Efficient and Site-Specific $^{125}$I-Radioiodination of Bioactive Molecules Using Oxidative Condensation Reaction

Sajid Mushtaq,†‡ You Ree Nam,† Jung Ae Kang,† Dae Seong Choi, † and Sang Hyun Park*,†‡

†Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup, Jeonbuk 56212, Republic of Korea
‡Department of Radiation Biotechnology and Applied Radioisotope Science, Korea University of Science and Technology, Daejeon 34113, Republic of Korea

Supporting Information

ABSTRACT: In this report, the novel and site-specific radioiodination of biomolecules by using aryl diamine and alkyl aldehyde condensation reaction in the presence of a Cu(II) catalyst under ambient conditions was reported. $^{125}$I-labeled alkyl aldehyde was synthesized using a tin precursor with a high radiochemical yield (72 ± 6%, n = 5) and radiochemical purity (>99%). The utility of the radioiodinated precursor was demonstrated through aryl diamine-installed cRGDK(C) peptide and human serum albumin (HSA). Radioiodinated cRGDK(C) peptide and HSA protein were synthesized with high radiochemical yields and purity. $^{125}$I-HSA protein showed excellent in vivo stability and negligible thyroid uptake as compared with directly radioiodinated HSA by using the tyrosine group. Excellent reaction kinetics and the in vitro and in vivo stabilities of $^{125}$I-labeled alkyl aldehyde have suggested the usefulness of the strategy for the radioiodination of bioactive molecules.

INTRODUCTION

Among the various available radioisotopes, radioiodine is the most extensively used for radiolabeling of biologically active macro and micromolecules.1–3 Radioiodine can be used for positron emission tomography (PET) imaging (124I),4 single-photon emission computed tomography (SPECT) imaging (123/125I),5,6 treatment of various cancers (131I),7 and pharmacokinetics and biodistribution studies of newly designed therapeutic drugs (125I).8 Several methodologies have been reported in the literature for the efficient and chemoselective iodination of biomolecules.11,12 The most frequently used strategy is direct radioiodination via the tyrosine group in the selected peptides and proteins by using some strong oxidizing agents.13 The use of an electrophilic aromatic substitution reaction on the tyrosine group provides a high radiochemical yield within a short time.14 However, radioiodine attached to biomolecules are usually unstable inside a living body. In vivo deiodination and the accumulation of free radiiodine in the blood, thyroid, and other organs results in a high background signal and poor image quality.15 Moreover, strong oxidizing agents can damage and/or alter the bioactivity of radiolabeled biomolecules.16 To address these issues, many radioiodinated maleimide and N-succinimidyl ester-based prosthetic groups have been synthesized for thiol and amino group coupling reactions, respectively (Scheme 1).17–19 Unfortunately, these prosthetic groups cannot provide chemoselective, fast, and high-yield radiolabeling.16 Recently, conventional bioconjugation methods including radioiodinated azide–alkyne cycloaddition,20 Staudinger–Bertozzi ligation,21 and inverse electron-demand Diels–Alder ligation between trans-cyclooctene (TCO)-conjugated biomolecules and radioiodinated tetrazine were utilized for radioiodination.22 The bioconjugation reactions based on azide–alkyne cycloaddition and Staudinger–Bertozzi ligation demonstrated slow reaction kinetics. The reaction between TCO and radioiodinated tetrazine is fast but it provides a mixture of radioiodinated products, which may not be acceptable to regulatory authorities for clinical application.22 Therefore, the search for more efficient, easy to apply, and clean bioconjugation reactions is still in demand to satisfy diverse applications. Moreover, expansion can provide additional and better choices when multiple reactions are used to modify the biomolecules.

For several decades, benzimidazole molecules have been widely applied in organic synthesis, and various strategies have been developed for the construction of benzimidazole.23–26 Previously published reports have suggested that o-phenylenediamine and alkyl aldehyde can react quickly in the presence of oxygen and a catalytic amount of copper to provide highly stable benzimidazole with a high yield at room temperature (rt).27,28 In this report, a new radioiodination strategy using an aryl diamine and alkyl aldehyde condensation reaction has been demonstrated. The condensation reactions between aryl...
diamine and radioiodinated alkyl aldehyde have been tested. The cRGD peptide and human serum albumin (HSA) protein were used as the model substrates.\textsuperscript{22} The biodistribution data for radioiodinated HSA have been acquired to confirm the in vivo stability against deiodination. Among the various available radioiodine, \textsuperscript{125}I has been selected because of its easy commercial availability and suitable half-life (59.4 days), which allows repetitive experiments to optimize the radio-labeling procedure.\textsuperscript{29}

