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Synthesis and evaluation of macrocyclic amino acid derivatives for tumor imaging by gallium-68 positron emission tomography

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ABSTRACT

^{68}Ga PET imaging in clinical oncology represents a notable development because the availability of ^{68}Ga is not dependent on a cyclotron. Furthermore, labeled amino acid derivatives have been proven to be useful for the imaging many tumor types. In the present study, we synthesized β-aminoalanine, γ-aminohomoalanine, and lysine conjugates of macrocyclic bifunctional chelating agents, such as, NOTA (**1a–c**) and DOTA (**2a–c**). The compounds produced were found to be potential useful as ^{68}Ga-PET imaging agents. In particular, they showed high tumor uptakes in vitro and in vivo, and had high labeling yields and excellent stabilities. The co-ordination chemistry of NOTA-monoamide compound **1a** was studied by multinuclear NMR. In vitro studies showed that the synthesized compounds were taken up by cancer cells more than controls (^{68}Ga-NOTA and ^{68}Ga-DOTA). Furthermore, in vivo studies showed that they have high tumor to muscle and tumor to blood ratios, and small-animal PET imaging revealed high tumor uptakes as compared with other organs, and high bladder activities, indicating rapid renal excretion. These results might motivate the use of ^{68}Ga amino acid PET for tumor diagnosis.

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1. Introduction

Positron emission tomography (PET) has been extensively used during the past three decades for the functional characterization of tumors because of its high sensitivity. Furthermore, 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) is still the most widely used PET agent in clinical oncology.[1–3] However, interpretations of [^{18}F]FDG uptakes in solid tumors are often complicated by high uptakes caused by non-neoplastic processes, such as, inflammation and normal brain activity.[4,5] Thus, the development of new PET radiopharmaceuticals to complement the use of [^{18}F]FDG is an active research topic. A number of studies have indicated that PET imaging using radiolabeled amino acids better defines tumor boundaries within the normal brain than [^{18}F]FDG PET, and thus, allows more thorough treatment planning.[6,7] Conventionally, the amino acids used for PET are labeled with cyclotron-produced ^{11}C or ^{18}F, and include, L-[^{11}C]methionine (Met), [^{18}F]fluoroethyl-L-tyrosine (FET), and 1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid ([^{18}F]FACBC).[8–10] Apart from their usefulness for detecting brain and systemic tumors, labeled non-natural amino acids have the advantage that they are metabolized relatively slowly in vivo, which can simplify kinetic analyses of amino acid uptakes.

^{68}Ga-PET imaging represents an important development in functional and metabolic imaging because ^{68}Ga is readily available from the ^{68}Ge/^{68}Ga-generator system.[11,12] Furthermore, ^{68}Ga is an excellent positron emitter, with a high positron emission (89%) and a low photon emission (1077 keV, 3.22%).[13] For these reasons, we undertook the development of ^{68}Ga-labeled amino acid derivatives for cancer PET.

In order to deliver ^{68}Ga-labeled amino acid agents to target sites, it is important that amino acid transporters recognize the amino acids used. Many radiometal labeled small molecules have been designed to target transporters, receptors, or enzymes, but are ineffective for imaging purposes due to a loss of molecular recognition.[14,15] However, a well-designed study on the take-up of ^{99m}Tc-labeled amino acid derivative by amino acid transporters opened up the possibility of using amino acids with metal chelating groups.[16]

Bifunctional chelating agents (BCA), such as, 1,4,7,10-tetra-azacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) have been widely used in the developmental research for radiometal based imaging agents.[17–20] A large number of studies have been conducted on

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the conjugation of DOTA to peptides and other biomolecules via one of its carboxylate containing pendent arms. Studies on ^{68}Ga-labeled 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid (DO2A) derivatives of tyrosine was reported,[21] in which size of the amino acid pendant arm was bigger than our previous compounds those include DO2A and 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A) derivatives of alanine and homoalanine derivatives.[22] Co-ordination geometries of DO2A or DO3A complexes were different from corresponding NOTA or DOTA derivatives. Also, in the present study, we tried to keep the size of amino acid pendant arm as small as possible for the better molecular recognition. Furthermore, all α-amino groups were left intact to allow the derivatives to mimic natural amino acids.

On the other hand, in the case of NOTA, most of the modifications described to date have been restricted to conjugation to a methylene group or to a cyclic ring in a pendant arm, which involves cumbersome synthetic steps.[20] Though recent studies on conjugation of peptides to NOTA via one of carboxylate pendant arms opens the possibility of direct linkage,[23] there was no detailed studies on co-ordination chemistry of complexes formed from these modification. Recently we reported the detailed co-ordination studies and effect of pH on co-ordination polyhedron formation in Ga-complexes of one of carboxylate pendant arm modified NOTA derivatives.[24,25] In the present study we extended this application by synthesizing β-aminoalanine, γ-aminohomoalanine, and lysine conjugates of NOTA and DOTA on one of their carboxylate pendent arms (Fig. 1). The structure of Ga-complexed NOTA-aminoalanine (**1a**) in aqueous solution was confirmed by multinuclear magnetic resonance spectroscopy. Furthermore, we labeled the conjugates with ^{68}Ga obtained from ^{68}Ge/^{68}Ga-generator, and in vitro and in vivo biological properties were studied.

2. Results and discussion

2.1. Chemistry

The protected β-amino-L-alanine (**6**) was synthesized from commercially available *N*-*tert*-butyl-L-serine methyl ester (**3**) using a combination of reported methods with some modification (Scheme 1).[26–28] Treatment of **4** with sodium azide in dimethylformamide (DMF) resulted in the formation of **5**. Although tetrabutylammonium bromide (TBAB) was used as a catalyst in a previous study,[28] we did not use it because it caused a significant elimination reaction even when present in trace amounts. The catalytic reduction of **5** led to the formation of **6**, which was stable in a refrigerator for several days.

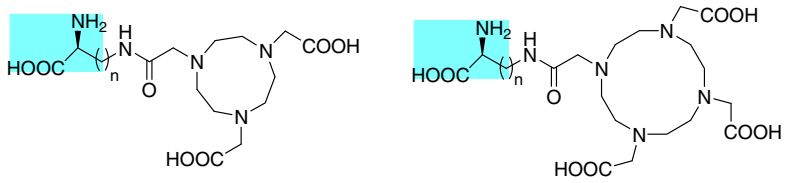
Because of the high polarities of NOTA and DOTA, their conjugation with **6** was performed in aqueous solution using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl) as a coupling reagent. Active ester formation was achieved over 30 min in the pH range 4.5–5.0, and amine coupling was achieved by increasing the pH to ~8 for another 30 min to obtain the alanine conjugates **7a** and **8a**. For NOTA and DOTA, the pH of the reaction was controlled using organic bases, such as, diisopropylethylamine (DIPEA) or triethylamine, which resulted in higher levels of conjugation than were achieved when an inorganic base, such as, 1 N NaOH was used. However, a sudden increase to pH 8 resulted in no reaction or a poor yield. When the reaction was carried out at pH 6, a di-conjugated product was obtained for NOTA (ESI^+, m/z = 704). Furthermore, when we increased the equivalence of **6** from 1.5 to 2, the yield of conjugation reaction was increased. Hydrolysis of the protected esters using LiOH followed by hydrochloric acid resulted in the formations of **1a** and **2a** as hydrochloride salts.

Protected lysine (**11**) was synthesized from commercially available N^{α}-Boc-N^{ε}-benzyloxycarbonyl-lysine (**9**) by *tert*-butyl esterification and subsequent deprotection by catalytic hydrogenation (Scheme 2).[29,30] Esterification reaction using perchloric acid ($HClO_4$) in *tert*-butyl acetate produced **10** at a low yield (~20%). However, when the reaction was carried out using Boc anhydride in *tert*-butanol in the presence of 20 mol % of 4-dimethylamino pyridine (DMAP; a catalyst), the yield was greatly increased to ~90%.

Another protected γ-amino-L-homoalanine derivative (**12**) was purchased for conjugation with NOTA or DOTA. Conjugation of **11** or **12** with one of the pendent carboxyl groups of NOTA or DOTA to give **7b–c** and **8b–c** was performed in a solvent mixture (water/MeCN = 50:50, v/v) using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as a coupling agent and pyridine as a base. Further hydrolysis and deprotection using hydrochloric acid in dioxane resulted in the formation of **1b–c** and **2b–c** as hydrochloric acid salts. All final products were purified by RP–HPLC.

