Published in final edited form as:

Nucl Med Biol. 2009 January ; 36(1): 39–45. doi:10.1016/j.nucmedbio.2008.10.010.

Synthesis and Biodistribution of Lipophilic Monocationic Gallium Radiopharmaceuticals Derived from *N,N'*-bis(3-aminopropyl)-*N,N'*-dimethylethylenediamine: Potential Agents for PET Myocardial Imaging with ^{68}Ga

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Abstract

Introduction—In locations that lack nearby cyclotron facilities for radionuclide production, generator-based ^{68}Ga -radiopharmaceuticals might have clinical utility for positron emission tomography (PET) studies of myocardial perfusion and other physiologic processes.

Methods—The lipophilic, monocationic ^{67}Ga -labeled gallium chelates of five novel hexadentate bis(salicylaldimine) ligands, the bis(salicylaldimine), bis(3-methoxysalicylaldimine), bis(4-methoxysalicylaldimine), bis(6-methoxysalicylaldimine), and bis(4,6-dimethoxysalicylaldimine) of *N,N'*-bis(3-aminopropyl)-*N,N'*-dimethylethylenediamine (BAPDMEN), were prepared. The structure of the unlabeled $[\text{Ga}(\text{4-MeOsAl})_2\text{BAPDMEN}]^+\text{PF}_6^-$ salt was determined by X-ray crystallography, and the biodistribution of each of the ^{67}Ga -labeled gallium chelates determined in rats following i.v. administration and compared to the biodistribution of ^{86}Rb rubidium chloride.

Results—The $[\text{Ga}(\text{4-MeOsAl})_2\text{BAPDMEN}]^+\text{PF}_6^-$ complex exhibits the expected pseudo-octahedral $\text{N}_4\text{O}_2^{2-}$ coordination sphere about the Ga^{3+} center with a trans-disposition of the phenolate oxygen atoms. All five of the ^{67}Ga -radiopharmaceuticals were found to afford the desired myocardial retention of the radiogallium. The $^{67/68}\text{Ga}[\text{Ga}(\text{3-MeOsAl})_2\text{BAPDMEN}]^{1+}$ radiopharmaceutical appears to have the best properties for myocardial imaging, exhibiting 2% of the injected dose in the heart at both 1-minute and 2-hours post-injection and very high heart/non-target ratios (heart/blood ratios of 7.6 ± 1.0 and 54 ± 10 at 1-min and 120-min, respectively; heart/liver ratios of 1.8 ± 0.4 and 39 ± 3 at 1-min and 120-min, respectively).

Conclusions—Most of these new agents, particularly $^{67/68}\text{Ga}[\text{Ga}(\text{3-MeOsAl})_2\text{BAPDMEN}]^{1+}$, would appear superior to previously reported bis(salicylaldimines) of *N,N'*-bis(3-aminopropyl) ethylenediamine as candidates for PET imaging of the heart with ^{68}Ga .

Keywords

Gallium-68; Positron Emission Tomography (PET); Radiopharmaceuticals; Myocardial Imaging

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INTRODUCTION

The $^{68}\text{Ge}/^{68}\text{Ga}$ parent/daughter radionuclide generator is a potential source of positron emission tomography (PET) radiopharmaceuticals in the absence of a nearby cyclotron facility [1–6]. The 271-day half-life [7] of the ^{68}Ge parent gives this generator a long shelf-life, while the 68-minute half-life [7] of the positron-emitting ^{68}Ga daughter is adequate for radiopharmaceutical synthesis. The ^{68}Ga half-life can potentially be exploited in PET data collections over an extended time frame, either for improving counting statistics in images of a tracer that is trapped in tissue, or for kinetic modeling with an agent exhibiting more complicated pharmacokinetics. Numerous gallium-68 radiopharmaceuticals have been reported [1–6,8–21] and some have found use in human studies [22–27].

The clinical importance of myocardial perfusion imaging for detection of coronary artery disease has motivated attempts to develop ^{68}Ga -labeled PET agents that could substitute for the widely used $^{99\text{m}}\text{Tc}$ SPECT heart agents, Cardiolite® ($^{99\text{m}}\text{Tc}$]Tc-sestamibi) and Myoview™ ($^{99\text{m}}\text{Tc}$]Tc-tetrofosmin). However, identification of suitable lipophilic ^{68}Ga -radiopharmaceuticals for perfusion imaging has proven somewhat elusive [8–15]. High, and ideally complete, first-pass extraction of an agent from blood into tissue is desired, since this will assure that the distribution of tracer maps regional tissue perfusion.

