pm$ 0.00	0.13 $\pm$ 0.03	0.10 $\pm$ 0.02
Lung	2.22 $\pm$ 0.67	0.60 $\pm$ 0.26	0.36 $\pm$ 0.05	0.30 $\pm$ 0.14
Kidney	5.48 $\pm$ 0.93	3.60 $\pm$ 0.56	1.74 $\pm$ 0.21	0.96 $\pm$ 0.14
Spleen	2.23 $\pm$ 0.66	0.58 $\pm$ 0.13	0.44 $\pm$ 0.07	0.45 $\pm$ 0.02
Liver	5.00 $\pm$ 0.67	2.44 $\pm$ 0.24	1.50 $\pm$ 0.20	1.08 $\pm$ 0.06
Skin	0.24 $\pm$ 0.05	0.15 $\pm$ 0.03	0.09 $\pm$ 0.02	0.07 $\pm$ 0.01
Brain	0.04 $\pm$ 0.01	0.02 $\pm$ 0.00	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00
Heart/tissue ratio				
Heart/blood	5.20 $\pm$ 1.14	12.1 $\pm$ 1.32	17.9 $\pm$ 3.43	13.3 $\pm$ 1.49
Heart/liver	0.60 $\pm$ 0.11	0.70 $\pm$ 0.09	0.83 $\pm$ 0.15	0.74 $\pm$ 0.08
Heart/muscle	20.0 $\pm$ 3.81	12.1 $\pm$ 1.00	9.61 $\pm$ 2.53	8.00 $\pm$ 1.83
[$^{99\text{m}}\text{Tc}$]sestamibi				
Blood	0.17 $\pm$ 0.01	0.03 $\pm$ 0.00	0.02 $\pm$ 0.00	0.02 $\pm$ 0.00
Heart	2.65 $\pm$ 0.16	2.19 $\pm$ 0.16	2.43 $\pm$ 0.17	2.72 $\pm$ 0.15
Muscle	0.13 $\pm$ 0.03	0.13 $\pm$ 0.04	0.12 $\pm$ 0.03	0.17 $\pm$ 0.05
Lung	1.43 $\pm$ 0.28	0.53 $\pm$ 0.05	0.42 $\pm$ 0.08	0.31 $\pm$ 0.02
Kidney	7.60 $\pm$ 0.56	4.30 $\pm$ 0.32	2.24 $\pm$ 0.08	1.80 $\pm$ 0.34
Spleen	2.66 $\pm$ 1.03	1.30 $\pm$ 0.35	1.03 $\pm$ 0.08	0.74 $\pm$ 0.23
Liver	2.60 $\pm$ 0.28	1.41 $\pm$ 0.26	0.60 $\pm$ 0.14	0.30 $\pm$ 0.14
Skin	0.28 $\pm$ 0.06	0.17 $\pm$ 0.05	0.18 $\pm$ 0.02	0.18 $\pm$ 0.03
Brain	0.04 $\pm$ 0.00	0.03 $\pm$ 0.00	0.02 $\pm$ 0.00	0.02 $\pm$ 0.00
Heart/tissue ratio				
Heart/blood	15.6 $\pm$ 1.31	73.0 $\pm$ 5.33	122 $\pm$ 8.53	136 $\pm$ 7.50
Heart/liver	1.01 $\pm$ 0.12	1.60 $\pm$ 0.31	4.05 $\pm$ 0.99	9.06 $\pm$ 4.25
Heart/muscle	20.4 $\pm$ 4.86	16.9 $\pm$ 5.34	20.2 $\pm$ 5.24	17.0 $\pm$ 5.08