The complex between **1a** and gallium(III) was prepared in aqueous solution to study the co-ordination chemistry of the metal complex with mono carboxyl modified NOTA by adding stoichiometric amounts (1:1) of **1a** and gallium(III) nitrate at pH 3. We were unable to produce a crystal of the chelate and did not perform the crystallography study. Instead, the structure and dynamics of the chelate were studied in aqueous solution using homonuclear proton correlation and ^{71}Ga NMR (Fig. 2). The ^{1}H NMR spectrum of the complex showed the methylene protons of pendent arms as singlets at δ 3.63 and 3.64, suggesting the participation of the amide groups of modified pendent arms during complex formation. The downfield shifts of the α-hydrogen (Ha) and the two β-hydrogens (Hb) of alanine versus pure alanine suggested the absence of an imide bond, and hence, co-ordination via an amide –NH bond with metal in the complex. The $-CH_2N-$ ring protons produced multiplets centered at δ 2.97, 3.04, and 3.30, which contrasted with those of the Ga(NOTA) system which produced symmetrical multiplets centered at δ 3.23 and 3.51 due to the methylene protons of the diastereotopic ring.[31] During H–H COSY analysis, along with coupling between axial and equatorial hydrogens of ring methylene groups (H1, H3 and H2, H4), as was observed for Ga-NOTA complex,[32] we also observed coupling between β-hydrogens (Hb) and the pendent methylene group hydrogen (Hc) and coupling between the axial hydrogens of ring methylenes (Fig. 2A).

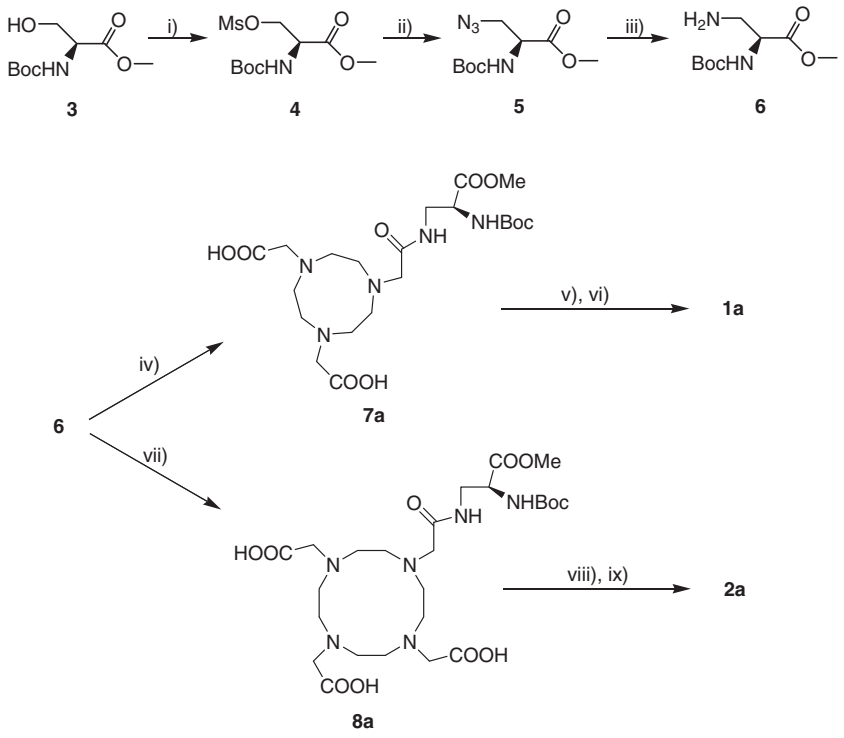
The ^{71}Ga NMR spectrum of an aqueous solution (D_2O) of the complex at pH 7 consisted of a relatively narrow singlet at



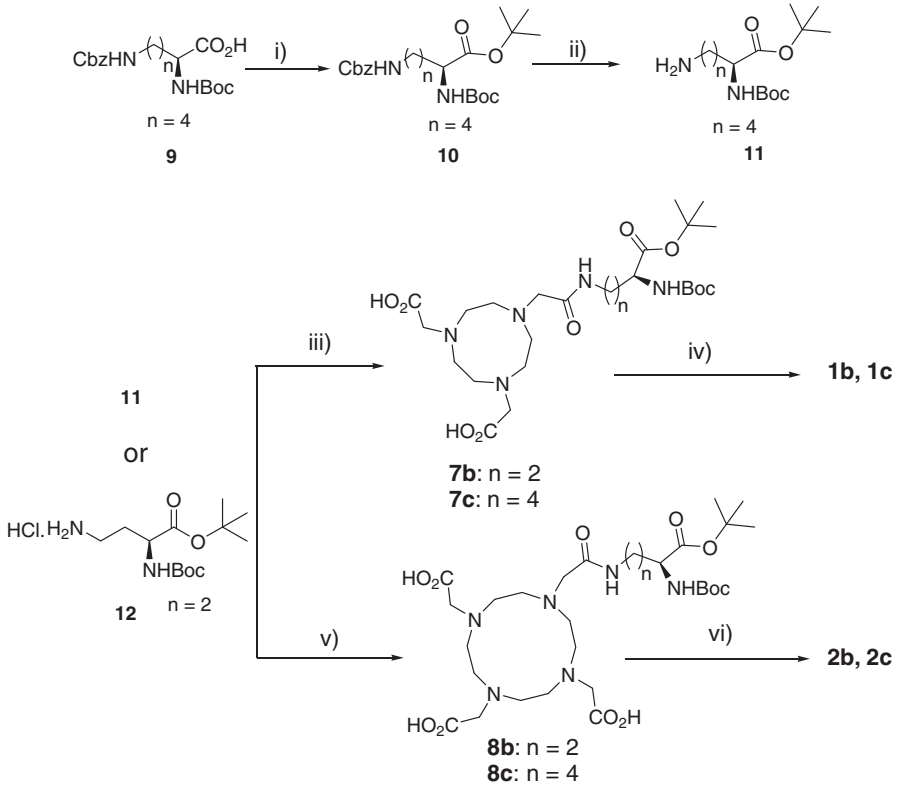
1a: n = 1
1b: n = 2
1c: n = 4

2a: n = 1
2b: n = 2
2c: n = 4

Figure 1. Synthesized amino acid derivatives studied the present work: (n = 1, β-aminoalanine; n = 2, γ-aminohomoalanine; and n = 4, ε-aminolysine derivatives).



Scheme 1. Synthesis of BCA–β-aminoalanine conjugates. Reagents and conditions: (i) MsCl, Et_3N, CH_2Cl_2; (ii) NaN_3, DMF; (iii) Pd–C (10%), H_2, 1 atm, EtOH; (iv) NOTA, HOBT, EDC, DIPEA, water/MeOH, pH 5.0–8.0; (v) LiOH, water, rt; (vi) 30% HCl; (vii) DOTA, EDC, HOBT, DIPEA, water/MeOH, pH 4.5–8.0; (viii) LiOH, water; (ix) 30% HCl. (Boc, *tert*-butyl carbonate; Ms, methanesulfonyl.)



Scheme 2. Synthesis of BCA–γ-aminohomoalanine and BCA–lysine conjugates. Reagents and conditions: (i) $(Boc)_2O$, *tert*-BuOH, DMAP; (ii) Pd–C/H_2 (10%), EtOH; (iii) NOTA, HOBT, DCC, pyridine, water/MeCN (1:1); (iv) 4 M HCl/1,4-dioxane; (v) DOTA, DCC, HOBT, pyridine, water/MeCN (1:1); (vi) 4 M HCl/1,4-dioxane. (Boc, *tert*-butyl carbonate.)