### RESULTS AND DISCUSSION

#### Radiosynthesis of \textsuperscript{125}I-Labeled Aldehyde \([\text{\textsuperscript{125}I}] 5\)

The radiosynthesis of \textsuperscript{125}I-labeled aldehyde \([\text{\textsuperscript{125}I}] 5\) is shown in Scheme 3. An alkyl chain containing aldehyde has been synthesized by considering its importance for the efficient production of the corresponding benzimidazole. For the identification and characterization of radioiodinated \([\text{\textsuperscript{125}I}] 5\), nonradioactive analogue \textsuperscript{4}-iodo-N-(4-oxobutyl)benzamide \(4\) has been synthesized by the coupling of \textsuperscript{4}-aminobutyraldehyde diethyl acetal and \textsuperscript{4}-iodobenzoic acid under basic conditions and then subsequent reduction under acidic conditions in the next step (Scheme 2). Radioiodination of \(6\) was accomplished using \([\text{\textsuperscript{125}I}]{\text{Na}}\) and chloramine-T as an oxidizing agent (Scheme 3). The radioiodination reaction was terminated by adding aqueous sodium metabisulphite. After high performance liquid chromatography (HPLC) purification of the crude product, \([\text{\textsuperscript{125}I}] 5\) was obtained with a high radiochemical yield (72 ± 6%, \(n = 5\)). The specific activity was 45 GBq/\(\mu\)mol and chemical purity was more than 99%. A HPLC chromatogram of the crude mixture clearly showed the major product \([\text{\textsuperscript{125}I}] 5\) at 22.6 min (Figure S16). Radiolabeling reaction was performed by using varying amounts of radioactivity (100 \(\mu\)Ci to 1 mCi), however, the radiochemical results were consistent. The radioiodinated compound \([\text{\textsuperscript{125}I}] 5\) was found to be stable in the refrigerator (4 °C) for more than 6 months and did not undergo hydrolysis, unlike already used active ester-based prosthetic groups (Bolton–Hunter-reagent).\textsuperscript{8} Compound \([\text{\textsuperscript{125}I}] 5\) was found to be stable in different media, including phosphate-buffered saline (PBS), saline, and mouse serum at 37 °C for more than 24 h, as determined using radio-HPLC (Figure S17).

#### Radiosynthesis of Compound \([\text{\textsuperscript{125}I}] 7\)

Aldehyde diamine coupling reactions were carried out using radioiodinated compound \([\text{\textsuperscript{125}I}] 5\) and diamine group containing compound \(4\). An aryl diamine ring supplemented with the electron-withdrawing group (–OCH\textsubscript{2}–) has been selected to accelerate the coupling reaction (Scheme 1). Before the radiosynthesis of the target compound \([\text{\textsuperscript{125}I}] 7\), a nonradioactive analogue \(7\) was synthesized for the HPLC identification and characterization of \([\text{\textsuperscript{125}I}] 7\). Compound \(7\) was synthesized by stirring compounds \(4\) and \(5\) in the presence of oxygen and a catalytic amount of copper sulfate at rt (Scheme 2). The radiolabeling reaction was performed by mixing various concentrations of \(5\)-[(3,4-diaminophenoxy)-N-\((2\text{-}(2,5\text{-dioxo}-2,5\text{-dihydro-1H-pyrrol-1-yl})\text{ethyl})\text{pentanamide} \(4\) (5, 25, and 50 \(\mu\)M) with 100 \(\mu\)Ci of \([\text{\textsuperscript{125}I}] 5\) at rt. The reaction was monitored at different time...
were obtained using 50 and 25 μM of 4, respectively, within 30 min and at rt (entries 2 and 3). In addition, more than 80% of \([^{125}\text{I}]\ 5\) was converted into \([^{125}\text{I}]\ 7\) using 5 μM of substrate 4 in 30 min (entry 4). The reaction was slow at 5 min although we used 50 μM of precursor 4 (entry 1), however, a high radiochemical yield was observed 30 min post incubation (entry 3). The radiochemical yields and reaction kinetics were found to be comparable to many bioconjugate reactions given in the literature.28 These results encouraged us to label the model protein using a diamine and aldehyde coupling reaction.

**Radioiodination of HSA \([^{125}\text{I}]\ 9\).** To determine the radiolabeling efficiency of an aryl diamine alkyl aldehyde coupling reaction, \([^{125}\text{I}]\ 5\) was treated with aryl diamine containing HSA protein 9 (Scheme 5). The HSA protein not only serves as a drug-delivery carrier but also enhances the blood circulation time of rapidly clearing drugs.31−34 It is readily available against chemical modifications and can be stable at different pH levels (4−9) and at high temperature (60°C).
For successful noninvasive sentinel lymph node imaging, 68Ga, 99mTc, and 18F-labeled HSA have been synthesized and used. In this experiment, we used maleimide–cysteine-34 conjugation instead of N-succinimidyl ester–lysine based coupling on HSA because it offers a better diamine installed HSA protein with minimum alteration of the protein structure and a very high purity product. In our previous study, for the synthesis of TCO-modified HSA, TCO succinimidyl ester was used, which caused changes in the structure and biological activity of modified HSA. This is because lysine groups are abundant in the HSA protein, and TCO succinimidyl ester group addition can go in an uncontrolled fashion. The maleimide-cysteine reaction used in this study showed clear advantage over previous results.

