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**Preparation and evaluation of a radiogallium complex-conjugated
bisphosphonate as a bone scintigraphy agent**

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Keywords:

Gallium; PET; DOTA; bisphosphonate; bone

Abstract**Introduction:**

^{68}Ga is a radionuclide of great interest as a positron emitter for PET. To develop a new bone-imaging agent with radiogallium, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was chosen as a chelating site and Ga-DOTA complex-conjugated bisphosphonate, which has a high affinity for bone, was prepared and evaluated. Although we are interested in developing ^{68}Ga labeled bone imaging agents for PET, in these initial studies ^{67}Ga was used because of its longer half-life.

Methods:

DOTA-conjugated bisphosphonate (DOTA-Bn-SCN-HBP) was synthesized by conjugation of

2-(4-isothiocyanatebenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (*p*-SCN-Bn-DOTA) to

4-amino-1-hydroxybutylidene-1,1-bisphosphonate (alendronate).

^{67}Ga -DOTA-Bn-SCN-HBP was prepared by coordination with ^{67}Ga , and its *in vitro* and *in vivo* evaluations were performed.

Results:

^{67}Ga -DOTA-Bn-SCN-HBP was prepared with a radiochemical purity of over 95% without purification. ^{67}Ga -DOTA-Bn-SCN-HBP had great affinity for hydroxyapatite in binding assay. In biodistribution experiments, ^{67}Ga -DOTA-Bn-SCN-HBP accumulated in bone rapidly but was hardly observed in tissues other than bone. Pretreatment of an excess amount of alendronate inhibited the bone accumulation of ^{67}Ga -DOTA-Bn-SCN-HBP.

Conclusions:

^{67}Ga -DOTA-Bn-SCN-HBP showed ideal biodistribution characteristics as a bone-imaging agent. These findings should provide useful information on the drug design of bone-imaging agents for PET with ^{68}Ga .

1. Introduction

Over the last thirty years, complexes of ^{99m}Tc with bisphosphonate, such as methylene diphosphonate (^{99m}Tc -MDP) and hydroxymethylene diphosphonate (^{99m}Tc -HMDP), have been widely used as radiopharmaceuticals for the imaging of bone disorders such as metastatic bone disease, Paget's disease, osteoporotic fractures, etc [1-4].

Bisphosphonate analogs have a high affinity for hydroxyapatite and accumulate in bone because their phosphonate groups bind to the Ca^{2+} of hydroxyapatite crystals [5]. In the cases of ^{99m}Tc -MDP and ^{99m}Tc -HMDP, the phosphonate groups coordinate with technetium [6], which might decrease the inherent accumulation of MDP and HMDP in bone. Thus, we hypothesized that the bone affinity of technetium-99m labeled bisphosphonate would be increased by the design of a bisphosphonate in which the phosphonate groups do not coordinate with technetium-99m. To enable imaging at an earlier time after injection, we and other groups have designed technetium-99m complex-conjugated bisphosphonate compounds based on the concept of bifunctional radiopharmaceuticals [7-10]. As we expected, some of the novel technetium-99m complex-conjugated

bisphosphonate compounds showed superior biodistribution compared with previous compounds. This drug concept is applicable not only to technetium complex radiopharmaceuticals but also to other metals, such as rhenium [11-18], lutetium [19], yttrium [20], gadolinium [21], and so on.

^{68}Ga is one of the greatest practical and interesting radionuclides for clinical positron emission tomography (PET) because of its radiophysical properties ($T_{1/2} = 68$ min) [22]. ^{68}Ga is a generator-produced nuclide and can be eluted at any time on demand. Specifically, it does not require an on-site cyclotron. In principle, the long half-life of the parent nuclide ^{68}Ge ($T_{1/2} = 270.8$ days) provides a long life-span generator.

In this study, to develop a new tracer with radiogallium for the imaging of bone disorders such as bone metastases, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was chosen as a chelating site because it has been well known that Ga forms a stable complex with DOTA. Therefore, Ga-DOTA-conjugated bisphosphonate was designed, and evaluated by *in vitro* and *in vivo* experiments. Although we are interested in developing ^{68}Ga labeled bone imaging agents for PET, in these initial studies ^{67}Ga was used because of its

longer half-life.