δ = 170 ppm ($\omega_{1/2}$ = 286 Hz) (Fig. 2B). In the pH range studied (0–8), the signals corresponding to the free cation $[Ga(H_2O)_6]^{3+}$ (δ = 0 ppm) or $[Ga(OH)_4]^-$ (δ = 222 ppm)[33] were not observed, which implied that complex was stable within this pH range. Also,

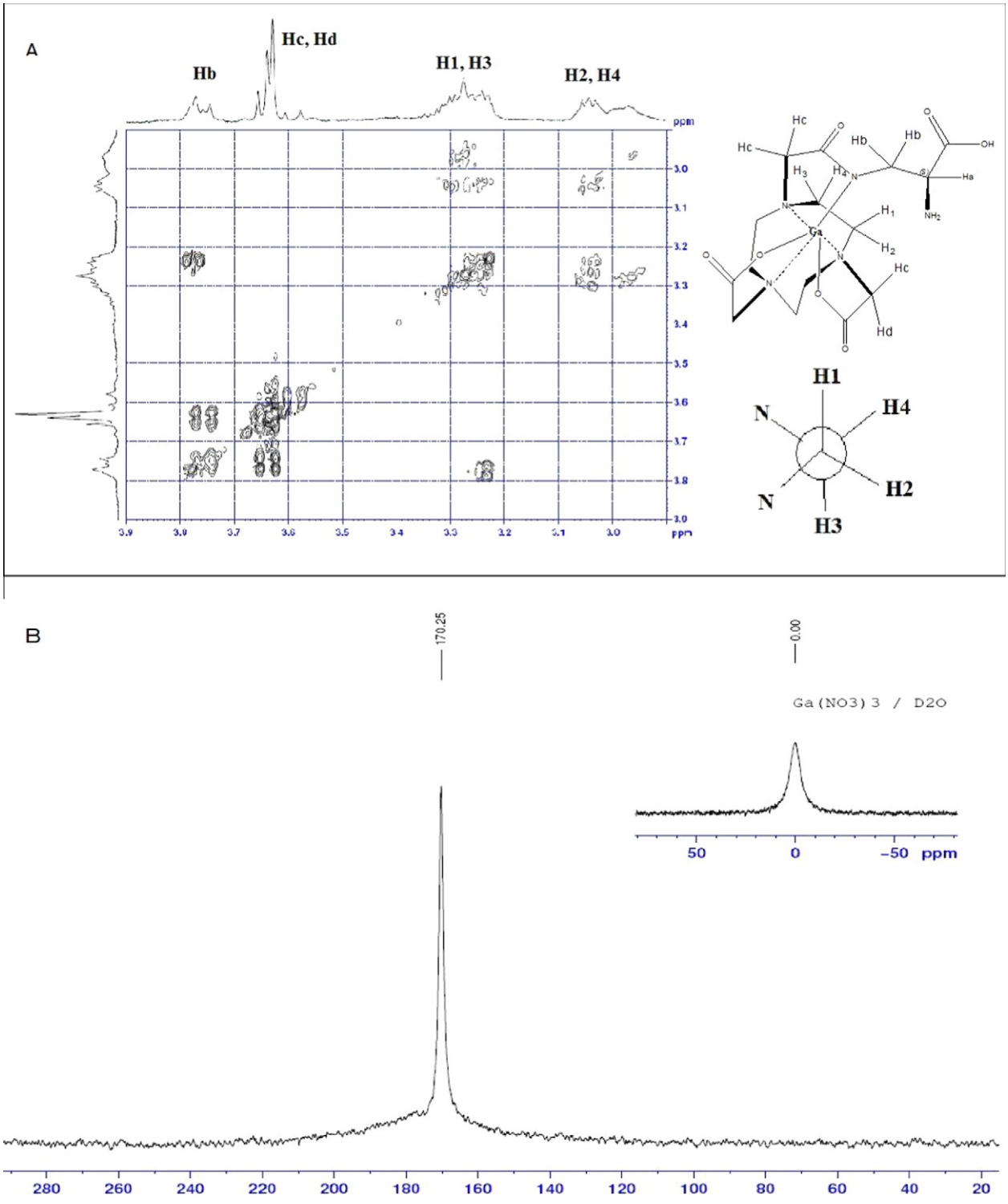


Figure 2. Spectral studies of the Ga-NOTA-ala complex: (A) ^{1}H–^{1}H COSY NMR spectrum (600 MHz, D_2O, pH 7, 298 K) from δ 2.6–4.0 region; (B) ^{71}Ga NMR spectrum (600 MHz, D_2O, pH 7, 298 K). A small figure shows a portion of the ^{71}Ga NMR spectrum of $Ga(NO_3)_3$ in aqueous solution under similar conditions.

the δ value of the complex was close to that of the Ga-NOTA complex (δ = 171 ppm),[32] which suggested a symmetry similar to that of the N_4O_2 bound complex. ^{71}Ga NMR signal peak widths are determined by quadrapolar relaxation induced by electric field gradients in the distorted Ga^{3+} co-ordination polyhedron, and thus, can be used as a sensitive measure of degree of distortion in solution, after correction for changes of rotational correlation time.[33] The peak width of Ga-**1a** was between Ga(NOTA) ($\omega_{1/2}$ = 210 Hz) and Ga-NOTP ($\omega_{1/2}$ = 434 Hz) (NOTP-1,4,7-triazacyclononane-1,4,7-tris(methylenephosphonate)),[33] indicating the presence of a stable Ga-**1a** complex in aqueous solution, possibly with octahedral or pseudo-octahedral geometry.

2.2. Radiochemistry

Pure **1a–c** and **2a–c** were labeled with ^{68}Ga eluted from a titanium dioxide based $^{68}Ge/^{68}Ga$-generator. DOTA derivatives were labeled in a boiling water bath for 10 min, whereas NOTA derivatives

were labeled at room temperature. The labeling procedure, including the purification, was straightforward and products were analyzed by ITLC-SG (Instant Thin Layer Chromatography-Silica Gel) eluted with 0.1 M sodium carbonate solution. The R_f value of free ^{68}Ga was 0.0 and labeled derivatives moved with the solvent front (see Supplementary Fig. 1). NOTA and DOTA also were labeled with ^{68}Ga as controls for the in vitro protein binding and cell uptake studies. The labeling efficiency was above 95% for all compounds, and no free ^{68}Ga was found after purification. The specific activities of the purified ^{68}Ga-labeled compounds fell in the range 1.94–9.21 GBq/μmol. Stability studies of labeled compounds at room temperature confirmed their stability for more than 4 h (see Supplementary Fig. 2).

2.3. In vitro studies

Protein binding importantly determines the biodistributions of labeled agents, because protein bound fractions cannot be transported into target cells before they are released from the protein. A protein binding studies were performed after incubating the labeled agents with human serum at 37 °C, and then bound and unbound fractions were separated using a PD-10 size exclusion column. All NOTA derivatives, ^{68}Ga-**1a**, ^{68}Ga-**1b**, and ^{68}Ga-**1c** showed lower levels of protein binding than the corresponding DOTA derivatives, ^{68}Ga-**2a**, ^{68}Ga-**2b**, and ^{68}Ga-**2c**, respectively (Table 1). Of these, ^{68}Ga-**1b** showed the lowest level of protein binding (1.71% at 10 min and 1.44% at 1 h), and ^{68}Ga-**2a** showed the highest level of binding (9.87% at 10 min and 19.81% at 1 h). Moreover, only the three DOTA derivatives, showed significant increases in protein binding with time. Although not fully proven by the present study, the results can be postulated based on chelate stabilities. It is known that NOTA forms much more stable chelate with gallium than DOTA,[31,34,35] and thus, ^{68}Ga might be transchelated to serum transferrin from the less stable chelates of DOTA and its derivatives.

The preliminary evaluation of these labeled amino acid derivatives in Hep3B (a human hepatoma cell-line) and CT-26 (a mouse colon cancer cell-line) cells showed significantly higher uptakes that the control compounds (^{68}Ga-NOTA and ^{68}Ga-DOTA) (Fig. 3). ^{68}Ga-**1a** showed higher uptake in both cell lines as compare with the other NOTA derivatives. However, in the case of DOTA derivatives, ^{68}Ga-**2a** showed highest uptake in Hep3B cells, and ^{68}Ga-**2b** (a homoalanine derivative) showed highest uptake in CT-26 cells. These different uptakes could be due to different expressions of amino acid transporters in these cells, because one amino acid can be transported by several different amino acid transporters

2.4. In vivo studies

A biodistribution study of the ^{68}Ga-labeled agents at 10, 30, 60, and 120 min after tail vein injection in mice bearing human colon cancer CT-26 xenografts was performed. Tumor uptakes were higher than those of most organs, except kidneys, at 120 min post-injection (see Supplementary Tables 1–6). Tumors to blood ratios were similar for all six derivatives up to 60 min post-injection, but differed at 120 min (Fig. 4A). Tumor to blood ratios of the NOTA-amino acid derivatives (^{68}Ga-**1a**, ^{68}Ga-**1b**, and ^{68}Ga-**1c**) were higher than those of the DOTA-amino acid derivatives (^{68}Ga-**2a**, ^{68}Ga-**2b**, and ^{68}Ga-**2c**) at 2 h post-injection, which might have been caused by higher serum protein binding by DOTA derivatives (Fig. 4A). Tumors to muscle ratios were not similar for all six agents up to 60 min post-injection. However, ^{68}Ga-**2c** demonstrated a higher tumor to muscle ratio than ^{68}Ga-**1c** at 2 h, which reversed the order observed at 30 min post-injection (Fig. 4B). Highest tumor to muscle ratio was achieved by ^{68}Ga-**1c** (4.09 ± 0.81), and this was followed by ^{68}Ga-**1b** (2.72 ± 1.09) and ^{68}Ga-**2a** (2.48 ± 0.71) at 30 min post-injection (Fig. 4B).