For the chemical installation of the aryl diamine group to the HSA, a 4 molar excess amount of maleimide containing aryl diamine 4 was incubated with HSA in PBS of pH 7.5 at 25 °C for 10 h (Scheme 5). At the end of the reaction, the mixture was passed through a PD-10 (size exclusion) column to get chemically installed aryl diamine HSA. The matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analysis confirmed the purity and characterization of the desired product. The results showed that 1.3 aryl diamine groups were conjugated with one HSA protein at rt (Figure S14).

To determine the efficiency of an alkyl aldehyde aryl diamine condensation reaction, various concentrations of aryl diamine-installed HSA were incubated with 100 μCi of 125I-labeled alkyl aldehyde [125I] 5 in the presence of a catalytic amount of CuSO4. The radiochemical yields were determined using radio-thin-layer chromatography (TLC) and are summarized in Table 2. The conversion yield was concentration-dependent and more than a 94% radiochemical yield was obtained for 50 μM aryl diamine-modified HSA 9 within 2 h (entry 3). Under the same reaction conditions, 89 and 67% radiochemical yields were obtained for 25 and 5 μM of aryl diamine-modified HSA, respectively (entries 4 and 5). In the control experiment, [125I] 5 was incubated with nonmodified pure HSA under the same conditions, but no nonspecific interactions were observed.

For the radiolabeling and in vitro biodistribution studies, HSA was radiolabeled with 1 mCi of 125I-labeled alkyl aldehyde [125I] 5 for 2 h in the presence of a catalyst. The crude mixture was purified using a PD-10 desalting column to provide 125I-labeled HSA ([125I] 9) with 90% isolated radiochemical yield and more than 99% radiochemical purity. To compare the in vivo behavior, HSA was also radioiodinated via a tyrosine ring using [125I] NaI and chloramine-T as the oxidizing agent. 125I-HSA ([125I] 10) was synthesized with an 83% radiochemical yield and more than 99% radiochemical purity after purification through the PD-10 column.

Table 2. In vitro radiolabeling results of aryl diamine-conjugated HSA 9 using [125I] 5

| entry | concentration of 9 (μmol) | time (h) | % RCY |
|-------|--------------------------|---------|-------|
| 1     | 50                       | 0,5     | 54    |
| 2     | 50                       | 1       | 63    |
| 3     | 50                       | 2       | 94    |
| 4     | 25                       | 2       | 89    |
| 5     | 5                        | 2       | 67    |

Reagents and conditions: (i) PBS, rt, pH 7.5, 10 h and (ii) [125I] 5, Cu2+, air, rt.

(5°C for 10 h). For successful noninvasive sentinel lymph node imaging, 68Ga, 99mTc, and 18F-labeled HSA have been synthesized and used. 35 In this experiment, we used maleimide–cysteine-34 conjugation instead of N-succinimidyl ester–lysine based coupling on HSA because it offers a better diamine installed HSA protein with minimum alteration of the protein structure and a very high purity product. In our previous study, for the synthesis of TCO-modified HSA, TCO succinimidyl ester was used, which caused changes in the structure and biological activity of modified HSA. This is because lysine groups are abundant in the HSA protein, and TCO succinimidyl ester group addition can go in an uncontrolled fashion. The maleimide-cysteine reaction used in this study showed clear advantage over previous results.

