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RESEARCH ARTICLE

Synthesis and stability studies of Ga-67 labeled phosphonium salts

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Delocalized lipophilic cations such as tri- and tetra-arylphosphonium are able to diffuse across the mitochondrial membrane, which allows them to selectively accumulate in cells with a high transmembrane potential ($\Delta\Psi_m$). The mitochondrial membrane potential of cancer cells and cardiomyocytes has been reported to be significantly higher than that of normal epithelial cells. This feature can be exploited for the selective accumulation of phosphonium derivatives for the purposes of molecular imaging using radionuclides. Four structurally related Ga(III)-phosphonium salts were synthesized and fully characterized and found to be modest in toxicity toward T98G human glioblastoma cells ($IC_{50} > 4$ mM). High-activity (100 MBq) analogs containing Ga-67 were also synthesized and their stabilities in phosphate-buffered saline and human serum were determined.

KEYWORDS

cancer, mitochondria, PET chemistry, phosphonium

1 | INTRODUCTION

Delocalized lipophilic cations (DLCs) such as tri- and tetra-arylphosphonium are able to diffuse across the mitochondrial membrane as its membrane potential is negative and the process occurs without the aid of endogenous transporters.[1–6] DLCs have been shown to selectively accumulate in cells with high transmembrane potential ($\Delta\Psi_m$).[7–9] For example, the difference in $\Delta\Psi_m$ between the colon carcinoma cell line CX-1 and the control green monkey kidney epithelial cell line (CV-1) is reported to be approximately 60 mV (163 mV in tumor cells vs 104 mV in normal cells).[9] By exploiting the difference in $\Delta\Psi_m$ between the 2 mammalian cell lines, several researchers have reported the synthesis of several Cu-64 labeled phosphonium complexes for positron emission tomography (PET) imaging, some of which have shown a high selectivity for tumors.[10–12]

The incorporation of radiolabels such as H-3, C-11, F-18, and I-125 into phosphonium salts have also proved useful for medical (PET and SPECT) imaging. For example, tritiated phosphonium cations such as [^{3}H]-tetraphenylphosphonium have been widely used as *in vitro* tumor probes,[13] and [^{11}C]-triphenylmethylphosphonium has been used to determine the kinetics and tumor selectivity in canine brain glioma by PET imaging.[14] 4-([^{18}F]-fluorophenyl)triphenylphosphonium is another probe developed as a potential myocardial blood flow agent for PET imaging.[15] More recently, [^{125}I]-*p*-iodobenzyl triphenyl phosphonium, [^{125}I]-*p*-iodobenzyl dipropylphenyl phosphonium, and [^{125}I]-*p*-iodobenzylmethyldiphenylphosphonium were also investigated as potential myocardial SPECT imaging agents, and all these agents showed good heart tissue uptake.[16]

Most phosphonium compounds have used several synthetic steps following radiolabeling, an inefficient strategy when the short half-lives of many radioisotopes are considered.[17] This reduces the overall radiochemical yield and limits potential radionuclides to those with longer half-lives.

Previously, our research group has investigated similar Gd(III) phosphonium salts as potential tumor mitochondrial targeting agents for neutron capture and photon activation therapies.[18,19] *In vitro* studies of these compounds confirmed their modest toxicity ($IC_{50} > 1$ mM), high uptake by T98G human glioblastoma cells (>1500 ng Gd/mg protein), and high selectivity of tumor to normal cells compared with the control noncancerous SVGp12 fetal glial cell line. All these factors make the phosphonium compounds good potential imaging agents with radiometals such as Ga-67. The primary aim of this study was to develop a rapid method for the synthesis of structurally related Ga-67 phosphonium-based agents in high radiochemical yield and purity for PET imaging. Herein, we report the synthesis of 4 structurally related Ga(III)-phosphonium salts and radiolabeled analogs

containing Ga-67. Their stabilities in phosphate-buffered saline (PBS) and human serum were also determined.

2 | RESULTS AND DISCUSSION

2.1 | Syntheses

Four structurally related phosphonium ligands **L1** to **L4** containing a phosphonium center, a xylyl linker and the macrocycle DO3A (1,4,7,10-tetraazacyclododecane,-*N*,*N*′,*N*″-triacetic acid) were synthesized[18,19] according to Scheme 1 and purified using reversed-phase high-pressure liquid chromatography (HPLC) on a C18 column. The "cold" Ga complexes were synthesized in high purity by addition of $GaCl_3$ to ligands **L1** to **L4** in aqueous solution.