Animal PET imaging in mice bearing human colon cancer CT-26 xenografts was also performed. Images were obtained at 30 min after the intravenous injection of the six labeled agents through a tail vein (Fig. 5). High levels of bladder activity were observed for all agents, indicating rapid renal excretion. Tumor uptakes were visualized by PET, but bladder and kidney uptakes were higher for all six agents. For quantitative analysis, percentages of injected dose per tissue gram (% ID/g) were obtained for tumor and normal tissues (Fig. 6). ^{68}Ga-**1b** demonstrated the highest tumor to non-tumor ratio (12.3 ± 0.05), followed by ^{68}Ga-**2a** (5.9 ± 0.4) and ^{68}Ga-**2b** (4.3 ± 0.35) (Fig. 6). The above results suggest that ^{68}Ga-**1b** is the best proposition for tumor imaging by PET.

Table 1
Results of the protein binding study in human serum at 37 °C

Derivatives	Incubation time	
	10 min	1 h
^{68}Ga-NOTA	2.08 ± 0.01	2.67 ± 0.04
^{68}Ga-DOTA	2.04 ± 0.15	3.32 ± 0.24
^{68}Ga-**1a**	3.07 ± 0.14	3.77 ± 0.06
^{68}Ga-**2a**	9.87 ± 0.49	19.81 ± 0.54
^{68}Ga-**1b**	1.71 ± 0.41	1.44 ± 0.33
^{68}Ga-**2b**	6.40 ± 0.54	16.60 ± 0.92
^{68}Ga-**1c**	2.33 ± 0.47	2.64 ± 0.65
^{68}Ga-**2c**	2.44 ± 0.35	15.57 ± 0.28

Values represent bound fractions (%).

3. Conclusion

^{68}Ga-labeled amino acid derivatives have great potential for cancer PET imaging. In terms of the design of amino acid derivatives, a small R group is important for the recognition and transportation of a derivative by amino acid transporters. The most challenging factor regarding the design of these derivatives is that ^{68}Ga chelation requires a substantial R group. In the present study, we deliberately designed amino acid derivatives with small R groups, such as, NOTA-monoamide derivatives, and our NMR findings show that these derivatives form stable complexes with gallium. Furthermore, all six derivatives efficiently labeled by ^{68}Ga and showed low serum protein binding. Our in vitro cancer cell uptake study demonstrated that the six Ga chelates have significantly higher tumor uptakes than the ^{68}Ga-NOTA and DOTA controls. Moreover, our in vivo biodistribution and PET studies showed fast renal excretion and blood clearance, low nonspecific uptakes in normal organs except kidneys, and relatively high uptakes for all six agents in tumor tissues. These results suggest the possibility of using NOTA-monoamide derivatives for ^{68}Ga-labeled agents, when molecular size and complex stability are issues.

4. Experimental

4.1. General

DOTA and NOTA were purchased from ChemaTech (France), HPLC-grade MeCN was from Fischer Scientific Ltd (Seoul), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) from Fluka (Germany). Protected γ-amino homoalanine (4-amino-2-*tert*-butoxycarbonylamino-butyric acid *tert*-butyl ester) was purchased from Watanabe Chemical Industries (Japan). All other chemical reagents were purchased from Sigma Aldrich (St. Louis, USA). NMR spectra were recorded on a Jeol 300 JNM spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C). ^{1}H–1H COSY and ^{71}Ga NMR Spectra were recorded on a Bruker Avanee 600 spectrometer (Bruker, Germany; 600 MHz for COSY and

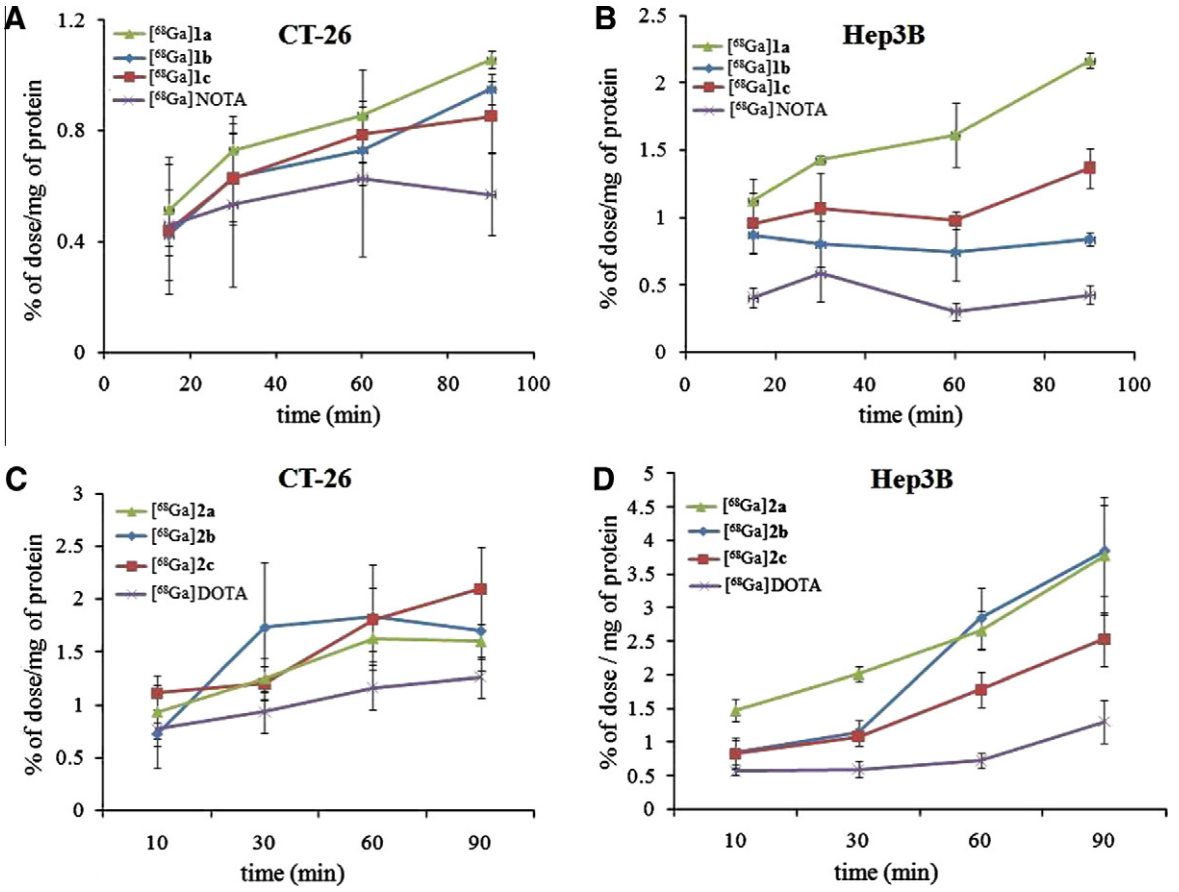


Figure 3. In vitro tumor cell uptake studies of ^{68}Ga-labeled amino acid derivatives, in which ^{68}Ga-NOTA and ^{68}Ga-DOTA were used as controls: (A) and (B) are results obtained for ^{68}Ga-labeled NOTA-amino acid derivatives (**1a–c**). (C) and (D) are results for DOTA-amino acid derivatives (**2a–c**). Uptake values are expressed as percentages of administered dose.