For the chemical installation of the aryl diamine group to the HSA, a 4 molar excess amount of maleimide containing aryl diamine 4 was incubated with HSA in PBS of pH 7.5 at 25 °C for 10 h (Scheme 5). At the end of the reaction, the mixture was passed through a PD-10 (size exclusion) column to get chemically installed aryl diamine HSA. The matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analysis confirmed the purity and characterization of the desired product. The results showed that 1.3 aryl diamine groups were conjugated with one HSA protein at rt (Figure S14). To determine the efficiency of an alkyl aldehyde aryl diamine condensation reaction, various concentrations of aryl diamine-installed HSA were incubated with 100 μCi of 125I-labeled alkyl aldehyde [125I] 5 in the presence of a catalytic amount of CuSO4. The radiochemical yields were determined using radio-thin-layer chromatography (TLC) and are summarized in Table 2. The conversion yield was concentration-dependent and more than a 94% radiochemical yield was obtained for 50 μM aryl diamine-modified HSA 9 within 2 h (entry 3). Under the same reaction conditions, 89 and 67% radiochemical yields were obtained for 25 and 5 μM of aryl diamine-modified HSA, respectively (entries 4 and 5). In the control experiment, [125I] 5 was incubated with nonmodified pure HSA under the same conditions, but no nonspecific interactions were observed.
The biodistribution study suggested that 125I-labeled HSA ([125I] 9) is highly stable against in vivo deiodination, with 59.98% ID/g at 24 and 36 h post iv injection, respectively. The overall isolated radiochemical yield and in vivo stability of alkyl aldehyde aryl diamine condensation reaction is far better as compared with previously reported ligation. These results suggest the high instability of radioiodine on the tyrosine ring in vivo. Moreover, the [125I] 5 structure is proved to be stable and altogether different from iodotyrosine, which can undergo easy deiodination in vivo. The overall isolated radiochemical yield and in vivo stability of alkyl aldehyde aryl diamine condensation reaction is far better as compared with the direct radioiodination of tyrosine using some harsh oxidizing agents such as chloramine-T, which can damage the protein and decrease the bioactivity of the radiolabeled protein. The aryl diamine and alkyl aldehyde condensation reaction can be used for the radioiodination of many other small and large bioactive molecules. Moreover, the in vitro and in vivo stabilities of precursor [125I] 5 are quite high and it is normally unreacted toward all functional groups in biologically active proteins.

Experimental Section
Materials and Instruments. [125I]NaI with a 10 mCi activity in an aqueous solution of 0.1 M NaOH was supplied by PerkinElmer Republic of Korea. All chemicals including 4-amino-3-nitrophenol, methyl 5-bromovalerate, zinc dust (<10 μm) N-(2-aminoethyl)maleimide trifluoroacetate, chloramine-T trihydrate, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), DIPEA, and HSA were purchased from Sigma-Aldrich. Cysteine containing the cRGD peptide was purchased from Peptide International. All chemicals were pure and used without further purification step. HPLC experiments were performed using an Agilent Technologies 1290 infinite analytical HPLC system (Eclipse XDB-C18, 4.6 × 250 mm, 5 μm) and 1260 infinite preparative HPLC system (Eclipse XDB-C18, 21.2 × 150 mm, 7 μm). Solvent A (0.1% formic acid in deionized water) and solvent B (0.1% formic acid in acetonitrile) were used for the HPLC analysis and purification. All nuclear magnetic resonance (13C NMR and 1H NMR) spectra were acquired using a JEOL 500 MHz spectrometer with DMSO-d6 acetone-d6 or chloroform-d (CDCl3) as a solvent. Chemical shifts are given as δ (ppm) relative to tetramethylsilane (0.0 ppm) as an internal standard; multiplicities are given as singlet (s), doublet (d), doublet-of-doubles (dd), or multiplet (m). Agilent ESI-TOF analyzer and 4800 MALDI TOF/TOF Analyzer-(AB SCIEX) were used for mass spectroscopy.

Synthesis of Di-tert-butyl-(4-hydroxy-1,2-phenylene)-dicarbamate 1. To the mixture of HCl-activated Zn dust (4.2 g, 61.0 mmol) in methanol (30 mL), 4-amino-3-nitrophenol (2 g, 12.9 mmol) was added at 0 °C. To start a reaction, 10 mL of formic acid was added drop-wise. The reaction mixture was carried out at rt for 1 h before filtration. The excess amount of solvent was removed under reduced pressure. The solid residue was redissolved in methanol (15 mL), and di-tert-butyl dicarbonate (7.0 g, 32.0 mmol) was added to the reaction mixture. The reaction was carried out at rt for 3 days. At the end of the reaction, methanol was removed under reduced pressure, and the compound was dissolved in chloroform and washed with deionized water. The combined organic layer was dried using anhydrous sodium sulfate (Na2SO4) and concentrated. The crude product was purified using a silica gel column (ethyl acetate/hexane = 1:2) to give di-tert-butyl-(4-hydroxy-1,2-phenylene)dicarbamate (2.2 g, 6.79 mmol, 52%) as a white solid. 1H NMR (500 MHz, acetone-d6): 8 8.217 (br, 1H), 7.727 (br, 1H), 7.236 (s, 1H), 7.190 (s, 1H), 7.129 (s, 1H). 13C NMR (125 MHz, acetone-d6): 155.246, 154.502, 153.167, 133.670, 126.641, 121.146, 110.702, 109.214, 6911.
Synthesis of Methyl 5-(3,4-Bis((tert-butoxycarbonyl)amino)phenoxy)pentanoate 2. To the mixture of intermediate precursor 1 (2.0 g, 6.17 mmol) and methyl 5-bromovalerate (1.7 g, 9.2 mmol) in 20 mL of DMF, potassium carbonate (K2CO3) (2.5 g, 18.51 mmol) was added gradually. The reaction was carried out at 25 °C for 1 day. After the consumption of the starting material, the crude mixture was dissolved in chloroform and washed with water to remove the excess amount of DMF and other side-products. The crude product was then dried using anhydrous sodium sulfate (Na2SO4), concentrated under a reduced pressure, and purified by using a silica gel column (ethyl acetate/hexane = 1:4) to give 2 as a brown oil (1.9 g, 4.33 mmol, 70%). 1H NMR (500 MHz, CDCl3): δ 7.137 (br, 1H), 6.904 (br, 1H), 6.574 (dd, J1 = 8.5 Hz, J2 = 2.5 Hz, 1H), 3.928 (t, J = 6.6 Hz, 2H), 3.65 (s, 3H), 2.369 (t, J = 7.3 Hz, 2H), 1.70–1.78 (m, 6H), 1.495 (s, 9H) and 1.482 (s, 9H). 13C NMR (125 MHz, CDCl3): 174.027, 171.041, 157.459, 154.683, 153.243, 134.319, 126.850, 110.750, 108.346, 80.780, 80.685, 67.672, 51.631, 33.747, 28.672, 28.367, 21.681; HRMS: [M + Na]+ found, 461.2263; calculated, 461.1985.