We have previously investigated [8–10] neutral gallium-68 radiopharmaceuticals with tripodal hexadentate *tris*(salicylaldimine) ligands, identifying some $^{67/68}\text{Ga}$ -tracers capable of producing extremely high myocardial uptake and very high heart/blood ratios in the rat at 1 minute post-injection (~2.4% of the injected dose in myocardium at 1 minute with heart/blood ratios as high as 17:1) [10]. In the dog, excellent myocardial images were obtained with [^{68}Ga]Ga[(sal)₃tame-O-*iso*-Bu], *without* blood-pool subtraction, that compare favorably to blood-pool-subtracted ^{15}O -water images [10]. Unfortunately, all of the studied neutral ^{68}Ga -compounds of this type were found to clear rather rapidly from myocardium [8–10], which we believe undermines their potential clinical utility. A ^{68}Ga radiopharmaceutical for imaging the heart with PET may be much more attractive if, following very high first-pass myocardial extraction, the radiolabel is retained in myocardium, thereby allowing use of long image acquisition periods for improved image quality.

With the goal of obtaining lipophilic gallium radiopharmaceuticals that not only exhibit high heart uptake following intravenous administration, but also afford myocardial retention of the ^{68}Ga -radiolabel (similar to the myocardial retention observed with the lipophilic monocationic $^{99\text{m}}\text{Tc}$ -myocardial perfusion agents, Cardiolite® and Myoview™), we have also prepared and evaluated a series of *monocationic* Ga(III) complexes with hexadentate *bis*(salicylaldimine) ligands [11–13]. The compounds studied in this regard have been *bis*(salicylaldimines) derived from *N,N'*-*bis*(3-aminopropyl)ethylenediamine (BAPEN) that provide a $\text{N}_4\text{O}_2^{2-}$ coordination sphere for the octahedral Ga^{3+} ion. Such chelates consistently provided the desired myocardial retention of radiogallium, but myocardial uptake was always less than 1.1% of the injected dose in the rat model [12]. A PET study with [^{68}Ga]Ga[(4,6-MeO₂sal)₂BAPEN]¹⁺ in the dog clearly delineated the heart with good heart-to-blood and heart-to-lung contrast in images similar to those observed in a ^{15}O -water perfusion study performed immediately prior to injection of the ^{68}Ga radiopharmaceutical [11]. Tracer retained in myocardium, as well as excreted in bile, appears to be the intact gallium radiopharmaceutical [13]. However, while the 1% tracer uptake in myocardium observed with these compounds is substantial, it remains slightly lower than the myocardial uptake of $^{99\text{m}}\text{Tc}$ -Sestamibi in this rat model [11].

The utility of a ^{68}Ga -radiopharmaceutical for myocardial perfusion imaging would seemingly be improved with: (*i*) heart uptake in the range of 2–3% of the injected dose, suggesting very

high 1st-pass myocardial extraction; (ii) prolonged myocardial retention of the extracted ⁶⁸Ga radiotracer; and (iii) high heart/blood, heart/lung, and heart/liver ratios. The ability of the monocationic gallium bis(salicylaldimine) radiopharmaceuticals to provide high heart/liver contrast appears to derive largely from their susceptibility to transport by the *MDR1* P-glycoprotein, which is expressed on the biliary surface of hepatocytes and can speed tracer clearance from liver to bile [28–30].

We report here the synthesis and biodistribution of a related series of gallium bis(salicylaldimine) radiopharmaceuticals derived from *N,N'*-bis(3-aminopropyl)-*N,N'*-dimethylethylenediamine (BAPDMEN) (Figure 1).

MATERIALS AND METHODS

The reagents were purchased from Aldrich Chemical Co. (St. Louis, MO) and used without further purification unless otherwise stated. Gallium(III)-acetylacetonate [Ga(acac)₃] was purchased from Strem Chemical Co. (Newburyport, MA). ¹H NMR spectra were obtained on a Bruker 300 MHz spectrometer. [⁶⁷Ga]Gallium chloride in HCl solution was obtained from Mallinckrodt Medical, Inc. (St. Louis, MO). No-carrier-added [⁸⁶Rb]rubidium chloride was purchased from DuPont/NEN Research Products (N. Billerica, MA). Radiochromatograms were analyzed with a Berthold Tracemaster 20 Automatic TLC Linear-Analyzer.

Radiopharmaceutical biodistribution in rats was determined as described previously following intravenous injection (1–5 μCi or 0.037–0.185 MBq in 0.1–0.2 mL) via the femoral vein of ether-anesthetized male Sprague-Dawley rats [10–12]. Blood was assumed to account for 7% of total body mass, and muscle to account for 42% of total body mass [31]. All animal studies were carried out in accordance with procedures approved by the Purdue Animal Care and Use Committee.