2.2 | Radiolabeling studies

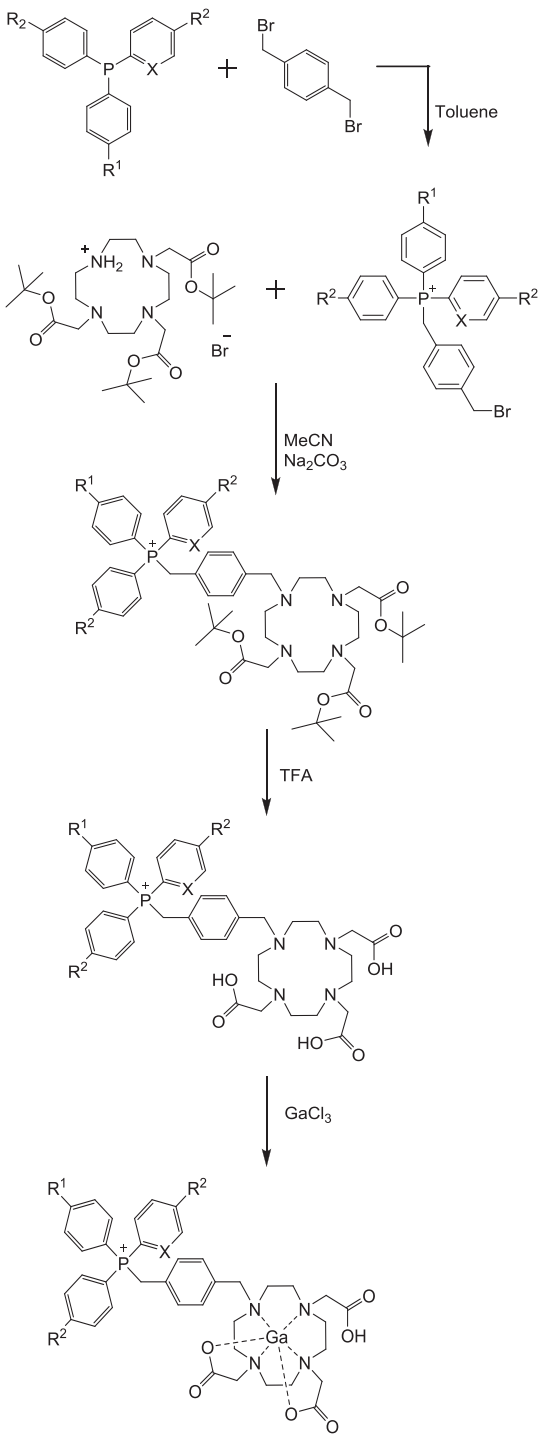
Ligands **L1** to **L4** were efficiently complexed with $^{67}Ga^{3+}$ under typical conditions (80°C, 10 minutes), giving radiochemical yields (based on radio-HPLC) of >95% for all complexes (Table 1).

The Ga-67 radiolabeled complexes containing **L1** to **L4** were purified using a C18 SPE and optimally eluted (>90% recovery of activity) in a 1:1 mixture of EtOH/0.9% saline solution. Ethanol content of <40% gave poor recoveries of the more lipophilic complexes **^{67}Ga·L2** and **^{67}Ga·L4**. Removal of the EtOH gave a suitably formulated product for stability and serum protein binding studies. In all cases, except for **^{67}Ga·L3**, the final product purity was >95%, as determined by radio-HPLC. An uncharacterized minor impurity (<2%) in **L3** appears to complex ^{67}Ga preferentially. The modification of reaction parameters (time, temperature, and ligand stoichiometry) had little effect on the product distribution. Attempts to purify **^{67}Ga·L3** by the above-mentioned method also proved problematic. Although adequate separation of the 2 products was achieved, the overall recovery of injected activity was <30%. Recoveries were significantly improved by the addition of **^{nat}Ga·L3** to the injections, but this product was unsuitable for the stability studies.

2.3 | Stability studies

The stabilities of the Ga-67 radiolabeled complexes containing **L1** to **L4** were examined in Dulbecco's PBS (dPBS). Data for all the time points examined in this study are presented in Table 2. All of the radiolabeled complexes demonstrated good stability in dPBS. HPLC chromatograms indicated minor loss of $^{67}Ga^{3+}$ from the complexes as well as the formation of polar fragments, presumably formed by the cleavage of the ^{67}Ga·DO3A-xylyl moiety from the phosphonium salt.

Studies involving human serum allowed for the collection of 2 sets of data: serum protein binding (Table 3) and stability in human serum (Table 4). Complexes **^{67}Ga·L1, ^{67}Ga·L2,**



1: R^1= I, R^2= H, X=CH; **2**: R^1= MeO, R^2= MeO, X=CH
3:R^1= H, R^2= H, X=N; **4**: R^1= Me, R^2= Me, X=CH

SCHEME 1 Synthesis of Complexes 1–4.

and **^{67}Ga·L4** all showed the rapid (>1 hour) association of 5% to 6% of their activity with serum proteins (Table 3). This association increased slightly over time, but it appears to be a system of limited capacity. At the 24-hour time point, 2% to 3% of the activity was associated with human serum albumin proteins.

The stability of the ^{67}Ga complexes in human serum was examined by removing the majority of proteins (>99%) by

TABLE 1 Radiochemical yield and purity and SPE purification recovery of Ga-67 complexes containing **L1** to **L4**

Complex	Radiochemical yield (HPLC)[a]	Radiochemical purity[a,b]	SPE purification recovery (%)
^{67}Ga·L1	98.3 ± 0.5 (n = 6)	95.6 ± 0.9 (n = 3)	98.2 ± 0.9 (n = 3)
^{67}Ga·L2	97.2 ± 0.5 (n = 6)	97.4 ± 1.2 (n = 3)	94.4 ± 2.4 (n = 3)
^{67}Ga·L3	97.6 ± 0.4 (n = 3)	91.7 ± 1.8 (n = 3)	95.6 ± 1.4 (n = 3)
^{67}Ga·L4	96.9 ± 0.6 (n = 3)	98.5 ± 0.4 (n = 3)	91.5 ± 1.0 (n = 3)

[a]Based on HPLC peak integrations.

[b]After SPE purification.

TABLE 2 Stability of Ga-67 complexes with **L1** to **L4** in dPBS at all time points

Complex	1 h	2 h	4 h	24 h
^{67}Ga·L1	93.5	94.4	94.0	88.2
^{67}Ga·L2	94.7	96.1	95.8	92.5
^{67}Ga·L3	100	100	99.8	99.7
^{67}Ga·L4	96.9	97.1	96.2	93.9

ultracentrifugation using a 3000 MWCO filter, providing a filtrate that was suitable for injection on a standard C18 HPLC column. Table 4 shows the stability of these complexes at all time points. Two of the complexes (**^{67}Ga·L1** and **^{67}Ga·L3**) showed good stability for up to 2 hours. All complexes showed some degradation after 24 hours, but **^{67}Ga·L3** remained mostly intact.