Synthesis of Di-tert-butyl-4-(((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-5-oxopentyl)oxy)-1,2-phenylene)dicarbamate 3. To the water/dioxane (1:3, 4.8 mL), intermediate precursor 1 (2.0 g, 6.17 mmol) and methyl 5-butoxycarbonyl)benzo[1,2-c]imidazole-2-yl)propyl)-4-iodobenzamide 7. To a solution of 4-iodobenzoic acid (0.5 g, 2.02 mmol) in DMF, TCEP (5.5 mg, 0.02 mmol) and DIPEA (0.75 g, 5.8 mmol) were added at 0 °C, and a reaction was performed at 25 °C for 18 h. The reaction mixture was filtered through a silica gel column and ethyl acetate/hexane (2:1) was used to purify the crude product, giving titled compound 5 (0.33 g, 1.0 mmol, 53%). 1H NMR (500 MHz, DMSO-d6): δ 9.872 (t, J = 6.3 Hz, 2H), 7.772 (d, J = 8.0 Hz, 1H), 7.499 (d, J = 8.0 Hz, 1H), 3.470 (t, J = 7.3 Hz, 2H), 2.629 (t, J = 6.5 Hz, 2H), 1.990–1.925 (m, 6H); 13C NMR (125 MHz, DMSO-d6): 202.423, 167.341, 137.648, 133.623, 129.111, 98.302, 42.017, 23.913, 21.681; HRMS: [M + Na]+ found, 317.9940; calculated, 317.9913.

Synthesis of N-(4-Oxobutyl)-(4-(tributylstannyl)-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-5-oxopentyl)oxy)-1H-benzo[djimazole-2-yl)propyl)-4-iodobenzamide 7. To a stirring solution of 5 (200 mg, 0.61 mmol) in 15 mL of 1, 4-dioxane, tetrakis(triphenylphosphine) palladium (72 mg, 0.60 mmol) and bis(trifluoracetyle)acetic acid (2 mL) and dichloromethane was added, and a reaction was performed at 25 °C for 2.5 h. The reaction was terminated by adding diluted 1 M HCl (6 mL). The crude product was dissolved in dichloromethane and washed with distilled water, dried over Na2SO4, and concentrated under reduced pressure. The dried residue was redissolved in tetrahydrofuran/H2O (3:1, 15 mL) and stirred with concentrated HCl (3.5 mL) for an additional 2.5 h. The crude product was then dried with ethyl acetate/hexane (2:1) to give a white solid. The crude mixture was added, and a reaction was performed at 25 °C for 2.5 h. The reaction was terminated by adding diluted 1 M HCl (6 mL). The crude product was dissolved in dichloromethane and washed with distilled water, dried over Na2SO4, and concentrated under reduced pressure. The dried residue was redissolved in tetrahydrofuran/H2O (3:1, 15 mL) and stirred with concentrated HCl (3.5 mL) for an additional 2.5 h. The crude product was extracted with CH2Cl2, dried, and concentrated. A silica gel column and ethyl acetate/hexane (2:1) was used to purify the crude product, giving titled compound 5 (0.33 g, 1.0 mmol, 53%). 1H NMR (500 MHz, DMSO-d6): δ 9.872 (t, J = 6.3 Hz, 2H), 7.772 (d, J = 8.0 Hz, 1H), 7.499 (d, J = 8.0 Hz, 1H), 3.470 (t, J = 7.3 Hz, 2H), 2.629 (t, J = 6.5 Hz, 2H), 1.990–1.925 (m, 6H); 13C NMR (125 MHz, DMSO-d6): 202.423, 167.341, 137.648, 133.623, 129.111, 98.302, 42.017, 23.913, 21.681; HRMS: [M + H]+ found, 317.9940; calculated, 317.9913.
gradient: 0–2 min: 95%A/5%B; a linear gradient to 35%A/65%B from 95%A/5%B; 2–25 min; Rt: 14.6 min). MALDI-TOF: \([M + H]^+\) calc'd for \(C_{10}H_{22}N_{10}O_{12}S^2\), 1053.4991; found, 1053.5125.

