^{68}Ga-Labeled 2-[3-(1-carboxy-5-{7-[5-carboxy-5-(3-phenyl-2-{3-phenyl-2-[2-(4,7,10-tris-carboxymethyl-1,4,7,10-tetraazacyclododec-1-l)acetylamino]propionylamino}propionylamino)pentylcarbamoyl]heptanoylamino}pentyl)ureido]pentanedioic acid

[^{68}Ga]6

Arvind Chopra, PhD[1]

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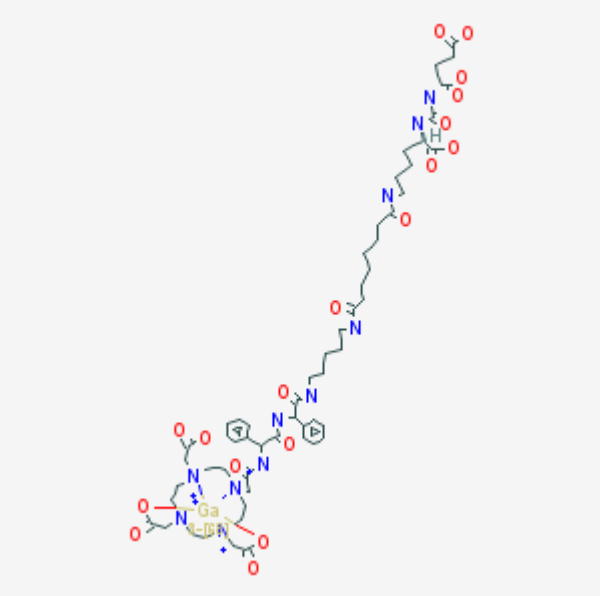
Chemical name:	^{68}Ga-Labeled 2-[3-(1-carboxy-5-{7-[5-carboxy-5-(3-phenyl-2-{3-phenyl-2-[2-(4,7,10-tris-carboxymethyl-1,4,7,10-tetraazacyclododec-1-l)acetylamino]propionylamino}propionylamino)pentylcarbamoyl]heptanoylamino}pentyl)ureido]pentanedioic acid
Abbreviated name:	[^{68}Ga]**6**
Synonym:	
Agent Category:	Compound
Target:	Prostate-specific membrane antigen (PSMA)
Target Category:	Antigen
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{68}Ga
Activation:	No



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Studies:	• *In vitro* • Rodents	Click on the above structure for additional information on [^{68}Ga]**6** in PubChem.

Background

[PubMed]

The prostate-specific membrane antigen (PSMA) is a type II membrane glycoprotein that is present primarily in the prostate and is overexpressed during all stages of the androgen-insensitive or the metastatic cancer of this organ (1). In addition, PSMA is expressed at lower levels (compared to the prostate) in the neovasculature of some solid tumors (for details, see Elsasser-Beile et al. (1)). Because of its high expression during the development and progression of a malignancy, PSMA is considered to be a good target for the imaging and treatment of prostate cancer (2). Several investigators have reported the use of radio-halogenated (e.g., ^{18}F, ^{125}I, etc.) and ^{99m}Tc-labeled small molecule inhibitors for the imaging of PSMA (3). Among these radionuclides, ^{18}F is the most frequently used to label diverse positron emission tomography (PET) agents for diagnostic imaging because its addition to a molecule (usually by the replacement of a hydrogen atom) does not alter its chemical properties; however, due to its short half-life (~110 min), an on-site cyclotron is needed to produce this radio-halogen (4). Recently ^{68}Ga (half-life, ~68 min) has been suggested to be a suitable alternative to the use of ^{18}F for the production of PET imaging probes because ^{68}Ga is easy to produce with a ^{68}Ge/^{68}Ga generator system that does not have to be on-site. However, it is necessary to mention that currently no ^{68}Ge/^{68}Ga generator system has been approved by the United States Food and Drug Administration (Eckelman: personal communication). In addition, ^{68}Ga has been successfully used by some investigators to study cancer and tumor biology (4, 5). On the basis of these observations, and as an extension of their earlier work (Banerjee et al. developed two ^{68}Ga-labeled, urea-based inhibitors of PSMA ([^{68}Ga]-labeled 2-[3-(1-carboxy-5-{7-[5-carboxy-5-(3-phenyl-2-{3-phenyl-2-[2-(4,7,10-tris-carboxymethyl-1,4,7,10-tetraazacyclododec-1-l)acetylamino]propionylamino}propionylamino)pentylcarbamoyl]