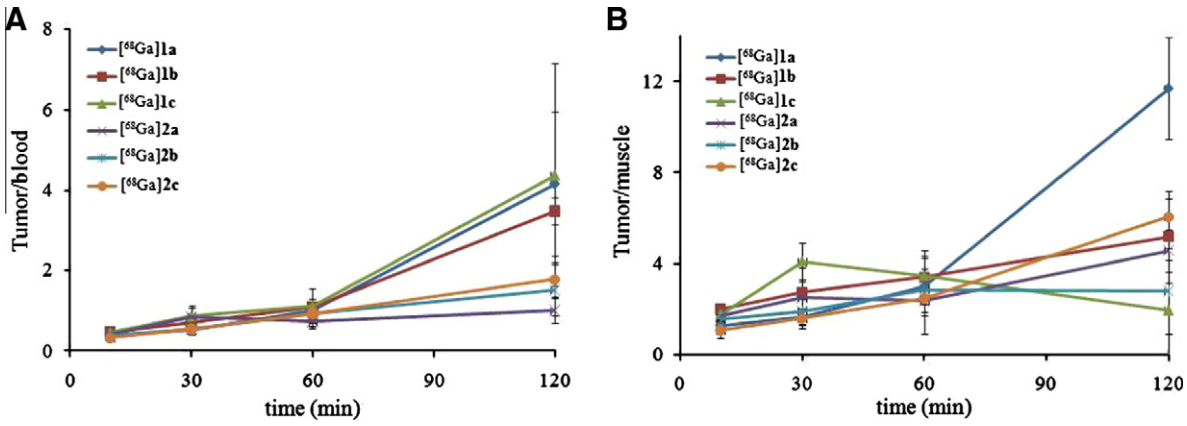


Figure 4. Biodistribution studies of ^{68}Ga-labeled compounds in mice bearing a CT-26 xenograft. Mice were sacrificed after a tail vein injection at different times (10, 30, 60, and 120 min): (A) tumor to blood ratio and (B) tumor to muscle ratio.

183 MHz for ^{71}Ga). ^{1}H resonance shifts were measured relative to tetramethylsilane (TMS) and ^{67}Ga chemical shifts relative to $[Ga(H_2O)_6]^{3+}$ in a 0.1 M $Ga(NO_3)_3$ D_2O solution. To calculate the specific rotations, a Jasco P-1020 Polarimeter was used with sample cell strength of 2 mL (Easton, MD, USA). Compounds were purified on an XTerra® prep RP18 (19 × 250 mm) preparative column (Waters Corporation, Milford, USA). The solvent systems used were; solvent A (0.01% HCl in H_2O) and solvent B (MeCN). The flow rates were 1 mL/min for analytical HPLC and 7 mL/min for preparative HPLC, at the indicated linear gradients. Electrospray ionization mass spectra (ESI-MS) were acquired using a Waters ESI ion trap spectrometer using positive and negative ion detection. Samples were diluted 100 times with methanol and injected directly into the source. To obtain chemical ionization mass spectra, a JEOL JMS 600 spectrometer was used. Fast atomic bombardment (FAB^+) ionization mass spectra were acquired on JEOL JMS-600W Agilent 6890 series spectrometer (Tokyo, Japan) in positive ion mode.

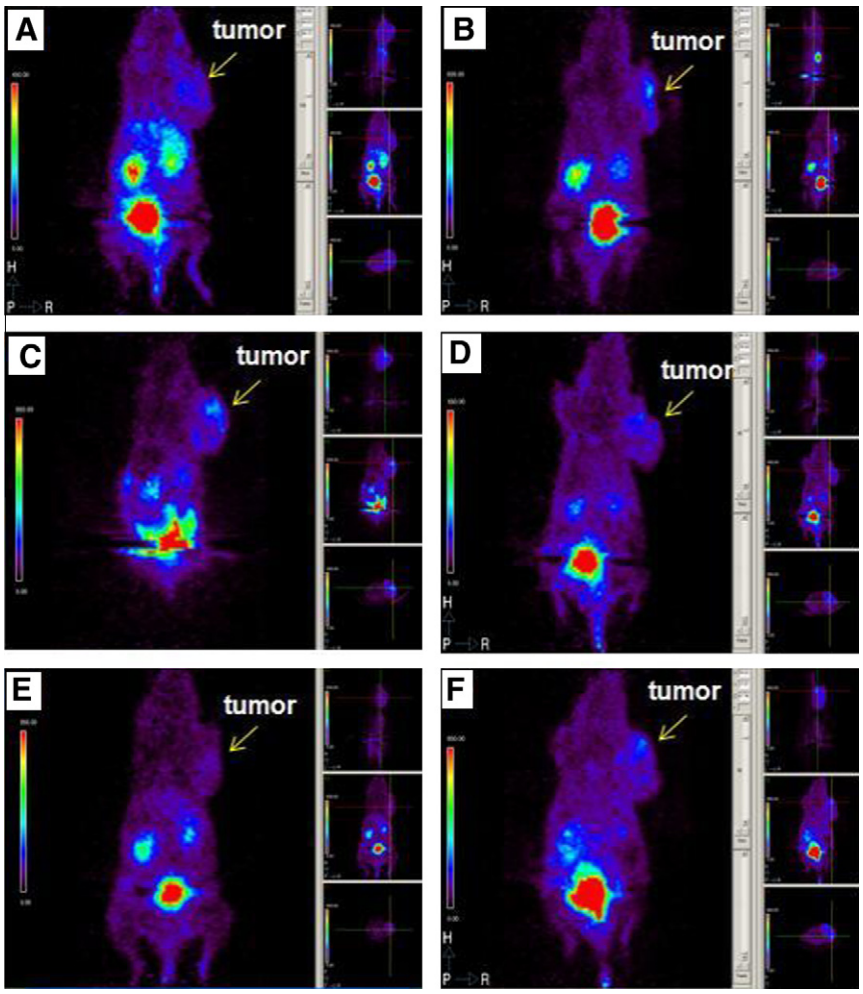


Figure 5. Small animal PET images of ^{68}Ga labeled amino acid derivatives. Images were recorded 30 min after tail vein injection into mice bearing a CT-26 xenograft: (A) ^{68}Ga-**1a**, (B) ^{68}Ga-**2a**, (C) ^{68}Ga-**1b**, (D) ^{68}Ga-**2b**, (E) ^{68}Ga-**1c**, and (F) ^{68}Ga-**2c**.

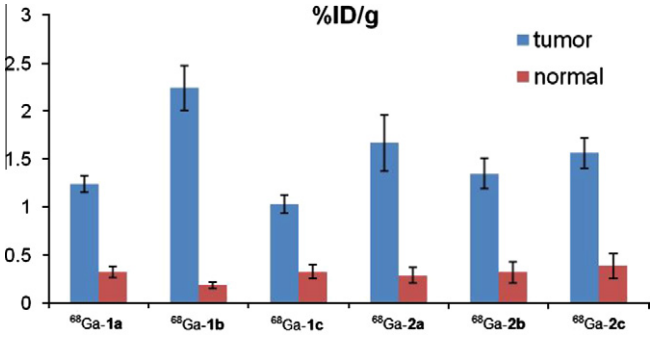


Figure 6. The values of % ID/g obtained for tumor and normal organ (muscle) from animal-PET images.

4.2. (*S*)-2-*tert*-Butoxycarbonylamino-3-methanesulfonyloxy-propionic acid methyl ester (4)

To a solution of commercially available *N*-*tert*-butyl-L-serine methyl ester, **3** (2 g, 9.1 mmol) and triethylamine (1 g, 10 mmol) in dichloromethane (50 mL) were added. Methane sulfonyl chloride (1.15 g, 10 mmol) was added slowly under cooling on ice. The reaction mixture was maintained at 0 °C for 30 min with stirring, and after checking reaction completion by TLC (Hexane/EtOAc: 7:3) using $KMnO_4$ solution as a staining agent, water (25 mL) was added with stirring. Two layers separated and the aqueous layer was extracted twice with dichloromethane (25 mL). Organic layers were pooled, washed with brine, and dried over anhydrous sodium sulfate. After filtration, the organic phase was evaporated to give **4** as colorless oil (2.2 g, 81% yield).

$[\alpha]_D^{20.7}$ +22 (*c* 0.98, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.49 (br, 1H), 4.53 (dd, *J* = 9.6, 3.3 Hz, 2H), 3.81 (s, 3H), 3.03 (s, 3H), 1.48 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.3, 154.9, 68.9, 63.1, 52.9, 37.2, 28.1. MS (APCI$^+$), *m/z* 298.1 $(M+H)^+$.