Cold Synthesis

***N,N*-bis(3-aminopropyl)-*N,N'*-dimethylethylenediamine (BAPDMEN)**—To 1.7 g (5 mmol) of KOH in 50 mL of absolute ethanol was added 10 g (11 mmol) of *N,N'*-dimethylethylenediamine. The mixture was refluxed under nitrogen at 70°C for 2 h, following which 60 g (22 mmol) of *N*-3-bromopropylphthalimide was added. This cloudy mixture was refluxed for 16 h. The hot solution was then filtered and the solvent removed by rotary evaporation to give 20 g of a yellow semisolid. Four hundred mL of 6 N HCl was slowly added to the isolated semisolid, and the mixture was refluxed for about 18 h. A clear solution was obtained, and upon cooling to 0°C, a white crystalline solid precipitated. The solution was filtered and the filtrate evaporated on a rotary evaporator to give 20 g of white solid. This solid was neutralized with NaOH solution to give two layers. The upper layer was collected and dried over NaOH. The pure product was obtained as a clear oil (2.2 g, 20% yield) by fractional vacuum distillation at 93°C (0.12 torr). ¹H NMR (CDCl₃): δ (ppm) 1.38 (s, br, NH₂, 4 H); 1.57 (quintet, CH₂CH₂CH₂, 4 H); 2.18 (s, N-CH₃, 6 H); 2.36 (t, H₂N-CH₂, 4 H); 2.42 (s, NCH₂CH₂N, 4 H); 2.68 (t, CH₂-NCH₃, 4 H).

H₂[(sal)₂BAPDMEN] (1)—The bis(salicylaldimine) of BAPDMEN was obtained by mixing a solution of salicylaldehyde (370 mg; 3.03 mmol) in 15 mL of dry methanol, with 300 mg BAPDMEN (1.45 mmol) in 15 mL of dry methanol. The mixture was refluxed for 40 min and then stirred until cooled to 25 °C. A yellow oil was obtained after the solvent was removed by rotary evaporation. This yellow oil was dissolved in diethyl ether, the solution was filtered to remove unreacted salicylaldehyde, and then cooled to 0 °C for 2 days. The resultant solution was filtered and the solvent was removed by rotor evaporation and dried under vacuum to give yellow to orange oil (0.3 g, 65% yield). ¹H NMR (CDCl₃): δ (ppm) 1.67 (quintet,

$\text{CH}_2\text{CH}_2\text{CH}_2$, 4 H); 2.23 (s, CH_3 , 6 H); 2.43 (t, $\text{CH}_2\text{-N}$, 4 H); 2.46 (s, $\text{NCH}_2\text{CH}_2\text{N}$, 4 H); 3.61 (t, $\text{CH}_2\text{-N=}$, 4 H); 6.8–7.3 (m, C_6H_4 , 8 H); 8.33 (s, CH=N , 2 H); 13.6 (s, br, OH , 2 H).

$\text{H}_2[(4\text{-MeOsal})_2\text{BAPDMEN}]$ (2**)**—The bis(4-methoxysalicylaldehyde) of BAPDMEN was obtained by reaction of BAPDMEN (400 mg; 1.98 mmol) with 605 mg (3.98 mmol) 2-hydroxy-4-methoxy benzaldehyde in a manner analogous to **1**, allowing isolation of 0.4 g (80 % yield) of **2** as a yellow oil. ^1H NMR (CDCl_3): δ (ppm) 1.78 (quintet, $\text{CH}_2\text{CH}_2\text{CH}_2$, 4 H); 2.19 (s, CH_3 , 6 H); 2.37 (t, $\text{CH}_2\text{-N}$, 4 H); 2.42 (s, $\text{NCH}_2\text{CH}_2\text{N}$, 4 H); 3.50 (t, $\text{CH}_2\text{-N=}$, 4 H); 3.76 (s, OCH_3 , 6 H); 6.3–7.0 (m, C_6H_3 , 6 H); 8.1 (s, CH=N , 2 H); 14.0 (s, br, OH , 2 H).

$\text{H}_2[(4,6\text{-MeOsal})_2\text{BAPDMEN}]$ (3**)**—The bis(4,6-dimethoxysalicylaldehyde) of BAPDMEN was obtained by reaction of BAPDMEN (400 mg; 1.98 mmol) with 725 mg (3.96 mmol) 4,6-dimethoxysalicylaldehyde, yielding 0.45 g (85% yield) of **3**. ^1H NMR (CDCl_3): δ (ppm) 1.78 (quintet, $\text{CH}_2\text{CH}_2\text{CH}_2$, 4 H); 2.19 (s, CH_3 , 6 H); 2.37 (t, $\text{CH}_2\text{-N}$, 4 H); 2.42 (s, $\text{NCH}_2\text{CH}_2\text{N}$, 4 H); 3.46 (t, $\text{CH}_2\text{-N=}$, 4 H); 3.73 (s, OCH_3 , 12 H); 5.3–6.0 (m, C_6H_2 , 4 H); 8.3 (s, CH=N , 2 H); 14.1 (s, br, OH , 2 H).