2. Materials and methods

2.1. Materials

Proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectra were recorded on a JEOL JNM-ECS600 spectrometer (JEOL Ltd., Tokyo, Japan) and the chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. Electrospray ionization mass spectra (ESI-MS) were obtained with an LCQ (Thermo Fisher Scientific, Waltham, MA). ^{67}Ga was supplied by Nihon Medi-Physics Co., Ltd. (Tokyo, Japan). TLC analyses were performed with silica plates (Art 5553, Merck KGaA, Darmstadt, Germany). Cellulose acetate electrophoresis (CAE) (Separax-SP; Joko Co. Ltd., Tokyo, Japan) was run at an electrostatic field of 1.0 mA/cm for 20 minutes in veronal buffer ($I = 0.06$, pH 8.6). 2-4-Isothiocyanatobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (*p*-SCN-Bn-DOTA) was purchased from Macrocyclics (Dallas, TX). Other reagents were of reagent grade and used as received.

2.2. Synthesis of

4-[3-(4-hydroxy-4,4-bisphosphonobutyl)thioureido]benzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA-Bn-SCN-HBP (**3**))

(Scheme 1)

4-Amino-1-hydroxybutylidene-1,1-bisphosphonate (alendronate (**2**)) (2.5 mg, 10 μ mol), which was synthesized according to procedures described previously [13], was suspended in 150 μ L of distilled water and then triethylamine (30 μ L, 0.2 mmol) was added to the suspension. After a few seconds of being stirred at room temperature, the suspension became clear. *p*-SCN-Bn-DOTA (**1**) (5.5 mg, 10 μ mol) was dissolved in 100 μ L of DMF and then added dropwise to the reaction mixture. After the reaction mixture was incubated for 15 hours at 37°C, the mixture was purified by RP-HPLC performed with a Hydrosphere C18 column (10 \times 150 mm, YMC, Kyoto, Japan) at a flow rate of 4 mL/min with a gradient mobile phase of 20% methanol in water with 0.1% trifluoroacetic acid to 50% methanol in water with 0.1% trifluoroacetic acid for 20 min. Chromatograms were obtained by monitoring UV absorption at a wavelength of 254 nm. The fraction containing DOTA-Bn-SCN-HBP was determined by mass

spectrometry and collected. The solvent was removed by lyophilization to provide DOTA-Bn-SCN-HBP (**3**) (1.6 mg, 20.0%) as a white powder.

^1H NMR (D_2O): δ 1.89-1.99 (m, 4H), 2.90-3.94 (m, 27H), 7.28 (d, 2H), 7.33 (d, 2H).

MS (ESI) calcd for $\text{C}_{28}\text{H}_{46}\text{N}_6\text{O}_{15}\text{P}_2\text{S}$: $(\text{M} - \text{H})^-$ m/z 799. Found: 799.

2.3. Preparation of ^{67}Ga -DOTA-Bn-SCN-HBP

DOTA-Bn-SCN-HBP (**3**) (50 μg) was dissolved in 75 μL of 0.2 M ammonium acetate buffer (pH 5.0) and then 25 μL of $^{67}\text{GaCl}_3$ solution in 0.01 M HCl was added to the DOTA-Bn-SCN-HBP. The mixture was reacted at 95°C for 30 minutes. ^{67}Ga -DOTA was prepared by the same procedure using DOTA instead of DOTA-Bn-SCN-HBP. The radiochemical purities were confirmed by CAE and TLC.

2.4. *In vitro* stability

To evaluate the stability of ^{67}Ga -DOTA-Bn-SCN-HBP in buffered-solution, ^{67}Ga -DOTA-Bn-SCN-HBP (100 μL) was diluted with 0.1 M phosphate buffer (pH 7.4, 400 μL) and the solution was incubated at 37°C. After 1, 3, 6, and 24 hours of incubation, samples were drawn and

radioactivity was analyzed by CAE and TLC.