Synthesis of Diamine-Conjugated HSA 9. HSA (350 μM) was dissolved in 100 μL of phosphate buffer solution of pH 7.5. Intermediate compound 4 (15 mM) in 10 μL of DMSO was added to the above solution. After 10 h, the crude product was diluted in 2.5 mL of deionized water and passed through a pre-conditioned PD-10 desalting column. The compound was extracted with 3.5 mL of phosphate buffer and centrifuged (5500 rpm, 10 min) using a 30 kDa centrifugal filter to obtain pure diamine-conjugated HSA. Pure diamine-conjugated HSA protein was characterized by MALDI-TOF, and the concentration was determined through a protein analysis and quantification system (Millipore).

Radioiodination of 125I-Labeled Alkyl Aldehyde \([^{125}\text{I}]\) 5. To the solution of \(N-(4\text{-oxobutyl})-4\text{-}((\text{tributylstannyl})-benzamide 6 (1 mg) in 100 μL of DMSO, chloramine-T solution (1 mg, 10 μL of H₂O₂, acetic acid (3 μL), and \([^{125}\text{I}]\)NaI solution (1.7 mCi, 10 μL) in 0.1 M NaOH was added sequentially. The reaction was performed at 25 °C for 10 min and terminated using a sodium metabisulfite solution (Na₂S₂O₅) (2 mg in 20 μL of H₂O). The mixture was purified using a preparative HPLC system (flow rate: 10 mL/min, eluent gradient: 0–2 min: 95%A/5%B; 2–13 min: a linear gradient to 65%A/35%B from 95%A/5%B; 13–25 min: a linear gradient to 35%A/65%B from 65%A/35%B; 25–30 min: a linear gradient to 0%A/100%B from 35%A/65%B, Rt: 22.6 min) and 1.2 mCi of 125I-labeled product \([^{125}\text{I}]\) 5 were obtained (72% of radiochemical yield, n = 5). The radiochemical purity was more than 99% as determined using an analytical HPLC (flow rate: 1 mL/min, eluent gradient: 0–2 min: 95%A/5%B; 2–13 min: a linear gradient to 65%A/35%B from 95%A/5%B; 13–25 min: a linear gradient to 35%A/65%B from 65%A/35%B; 25–30 min: a linear gradient to 0%A/100%B from 35%A/65%B, Rt: 22.6 min).

Radiosynthesis of Compound \([^{125}\text{I}]\) 7. To a mixture of 5-(3,4-diaminophenoxyl)-N-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)pentanamide 4 (50 μL in DMSO), 10 μL of copper sulfate (CuSO₄) (0.1 equiv) and 100 μCi of 125I-labeled alkyl aldehyde \([^{125}\text{I}]\) 5 (40 μL in DMSO) were added sequentially. The final reaction volume was 100 μL, and the actual concentration of 4 was 50, 25, or 5 μM. The labeling reaction was conducted at rt in the presence of oxygen. The reaction was monitored at different time points using a radio-HPLC system. The radiochemical yield of the reaction was measured through integration of a radio-HPLC chromatogram (system flow rate: 1 mL/min, gradient: 0–2 min: 95%A/5%B; 2–13 min: a linear gradient to 65%A/35%B from 95%A/5%B; 13–25 min: a linear gradient to 35%A/65%B from 65%A/35%B; 25–30 min: a linear gradient to 0%A/100%B from 35%A/65%B). The observed radiochemical yields are summarized in Table 1.

Radioiodination of the cRGD Peptide \([^{125}\text{I}]\) 8. The procedure for radioiodination of the cRGD peptide \([^{125}\text{I}]\) 8 was the same as that adopted for \([^{125}\text{I}]\) 7. The radiolabeling reaction was monitored at different time points using a radio-HPLC system (system flow rate: 1 mL/min, gradient: 0–2 min: 95%A/5%B; a linear gradient to 35%A/65%B from 95%A/5%B; 2–25 min; Rt: 17.7 min). The observed radiochemical yields are summarized in Table 1.