$\text{H}_2[(3\text{-MeOsal})_2\text{BAPDMEN}]$ (4**)**—The bis(3-methoxysalicylaldehyde) of BAPDMEN was obtained by reaction of BAPDMEN (100 mg; 0.5 mmol) with 151 mg (1.0 mmol) of 3-methoxysalicylaldehyde, yielding 0.38 g (80 % yield) of **4** as a yellow oil. ^1H NMR (CDCl_3): δ (ppm) 1.78 (quintet, $\text{CH}_2\text{CH}_2\text{CH}_2$, 4 H); 2.14 (s, CH_3 , 6 H); 2.35 (t, $\text{CH}_2\text{-N}$, 4 H); 2.34 (s, $\text{NCH}_2\text{CH}_2\text{N}$, 4 H); 3.54 (t, $\text{CH}_2\text{-N=}$, 4 H); 3.81 (s, OCH_3 , 6 H); 6.3–7.0 (m, C_6H_3 , 6 H); 8.23 (s, CH=N , 2 H); 13.4 (s, br, OH , 2 H).

$\text{H}_2[(6\text{-MeOsal})_2\text{BAPDMEN}]$ (5**)**—The bis(6-methoxysalicylaldehyde) of BAPDMEN was obtained by reaction of BAPDMEN (25 mg; 0.13 mmol) with 38 mg (0.26 mmol) 6-methoxysalicylaldehyde, providing 70 mg (60 % yield) of **5** as a yellow oil. ^1H NMR (CDCl_3): δ (ppm) 1.78 (quintet, $\text{CH}_2\text{CH}_2\text{CH}_2$, 4 H); 2.22 (s, CH_3 , 6 H); 2.48 (t, $\text{CH}_2\text{-N}$, 4 H); 2.49 (s, $\text{NCH}_2\text{CH}_2\text{N}$, 4 H); 3.54 (t, $\text{CH}_2\text{-N=}$, 4 H); 3.76 (s, OCH_3 , 6 H); 6.0–7.0 (m, C_6H_3 , 6 H); 8.7 (s, CH=N , 2 H); 14.0 (s, br, OH , 2 H).

$[\text{Ga}(4\text{-MeO-sal})_2\text{BAPDMEN}]\text{PF}_6^-$ —To 15 mL of a methanol solution of **2** (142 mg, 0.3 mmol) was added $\text{Ga}(\text{acac})_3$ (110 mg, 0.3 mmol) in 15 mL of warm methanol. The mixture was refluxed for 2 hr, after which 150 mg of KPF_6 in 1 mL of water was added to the hot methanol solution. A white solid precipitate was obtained on cooling to room temperature. Crystalline product was obtained by dissolving the white solid in 3 mL acetonitrile, then the solution filtered, layered with diethyl ether, and cooled to 0 °C (80% yield). The ESI fast atom bombardment (FAB) mass spectrum of the product shows the parent ion peak at $m/e = 537.21$ (expected = 537.20) for $[\text{C}_{26}\text{H}_{44}\text{N}_4\text{O}_4\text{Ga}]^{1+}$.

The structure of $[\text{Ga}(4\text{-MeO-sal})_2\text{BAPDMEN}]\text{PF}_6$ was confirmed by X-ray crystallography. Preliminary examination and data collection for $[\text{Ga}(4\text{-MeO-sal})_2\text{BAPDMEN}]\text{PF}_6$ were performed with $\text{Cu K}\alpha$ radiation ($\lambda = 1.54184 \text{ \AA}$) on an Enraf-Nonius CAD4 computer controlled kappa axis diffractometer equipped with a graphite crystal, incident beam monochromator. Crystal data for $\text{C}_{26}\text{H}_{36}\text{N}_4\text{O}_4\text{F}_6\text{PGa}$: $a = 10.701 (1) \text{ \AA}$, $b = 13.310 (2) \text{ \AA}$, $c = 20.723 (2) \text{ \AA}$; $\beta = 93.27 (6)^\circ$; $V = 2946.8 (8) \text{ \AA}^3$; $Z = 4$ in monoclinic space group $\text{P}2_1/\text{n}$; $T = 293 \text{ K}$; $\text{calc} = 1.54 \text{ g cm}^{-3}$; $\mu = 24.75 \text{ cm}^{-1}$; $R(F) = 4.7\%$, $R_w(F) = 6\%$. The structure was solved using the Patterson heavy-atom method which revealed the position of the Ga atom. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were located and added to the structure factor calculations but their positions were not refined. Complete details are provided in the deposited supporting material.

