

**Electronic Supplementary Information (ESI) for:**

**Development of a novel radiotheranostic platform with a DOTA-based  
trifunctional chelating agent**

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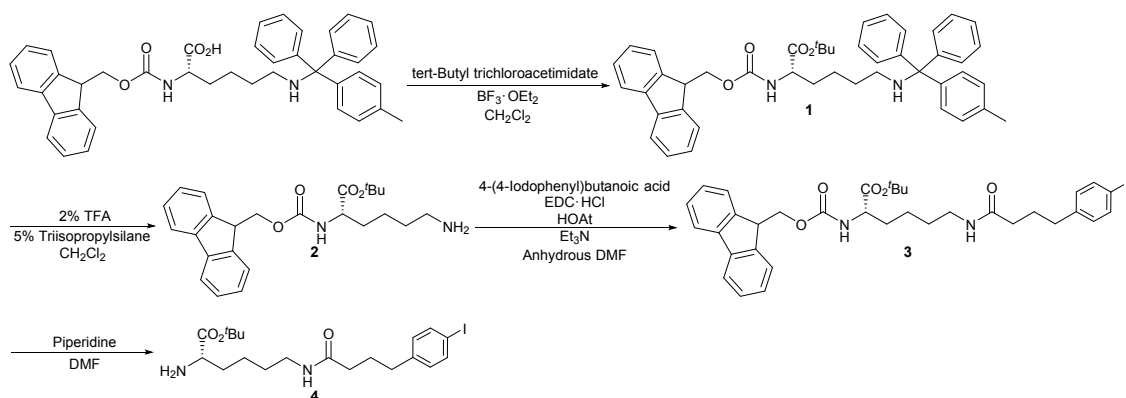
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## 1. General remarks

All reagents were obtained commercially and used without further purification, unless otherwise indicated. [ $^{111}\text{In}$ ] $\text{InCl}_3$  was obtained from Nihon Medi-Physics (Tokyo, Japan). High-resolution mass spectrometry (HRMS) was conducted with a liquid chromatography/mass spectrometry ion trap time-of-flight (LCMS-IT-TOF) mass spectrometer (Shimadzu, Kyoto, Japan), and MS was conducted with an LCMS-2020 system (Shimadzu, Kyoto, Japan).  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-ECS400 system (JEOL, Tokyo, Japan) with tetramethyl silane used as an internal standard. Coupling constants are reported in Hertz. Multiplicity was defined as singlet (s), doublet (d), triplet (t), or multiplet (m). Reversed-phase high-performance liquid chromatography (RP-HPLC) was performed using a Shimadzu system (an LC-20AT pump with an SPD-20A ultraviolet (UV) detector,  $\lambda = 254$  nm) with a Cosmosil  $\text{C}_{18}$  column (5 $\text{C}_{18}$ -AR-II,  $4.6 \times 150$ ,  $10 \times 250$ , or  $20 \times 250$  mm; Nacalai Tesque, Kyoto, Japan).

## 2. Chemistry



### *tert*-Butyl $N^2$ -(((9*H*-fluoren-9-yl)methoxy)carbonyl)- $N^6$ -(diphenyl(*p*-tolyl)methyl)- $L$ -lysinate (**1**)

To a solution of  $N^2$ -[(9*H*-fluoren-9-ylmethoxy)carbonyl]- $N^6$ -[(4-methylphenyl)diphenylmethyl]- $L$ -lysine (1,000 mg, 1.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) were added *tert*-butyl trichloroacetimidate (699.5 mg, 3.2 mmol) and  $\text{BF}_3 \cdot \text{OEt}_2$  (25  $\mu\text{L}$  as a catalyst). The mixture was stirred at room temperature for 42 h. The solvent was removed, and the residue was washed with  $\text{H}_2\text{O}$  (100 mL) and extracted with an EtOAc/hexane

mixture (1/5, 100 mL  $\times$  2). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and filtrated. The filtrate was evaporated, and the residue was purified by silica gel chromatography (EtOAc/hexane = 25/75) to give 569 mg of **1** (52% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.69 (d,  $J$  = 7.3 Hz, 2H), 7.56 (d,  $J$  = 7.8 Hz, 2H), 7.45 (d,  $J$  = 7.8 Hz, 4H), 7.33 (d,  $J$  = 8.2 Hz, 4H), 7.26-7.20 (m, 6H), 7.16-7.11 (m, 2H), 7.04 (d,  $J$  = 7.8 Hz, 2H), 5.39 (d,  $J$  = 8.2 Hz, 1H), 4.35 (d,  $J$  = 6.9 Hz, 2H), 4.27-4.22 (m, 1H), 4.18 (t,  $J$  = 6.9 Hz, 1H), 2.26 (s, 3H), 2.19-2.07 (m, 2H), 1.82-1.75 (m, 1H), 1.64-1.56 (m, 1H), 1.50-1.47 (m, 4H), 1.44 (s, 9H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.6 (1C), 155.7 (1C), 146.3 (2C), 143.7 (2C), 143.2 (1C), 141.1 (2C), 135.4 (1C), 128.4-128.3 (8C), 127.6-127.5 (6C), 126.9 (2C), 126.0 (2C), 125.0 (2C), 119.8 (2C), 81.7 (1C), 70.5 (1C), 66.7 (1C), 60.2 (1C), 47.1 (1C), 43.2 (1C), 32.7 (1C), 30.4 (1C), 27.8 (3C), 22.8 (1C), 20.7 (1C). HRMS (electrospray ionization [ESI])  $m/z$  calculated for C<sub>45</sub>H<sub>49</sub>N<sub>2</sub>O<sub>4</sub><sup>+</sup>, 681.3687 [M + H]<sup>+</sup>; found, 681.3677