To evaluate the stability of ^{67}Ga -DOTA-Bn-SCN-HBP in plasma, ^{67}Ga -DOTA-Bn-SCN-HBP (50 μL) was diluted 10-fold with freshly prepared murine plasma (450 μL), and solutions were incubated at 37°C for 24 hours. After 1, 3, 6, and 24 hours incubation, samples (40 μL) were drawn and loaded onto Microcon YM-30 filters (MILLIPORE, Billerica, MA) and centrifuged at 14000g for 12 minutes at room temperature for separation of proteins. Filtrates of radioactivity were analyzed by CAE and TLC.

2.5. Hydroxyapatite-binding assays

Hydroxyapatite-binding assays were performed according to procedures described previously with slight modification [23]. In brief, hydroxyapatite beads (Bio-Gel; Bio-Rad, Hercules, CA) were suspended in Tris/HCl-buffered saline (50 mM, pH 7.4) at 1 mg/mL, 10 mg/mL, and 25 mg/mL. For the solutions of ^{67}Ga -labeled compounds (^{67}Ga -DOTA-Bn-SCN-HBP and ^{67}Ga -DOTA), the ligand concentrations were adjusted to 19.5 μM . Two hundred microliters of each solution of ^{67}Ga -labeled compound was added to 200 μL of the hydroxyapatite

suspension, and the samples were gently shaken for 1 hour at room temperature. This period was used because it has been reported that 1 h is sufficient to attain binding equilibrium [23]. After centrifugation at 10,000g for 5 minutes, the radioactivity of the supernatant was measured with an auto well gamma counter (ARC-7010B, Aloka, Tokyo, Japan). Control experiments were performed using the same procedure but without the hydroxyapatite beads. In the control experiments, we confirmed that the radioactivity adsorbed to the vials was less than 0.1%. The rate of binding was determined as follows:

$$\text{Hydroxyapatite binding (\%)} = (1 - [\text{radioactivity of supernatant of each sample}] / [\text{radioactivity of supernatant in the respective control}]) \times 100$$

2.6. Biodistribution experiments

Experiments with animals were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Kanazawa University. Biodistribution experiments were performed with an intravenous administration of each diluted tracer solution (37 kBq / 100 μ L) to 6-week-old male ddY mice (27-32 g, Japan SLC, Inc., Hamamatsu, Japan).

To investigate the effect of an excess amount of bisphosphonate on biodistribution, alendronate (40 mg/kg) was intravenously administered to mice 1 minute before the intravenous injection of

^{67}Ga -DOTA-Bn-SCN-HBP. Four or five mice each were sacrificed by decapitation at 10, 60, and 180 minutes postinjection of the radiotracer.

Tissues of interest were removed and weighed. The complete left femur was isolated as a representative bone sample. The levels of radioactivity in these tissues were determined with an auto well gamma counter and corrected for background radiation and physical decay during counting.

2.7. Statistical analysis

Data are expressed as mean \pm standard deviation, where appropriate.

The results were statistically analyzed using unpaired Students' *t* test; *p* = 0.05 was set as the level of significance.

3. Results

3.1. Preparation of ^{67}Ga -labeled compounds

DOTA-Bn-SCN-HBP (**3**) as a precursor was synthesized by a

coupling reaction between *p*-SCN-Bn-DOTA (1) and alendronate (2) according to Scheme 1. ^{67}Ga labeling was performed by complexation between DOTA-Bn-SCN-HBP or DOTA and ^{67}Ga . The radiochemical purities of ^{67}Ga -DOTA-Bn-SCN-HBP and ^{67}Ga -DOTA were each over 95% without purification.

3.2. *In vitro* stability

Figure 1 shows the stability of ^{67}Ga -DOTA-Bn-SCN-HBP in buffered solution and plasma. After 24 hours of incubation in buffered solution, no decomposition of ^{67}Ga -DOTA-Bn-SCN-HBP was observed. After 24 hours of incubation in plasma, $80.2 \pm 1.0\%$ of ^{67}Ga -DOTA-Bn-SCN-HBP remained intact.

3.3. *Hydroxyapatite-binding assay*

Figure 2 shows the percentages of ^{67}Ga -DOTA-Bn-SCN-HBP and ^{67}Ga -DOTA as a negative control bound to hydroxyapatite beads. With an increase in the amount of hydroxyapatite, the rate of binding of ^{67}Ga -DOTA-Bn-SCN-HBP rose. In contrast, ^{67}Ga -DOTA did not bind to the

hydroxyapatite beads at all.