[⁶⁷Ga]Ga-Bis(salicylaldimine) Radiopharmaceuticals

Each of the hexadentate bis(salicylaldimine) ligands was used to prepare the corresponding [⁶⁷Ga]Ga-bis(salicylaldimine) radiopharmaceutical following the general procedure described previously [11,12]. Briefly, the dilute HCl solution of ⁶⁷Ga³⁺ (3.7 MBq; 0.1 mCi) was evaporated to dryness in a test tube while heating under a flow of N₂. The radioactive ⁶⁷Ga³⁺ was immediately redissolved in ethanol containing 0.002 wt% acetylacetone, transferred to a clean test tube, and then 1 mg of the bis(salicylaldimine) ligand (ca. 10mg/mL ethanol) added. The mixture was heated in a water bath at 70°C for 20 minutes to form the gallium(III) complex. The reaction mixture was then diluted to 5–10% ethanol with saline and filtered through a sterile 0.2 μm polytetrafluoroethylene filter before use. The radiochemical purity of the product was evaluated by thin-layer chromatography on C₁₈-silica gel plates eluted with methanol containing 10% saline.

The octanol/water partition coefficients of the ⁶⁷Ga-radiotracers were measured between 1-octanol and isotonic TRIS-buffered saline (pH 7.4). The octanol phase from the partitioning was repartitioned three times with fresh buffer to insure that trace hydrophilic ⁶⁷Ga impurities did not alter the calculated *P* values. The reported *log P* values (Table 1) are the mean (± standard deviation) of the second, third and fourth (or third and fourth) (*n* = 4–6).

RESULTS AND DISCUSSION

Cold Chemistry

A series of five hexadentate bis(salicylaldimine) ligands were prepared using the *N,N'*-bis(3-aminopropyl)-*N,N'*-dimethylethylenediamine (BAPDMEN) tetraamine backbone (Figure 1 and Table 1). These differ from the *N,N'*-bis(3-aminopropyl)ethylenediamine (“BAPEN”) ligands examined previously [11–13] by the addition of methyl substituents on each of the two nitrogen atoms of the central ethylenediamine fragment. The X-ray structure of the 4,6-dimethoxysalicylaldimine derivative, [⁶⁷Ga][Ga-2]¹⁺•PF₆¹⁻, (Figure 2) confirms that these ligands retain their ability to bind the nearly octahedral Ga(III) center as hexadentate ligands in which the phenolic oxygen atoms adopt a *trans*-geometry. The Ga-N_{imine} and Ga-O bond distances of 2.05 Å, and 1.93 Å, respectively, are similar to the values seen in other gallium (III) bis(salicylaldimine) chelates derived from a *N,N'*-bis(3-aminopropyl)ethylenediamine backbone [12,28], while the Ga-N_{amine} distances of 2.14 Å are slightly longer than those seen in the related gallium chelates lacking the N-methyl substituents [12,28].

Radiochemistry

While it is ultimately ⁶⁸Ga that is of the greatest interest as a radiopharmaceutical label, ⁶⁷Ga was employed as a surrogate in the present studies due to the experimental convenience offered by its longer physical half-life. The ⁶⁷Ga-labeled gallium chelates of the five new salicylaldimine ligands (Figure 1 and Table 1) were obtained with >93% radiochemical purity and were used without further purification. Each bis(salicylaldimine) radiopharmaceutical migrated as single radioactive peak with *R_f* values in the range 0.3–0.6 on C18-plates developed with methanol containing 10% saline, conditions under which the [⁶⁷Ga]Ga(acac)₃ precursor remains at the origin (*R_f* = 0).

The lipophilicity of the five BAPDMEN chelates of ⁶⁷Ga, assessed via their octanol/water partition coefficients, spanned *log P* values from 1.4 to 3.0 (Table 1), which is the range where optimum myocardial uptake was observed in previous studies of gallium bis(salicylaldimine) chelates derived from the BAPEN tetraamine backbone [12]. For the salicylaldimine, 4-methoxysalicylaldimine, and 4,6-dimethoxysalicylaldimine chelates of the BAPDMEN backbone, where the corresponding BAPEN derivatives were previously studied, it was found that each additional *N*-methyl substituent of the BAPDMEN backbone (*i.e.*, methyl groups

replacing the amino N-H of the BAPEN ligands) increases $\log P$ by approximately 0.4. This is in keeping with the substituent effect one would predict from the work of Hansch on the variation of octanol/water partition coefficients in simple organic molecules bearing aliphatic amine substituents [32,33].

Biodistribution of ^{67}Ga -chelates vs. $^{86}\text{RbCl}$

The biodistribution of each of the ^{67}Ga -chelates was determined in rats at 1-minute and 2-hours following intravenous injection (Tables 2–6). For reference, the biodistribution of $^{86}\text{Rb}^+$ was similarly evaluated (Table 7), since the aqueous $^{86}\text{Rb}^+$ cation is an FDA-approved agent for PET assessment of myocardial perfusion [34,35]. The data shown is expressed as a percentage of the injected dose per organ, allowing direct assessment of total delivery to the heart and other major organs. The corresponding values for the percentage of the injected dose per gram of tissue (wet mass) are provided for reference in the electronic “Supporting Data.” The tracers are all rapidly cleared from blood following intravenous administration (Tables 2–7).