***tert-Butyl N<sup>2</sup>-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysinate (2)***

Compound **1** (569 mg, 0.84 mmol) was dissolved in a mixture of trifluoroacetic acid (TFA) (100  $\mu$ L), triisopropylsilane (250  $\mu$ L), and CH<sub>2</sub>Cl<sub>2</sub> (4.65 mL). The solution was stirred at room temperature for 6 h. The solvent was removed, and the residue was purified by silica gel chromatography (MeOH/CHCl<sub>3</sub> = 19/81) to give 355 mg of **2** (100% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.71 (d,  $J$  = 7.6 Hz, 2H), 7.56 (d,  $J$  = 7.1 Hz, 2H), 7.35 (t,  $J$  = 7.6 Hz, 2H), 7.27 (t,  $J$  = 7.6 Hz, 2H), 5.72 (d,  $J$  = 8.0 Hz, 1H), 4.33 (d,  $J$  = 7.1 Hz, 2H), 4.16 (t,  $J$  = 6.6 Hz, 2H), 3.38 (s, 2H), 1.77-1.56 (m, 4H), 1.43 (s, 9H), 1.27-1.13 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.5 (1C), 156.2 (1C), 143.6 (2C), 141.1 (2C), 127.6 (2C), 127.0 (2C), 125.0 (2C), 119.8 (2C), 82.3 (1C), 66.9 (1C), 54.1 (1C), 46.9 (1C), 39.5 (1C), 31.1 (1C), 27.7 (3C), 26.8 (1C), 22.1 (1C). HRMS (ESI)  $m/z$  calculated for C<sub>25</sub>H<sub>33</sub>N<sub>2</sub>O<sub>4</sub><sup>+</sup>, 425.2435 [M + H]<sup>+</sup>; found, 425.2437

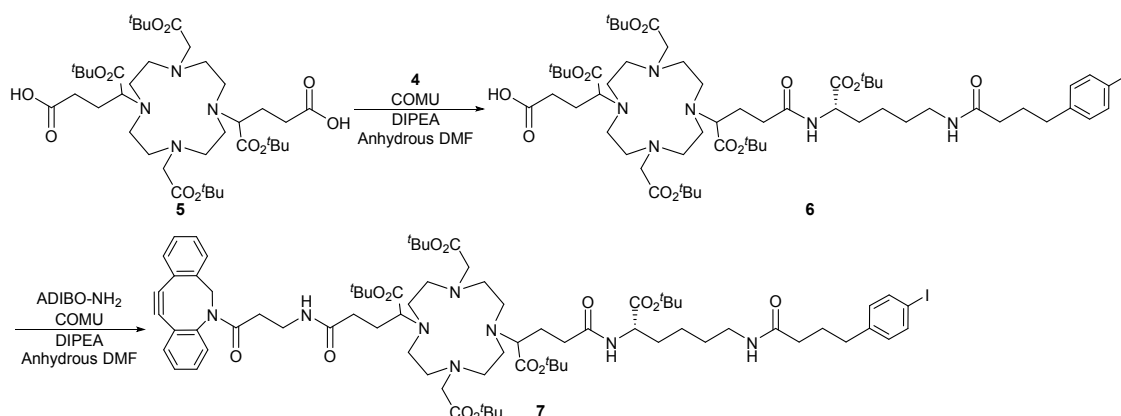
***tert-Butyl N<sup>2</sup>-(((9H-fluoren-9-yl)methoxy)carbonyl)-N<sup>6</sup>-(4-(4-iodophenyl)butanoyl)-L-lysinate (3)***

To a solution of 4-(4-iodophenyl)butanoic acid (364 mg, 1.25 mmol) in anhydrous *N,N*-dimethylformamide (DMF) (3 mL) were added 1-ethyl-3-(dimethylaminopropyl)carbodiimide (EDC) hydrochloride (320 mg, 1.7 mmol) and 1-