4.3. (*S*)-3-Azido-2-*tert*-butoxycarbonylamino-propionic acid methyl ester (5)

To a solution of **4** (2.2 g, 8.5 mmol) in DMF (60 mL), sodium azide (1.4 g, 21 mmol) was added stepwise with stirring. The reaction mixture was then heated to 50 °C for 30 min, and cold water (200 mL) was added with stirring. The reaction mixture was then extracted with EtOAc (60 mL). The aqueous layer was further extracted with EtOAc (60 mL) two times. The organic layers were pooled, washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a light yellow oil. Further purification was performed by flash chromatography on a silica gel column. Elution with 1:5 ethyl acetate/hexane and evaporation under reduced pressure gave **5** as colorless oil (1 g, 56% yield).

$[\alpha]_D^{20.7}$ +26 (*c* 0.99, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 5.37 (br, 1H, –N*H*), 4.42 (t, 1H), 3.73 (s, 3H), 3.66 (d, *J* = 3.3 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2, 155.0, 80.4, 53.4, 52.8, 59.7, 28.1. MS ($APCI^+$), *m/z* 245.1 $(M+H)^+$.

4.4. (*S*)-3-Amino-2-*tert*-butoxycarbonylamino-propionic acid methyl ester (6)

A solution of azide **5** (1 g, 4.08 mmol) and 10% Pd–C (60 mg) in 10 mL of absolute ethanol was stirred at room temperature under 1 atm of hydrogen for 90 min. The catalyst was removed by filtration through a Celite filter, which was then washed with ethanol. Filtrates were pooled and concentrated under reduced pressure to give amine **6** as a colorless oil (700 mg, 79% yield).

$[\alpha]_D^{19}$ −18 (*c* 0.5, EtOH). ^1H NMR (300 MHz, $CDCl_3$): δ 5.42 (br, 1H, –N*H*), 4.24 (br, 1H), 3.73 (s, 3H), 3.06 (d, *J* = 12.3 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2, 155.7, 80.3, 53.9, 52.7, 42.5, 28.3. MS (CI^+), *m/z* 219.39 $(M+H)^+$.

4.5. 6-Benzoylamino-2-*tert*-butoxycarbonylamino-hexanoic acid *tert*-butyl ester (10)

To a solution of Boc anhydride (0.4 g, 1.84 mmol) and **9** (0.5 g, 1.31 mmol) in *tert*-butanol (2.5 mL), 4-dimethylaminopyridine (DMAP) (0.3 equiv) in *t*-butanol (0.5 mL) was added at room temperature. After stirring for 9 h, the solvent was removed under reduced pressure and the residue was purified by column chromatography (EA/Hexane: 3:7) to obtain **10**.

$[\alpha]_D^{20}$ +3.85 (*c* 1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.38–7.30 (m, 5H, –Ar), 5.08 (s, 2H), 4.86 (s, 1H), 4.16–4.11 (m, 1H), 3.19 (q, *J* = 6.6, 6.3 Hz, 2H), 1.75–1.49 (4H, m), 1.45 (9H, s), 1.43 (9H, s), 1.28–1.22 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.9, 155.5, 81.9, 79.6, 53.9, 40.7, 32.6, 30.9, 30.0, 28.4, 28.0, 22.5, 21.7. MS (ESI^+), *m/z* 437.2 $(M+H)^+$, 895.4 $(2\,M+Na)^+$.

4.6. 6-Amino-2-*tert*-butoxycarbonylamino-hexanoic acid *tert*-butyl ester (11)

To a solution of **10** (0.4 g, 0.92 mmol) in ethanol (2.5 mL), Pd–C (10%) was added. The reaction mixture was stirred at room temperature under a hydrogen atmosphere. After 3 h, the reaction mixture was filtered, washed with methylene chloride, and solvent was evaporated under reduced pressure to give **11**.

$[\alpha]_D^{20}$ +4.18 (*c* 1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 5.14 (d, 1H, –N*H*), 4.15–4.13 (m, 1H), 2.87–2.84 (m, 2H), 1.94–1.56 (m, 4H), 1.46 (9H, s), 1.43 (9H, s), 1.25–1.21 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.9, 155.5, 81.8, 79.6, 58.0, 53.7, 40.5, 32.4, 29.8, 28.2, 27.9, 22.4, 18.3; MS (ESI^+), *m/z* 302.9 $(M+H)^+$, 605.4 $(2\,M+H)^+$.

4.7. General procedure for the synthesis of compounds 7a and 8a

To a solution of DOTA or NOTA (0.05 g) and HOBT (1 equiv) in water (1.5 mL), **6** (2 equiv) in methanol (0.5 mL) was added slowly. The reaction mixture was cooled on ice and it pH adjusted to 4.5–5.0 using an organic base, such as, *N,N*-diisopropylethylamine. EDC·HCl (1.5 equiv) in water (0.5 mL) was added slowly to the mixture, which was then stirred for 30 min with cooling. Its pH was then elevated to 8 by adding *N,N*-diisopropylethylamine, and it was stirred for a further 30 min at room temperature. Reaction completion was monitored by HPLC and mass analysis (ESI^+). Crude products were purified by preparative RP–HPLC (100% of A for 5 min and 0–50% of B for 25 min) to afford compounds **7a** or **8a** (~50%). (HPLC, t_R = 17.2 min for **7a**, 14.1 min for **8a**); MS (ESI^+), *m/z* 504.2 $(M+H)^+$ for **7a**, 605.3 $(M+H)^+$ for **8a**.

4.8. General procedure for synthesizing compounds 1a and 2a

To a solution of **7a** or **8a** in water (0.5 mL), lithium hydroxide (4 equiv) was added slowly under cooling on ice. The reaction mixture was then maintained at room temperature for 4 h. The completion of hydrolysis was monitored by HPLC (t_R shifted from 17.2 to 14.6 min for **7a** and from 14.1 to 12.6 min for **8a**) and mass analysis. After reaction completion, the mixture was neutralized slowly and it pH was further reduced to around 1 by adding 30% HCl. The mixture was then stirred at room temperature until the reaction was complete by HPLC and mass analysis. The crude product was purified by preparative RP–HPLC (100% of A for 5 min and 0–50% of B for 25 min) to afford compounds **1a** or **2a** as white solid.

4.8.1. (*S*)-2-Amino-3-[2-(4,7-bis-carboxymethyl-1,4,7 triazonan-1-yl)-acetylamino]-propionic acid·HCl (1a)

Yield: 75%. $[\alpha]^{17.9}$ −14 (*c* 0.1, MeOH). ^1H NMR (300 MHz, D_2O): δ 4.09 (t, *J* = 1.5 Hz, 1H), 3.90 (s, 4H), 3.72 (s, 2H), 3.68 (d, *J* = 5.1 Hz, 1H), 3.64 (d, *J* = 6.2 Hz, 1H), 3.38 (s, 4H), 3.28 (br, 4H), 3.14 (br, 4H). ^{13}C NMR (75 MHz, D_2O): δ 173.4, 172.2, 170.4, 58.4, 57.6, 53.7, 51.9, 51.0, 50.7, 39.7. MS (ESI^+), *m/z* 390.2 $(M+H)^+$: HRMS: 390.1989 observed, 390.1989 calcd.

4.8.2. (*S*)-2-Amino-3-[2-(4,7,10-tris-carboxymethyl-1,4,7,10 tetraaza-cyclododec-1-yl)-acetylamino]-propionic acid·HCl (2a)

Yield: 67%. $[\alpha]_D^{19.5}$ −12 (*c* 0.2, MeOH). ^1H NMR (300 MHz, D_2O): δ 3.96 (q, *J* = 9.6, 9.36 Hz, 1H), 3.73 (s, 4H), 3.62 (s, 2H), 3.50 (d, *J* = 7.2 Hz, 2H), 3.45 (d, *J* = 7.2 Hz, 2H), 3.30–2.83 (m, 12H), 2.99 (br, 4H). ^{13}C NMR (75 MHz, D_2O): δ 169.6, 168.8, 54.8, 49.9, 49.6, 49.0, 48.7, 48.4, 48.2, 40.3, 39.7. MS (ESI^+), *m/z* 491.3 $(M+H)^+$: HRMS: 491.2456 observed, 491.2466 calcd.