All of these lipophilic monocationic ^{67}Ga -agents afford significant myocardial uptake, and retention, of the radiolabel. With the exception of the most lipophilic, the 4,6-dimethoxy chelate $^{67}\text{Ga}[\text{Ga-3}]^{1+}$, the new ^{67}Ga agents all provide higher myocardial uptake than observed with the structurally analogous radiopharmaceuticals derived from the BAPEN tetraamine backbone [11,12], with myocardial uptake of the 3-methoxy- derivative, $^{67}\text{Ga}[\text{Ga-4}]^{1+}$, reaching 2% of the injected dose (Table 5). The latter level of myocardial uptake exceeds that which we have previously reported for the $^{99\text{m}}\text{Tc}$ myocardial perfusion tracer Cardiolite® ($^{99\text{m}}\text{Tc}$ -sestamibi) in the rat model [11], but remains below the maximum myocardial uptake observed with $^{86}\text{Rb}^+$ (Table 7) and the neutral $^{67/68}\text{Ga}$ gallium tris(salicylaldimine) chelates [10]. Nevertheless, the myocardial retention of the $^{67/68}\text{Ga}[\text{Ga-4}]^{1+}$ radiopharmaceutical would make this the more attractive candidate for clinical use in PET myocardial imaging, since extended image acquisition periods could be used to improve counting statistics.

High myocardial uptake and retention are not the sole determinants of the quality expected in PET myocardial images — one also needs high heart/blood, heart/lung, and heart/liver contrast. Heart/blood and heart/lung contrast are observed to vary among the five new ^{67}Ga -radiopharmaceuticals studied (Table 8), but for at least the salicylaldimine ($^{67}\text{Ga}[\text{Ga-1}]^{1+}$), 4-methoxysalicylaldimine ($^{67}\text{Ga}[\text{Ga-2}]^{1+}$), and 3-methoxysalicylaldimine ($^{67}\text{Ga}[\text{Ga-4}]^{1+}$) tracers, would appear quite adequate for myocardial imaging using PET and ^{68}Ga . There is also considerable variation in the level of heart/liver contrast (Table 8 and Figure 3), with the 3-methoxy complex, $^{67}\text{Ga}[\text{Ga-4}]^{1+}$, providing outstanding heart/liver contrast due to its extremely efficient clearance from liver into bile. The rapid hepatobiliary clearance of $^{67}\text{Ga}[\text{Ga-4}]^{1+}$ is presumed to indicate this agent to be the most effectively transported by the *MDR1* P-glycoprotein of hepatocytes, and is consistent with our prior finding that 3-methoxy- and 3-ethoxy-substituted Ga-chelates appeared to be the best *MDR1* Pgp-transport substrates obtained using the *bis*(2,2-dimethyl-3-aminopropyl)ethylenediamine (Me_4BAPEN) ligand backbone [28].

CONCLUSIONS

The results seen with some of these new ^{67}Ga -chelates, particularly the 3-methoxysalicylaldimine derivative, $^{67}\text{Ga}[\text{Ga-4}]^{1+}$, suggest that the corresponding ^{68}Ga -radiopharmaceuticals could be superior to [$^{99\text{m}}\text{Tc}$]Tc-sestamibi as markers of myocardial perfusion. However, it must be noted that the extrapolation of such tracer performance from rodents to humans can be fraught with pitfalls, with species-dependent binding to plasma proteins potentially altering the efficiency of radiopharmaceutical delivery to myocardium [36]. Further work will be required to define whether, and how, the biodistribution and pharmacokinetics of such agents varies between species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by grants R01-CA46909 and R01-CA092403 awarded by the U.S. Public Health Service.

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Figure 1.

General structural formula for the hexadentate bis(salicylaldiminato) ligands derived from *N,N'*-Bis(3-AminoPropyl)-*N,N'*-DiMethyl-ethylenediamine (BAPDMEN, incorporating “en” as the standard acronym for the ethylenediamine fragment). The substituents R₃, R₄, and R₆ are either –H or –OCH₃, as specified in Table 1. These ligands bind to the octahedral Ga(III) ion via the two phenolate O atoms, the two imine N atoms, and the two amine N atoms.

Figure 2.