The metabolic profile of the complexes **^{67}Ga·L1**, **^{67}Ga·L2**, **^{67}Ga·L3**, and **^{67}Ga·L4** were investigated using radio-HPLC after 24 hours (Figure 1). Because the complexes are metabolizing in serum, it is unclear if the binding is associated with the breakdown products or the original complexes. The main metabolite of these complexes mainly shows up in 2 regions (1.5–2.3 and 2.4–3.7 minutes) of the HPLC chromatograms. The low retention time points to the metabolites being polar in nature likely formed from the cleavage of the phosphonium group, presumably at the xylyl-bridging group. The first region in the radio-HPLC chromatogram comprises unbound ^{67}Ga and ^{67}Ga(DO3A), and the second region comprises ^{67}Ga(DO3A)-xylyl metabolites.

3 | CONCLUSION

We have synthesized and evaluated the stability of 4 new Ga-67-labeled phosphonium complexes (**^{67}Ga·L1**, **^{67}Ga·L2**, **^{67}Ga·L3**, and **^{67}Ga·L4**). All complexes were stable in PBS but showed signs of metabolic degradation after 24 hours in human serum. The limited solution stability of the complexes after extended periods allows for possible future application to the shorter-lived Ga-68 isotope.[17] PET has several technical merits over SPECT, such as higher spatial resolution and more accurate attenuation correction. Ga-68 is an excellent positron emitter (511-keV annihilation radiation; $t_{1/2}$ = 68 minutes) suitable for PET imaging and is available from a Ge-68/Ga-68 generator, which renders it independent of an on-site cyclotron. The short overall time required for labeling and purification using the method reported here allows for future imaging applications involving selected Ga-68 complexes.

TABLE 4 Stability of Ga-67 complexes with **L1** to **L4** in human serum at all time points

Complex	1 h	2 h	4 h	24 h
^{67}Ga·L1	84.45	76.30	73.19	56.26
^{67}Ga·L2	90.70	49.77	44.56	28.16
^{67}Ga·L3	90.78	92.21	91.06	72.49
^{67}Ga·L4	92.08	54.67	43.04	14.40

4 | EXPERIMENTAL

4.1 | Materials and methods

Distilled water was used for all experiments requiring water. THF and MeCN were dried before use by following the methods of Armarego and Chai.[20] THF was dried over sodium wire and freshly distilled from benzophenone ketyl. Anhydrous MeCN was freshly distilled from CaH_2. All other solvents were used without further purification.

All precursor chemicals were commercially available. 1,4,7,10-Tetraazacyclododecane (cyclen) was purchased from Nowapharm (China). Gallium(III) chloride was purchased from Strem. All other chemicals were purchased from Sigma-Aldrich Co.

Column chromatography was conducted on Grace Davison LC60A 40–63 μm silica column. Thin-layer chromatography was conducted on Merck Kieselgel 60 F_{254} aluminum back plates. Visualization of plates was achieved by using a 254 nm light.

TABLE 3 Binding of Ga-67 complexes with **L1** to **L4** to human serum proteins at all time points

Complex	1 h		2 h		4 h		24 h	
	HSA	Other	HSA	Other	HSA	Other	HSA	Other
^{67}Ga·L1	0	4.73	0	4.72	0	6.90	1.95	5.44
^{67}Ga·L2	0	5.97	0	6.59	0	6.93	3.08	7.38
^{67}Ga·L3	0	0	0	0	0	0	2.20	0
^{67}Ga·L4	0	5.77	0	5.75	0	6.08	2.98	7.78

HSA indicates human serum albumin.

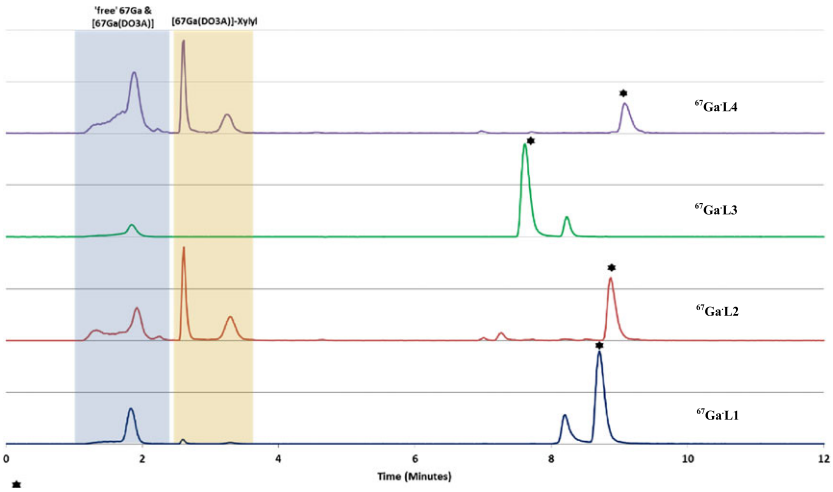


FIGURE 1 Radio-HPLC chromatograms of serum ultrafiltrates after 24 hours

4.2 | Instrumentation

All ^{1}H, $^{13}C\{^{1}H\}$, and $^{31}P\{^{1}H\}$ NMR spectra were recorded on a Bruker Avance200 (^{1}H at 200 MHz and ^{13}C at 50 MHz), a Bruker Avance300 (^{1}H at 300 MHz, ^{13}C at 75 MHz, and ^{31}P at 121 MHz), or a Bruker Avance^{III}500 (^{1}H at 500 MHz, ^{13}C at 125 MHz, and ^{31}P at 202 MHz) NMR spectrometer at 300 K. All NMR signals (δ) are reported in parts per million. ^{1}H NMR spectra recorded in $CDCl_3$ were referenced to TMS and to their residual solvent peaks in all other solvents. $^{13}C\{^{1}H\}$ NMR spectra were referenced to their solvent peaks (except for D_2O). $^{13}C\{^{1}H\}$ NMR spectra in D_2O were referenced according to an internal standard of TMS (25.145020 MHz). $^{31}P\{^{1}H\}$ NMR spectra were referenced according to internal standards of H_3PO_4 (40.480742 MHz). Coupling constants ($^{n}J_{ij}$) are reported in Hz. Peak multiplicities have been abbreviated as s (singlet), d (doublet), t (triplet), br (broad), and m (multiplet—unassignable multiplicity).