3.4. Biodistribution experiments

The biodistributions of ^{67}Ga -DOTA-Bn-SCN-HBP and ^{67}Ga -DOTA in normal mice are presented in Tables 1 and 3, respectively.

^{67}Ga -DOTA-Bn-SCN-HBP showed a high degree of accumulation in bone soon after injection, and almost all other radioactivity was quickly excreted via the kidneys. Consequently, radioactivity was scarcely observed in any tissue without bone at 60 minutes after injection. Meanwhile, ^{67}Ga -DOTA did not accumulate in bone, and almost all injected radioactivity was quickly excreted via the kidneys.

The biodistributions of ^{67}Ga -DOTA-Bn-SCN-HBP with pretreatment of alendronate (40 mg/kg) in normal mice is presented in Table 2.

Pretreatment of alendronate inhibited bone accumulation of

^{67}Ga -DOTA-Bn-SCN-HBP and caused an increase in radioactivity levels in most organs except bone, muscle, and brain.

4. Discussion

In our previous study, we synthesized a similar ligand, DOTA-HBP, which is a DOTA-conjugated bisphosphonate, by coupling the reaction between DOTA-NHS-ester and alendronate, and prepared ^{90}Y -DOTA-HBP [20]. Because ^{90}Y -DOTA-HBP showed superior biodistribution in mice as a bone-seeking radiopharmaceutical, in this study, we first tried to label the DOTA-HBP ligand with radiogallium. However, in the case of ^{67}Ga -labeled compounds, ^{67}Ga -DOTA-HBP and ^{67}Ga -DOTA were not retained in the RP-HPLC column. Thus, the separation of ^{67}Ga -DOTA-HBP and ^{67}Ga -DOTA as an impurity was impossible. It was reported that four nitrogen atoms of the macrocycle and two oxygen atoms from the carboxyl groups are involved in the complexation of gallium by DOTA [24]. In contrast, for ^{90}Y , it is assumed that four nitrogen atoms and four oxygen atoms from the carboxyl groups in DOTA are involved in the complexation. The difference of coordination structure might affect retention in the ODS column for RP-HPLC. In RP-HPLC analysis after the synthesis reaction of DOTA-HBP, the peaks of DOTA-HBP and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(*N*-hydroxysuccinimidyl ester) (DOTA-NHS-ester) were close. When

we purified DOTA-HBP by RP-HPLC, a subtle amount of DOTA-NHS-ester might have mixed with DOTA-HBP. As a result of DOTA-NHS-ester hydrolyzation to DOTA, ^{67}Ga -DOTA could mix as an impurity with ^{67}Ga -DOTA-HBP as a major labeled product.

In this study, the separation of a precursor with HPLC is easier than it was in a previous study because DOTA-Bn-SCN-HBP with a benzene ring is more lipophilic compared with DOTA-HBP. Because of the complete successful purification of the precursor with HPLC, a high radiochemical purity of ^{67}Ga -DOTA-Bn-SCN-HBP was achieved without purification after radiolabeling. The lack of a need for purification after radiolabeling could facilitate the making of a kit for radiopharmaceuticals and would be beneficial for clinical use.

Investigations of ^{68}Ga -labeled compounds for bone imaging were previously described in the 1970s [25,26]. In these reports, gallium was labeled with tripolyphosphate or ethylenediamine tetramethylene phosphonate (EDTMP). Since gallium can form a complex with tripolyphosphate or EDTMP, there is a possibility that ^{67}Ga coordinates with the phosphonate group in DOTA-Bn-SCN-HBP. To ascertain whether ^{67}Ga

is selectively complexed with only the DOTA moiety, a mixture containing DOTA and alendronate at equal mole concentrations was reacted under the same conditions as the labeling reaction of DOTA-Bn-SCN-HBP. Through an analysis of this reaction mixture, the ^{67}Ga -labeled product was found to be identical to that obtained from the reaction of only DOTA with ^{67}Ga . These findings suggest that ^{67}Ga is chelated with the DOTA moiety in DOTA-Bn-SCN-HBP.