ORTEP drawing illustrating the crystallographically-determined structure of the $[\text{Ga}(4\text{-MeOsai})_2\text{BAPDMEN}]^{1+}$ ($[\text{Ga-2}]^{1+}$) cation. Selected bond distances (Å): Ga–O(102) 1.9279(2); Ga–O(122) 1.929(2); Ga–N(1) 2.053(3); Ga–N(5) 2.139(3); Ga–N(8) 2.147(3); Ga–N(12) 2.045(3). Selected bond angles (degrees): O(102)–Ga–O(122) 179.4(1); O(102)–Ga–N(1) 88.7(1); O(102)–Ga–N(5) 92.1(1); O(102)–Ga–N(8) 87.3(1); O(102)–Ga–N(12) 90.9(1); O(122)–Ga–N(1) 91.7(1); O(122)–Ga–N(5) 88.4(1); O(122)–Ga–N(8) 92.4(1); O(122)–Ga–N(12) 88.5(1); N(1)–Ga–N(5) 86.1(1); N(1)–Ga–N(8) 169.6(1); N(1)–Ga–N(12) 102.8(1); N(5)–Ga–N(8) 84.4(1); N(5)–Ga–N(12) 170.6(1); N(8)–Ga–N(12) 86.9(1). (Numbers in parentheses are estimated standard deviations in the least significant digits.)

Figure 3. Heart/Liver Ratios for the [⁶⁷Ga]Ga-bis(salicylalimine) chelates and [^{99m}Tc]Tc-Sestamibi and [⁸⁶Rb]rubidium chloride reference tracers. The [^{99m}Tc]Tc-Sestamibi data shown are from reference 11.

Table 1

The bis(salicylaldehydato) ligands studied, and the lipophilicity of the corresponding ⁶⁷Ga-chelates. The substituents R₃, R₄, and R₆ are numbered as in Figure 1.

Free Ligand	Acronym of Deprotonated Ligand (L)	R ₃	R ₄	R ₆	log P [Ga-L] ¹⁺
1	[(sal) ₂ BAPDMEN] ²⁻	H	H	H	1.59 ± 0.03
2	[(4-MeOsal) ₂ BAPDMEN] ²⁻	H	OCH ₃	H	1.92 ± 0.01
3	[(4,6-MeOsal) ₂ BAPDMEN] ²⁻	H	OCH ₃	OCH ₃	2.97 ± 0.04
4	[(3-MeOsal) ₂ BAPDMEN] ²⁻	OCH ₃	H	H	1.39 ± 0.01
5	[(6-MeOsal) ₂ BAPDMEN] ²⁻	H	H	OCH ₃	2.56 ± 0.02

Table 2Biodistribution of [^{67}Ga][Ga-1] $^{1+}$ in Rats

Organ	Percentage of Injected Dose per Organ [*]	
	1 min	120 min
Blood	4.97 $\pm$ 0.11	0.99 $\pm$ 0.11
Heart	1.68 $\pm$ 0.06	1.81 $\pm$ 0.12
Lungs	1.44 $\pm$ 0.17	1.15 $\pm$ 0.11
Liver	16.9 $\pm$ 1.10	1.67 $\pm$ 0.26
Spleen	0.39 $\pm$ 0.07	0.37 $\pm$ 0.09
Kidney (1)	7.90 $\pm$ 0.14	2.38 $\pm$ 0.14
Brain	0.045 $\pm$ 0.001	0.030 $\pm$ 0.002
Muscle	6.73 $\pm$ 0.63	8.44 $\pm$ 1.97

* Values shown are the mean $\pm$ standard deviation ($n = 3$) of data from male rats, 158–172 g.

Table 3Biodistribution of [^{67}Ga][Ga-**2**] $^{1+}$ in Rats

Organ	Percentage of Injected Dose per Organ [*]	
	1 min	120 min
Blood	7.80 $\pm$ 0.16	1.34 $\pm$ 0.08
Heart	1.70 $\pm$ 0.14	1.60 $\pm$ 0.17
Lungs	1.31 $\pm$ 0.05	1.03 $\pm$ 0.15
Liver	28.0 $\pm$ 3.40	2.60 $\pm$ 0.16
Spleen	0.44 $\pm$ 0.04	0.35 $\pm$ 0.04
Kidney (1)	7.15 $\pm$ 0.70	3.42 $\pm$ 0.42
Brain	0.05 $\pm$ 0.01	0.03 $\pm$ 0.01
Muscle	7.49 $\pm$ 2.15	7.86 $\pm$ 2.03

^{*} Values shown are the mean $\pm$ standard deviation ($n = 3$) of data from male rats, 179–198 g.

Table 4Biodistribution of [^{67}Ga][Ga-3] $^{1+}$ in Rats

Organ	Percentage of Injected Dose per Organ [*]	
	1 min	120 min
Blood	23.0 $\pm$ 1.70	1.87 $\pm$ 0.40
Heart	0.85 $\pm$ 0.03	0.59 $\pm$ 0.03
Lungs	2.44 $\pm$ 0.22	0.71 $\pm$ 0.08
Liver	33.2 $\pm$ 1.10	2.79 $\pm$ 0.23
Spleen	0.68 $\pm$ 0.15	0.85 $\pm$ 0.30
Kidney (1)	6.13 $\pm$ 0.31	5.50 $\pm$ 0.37
Brain	0.104 $\pm$ 0.013	0.034 $\pm$ 0.006
Muscle	4.86 $\pm$ 0.79	3.91 $\pm$ 0.48

* Values shown are the mean $\pm$ standard deviation ($n = 3$) of data from male rats, 184–196 g.