Low-resolution ESI-MS were recorded on a Finnigan LCQ mass spectrometer. High-resolution ESI-FT-ICR-MS data were recorded on a Bruker 7.0T mass spectrometer.

4.3 | HPLC methods

All HPLC methods used a Waters HPLC system equipped with a UV/vis detector (λ = 254, 230 nm).

4.3.1 | Method 1

Preparative: A Waters C18 preparative (19 mm × 150 mm, 5 µm pore size) column was used. The flow rate was 7 mL/min. The mobile phase had a gradient 0 to 45 minutes, from 100% solvent A (0.1% TFA in water) and 0% solvent B (0.1% TFA in MeCN) to 0% solvent A and 100% solvent B.

Analytical: A Waters C18 analytical (4.6 mm × 150 mm, 5 µm pore size) column was used. The flow rate was 0.7 mL/min. The mobile phase had a gradient 0 to 45 minutes, from 100% solvent A (0.1% TFA in water) and 0% solvent B (0.1% TFA in MeCN) to 0% solvent A and 100% solvent B.

4.3.2 | Method 2

Semipreparative: An Atlantis T3 semipreparative (10 mm × 250 mm, 5 µm pore size) column was used. The flow rate was 3 mL/min. The mobile phase was isocratic 30% MeCN, 60% water and with 10% 0.1 M Na_2HPO_4 buffered at pH 7.

Analytical: An Atlantis T3 analytical (4.6 mm × 150 mm, 3 µm pore size) column was used. The flow rate was 0.8 mL/min. The mobile phase was isocratic with 0.01 M Na_2HPO_4 in 30% MeCN and 70% water buffered at pH 7.

4.3.3 | Method 3

Semipreparative: An Atlantis T3 semipreparative (410 mm × 250 mm, 5 µm pore size) column was used. The flow rate was 3 mL/min. The mobile phase was isocratic 25% MeCN, 65% water and with 10% 0.1 M Na_2HPO_4 buffered at pH 7.

Analytical: An Atlantis T3 analytical (4.6 mm × 150 mm, 3 µm pore size) column was used. The flow rate was 0.8 mL/min. The mobile phase was isocratic with 0.01 M Na_2HPO_4 in 20% MeCN and 80% water buffered at pH 7.

4.4 | *In vitro* cytotoxicity assays

In vitro cytotoxicity assays were performed using human glioblastoma multiforme (T98G) cells. The cytotoxicity of complexes was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded (density 1 × 10^4 cells per well) in growth medium (minimum essential medium Eagle supplemented with 10% fetal bovine serum, penicillin (100 U), streptomycin (100 µg/mL), and L-glutamine (2.5 mM, 100 µL) using 96-well plates and were allowed to adhere overnight at 37°C. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in the presence of **1** or the vehicle (control). Serial dilutions of the complex were added to triplicate wells. Maximum concentration (MaxC) for the experiments was 4 mM. After 72 hours, the MTT solution in PBS (30 µL, 0.17% w/v)

was added, and the incubation was continued. After a further 4 hours, the culture medium and excess MTT solution were removed and the resulting MTT-formazan crystals dissolved by addition of 150 μL DMSO. Cell viability was determined by measuring the absorbance at 600 nm using a Victor3V microplate reader (PerkinElmer). All readings were corrected for absorbance from wells containing the vehicle alone, and the level of MTT was expressed relative to the corresponding vehicle-treated controls as % viability.

5 | SYNTHESES

5.1 | General synthetic method 1: bromoxylyl phosphonium salt precursors

A solution of the phosphine in PhMe was added dropwise to a solution of *a,a* -dibromo-*m*-xylene in the same solvent. For the synthesis of **P3**, the resulting solution was heated at reflux for 4 hours, whereas for the synthesis of **P4**, the resulting solution was stirred at room temperature for 20 hours. The precipitate was filtered off and washed with PhMe and diethyl ether.

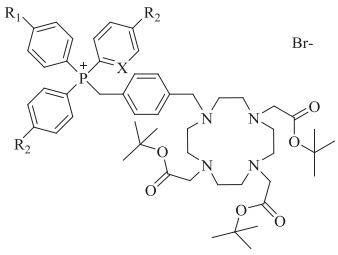
5.2 | (4-(Bromomethyl)benzyl)diphenyl(pyridin-2-yl) phosphonium bromide (P3)

Yield: 1.46 g (72.4%). ESI-MS: *m/z* 447.93 ($[M\text{-}Br^-]^+$). ^{1}H NMR ($CDCl_3$) δ 8.93 (d, 1H, Ph, $^2J_{HP}$ = 4.5 Hz), 8.39–8.34 (m, 1H, Ph), 8.09–8.07 (m, 1H, Ph), 7.80–7.74 (m, 11H, Ph), 7.15–7.07 (m, 4H, Ph), 5.48 (d, 2H, Ph, $^4J_{HP}$ = 15 Hz), 4.38 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 152.0 (d, Ph, $^1J_{CP}$ = 105 Hz), 144.8 (s, Ph), 143.3 (s, Ph), 138.4 (d, Ph, $^3J_{CP}$ = 21 Hz), 138.1 (d, Ph, $^3J_{CP}$ = 15 Hz), 135.2 (s, Ph), 134.6 (d, Ph, $^2J_{CP}$ = 39 Hz), 131.1 (d, Ph, $^1J_{CP}$ = 93 Hz), 131.6 (d, Ph, $^2J_{CP}$ = 39 Hz),130.1 (d, Ph, $^1J_{CP}$ = 48 Hz), 129.5 (s, Ph, $^3J_{CP}$ = 12 Hz), 128.3 (s, Ph), 127.5 (d, Ph, $^2J_{CP}$ = 33 Hz), 117.1 (s, Ph), 115.9 (s, Ph), 32.8 (s, CH_2), 29.5 (d, CH_2, $^1J_{CP}$ = 183 Hz). ^{31}P NMR ($CDCl_3$) δ 19.5 (s).