4.9. General procedure used to synthesize compounds 7b–c and 8b–c

To a solution of NOTA or DOTA (0.1 g) and HOBT (1 equiv) in water (3 mL), protected amino acids with free γ- or ε-amino group (**11** or **12**) (1 equiv) in acetonitrile (3 mL) was added. To this solution, DCC (1 equiv) in pyridine (17 mg/0.1 mL) was added slowly. The reaction mixture was maintained at room temperature with stirring for 24 h. After completion of the reaction, the mixture was filtered, and the filtrate was concentrated under reduced pressure to remove pyridine, and then purified by RP–HPLC (100% A for 5 min, 0–70% of B for another 25 min). The collected product fraction was lyophilized after removal of organic solvent. After lyophilization, products were obtained together Boc cleaved products.

4.10. General procedure used to synthesize compounds 1b–c and 2b–c

The products (**7b–c** and **8b–c**) were re-dissolved in a minimum amount of water (~1 mL) and 4 M hydrochloric acid in dioxane (4 mL) was added. The reaction mixture was maintained at room temperature for 6 h with stirring. Reaction completion was observed by mass analysis, and on completion, solvent was removed under reduced pressure and purified by RP–HPLC (0–40% of B for 20 min) to yield the final products as hydrochloride salts.

4.10.1. 2-Amino-4-[2-(4,7-bis-carboxymethyl-1,4,7 triazonan-1-yl)-acetylamino]-butyric acid·HCl (1b)

$[\alpha]_D^{19.5}$ −34.96 (*c* 0.35, CH_3OH). ^1H NMR (300 MHz, D_2O): δ 3.91 (s, 4H), 3.87–3.84 (m, 1H), 3.71 (s, 2H), 3.39–3.21 (m, 14H), 2.01–1.91 (m, 2H). ^{13}C NMR (75 MHz, D_2O): δ 172.0, 171.2, 170.8, 58.3, 57.5, 52.0, 51.5, 51.3, 51.0, 35.9, 29.7. MS (ESI^+), *m*/z 404.3 $(M+H)^+$: HRMS: 404.2149 observed, 404.2145 calcd.

4.10.2. 2-Amino-4-[2-(4,7,10-tris-carboxymethyl-1,4,7,10 tetraaza-cyclododec-1-yl)-acetylamino]-butyric acid·HCl (2b)

$[\alpha]_D^{19.5}$ −40.95 (*c* 0.3, CH_3OH). ^1H NMR (300 MHz, D_2O): δ 3.96–3.95 (d, *J* = 3.0 Hz, 2H), 3.60–2.60 (m, 25H), 2.04–1.92 (m, 2H). ^{13}C NMR (75 MHz, D_2O): δ 172.4, 171.2, 55.1, 54.9, 54.1, 51.9, 51.0, 36.6, 35.5, 30.1, 28.1. MS (ESI^+), *m/z* 505.2 $(M+H)^+$: HRMS: 505.2613 observed, 505.2622 calcd.

4.10.3. 2-Amino-6-[2-(4,7-bis-carboxymethyl-1,4,7 triazonan-1-yl)-acetylamino]-hexanoic acid·HCl (1c)

$[\alpha]_D^{19.5}$ −51.8 (*c* 0.2, CH_3OH). ^1H NMR (300 MHz, D_2O): δ 3.93 (s, 4H), 3.78 (s, 2H), 3.37–3.20 (m, 13H), 3.12–3.10 (m, 2H), 1.84–1.78 (br, 2H), 1.43–1.30 (m, 4H). ^{13}C NMR (75 MHz, D_2O): δ 172.7, 171.9, 58.9, 57.3, 53.4, 51.6, 51.4, 39.7, 29.9, 28.4, 22.2. MS (ESI^+), *m/z* 432.3 $(M+H)^+$: HRMS: 432.2455 observed, 432.2458 calcd.

4.10.4. 2-Amino-6-[2-(4,7,10-tris-carboxymethyl-1,4,7,10 tetraaza-cyclododec-1-yl)-acetylamino]-hexanoic acid·HCl (2c)

$[\alpha]_D^{19.5}$ −36.08 (*c* 0.3, CH_3OH). ^1H NMR (300 MHz, D_2O): δ 3.89 (t, *J* = 6.0 Hz, 2H), 3.70–2.65 (m, 25H), 1.76 (m, 2H), 1.38 (m, 4H). ^{13}C NMR (75 MHz, D_2O): δ 172.6, 172.4, 55.6, 54.9, 53.8, 53.3, 51.8, 49.2, 39.5, 29.9, 28.3, 26.9, 22.1. MS (ESI^+), *m/z* 533.3 $(M+H)^+$: HRMS: 533.2936 observed, 533.2935 calcd.

4.11. Synthesis of Ga-1a

The complex formed between **1a** and Ga^{3+} was prepared in aqueous solution by adding 1:1 stoichiometric amount of **1a** and gallium (III) nitrate. The pH of the solution was then adjusted to ~3 using 1 M NaOH solution and microfine pH test paper (Sigma–Aldrich, Hydrion™ Microfine™). The reaction mixture was stirred for 20 min, and reaction completion was monitored by mass analysis (ESI+). The complex obtained was purified by RP–HPLC [water (A)/EtOH; 0–40% B for 30 min]. After lyophilization of the collected product fractions, Ga-**1a** was analyzed.

^1H NMR (600 MHz, D_2O, pH ~7): δ 3.79–3.75 (m, 2H), 3.69–3.68 (m, 1H), 3.64 (s, 2H), 3.63 (s, 4H), 3.33–3.21 (m, 8H), 3.07–2.92 (m, 4H). ^{71}Ga NMR (600 MHz, D_2O, pH ~7): δ 170 ($\omega_{1/2}$ = 286 Hz). MS (ESI^+), *m/z* 456.1$(M+H)^+$.

4.12. General procedure used for the radiolabeling experiment

$^{68}GaCl_3$ (2.6–7.7 mCi) in hydrochloric acid (0.1 M) was obtained by $^{68}Ge/^{68}Ga$-generator elution. Radiolabeling of all amino acid derivatives (50 nmol each) was carried out at pH 3.1 by buffering reaction solutions with 1 M sodium acetate (pH 5). Reactions were performed for 10 min either at room temperature for NOTA derivatives or in a boiling water bath for DOTA derivatives. The control compounds NOTA and DOTA were also labeled using the same conditions. Labeling yields were determined by Instant Thin Layer Chromatography (ITLC SG. German Science, Ann Arbor, MI) in 0.1 M citric acid and saline solution. The radioactivity of strip was scanned using a TLC scanner (AR-2000, Bioscan, USA).

4.13. The stability study and protein binding assay

To check the stability of the labeled compounds they were placed in an incubator for 10, 30, 60, 120, and 240 min after labeling at room temperature. Extents of decomposition were checked by ITLC eluted with 0.1 M Na_2CO_3.

Protein binding assays were performed as previously described.[36] Labeled compounds were incubated with human serum (1 mL) at 37 °C for 10 or 60 min. After incubation, compounds in human serum were loaded onto a PD-10 column (pre-conditioned with 1 mL of 1% bovine serum albumin (BSA)/0.1 M DTPA), and eluted with phosphate buffered saline (PBS) solution into test tubes. Thirty 0.5 mL fractions were collected. The radioactivities of each fraction (expressed as cpm) were measured using a gamma scintillation counter (Packard, Canberra Co., USA). Two microliters of aliquots from each test tube were spotted onto filter paper and the presence of protein was checked by Coomassie blue dye staining. Percentage protein bindings were calculated using fraction activity curves.

4.14. Cell culture and transport assay

Hep3B (a human hepatoma cell-line) and CT-26 (a colon cancer cell-line) cells were obtained from the American Type Culture Collection (ATCC). Cells were grown in DMEM media (Welgene Inc., Korea). All media were supplemented with a 1% mixture of penicillin, streptomycin, and amphotericin B (10,000 IU/10 mg/25 μg/mL, Mediatech Inc., USA) and 10% fetal bovine serum (Welgene Inc., Korea). Cells were cultivated at 37° in a in 5% CO_2 incubator.

Cell uptake assays were performed after seeding about 1.2×10^5 cells/mL for CT-26 or 1.5×10^5 cells/mL for Hep3B in 24-multiwell culture plates 20 h beforehand. When cells were cultured to about 80% of confluence, transport assays were started by adding labeled derivatives or control compounds (10 μCi each) to 0.5 mL of media per well and then incubated at 37° in a CO_2 incubator. Media were then aspirated, and cells were washed twice with ice-cold Hank's balanced salt solution (HBSS, pH 7.3, Gibco, USA). After washing, cells were dissolved in 0.5% sodium dodecylsulfate. The radioactivities of harvested cells were measured using a gamma scintillation counter. Total protein concentrations in samples were determined using the bicinchoninic acid method (Pierce, USA).