Table 5Biodistribution of [^{67}Ga][Ga-4] $^{1+}$ in Rats

Organ	Percentage of Injected Dose per Organ [*]	
	1 min	120 min
Blood	5.3 ± 0.6	0.70 ± 0.11
Heart	2.0 ± 0.1	2.0 ± 0.1
Lungs	1.8 ± 0.2	1.1 ± 0.1
Liver	13 ± 2	0.56 ± 0.03
Spleen	0.45 ± 0.03	0.24 ± 0.05
Kidney (1)	7.5 ± 0.7	1.6 ± 0.24
Brain	0.057 ± 0.004	0.030 ± 0.002
Muscle	6.3 ± 0.3	7.8 ± 1.6

* Values shown are the mean ± standard deviation ($n = 3$) of data from male rats, 152–173 g.

Table 6Biodistribution of [^{67}Ga][Ga-5] $^{1+}$ in Rats

Organ	Percentage of Injected Dose per Organ [*]	
	1 min	120 min
Blood	15.0 $\pm$ 1.0	0.69 $\pm$ 0.07
Heart	1.43 $\pm$ 0.05	1.36 $\pm$ 0.02
Lungs	2.7 $\pm$ 0.2	0.96 $\pm$ 0.14
Liver	20 $\pm$ 4	2.2 $\pm$ 0.4
Spleen	0.57 $\pm$ 0.13	0.72 $\pm$ 0.13
Kidney (1)	7.57 $\pm$ 0.63	5.34 $\pm$ 0.72
Brain	0.09 $\pm$ 0.01	0.05 $\pm$ 0.01
Muscle	8.6 $\pm$ 1.5	7.4 $\pm$ 1.6

* Values shown are the mean $\pm$ standard deviation ($n = 3$) of data from male rats, 161–185 g.

Table 7Biodistribution of [^{86}Rb]RbCl in Rats

Organ	Percentage of Injected Dose per Organ [*]	
	1 min	120 min
Blood	3.61 $\pm$ 0.28	1.43 $\pm$ 0.08
Heart	2.86 $\pm$ 0.38	0.69 $\pm$ 0.02
Lungs	2.0 $\pm$ 0.3	1.11 $\pm$ 0.01
Liver	5.4 $\pm$ 0.8	14 $\pm$ 1
Spleen	0.32 $\pm$ 0.08	0.71 $\pm$ 0.13
Kidney (1)	6.7 $\pm$ 1.1	1.01 $\pm$ 0.04
Brain	0.08 $\pm$ 0.01	0.12 $\pm$ 0.01
Muscle	17 $\pm$ 4	42 $\pm$ 2

* Values shown are the mean $\pm$ standard deviation of data from male rats, 153–176 g; $n = 7$ at 1 minute, $n = 3$ at 120 minutes.

Myocardium-to-Non-target Tissue Ratios in Rats, Based on Radiotracer Biodistribution Calculated as a Percentage of the Injected Dose per Gram Tissue Wet Mass.

Table 8

Radiopharmaceutical	Heart/Blood		Heart/Lung		Heart/Liver	
	1 min	2 hrs	1 min	2 hrs	1 min	2 hrs
[⁶⁷ Ga][Ga-1] ¹⁺	6.5 ± 0.3	33.8 ± 4.9	2.13 ± 0.12	2.8 ± 0.4	1.13 ± 0.09	10.9 ± 2.2
[⁶⁷ Ga][Ga-2] ¹⁺	4.0 ± 0.4	23.3 ± 2.7	1.97 ± 0.29	2.6 ± 0.3	1.0 ± 0.2	7.2 ± 0.8
[⁶⁷ Ga][Ga-3] ¹⁺	0.68 ± 0.08	6.4 ± 1.3	0.58 ± 0.04	1.58 ± 0.08	0.33 ± 0.02	2.7 ± 0.3
[⁶⁷ Ga][Ga-4] ¹⁺⁻	7.6 ± 1.0	54 ± 10	1.85 ± 0.17	2.9 ± 0.4	1.8 ± 0.4	39.0 ± 3.0
[⁶⁷ Ga][Ga-5] ¹⁺	1.86 ± 0.08	37.9 ± 3.5	1.00 ± 0.05	2.4 ± 0.3	0.85 ± 0.22	7.2 ± 1.9
[^{99m} Tc]Tc-Sestamibi ^{1+*}	11.1 ± 0.2	208 ± 25 [†]	1.3 ± 0.2	6.5 ± 1.7 [†]	1.7 ± 0.1	5.3 ± 1.7 [†]

* From reference 11.

[†] Data from 60-minutes rather than 120-minutes post-injection.