5.3 | (4-(Bromomethyl)benzyl)tri-*p*-tolylphosphonium bromide (P4)

Yield: 0.89 g (94.9%). ESI-MS: *m/z* 535.00 ($[M\text{-}Br^-]^+$). ^{1}H NMR ($CDCl_3$) δ 7.62 (m, 9H, Ph), 7.60 (m, 6H, Ph), 7.58 (m, 1H, Ph), 7.55 (m, 1H, Ph), 7.37 (s, 4H, Ph), 7.05 (d, 1H, Ph, $^4J_{HP}$ = 1.16 Hz), 5.15 (d, 2H, CH_2, $^2J_{HP}$ = 14.52 Hz), 3.90 (s, 2H, CH_2), 3.90 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$) δ 146.3 (s, Ph), 137.9 (d, Ph, $^3J_{CP}$ = 15 Hz), 134.8 (s, Ph), 134.1 (d, Ph, $^2J_{CP}$ = 39 Hz), 131.7 (d, Ph, $^3J_{CP}$ = 21 Hz), 130.8 (d, Ph, $^1J_{CP}$ = 54 Hz), 129.2 (d, Ph, $^3J_{CP}$ = 9 Hz), 127.5 (d, Ph, $^2J_{CP}$ = 36 Hz), 118.2 (s, Ph), 117.0 (s, Ph), 114.0 (s, Ph), 112.8 (s, Ph), 32.7 (s, CH_2), 30.3 (d, CH_2, $^1J_{CP}$ = 189 Hz), 21.7 (s, CH_3). ^{31}P NMR ($CDCl_3$) δ 21.2 (s).

5.4 | General synthetic method 2: ^{t}butyl-protected phosphonium-DO3A ligands



C3: R_1= H, R_2= H, X=N
C4: R_1= Me, R_2= Me, X=CH

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A solution of DO3A-^{t}Bu$_3$·HBr, phosphonium salt, and Na_2CO_3 in MeCN was stirred at reflux for 20 hours. The mixture was filtered, the solvent was removed *in vacuo*, and the residue was recrystallized from acetone/diethyl ether to yield a colorless solid.

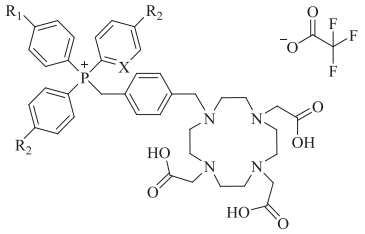
5.5 | Diphenyl(pyridin-2-yl)(4-((4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl) methyl)benzyl)phosphonium bromide (C3)

Yield: 1.24 g (84.6%). ESI-MS: *m/z* 880.52 ($[M\text{-}Br^-]^+$). ^{1}H NMR ($CDCl_3$) δ 8.17–8.07 (m, 2H, Ph), 7.78–7.64 (m, 2H, Ph), 7.53–7.42 (m, 8H, Ph), 7.22–7.19 (m, 2H, Ph), 7.10–7.07 (m, 2H, Ph), 5.35 (br, 2H, CH_2), 4.12 (s, 9H, CH_3), 3.59 (s, 2H, CH_3), 3.03–2.23 (br m, 25H, CH_2), 1.46 (s, 18H, CH_3), 1.45 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$) δ 173.5 (s, C = O), 172.6 (s, C = O), 134.7–128.5 (m, Ph), 83.0 (s, C), 82.8 (s, C), 56.1 (m, CH_2), 49.7 (m, CH_2), 28.1 (s, CH_3), 28.0 (s, CH_3). ^{31}P NMR ($CDCl_3$) δ 19.7 (s).

5.6 | Tri-*p*-tolyl(4-((4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl) benzyl)phosphonium bromide (C4)

Yield: 0.89 g (85.0%). ESI-MS: *m/z* 922.00 ($[M\text{-}Br^-]^+$). ^{1}H NMR ($CDCl_3$) δ 7.58–7.52 (m, 6H, Ph), 7.46–7.42 (m, 6H, Ph), 7.07–7.05 (m, 4H, Ph), 5.18 (br, 2H, CH_2), 3.69 (s, 9H, CH_3), 3.72 (s, 2H, CH_3), 3.05–2.28 (br m, 25H, CH_2), 1.49 (s, 18H, CH_3), 1.47 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$) δ 173.4 (s, C = O), 172.5 (s, C = O), 146.2 (s, Ph), 135.8 (s, Ph), 134.1 (d, Ph, $^3J_{CP}$ = 39 Hz), 131.3 (s, Ph), 130.9 (d, Ph, $^3J_{CP}$ = 54 Hz), 130.6 (s, Ph), 127.0 (d, Ph, $^2J_{CP}$ = 33 Hz), 114.9 (s, Ph), 113.7 (s, Ph), 82.8 (s, C), 82.4 (s, C), 58.0 (s, CH_2), 56.6 (s, CH_2), 55.8 (s, CH_2), 50.8 (m, CH_2), 49.1 (m, CH_2), 30.9 (d, CH_2, $^1J_{CP}$ = 195 Hz), 28.0 (s, CH_3), 27.8 (s, CH_3), 21.8 (s, CH_3). ^{31}P NMR ($CDCl_3$) δ 22.9 (s).

5.7 | General synthetic method 3: phosphonium-DO3A ligands



L3: R_1= H, R_2= H, X=N
L4: R_1= Me, R_2= Me, X=CH

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The coupled ligand **C3** or **C4** (0.5 g) was dissolved in trifluoroacetic acid (5 mL) and stirred at room temperature for 24 hours. The solvent was removed *in vacuo*, and the crude residue dissolved in H_2O (100 mL) and extracted with $CHCl_3$ (3 × 20 mL). The aqueous layer was reduced *in vacuo* to afford an off-white solid. This solid was then purified by reversed-phase HPLC (method 1), and the product fractions were lyophilized to afford a fluffy white solid.