Radioiodination of HSA \([^{125}\text{I}]\) 9. To a mixture of aryl diamine-conjugated HSA 9 (5–50 μM, 70 μL in 1× PBS), 20 μL of copper sulfate (CuSO₄) (0.1 equiv) and 100 μCi of 125I-labeled alkyl aldehyde \([^{125}\text{I}]\) 5 (10 μL in DMSO) were added. The final reaction volume was 100 μL, and the labeling reaction was conducted at rt in the presence of oxygen. The reaction was monitored at different time points using a radio-TLC system. The observed radiochemical yields are summarized in Table 1. For the tissue distribution study of radioiodinated HSA \([^{125}\text{I}]\) 9, aryl diamine-containing HSA 9 (50 μM, in 70 μL of PBS) was treated with 125I-labeled alkyl aldehyde \([^{125}\text{I}]\) 5 (1.0 mCi, 10 μL in DMSO) in the presence of CuSO₄ (20 μL, 0.1 equiv) at rt. After 2 h, the crude product was dissolved in saline and then purified using a PD-10 (desalting) column to give \([^{125}\text{I}]\) 9 of 90% radiochemical yield and more than 99% radiochemical purity.

Direct Radioiodination of HSA \([^{125}\text{I}]\) 10. To a solution of HSA (25 μM) in 100 μL of PBS (1× PBS, pH = 7.4), \([^{125}\text{I}]\) NaI (800 μCi, 5 μL) and chloramine-T oxidizing agent (1 mg, 10 μL of H₂O) were added at 4 °C. The reaction was carried out for 30 min at 4 °C and terminated using sodium metabisulfite solution (Na₂S₂O₅) (2 mg in 20 μL of H₂O). The product was purified using a PD-10 desalting column using saline as an eluent to give the final compound with a radiochemical yield >99% radiochemical purity.

In Vitro Stability Test for 125I-Labeled Alkyl Aldehyde \([^{125}\text{I}]\) 5. To evaluate the in vitro stability of a radiotracer, purified \([^{125}\text{I}]\) 5 (100 μL, 100 μCi) was incubated in 900 μL of saline, or 1× PBS, or mouse serum at 37 °C. The stability of \([^{125}\text{I}]\) 5 in each media was evaluated at the given time points (0.5, 3, 6, 8, and 24 h) using a radio-HPLC system.

Biodistribution of Radioiodinated HSA \([^{125}\text{I}]\) 9. For a biodistribution study, 5 male ICR mice (6 weeks old) were purchased from Orientbio Co., Ltd (Jeonbuk, Korea Republic). The ICR mice were divided into five groups so that each group contained five animals. Each mouse was injected with an aqueous solution of \([^{125}\text{I}]\) 9 or \([^{125}\text{I}]\) 10 (100 μL, 1 μCi) through the tail vein. After 0.5, 3, 6, 24, and 36 h post injection, a group of mice were sacrificed under isoflurane dose; the organs of interest (thyroid, lungs, stomach, heart, liver, kidneys, spleen, large intestines, and small intestine) and blood were collected. The collected blood and organs were weighed and the accumulated radioactivity was determined using a 1480 wizard 3, (PerkinElmer, USA) gamma counter. The final biodistribution data were reported in terms of the percentage injected dose per gram of organ or blood (% ID/g). All animal experimental procedures were approved by the Institutional Animal Ethical Committee and performed according to the guidelines prescribed by the committee.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00416.

NMR, HRMS, and HPLC data (PDF)

AUTHOR INFORMATION

Corresponding Author

E-mail: parksh@kaeri.re.kr. Phone: +82-63-570-3370. Fax: +82-63-570-3371 (S.H.P.).

ORCID

Sang Hyun Park: 0000-0001-8219-5283

Notes

The authors declare no competing financial interest.
ACKNOWLEDGMENTS

This work was supported by the Korea Atomic Energy Research Institute, Jeongeup, Republic of Korea.

ABBREVIATIONS

PET, positron emission tomography; SPECT, single photon emission computed tomography; HSA, human serum albumin; % ID/g, injected dose per gram of organ or blood