In this study, ^{67}Ga -DOTA did not bind HA *in vitro* and did not accumulate in bone *in vivo* at all (Fig. 2 and Table 3). Moreover, the pretreatment of an excess amount of alendronate, which is a bisphosphonate compound, greatly reduced the bone accumulation of ^{67}Ga -DOTA-Bn-SCN-HBP (Table 2). These results indicate that a bisphosphonate site in ^{67}Ga -DOTA-Bn-SCN-HBP plays a crucial role for the delivery of radiotracer to bone. The effect of the pretreatment of alendronate on the pharmacokinetics of ^{67}Ga -DOTA-Bn-SCN-HBP was not limited to bone. Actually, the radioactivity of most organs was increased. This could be caused by a delay in blood clearance. A previous report stated that there is a saturable transport mechanism for alendronate in renal tubular epithelial

cells [27]. The delay in blood clearance of ^{67}Ga -DOTA-Bn-SCN-HBP would be derived from saturation of the renal transport system by an excess amount of alendronate.

Recently, ^{68}Ga -EDTMP was re-evaluated by Mitterhauser *et al.* [28]. However, they stated that the advantage of ^{68}Ga -EDTMP over [^{18}F]-fluoride was not apparent and that the future clinical prospect of ^{68}Ga -EDTMP remained speculative. Meanwhile, PET/CT imaging of bone metastases with a ^{68}Ga -DOTA complex-conjugated bisphosphonate (BPAMD) based on a strategy similar to our study has been reported [29]. ^{68}Ga -BPAMD showed high uptake in osteoblastic metastases. Surprisingly, the maximal standardized uptake was 77.1 and 62.1 in the 10th thoracic and L2 vertebra vs. 39.1 and 39.2 for ^{18}F -fluoride PET, respectively. Accordingly, we suppose that ^{67}Ga -DOTA-Bn-SCN-HBP also could have a potential for the detection of osteoblastic bone metastases such as ^{68}Ga -BPAMD because both complexes have similar chemical structures and should have the same mechanism of bone adsorption. Actually, ^{67}Ga -DOTA-Bn-SCN-HBP showed superior biodistribution in normal mice. The results are comparable to those of $^{99\text{m}}\text{Tc}$ -HMDP (Tables 1 and 4), because it has long been used as a

radiopharmaceutical for bone scintigraphy. Furthermore, ^{67}Ga -DOTA-Bn-SCN-HBP might offer a more ideal structure-based drug design as a bone-seeking radiopharmaceutical because DOTA-Bn-SCN-HBP has a hydroxyl group (α -OH group) at a central carbon of the bisphosphonate structure. On the other hand, BPAMD does not have an α -OH group. Previous studies of bisphosphonates suggest that the presence of the α -OH group affects affinity for bone minerals [30]. In the design of radiometal complex-conjugated bisphosphonate derivatives, it has also been suggested that the α -OH group is effective in enhancing accumulation in bone [12].

In conclusion, a ^{67}Ga DOTA complex-conjugated bisphosphonate derivative, ^{67}Ga -DOTA-Bn-SCN-HBP, showed ideal biodistribution characteristics as a bone scintigraphy agent. These findings should provide useful information on the drug design of PET tracers with ^{68}Ga for diagnosis and as a monitor for the therapy of bone disorders, such as bone metastases.

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Table 1. Biodistribution of radioactivity after intravenous administration of ⁶⁷Ga-DOTA-Bn-SCN-HBP in mice.