Ligands **L1** and **L2** were prepared as reported previously.[18,19]

5.8 | Diphenyl(pyridin-2-yl)(4-((4,7,10-tris (carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl) methyl)benzyl)phosphonium trifluoroacetate (L3)

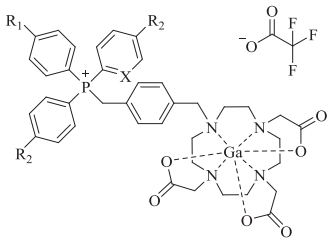
Yield: 0.37 g (86.5%). HPLC retention time (method 1) = 16.32 minutes. ESI-MS: *m/z* 836.93 ($[M\text{-}Br^-]^+$). ^{1}H NMR (D_2O) δ 7.90–7.87 (m, 2H, Ph), 7.80–7.75 (m, 2H, Ph), 7.61–7.50 (m, 8H, Ph,), 7.38–7.36 (m, 2H, Ph), 7.27–7.21 (m, 2H, Ph), 7.02–7.00 (m, 2H, Ph), 4.72 (d, 2H, CH_2, $^2J_{HP}$ = 14.7 Hz), 4.35 (br s, 2H, CH_2), 4.03 (br s, 2H, CH_2), 3.4–3.1 (br m, 20H, CH_2). ^{13}C NMR ($CDCl_3$) δ 162.1 (s, C = O), 162.6 (s, C = O), 152.0 (d, Ph, $^1J_{CP}$ = 75 Hz), 143.7 (s, Ph), 142.2 (s, Ph), 138.6 (d, Ph, $^2J_{CP}$ = 42 Hz), 134.2 (d, Ph, $^2J_{CP}$ = 39 Hz), 130.0 (s, Ph, $^1J_{CP}$ = 51 Hz), 118.2 (s, Ph), 116.4 (s, Ph), 115.2 (s, Ph), 114.4 (s, Ph), 110.5 (s, Ph), 57.1 (s, CH_2), 55.0 (s, CH_2), 53.2 (s, CH_2), 50.8 (m, CH_2), 48.5 (m, CH_2), 28.7 (d, CH_2, $^1J_{CP}$ = 192 Hz). ^{31}P NMR (D_2O) δ 22.8 (s).

5.9 | Tri-*p*-tolyl(4-((4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl)benzyl)phosphonium trifluoroacetate (L4)

Yield: 0.32 g (79.9%). HPLC retention time (method 1) = 21.17 minutes. ESI-MS: *m/z* 753.73 ($[M\text{-}Br^-]^+$). ^{1}H NMR (D_2O) δ 7.36–7.24 (m, 14H, Ph), 6.97–6.95 (m, 2H, Ph), 4.56 (d, 2H, CH_2, $^2J_{HP}$ = 15 Hz), 4.25 (br s, 2, CH_2), 4.02 (br s, 2, CH_2), 3.4–3.1 (br m, 20H, CH_2), 2.27 (s, 9 H, CH_3).^{13}C NMR (D_2O) δ 162.7 (s, C = O), 162.2 (s, C = O), 146.9 (s, Ph), 133.5 (d, Ph, $^2J_{CP}$ = 39 Hz), 132.0 (s, Ph), 131.3 (s, Ph), 130.6 (d, Ph, $^1J_{CP}$ = 51 Hz), 122.1 (s, Ph), 118.2 (s, Ph), 114.3 (d, Ph, $^2J_{CP}$ = 54 Hz), 113.0 (s, Ph), 110.5 (s, Ph), 57.2 (s, CH_2), 54.7 (s, CH_2), 53.0 (s, CH_2), 51.1 (m, CH_2), 49.5 (m, CH_2), 48.4 (m, CH_2), 29.7 (d, CH_2, $^1J_{CP}$ = 198 Hz), 20.8 (s, CH_3). ^{31}P NMR (D_2O) δ 21.3 (s).

5.10 | General synthetic method 4: "cold" Ga(III) complexes

.



1: R_1=I, R_2=H, X=CH
2: R_1=MeO, R_2=MeO, X=CHR
3: R_1=H, R_2=H, X=N
4: 1=Me, R_2=Me, X=CH

Equimolar amounts of the deprotected ligands **L1** to **L4** (50 mg) and $GaCl_3$ was stirred in water (5 mL) at room temperature for 72 hours. The reaction mixture was then purified by reversed-phase HPLC (method 1), and the product fractions were lyophilized to yield the product as a fluffy white powder.

5.11 | 4-Iodophenyl)diphenyl(4-((4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl) benzyl) phosphoniumgallium(III) trifluoroacetate (1)