REFERENCES

(1) Moon, C.; Kim, E. J.; Choi, D. B.; Kim, B. S.; Kim, S. H.; Choi, T. H. The Characterization of Anti-HER-2/neu Monoclonal Antibody using Different in vivo Imaging Techniques. Biomed. Sci. Lett. 2015, 21, 23–31.
(2) Ueda, M.; Fukushima, T.; Ogawa, K.; Kimura, H.; Ono, M.; Yamaguchi, T.; Ikehara, Y.; Saji, H. Synthesis and evaluation of a radioiodinated peptide probe targeting αvβ6 integrin for the detection of pancreatic ductal adenocarcinoma. Biochem. Biophys. Res. Commun. 2014, 445, 661–666.
(3) Yamasaki, K.; Zhao, S.; Nishimura, M.; Zhao, Y.; Yu, W.; Shimizu, Y.; Nishijima, K.-I.; Tamaki, N.; Takeda, H.; Kuge, Y. Radioiodinated BMIPP as a potential probe for hepatic fatty acid metabolism: Evaluation in mice at different feeding status. J. Nucl. Med. 2013, 54, 1209.
(4) Phillips, E.; Penate-Medina, O.; Zannonicco, P. B.; Carvajal, R. D.; Mohan, P.; Ye, Y.; Humm, J.; Gönen, M.; Kalaigian, H.; Schöder, H.; Strauss, H. W. Clinical translation of an ultrasmall inorganic optical-PEF imaging nanoparticle probe. Sci. Transl. Med. 2014, 6, 260ra149.
(5) Case, J.; Courter, S.; Van Vickle, S.; Bateman, T. Novel Approaches to Measuring 123I-labeled mIBG Heart-to-Mediastinal Intensities from PET Imaging: Comparison of Different Calculation Methods. J. Nucl. Med. 2015, 56, 407.
(6) Yang, Y.; Zhang, X.; Cui, M.; Zhang, J.; Guo, Z.; Li, Y.; Zhang, X.; Dai, J.; Liu, B. Preliminary Characterization and In Vivo Studies of Nanoparticles containing an azido function. J. Nucl. Med. 2016, 24, 2589–2594.
(7) Chari, M. A.; Shobha, D.; Sasaki, T. Room temperature synthesis of benzimidazole derivatives using reversible covalent hydroxide (II) and cobalt oxo (II) as efficient solid catalysts. Tetrahedron Lett. 2011, 52, 5575–5580.
(8) Panda, S. S.; Malik, R.; Jain, S. C. Synthetic approaches to 2-arylbenzimidazoles: A review. Curr. Org. Chem. 2012, 16, 1905–1919.
(9) Reddy, P. L.; Arundhati, M.; Rawat, D. S. CuI mediated expeditious synthesis of 2-substituted benzimidazoles as radiotracers for imaging tumors and thrombosis by SPECT. Bioconjugate Chem. 2016, 7, 1003–1006.
(10) Zhou, Y.; Chakraborty, S.; Liu, S. Radiolabeled cyclic RGD peptides as radiotracers for imaging tumors and thrombosis by SPECT. Theranostics 2011, 1, 58–82.
(11) Bertucci, C.; Domenici, E. Reversible and covalent binding of drugs to human serum albumin: methodological approaches and physiological relevance. Curr. Med. Chem. 2002, 9, 1463–1481.
(12) Mendez, C. M.; McClain, C. J.; Marsano, L. S. Albumin therapy nanoparticles mediated expedient synthesis of 2-substituted benzimidazoles using molecular oxygen as the oxidant. RSC Adv. 2016, 6, 53596–53601.
(13) McKay, C. S.; Finn, M. G. Click chemistry in complex mixtures: bioorthogonal bioconjugation. Chem. Biol. 2014, 21, 1075–1101.
(14) Hagimori, M.; Murakami, T.; Shimizu, K.; Nishida, M.; Ohshima, T.; Makai, T. Synthesis of radioiodinated probes to evaluate the biodistribution of a potent TRPC3 inhibitor. MedChemComm 2016, 7, 1003–1006.
(15) Zhou, Y.; Chakraborty, S.; Liu, S. Radiolabeled cyclic RGD peptides as radiotracers for imaging tumors and thrombosis by SPECT. Theranostics 2011, 1, 58–82.
(36) Cao, W.; Lu, X.; Cheng, Z. The Advancement of Human Serum Albumin-Based Molecular Probes for Molecular Imaging. *Curr. Pharm. Des.* 2015, 21, 1908−1915.
(37) Yang, M.; Hoppmann, S.; Chen, L.; Cheng, Z. Human serum albumin conjugated biomolecules for cancer molecular imaging. *Curr. Pharm. Des.* 2012, 18, 1023−1031.
(38) Yasumoto, K.; Takata, M.; Ueno, H.; Tomoda, F.; Inoue, H. Relation of circulating blood volume to left ventricular geometry in essential hypertension. *Hypertens. Res.* 2002, 25, 703−710.
(39) Henriksen, U. L.; Henriksen, J. H.; Bendtsen, F.; Møller, S. 99mTc-labelled human serum albumin cannot replace 125I-labelled human serum albumin to determine plasma volume in patients with liver disease. *Clin. Physiol. Funct. Imaging* 2013, 33, 211−217.
(40) Liu, Z.; Chen, X. Simple bioconjugate chemistry serves great clinical advances: albumin as a versatile platform for diagnosis and precision therapy. *Chem. Soc. Rev.* 2016, 45, 1432−1456.
(41) Sleep, D.; Cameron, J.; Evans, L. R. Albumin as a versatile platform for drug half-life extension. *Biochim. Biophys. Acta* 2013, 1830, 5526−5534.