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heptanoylamino}pentyl)ureido]pentanedioic acid ([^{68}Ga]**6**)) and [^{68}Ga]-labeled 2-{3-[5-(7-{1-benzyloxycarbonyl-5-[2-(4,7,10-tris-carboxymethyl-1,4,7,10-tetraazacyclododec-1-l)acetylamino]pentylcarbamoyl}-heptanoylamino)-1-carboxypentyl]ureido}pentanedioic acid ([^{68}Ga]**3**)) and evaluated them for biodistribution and the imaging of PSMA-expressing tumors in nude mice (3). This chapter details the results obtained with [^{68}Ga]**6**. Results obtained with [^{68}Ga]**3** are presented in a separate chapter of MICAD (www.micad.nih.gov) (6).

Other Sources of Information

PSMA-related chapters in MICAD.

Alternate names and other information regarding PSMA in the Human Protein Reference Database.

Clinical trials involving PSMA and PSMA inhibitors.

Gene information (Gene ID: 2346) for human PSMA in Entrez Gene.

PSMA1 variant 3 Protein and mRNA sequence.

Information on PSMA in Online Mendelian Inheritance in Man (OMIM).

Synthesis

[PubMed]

The synthesis of [^{68}Ga]**6** has been described in detail by Banerjee et al. (3). The radiochemical yield and purity of the labeled compound were reported to be 70.0% and >99.0%, respectively. The retention time of [^{68}Ga]**6** on analytical high-performance liquid chromatography (HPLC) was 22.5 min compared with a retention time of 25.0 min for [^{68}Ga]**3**. The specific activity of [^{68}Ga]**6** was reported to be between 3.0 and 6.0 MBq/nmol (81 and 162 μCi/nmol), which was similar to that of [^{68}Ga]**3**. After purification, the radiochemical was dried under vacuum and diluted in 0.9% saline to the desired tracer concentration and used in biodistribution and imaging studies. The stability of [^{68}Ga]**6** under *in vitro* or *in vivo* conditions was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Using unlabeled **6** and [^{68}Ga]**6** in a fluorescence-based PSMA inhibition assay, the inhibitory concentrations of these compounds were reported to be 1.23 and 0.44 nM, respectively (3). In comparison, the PSMA inhibitory concentrations for unlabeled **3** and [^{68}Ga]**3** were 2.9 and 29.0 nM, respectively.

Using a 1-octanol/water mixture with the shake flask method, the partition coefficient of [^{68}Ga]**6** was determined to be −3.9, which was similar to that of [^{68}Ga]**3** (3). This

indicated that both radiolabeled compounds had a lipophilic nature (3). On the basis of the HPLC analysis reported above, [^{68}Ga]**6** appeared to have a slightly higher lipophilicity than [^{68}Ga]**3**.