Yield: 11.8 mg (24.7%). HPLC retention time (method 1) = 19.38 minutes. ESI-FT-ICR-MS for $[M\text{-}CF_3CO_2]^+$ calculated *m/z* 903.12935; found 903.12859. ^{1}H NMR (D_2O) 8.08–8.06 (m, 2H, Ph), 7.91–7.90 (m, 2H, Ph), 7.71–7.69 (m, 8H, Ph), 7.35–7.31 (m, 4H, Ph), 7.07–7.06 (m, 2H, Ph), 4.84 (m, 2H, CH_2), 4.06–3.94 (m, 8H, CH_2), 3.57–3.54 (m, 6H, CH_2), 3.42–3.32 (m, 8H, CH_2), 2.98 (d, 2H, CH_2, $^2J_{HH}$ = 11.5 Hz). ^{13}C NMR (D_2O) δ 173.8 (s, C = O), 173.6 (s, C = O), 163.0 (d, Ph, $^1J_{CP}$ = 145 Hz), 139.1 (s, Ph, $^2J_{CP}$ = 50 Hz), 135.0 (d, Ph, $^3J_{CP}$ = 40 Hz), 134.0 (d, Ph, $^3J_{CP}$ = 40 Hz), 132.0 (d, Ph, $^5J_{CP}$ = 15 Hz), 131.3 (d, Ph, $^4J_{CP}$ = 25 Hz), 130.8 (d, Ph, $^4J_{CP}$ = 15 Hz), 130.0 (d, Ph, $^2J_{CP}$ = 50 Hz), 128.8 (d, Ph, $^3J_{CP}$ = 35 Hz), 119.8 (s, Ph), 117.5 (s, Ph), 117.1 (d, Ph, $^1J_{CP}$ = 55 Hz), 116.4 (d, Ph, $^2J_{CP}$ = 50 Hz), 115.2 (s, Ph), 112.9 (s, Ph), 103.4 (d, Ph, $^4J_{CP}$ = 15 Hz), 64.2 (s, CH_2), 63.3 (s, CH_2), 59.4 (s, CH_2), 57.3 (s, CH_2), 57.0 (s, CH_2), 54.5 (s, CH_2), 54.1 (s, CH_2), 29.1 (d, CH_2, $^1J_{CP}$ = 195 Hz). ^{31}P NMR (D_2O) δ 23.2 (s).

5.12 | Tris(4-methoxyphenyl)(4-((4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl) benzyl)phosphoniumgallium(III) trifluoroacetate (2)

Yield: 28.3 mg (58.5%). HPLC retention time (method 1) = 20.22 minutes. ESI-FT-ICR-MS for $[M\text{-}CF_3CO_2]^+$ calculated *m/z* 867.26440; found 867.26308. ^{1}H NMR (D_2O) δ 7.49–7.42 (m, 6H, Ph), 7.29–7.26 (m, 2H, Ph), 7.13–7.12

(m, 6H, Ph), 7.03–7.00 (m, 2H, Ph), 4.62 (d, 2H, CH_2, $^2J_{HP}$ = 14.7 Hz), 4.07–4.03 (m, 2H, CH_2), 3.94 (s, 6H, CH_3), 3.89 (s, 9H, CH_3), 3.5–3.3 (m, 14H, CH_2), 2.99 (d, 2H, CH_2, $^2J_{HH}$ = 10.2 Hz). ^{13}C NMR (D_2O) δ 173.7 (s, C = O), 164.3 (s, Ph), 163.1 (s, Ph), 162.6 (s, Ph), 135.8 (d, Ph, $^2J_{CP}$ = 45 Hz), 131.8 (s, Ph), 131.2 (s, Ph), 130.5 (s, Ph), 129.4 (s, Ph), 118.3 (s, Ph), 115.6 (d, Ph, $^1J_{CP}$ = 54 Hz), 114.4 (s, Ph), 108.8 (s, Ph), 107.6 (s, Ph), 59.4 (s, CH_2), 57.3 (s, CH_2), 57.0 (s, CH_2), 55.8 (s, CH_2), 54.5 (s, CH_2), 54.0 (s, CH_2). ^{31}P NMR (D_2O) δ 20.5 (s).

5.13 | Diphenyl(pyridin-2-yl)(4-((4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl) benzyl) phosphoniumgallium(III) trifluoroacetate (3)

Yield: 4.4 mg (9.2%). HPLC retention time (method 1) = 14.51 minutes. ESI-FT-ICR-MS for $[M\text{-}CF_3CO_2]^+$ calculated *m/z* 778.22795; found 778.22777. ^{1}H NMR (D_2O) δ 8.93–8.92 (m, 1H, Ph), 8.11–8.09 (m, 1H, Ph), 7.91–7.86 (m, 4H, Ph), 7.69–7.29 (m, 8H, Ph), 7.16–7.14 (m, 2H, Ph), 7.08–7.05 (m, 2H, Ph), 4.90–4.85 (m, 2H, CH_2), 4.05–3.93 (m, 8H, CH_2), 3.76–3.73 (m, 2H, CH_2), 3.55–3.29 (m, 12H, CH_2), 3.01–2.98 (m, 2H, CH_2). ^{31}P NMR (D_2O) δ 19.5 (s).

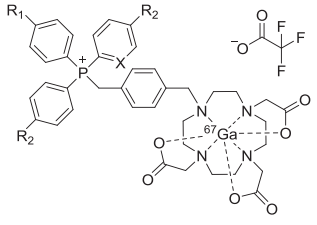
5.14 | Tri-*p*-tolyl(4-((4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl)benzyl) phosphoniumgallium(III) trifluoroacetate (4)

Yield: 26.0 mg (53.7%). HPLC retention time (method 1) = 20.87 minutes. ESI-FT-ICR-MS for $[M\text{-}CF_3CO_2]^+$ calculated *m/z* 819.27965; found 819.27947. ^{1}H NMR (D_2O) δ 7.76–7.60 (m, 12H, Ph), 7.39 (br s, 2H, Ph), 7.21 (br s, 2H, Ph), 4.41 (br s, 2H, CH_2), 4.04– 3.99 (m, 4H, CH_2), 3.5–3.1 (m, 24H, CH_2). ^{13}C NMR (D_2O) δ 163.1 (s, C = O), 162.7 (s, C = O), 133.0 (s, Ph), 131.3 (s, Ph), 131.0 (d, Ph, $^2J_{CP}$ = 36 Hz), 129.0 (d, Ph, $^1J_{CP}$ = 48 Hz), 128.7 (s, Ph), 118.1 (s, Ph), 114.2 (s, Ph), 57.3 (s, CH_2), 55.1 (s, CH_2), 53.0 (s, CH_2), 51.1 (s, CH_2), 49.4 (s, CH_2), 48.3 (s, CH_2), 29.6 (d, CH_2, $^1J_{CP}$ = 261 Hz). ^{31}P NMR (D_2O) δ 38.2 (s).