Tissue	Time after administration		
	10 min	1 h	3 h
Blood	1.69 (0.21)	0.13 (0.03)	0.10 (0.02)
Liver	0.46 (0.04)	0.16 (0.03)	0.19 (0.01)
Kidney	8.28 (2.66)	3.15 (1.65)	1.05 (0.21)
Intestine	0.38 (0.05)	0.11 (0.02)	0.11 (0.03)
Spleen	0.43 (0.04)	0.08 (0.02)	0.11 (0.01)
Pancreas	0.51 (0.06)	0.10 (0.05)	0.06 (0.01)
Lung	1.19 (0.17)	0.17 (0.03)	0.12 (0.01)
Heart	0.60 (0.11)	0.07 (0.03)	0.09 (0.03)
Stomach	0.23 (0.08)	0.05 (0.03)	0.05 (0.01)
Femur	17.44 (1.12)	22.28 (2.15)	23.53 (2.34)
Muscle	0.56 (0.17)	0.16 (0.11)	0.11 (0.04)
Brain	0.08 (0.05)	0.04 (0.06)	0.01 (0.00)
F/B ratio ^a	10.48 (1.88)	189.51 (59.75)	245.96 (70.65)

Data are expressed as % injected dose per gram tissue. Each value represents the mean (SD) of five animals.

^a Femur: blood ratio

Table 2. Biodistribution of radioactivity after intravenous administration of ^{67}Ga -DOTA-Bn-SCN-HBP in mice with pretreatment of alendronate (40 mg/kg).

Tissue	Time after administration		
	10 min	1 h	3 h
Blood	2.31 (0.38)*	0.60 (0.14)***	0.18 (0.04)**
Liver	2.33 (0.56)***	1.84 (0.32)***	1.39 (0.21)***
Kidney	17.68 (1.33)***	13.81 (1.74)***	11.91 (1.13)***
Intestine	0.58 (0.12)*	0.36 (0.06)***	0.29 (0.04)***
Spleen	1.39 (0.17)***	1.12 (0.42)***	1.02 (0.40)**
Pancreas	0.95 (0.09)***	0.54 (0.04)***	0.25 (0.08)***
Lung	5.23 (0.58)***	2.62 (0.69)***	1.54 (0.42)***
Heart	1.28 (0.32)**	0.47 (0.09)***	0.28 (0.05)***
Stomach	0.46 (0.11)**	0.61 (0.06)***	0.74 (0.13)***
Femur	7.51 (1.55)***	13.71 (1.59)***	15.16 (1.53)***
Muscle	0.68 (0.09)	0.25 (0.04)	0.15 (0.07)
Brain	0.06 (0.01)	0.02 (0.01)	0.01 (0.00)
F/B ratio ^a	3.36 (0.98)**	23.61 (4.61)***	85.61 (21.01)***

Data are expressed as % injected dose per gram tissue. Each value represents the mean (SD) of four animals.

^a Femur: blood ratio

* $p < 0.05$ vs. control (no pretreatment)

** $p < 0.01$ vs. control (no pretreatment)

*** $p < 0.001$ vs. control (no pretreatment)

Table 3. Biodistribution of radioactivity after intravenous administration of ⁶⁷Ga-DOTA in mice.

Tissue	Time after administration		
	10 min	1 h	3 h
Blood	2.60 (0.32)	0.05 (0.04)	0.00 (0.00)
Liver	0.58 (0.19)	0.06 (0.02)	0.04 (0.00)
Kidney	10.81 (4.52)	2.58 (1.74)	0.89 (0.12)
Intestine	0.51 (0.09)	0.06 (0.01)	0.11 (0.10)
Spleen	0.59 (0.13)	0.07 (0.02)	0.03 (0.01)
Pancreas	0.74 (0.15)	0.02 (0.01)	0.01 (0.01)
Lung	2.02 (0.35)	0.11 (0.04)	0.02 (0.42)
Heart	0.92 (0.06)	0.06 (0.01)	0.03 (0.01)
Stomach	0.33 (0.09)	0.03 (0.01)	0.03 (0.03)
Femur	1.22 (0.37)	0.21 (0.21)	0.07 (0.08)
Muscle	0.60 (0.16)	0.08 (0.11)	0.03 (0.01)
Brain	0.07 (0.02)	0.00 (0.00)	0.00 (0.00)
F/B ratio ^a	0.47 (0.13)	6.49 (6.96)	9.87 (17.10)

Data are expressed as % injected dose per gram tissue. Each value represents the mean (SD) of four animals.

^a Femur: blood ratio

Table 4. Biodistribution of radioactivity after intravenous administration of ^{99m}Tc -HMDP in mice^b.