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{68}Ga]**6** was studied in severe-combined immunodeficient (SCID) mice bearing PC-3-PIP (transfected with the PSMA gene and expressing high levels of the antigen) and PC-3-FLU (does not express PSMA) cell xenograft tumors (3). The mice were injected with the tracer through the tail vein and euthanized 5, 60, 120, and 180 min after the injection (n = 4 mice/time point) to determine the amount of radioactivity accumulated in the tumors and major organs. Results obtained from this study were presented as percent of injected dose per gram tissue (% ID/g). At 5 min post-injection (p.i.), the PSMA-expressing tumor had an uptake of 6.61 ± 0.55% ID/g that decreased to 1.80 ± 0.16% ID/g by 3 h p.i. Non-targeted organs such as kidneys, spleen, and lungs that contain high levels of PSMA showed a high uptake of 64.75 ± 12.00% ID/g, 5.17 ± 2.22% ID/g, and 4.59 ± 0.68% ID/g, respectively, at 5 min p.i. The radioactivity in these organs dropped to 10.04 ± 1.22% ID/g, 0.34 ± 0.09% ID/g, and 0.14 ± 0.03% ID/g, respectively, at 3 h p.i. Similar to [^{68}Ga]**3**, (see below for details), the clearance of radioactivity with [^{68}Ga]**6** from the kidneys was faster than from the PSMA-expressing tumors. In mice injected with [^{68}Ga]**6** PC-3-PIP, the tumor/muscle uptake ratio (T/M) increased from 4.17 at 30 min p.i. to 436.29 at 3 h p.i.; during the same time, the PC-3-FLU tumor T/M ratio increased from 1.67 at 30 min p.i. to 28.70 at 3 h p.i.

A biodistribution study was also performed with [^{68}Ga]**3** in SCID mice bearing the same tumors as described above (3). At 30 min p.i., the [^{68}Ga]**3** uptake of the PC-3-PIP tumors decreased gradually from 3.78 ± 0.09% ID/g to 1.10 ± 0.19% ID/g by 3 h p.i. (uptake in the PC-3-FLU tumors was 0.82 ± 0.20% ID/g at 30 min p.i. and 0.39 ± 0.02% ID/g at 3 h p.i.). During the same period, the label cleared rapidly from the kidneys, decreasing from 97.19 ± 16.07% ID/g at 30 min p.i. to 2.13 ± 0.11% ID/g at 3 h p.i. Except for the bladder, all other organs had <1.0% ID/g incorporation of radioactivity by 3 h p.i. The accumulation of radioactivity in the bladder was 8.96 ± 5.30% ID/g at 30 min p.i.; accumulation increased to 25.29 ± 8.63% ID/g at 60 min p.i. and decreased to 5.39 ± 2.98 at 3 h p.i. This indicated that the tracer was excreted mainly through the urinary route. With [^{68}Ga]**3**, the PC-3-PIP T/M increased from 8.30 at 30 min p.i. to 20.37 at 3 h p.i. In comparison, the T/M for the PC-3-FLU tumors was 1.80 at 30 min p.i. and increased to 7.34 at 3 h p.i.

For the PET imaging study, a single animal bearing LNCaP cell (expressing PSMA) tumors was injected intravenously with either [^{68}Ga]**3** or [^{68}Ga]**6** as described by Banerjee et al. (3). From the PET images it was clear that both tracers accumulated primarily in the PSMA-positive tumors and the kidneys (the kidneys are known to

express PSMA in the proximal renal tubules), similar to the observations made during the biodistribution study. In a blocking study, the tumor-bearing mice (the number of animals used per timepoint was not reported) were injected with 2-(phosphonomethyl)pentanedioic acid (2-PMPA) (50 mg/kg body weight), a known selective ligand of PSMA, and later the animals were injected with the respective tracers (time elapsed between the pretreatment with 2-PMPA and injection of the tracer was not reported). PET images of the animals were acquired at 30 and 60 min p.i., and it was observed that the PSMA-expressing tumors and the kidneys did not accumulate either tracer, suggesting that both radiochemicals bound specifically to the membrane antigen.

From these studies, the investigators concluded that both [^{68}Ga]**3** and [^{68}Ga]**6** bind specifically to PSMA and could be used for the imaging of PSMA-positive tumors in rodents (3). They also mentioned that more animal studies are necessary before these radiochemicals can be evaluated in the clinic.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

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