6 | RADIOLABELING STUDIES

6.1 | General synthetic method 5: Ga-67 complexes

.



^{67}Ga-L1: R_1=I, R_2=H, X=CH
^{67}Ga-L2: R_1=MeO, R_2=MeO, X=CH
^{67}Ga-L3: R_1=H, R_2=H, X=N
^{67}Ga-L4: R_1=Me, R_2=Me, X=CH

$^{67}GaCl_3$ in 0.1 M HCl and HEPES buffer (1.0 M) were mixed in a ratio of 2:1 in an acid-washed 1.5 mL Eppendorf tube. The pH was adjusted with 0.5 M KOH to 4 to 4.5, and 5 nmol of deprotected ligand (**L1–L4**) was added. The mixture was heated on a heating block at 80°C for 10 minutes. The crude product was purified by HPLC (method 2 for **^{67}Ga-L1**, **^{67}Ga-L2**, and **^{67}Ga-L4**; method 3 for **^{67}Ga-L3**). The purified fractions were diluted to 20 mL and loaded onto Oasis HLB (hydrophilic-lipophilic–balanced reversed-phase sorbent) sample extraction product and washed with water (3 × 3 mL). The compound was eluted off with a 1:1 mixture of EtOH/0.9% saline solution, which was then removed using a centrifuge evaporator.

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REFERENCES

1. Adams DM, Ji W, Barth RF, Tjarks W. Comparative *in vitro* evaluation of dequalinium B, a new boron carrier for neutron capture therapy (NCT). *Anticancer Res*. 2000;20:3395–3402.
2. Modica-Napolitano JS, Aprille JR. Delocalized lipophilic cations selectively target the mitochondria of carcinoma cells. *Adv Drug Deliv Rev*. 2001;49:63–70.
3. Rowe TC, Weissig V, Lawrence JW. Mitochondrial DNA metabolism targeting drugs. *Adv Drug Deliv Rev*. 2001;49:175–187.
4. Takasu K, Shimogama T, Saiin C, et al. Synthesis and evaluation of β-carbolinium cations as new antimalarial agents based on π-delocalized lipophilic cation (DLC) hypothesis. *Chem Pharm Bull*. 2005;53:653–661.
5. Modica-Napolitano JS, Nalbandian R, Kidd ME, Nalbandian A, Nguyen CC. The selective in vitro cytotoxicity of carcinoma cells by AZT is enhanced by concurrent treatment with delocalized lipophilic cations. *Cancer Lett*. 2003;198:59–68.
6. Galeano E, Nieto E, Garcia-Perez AI, et al. Effects of the antitumoural dequalinium on NB4 and K562 human leukemia cell lines. Mitochondrial implication in cell death. *Leuk Res*. 2005;29:1201–1211.
7. Johnson LV, Walsh ML, Chen LB. Localization of mitochondria in living cells with rhodamine 123. *Proc Natl Acad Sci U S A*. 1980;77:990–994.
8. Lampidis TJ, Bernal SD, Summerhayes IC, Chen LB. Selective toxicity of rhodamine 123 in carcinoma cells *in vitro*. *Cancer Res*. 1983;43:716–720.
9. Modica-Napolitano JS, Aprille JR. Basis for the selective cytotoxicity of rhodamine 123. *Cancer Res*. 1987;47:4361–4365.
10. Wang J, Yang CT, Kim YS, et al. 64Cu-Labeled triphenylphosphonium and triphenylarsonium cations as highly tumor-selective imaging agents. *J Med Chem*. 2007;50:5057-5069.
11. Yang CT, Kim YS, Wang J, et al. 64Cu-labeled 2-(diphenylphosphoryl)ethyl diphenylphosphonium cations as highly selective tumor imaging agents: effects of linkers and chelates on biodistribution characteristics. *Bioconjug Chem*. 2008;19:2008-2022.
12. Kim YS, Yang CT, Wang J, et al. Radiolabeled triphenylphosphonium cations as highly tumor-selective imaging agents: Effects of radiometals, bifunctional chelators and molecular charge. *J Med Chem*. 2008;51:2971-2984.
13. Madar I, Weiss L, Izbicki G. Preferential accumulation of (3)H-tetraphenylphosphonium in non-small cell lung carcinoma in mice: comparison with (99m)Tc-MIBI. *J Nucl Med*. 2002;43:234–238.
14. Madar I, Anderson JH, Szabo Z, et al. Enhanced uptake of [11C]-TPMP in canine brain tumor: a PET study. *J Nucl Med*. 1999; 40:1180–1185.
15. Shoup TM, Elmaleh DR, Brownell AL, et al. Evaluation of (4-[18F] Fluorophenyl)triphenylphosphonium ion. A potential myocardial blood flow agent for PET. *J Mol Imaging Biol*. 2011;13:511–517.

16. Sakai T, Saito Y, Takashima M, Ogawa M, Magata Y. Development of radioiodinated lipophilic cationic compounds for myocardial imaging. *Nucl Med Biol*. 2015;42:482–487.
17. Wadas TJ, Wong EH, Weisman GR, Anderson CJ. Coordinating radiometals of copper, gallium, indium, yttrium, and zirconium for PET and SPECT imaging of disease. *Chem Rev*. 2010;110:2858–2902.
18. Morrison D, Aitken J, De Jonge M, Ioppolo J, Harris HH, Rendina LM. High mitochondrial accumulation of new gadolinium(III) agents within tumour cells. *Chem Commun*. 2014;50:2252–2254.
19. Morrison D, Aitken J, De Jonge M, Issa F, Harris HH, Rendina LM. Synthesis and biological evaluation of a class of mitochondrially-targeted gadolinium (III) agents. *Chem Eur J*. 2014;20:16602–16612.
20. Armarego WLF, L CCL. *Purification of Laboratory Chemicals*. 7th ed. Waltham, MA: Butterworth-Heinemann;2013.

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