Tissue	Time after administration		
	10 min	1 h	3 h
Blood	1.63 (0.23)	0.15 (0.07)	0.07 (0.02)
Liver	0.48 (0.09)	0.14 (0.04)	0.13 (0.03)*
Kidney	10.78 (1.66)	5.69 (1.88)	0.85 (0.35)
Intestine	0.41 (0.05)	0.12 (0.02)	0.17 (0.04)*
Spleen	0.46 (0.08)	0.11 (0.03)	0.07 (0.02)*
Stomach	0.29 (0.08)	0.21 (0.20)	0.26 (0.28)
Femur	16.89 (2.12)	19.65 (1.76)	19.21 (2.08)*
Muscle	0.61 (0.15)	0.15 (0.06)	0.17 (0.12)
F/B ratio ^a	10.44 (1.49)	149.42 (67.11)	291.06 (61.64)

Data are expressed as % injected dose per gram tissue. Each value represents the mean (SD) of five animals.

^a Femur: blood ratio

^b Previously reported results [20]

* $p < 0.05$ vs. ^{67}Ga -DOTA-Bn-SCN-HBP

Figure Legends

Scheme 1. Synthesis of DOTA-Bn-SCN-HBP.

Figure 1. Stability of ^{67}Ga -DOTA-Bn-SCN-HBP in buffered solution (closed circles) and in plasma (open circles). Data are expressed as the mean \pm SD for three experiments.

Figure 2. Each column represents the binding of ^{67}Ga -DOTA (closed column) and ^{67}Ga -DOTA-Bn-SCN-HBP (open column) to hydroxyapatite beads, respectively. Data are expressed as the mean \pm SD for four experiments.

Scheme 1.

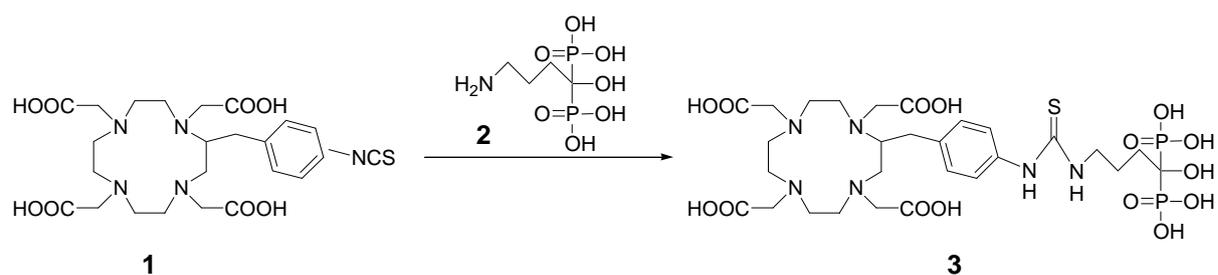


Figure 1.

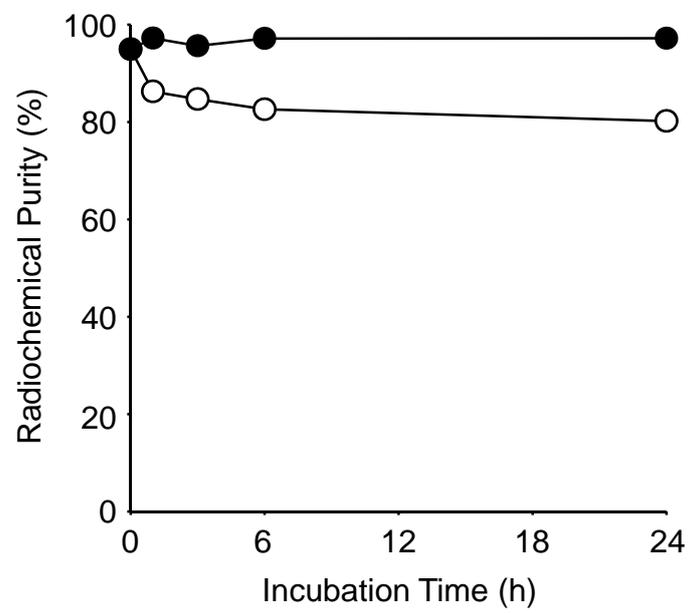


Figure 2.