4.15. Biodistribution in mice bearing colon cancer xenografts

All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee of the Clinical Research Institute at Seoul National University Hospital (an Association for the Assessment and Accreditation of Laboratory Animal Care accredited facility). In addition, the guidelines of the National Research Council for the care and use of laboratory animals (revised in 1996) were observed throughout. The human colon cancer cell-line CT-26 was grown in RPMI 1640 medium containing 10% fetal bovine serum and harvested with trypsin. Cells were washed with 10 mL of PBS by centrifugation (3000 rpm). Each balb/c mouse was injected subcutaneously with 2×10^5/0.1 mL of CT-26 cells in the right shoulder. After 13 days, labeled compounds (10 μCi/0.1 mL) were injected intravenously into each xenografted mouse through a tail vein. Mice were sacrificed at different times (10, 30, 60, and 120 min) after injection. Tumor, blood, muscle, and organs were obtained immediately and weighed. Counts were obtained using a γ-scintillation counter.

4.16. PET of mice bearing xenografts

Mice with tumors from CT-26 cell xenografts grown for 14 days were injected with a ^{68}Ga labeled amino acid derivative (0.6–0.8 mCi in 0.1 mL) through a tail vein. After inducing anesthesia with 2% isoflurane, PET images were obtained using a dedicated small-animal PET/CT scanner (GE Healthcare, Princeton, NJ, USA). The acquired three-dimensional emission data were reconstructed to temporally framed sonograms by Fourier rebinning using an ordered-subsets expectation maximization reconstruction algorithm without attenuation correction. Image visualization was performed with ASIPro software (Concorde Microsystems Inc.).

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Supplementary data

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References and notes

1. Conti, P. S. *Cancer Invest.* **1995**, *13*, 244.
2. Dichiro, G.; Patronas, N. J.; Delapaz, R. L.; Smith, B. H.; Milam, H.; Brooks, R. A.; Kornblith, P. L. *Ann. Neurol.* **1982**, *12*, 75.
3. Gupta, N. C.; Frick, M. P. C. A. *Cancer J. Clin.* **1993**, *43*, 235.
4. Chen, W. *J. Nucl. Med.* **2007**, *48*, 1468.
5. Ko, D.; Choi, J.; Song, Y.; Lee, S.; Kim, Y.; Lee, K.; Kim, B.; Lee, M. *Nucl. Med. Mol. Imaging* **2008**, *42*, 444.
6. Jager, P. L.; Vaalburg, W.; Pruim, J.; de Vries, E. G.; Langen, K. J.; Piers, D. A. *J. Nucl. Med.* **2001**, *42*, 432.
7. Ogawa, T.; Shishido, F.; Kanno, I.; Inugami, A.; Fujita, H.; Murakami, M.; Shimosegawa, E.; Ito, H.; Hatazawa, J.; Okudera, T.; Uemura, K.; Yasui, N.; Mineura, K. *Radiology* **1993**, *186*, 45.
8. Kim, S.; Chung, J. K.; Im, S. H.; Jeong, J. M.; Lee, D. S.; Kim, D. G.; Jung, H. W.; Lee, M. C. *Eur. J. Nucl. Med. Mol. Imaging* **2005**, *32*, 52.
9. Shoup, T. M.; Goodman, M. M. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 215.
10. Wester, H. J.; Herz, M.; Weber, W.; Heiss, P.; Senekowitsch-Schmidtke, R.; Schwaiger, M.; Stocklin, G. *J. Nucl. Med.* **1999**, *40*, 205.
11. Breeman, W. A.; Verbruggen, A. M. *Eur. J. Nucl. Med. Mol. Imaging* **2007**, *34*, 978.
12. Ehrhardt, G. J.; Welch, M. J. *J. Nucl. Med.* **1978**, *19*, 925.
13. Zhernosekov, K. P.; Filosofov, D. V.; Baum, R. P.; Aschoff, P.; Bihl, H.; Razbash, A. A.; Jahn, M.; Jennewein, M.; Rosch, F. *J. Nucl. Med.* **2007**, *48*, 1741.
14. Chen, X.; Li, L.; Liu, F.; Liu, B. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5503.
15. Storr, T.; Obata, M.; Fisher, C.; Bayly, S.; Green, D.; Brudzinska, I.; Mikata, Y.; Patrick, B.; Adam, M.; Yano, S. *Chem. Eur. J.* **2005**, 11.
16. Liu, Y.; Pak, J. K.; Schmutz, P.; Bauwens, M.; Mertens, J.; Knight, H.; Alberto, R. *J. Am. Chem. Soc.* **2006**, *128*, 15996.
17. Jeong, J. M.; Hong, M. K.; Chang, Y. S.; Lee, Y. S.; Kim, Y. J.; Cheon, G. J.; Lee, D. S.; Chung, J. K.; Lee, M. C. *J. Nucl. Med.* **2008**, *49*, 830.
18. Maecke, H. R.; Hofmann, M.; Haberkorn, U. *J. Nucl. Med.* **2005**, *46*, 172S.
19. Sabatino, G.; Chinol, M.; Paganelli, G.; Papi, S.; Chelli, M.; Leone, G.; Papini, A. M.; De Luca, A.; Ginanneschi, M. *J. Med. Chem.* **2003**, *46*, 3170.
20. Velikyan, I.; Maecke, H.; Langstrom, B. *Bioconjugate Chem.* **2008**, *19*, 569.
21. Burchardt, C.; Riss, P.; Zoller, F.; Maschauer, S.; Prante, O.; Kuwert, T.; Roesch, F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3498.
22. Shetty, D.; Jeong, J. M.; Ju, C. H.; Lee, Y. S.; Jeong, S. Y.; Choi, J. Y.; Yang, B. Y.; Lee, D. S.; Chung, J. K.; Lee, M. C. *Nucl. Med. Biol.*, in press.
23. Guerin, B.; Ait-Mohand, S.; Tremblay, M. C.; Dumulon-Perreault, V.; Fournier, P.; Benard, F. *Org. Lett.* **2010**, *12*, 280.
24. Shetty, D.; Choi, S. Y.; Jeong, J. M.; Hoigebazar, H.; Lee, Y. S.; Lee, D. S.; Chung, J. K.; Lee, M. C.; Chung, Y. K. *Eur. J. Inorg. Chem.*, in press.
25. Hoigebazar, L.; Choi, S. Y.; Choi, J. Y.; Shetty, D.; Lee, Y. S.; Lee, D. S.; Chung, J. K.; Lee, M. C.; Chung, Y. K. *J. Med. Chem.* **2010**, *53*, 6378.
26. McKillop, A.; Taylor, R.; Watson, R.; Lewis, N. *Synthesis* **1994**, 31.
27. Serrano, P.; Llebaria, A.; Delgado, A. *J. Org. Chem.* **2005**, *70*, 7829.
28. Zou, Y.; Fahmi, N. E.; Vialas, C.; Miller, G. M.; Hecht, S. M. *J. Am. Chem. Soc.* **2002**, *124*, 9476.
29. Glomb, M. A.; Pfahler, C. *J. Biol. Chem.* **2001**, *276*, 41638.
30. Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y. *Synthesis* **1994**, 1063.
31. Broan, C.; Cox, J.; Craig, A.; Kataky, R.; Parker, D.; Harrison, A.; Randall, A.; Ferguson, G. *J. Chem. Soc., Perkin Trans. 2* **1991**, 87.
32. Cole, E.; Copley, R.; Howard, J.; Parker, D.; Ferguson, G.; Gallagher, J.; Kaitner, B.; Harrison, A.; Royle, L. *J. Chem. Soc., Dalton Trans.* **1994**, 1619.
33. Prata, M. I.; Santos, A. C.; Geraldes, C. F.; de Lima, J. J. *J. Inorg. Biochem.* **2000**, *79*, 359.
34. Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1991**, *181*, 273.
35. Lee, J.; Garmestani, K.; Wu, C.; Brechbiel, M. W.; Chang, H. K.; Choi, C. W.; Gansow, O. A.; Carrasquillo, J. A.; Paik, C. H. *Nucl. Med. Biol.* **1997**, *24*, 225.
36. Jeong, J. M.; Kim, Y. J.; Lee, Y. S.; Lee, D. S.; Chung, J. K.; Lee, M. C. *Nucl. Med. Mol. Imaging* **2009**, *43*, 330.