hydroxy-7-azabenzotriazole (HOAt) (228 mg, 1.7 mmol) at 0 °C. The solution was stirred at 0 °C for 15 min. To the mixture were added **2** (355 mg, 0.84 mmol) and Et<sub>3</sub>N (232 µL, 1.7 mmol) at 0 °C. After being stirred at room temperature for 15 h, the solution was washed with H<sub>2</sub>O (100 mL) and extracted with an EtOAc/hexane mixture (1/5, 100 mL × 2). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and filtrated. The filtrate was evaporated, and the residue was purified by silica gel chromatography (EtOAc/hexane = 57/43) to give 226 mg of **3** (39% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.73 (d, *J* = 7.3 Hz, 2H), 7.60-7.50 (m, 6H), 7.37 (t, *J* = 7.3 Hz, 2H), 7.28 (t, *J* = 7.3 Hz, 2H), 6.83 (d, *J* = 8.2 Hz, 2H), 4.38-4.27 (m, 2H), 4.25-4.16 (m, 2H), 3.21-3.14 (m, 2H), 2.50-2.46 (m, 2H), 2.08 (t, *J* = 7.3 Hz, 2H), 1.91-1.84 (m, 2H), 1.73-1.57 (m, 2H), 1.47-1.29 (m, 13H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.8 (1C), 171.5 (1C), 156.0 (1C), 143.5 (2C), 141.0-140.9 (3C), 137.1 (2C), 130.3 (2C), 127.5 (2C), 126.9 (2C), 124.9 (2C), 119.8 (2C), 90.8 (1C), 82.0 (1C), 66.8 (1C), 53.9 (1C), 46.9 (1C), 38.9 (1C), 35.4 (1C), 34.4 (1C), 32.1 (1C), 28.6 (1C), 27.9 (3C), 26.7 (1C), 22.2 (1C). HRMS (ESI) *m/z* calculated for C<sub>35</sub>H<sub>42</sub>IN<sub>2</sub>O<sub>5</sub><sup>+</sup>, 697.2133 [M + H]<sup>+</sup>; found, 697.2130

***tert-Butyl N<sup>6</sup>-(4-(4-iodophenyl)butanoyl)-L-lysinate (4)***

Compound **3** (226 mg, 0.32 mmol) was dissolved in DMF (4 mL) and piperidine (1 mL). After being stirred at room temperature for 2 h, the solution was washed with H<sub>2</sub>O (100 mL) and extracted with an EtOAc/hexane mixture (1/5, 100 mL × 2). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and filtrated. The filtrate was evaporated, and the residue was purified by silica gel chromatography (MeOH/CHCl<sub>3</sub> = 7/93) to give 133 mg of **4** (87% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.58 (d, *J* = 8.2 Hz, 2H), 6.92 (d, *J* = 8.2 Hz, 2H), 3.31-3.27 (m, 1H), 3.26-3.20 (m, 2H), 2.60-2.55 (m, 2H), 2.15-2.11 (m, 2H), 1.96-1.88 (m, 2H), 1.74-1.65 (m, 2H), 1.56-1.48 (m, 2H), 1.44 (s, 9H), 1.43-1.40 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 175.2 (1C), 172.3 (1C), 141.1 (1C), 137.3 (2C), 130.5 (2C), 90.9 (1C), 80.9 (1C), 54.7 (1C), 39.1 (1C), 35.6 (1C), 34.6 (1C), 34.3 (1C), 29.2 (1C), 28.0 (3C), 26.8 (1C), 22.9 (1C). HRMS (ESI) *m/z* calculated for C<sub>20</sub>H<sub>32</sub>IN<sub>2</sub>O<sub>3</sub><sup>+</sup>, 475.1453 [M + H]<sup>+</sup>; found, 475.1453



**4,4'-(4,10-Bis(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)bis(5-(tert-butoxy)-5-oxopentanoic acid) (5)**

Compound **5** was synthesized from L-glutamic acid 5-benzyl ester and 1,4,7,10-tetraazacyclododecane according to previously described methods.<sup>1,2</sup>

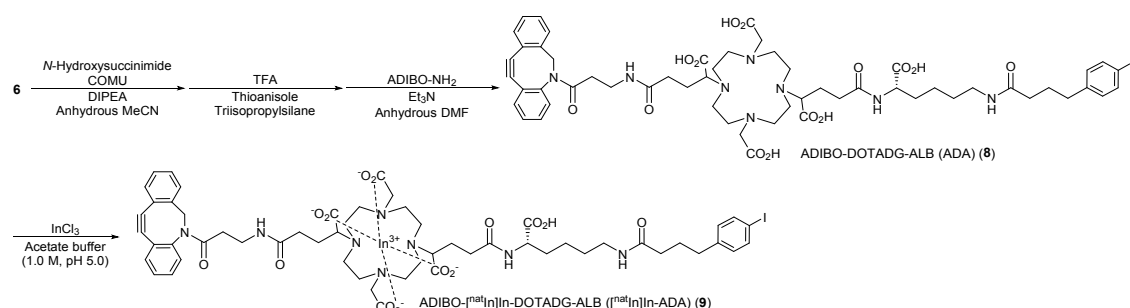
**4-(4,10-Bis(2-(tert-butoxy)-2-oxoethyl)-7-(1-(tert-butoxy)-5-(((S)-1-(tert-butoxy)-6-(4-(4-iodophenyl)butanamido)-1-oxohexan-2-yl)amino)-1,5-dioxopentan-2-yl)-1,4,7,10-tetraazacyclododecan-1-yl)-5-(tert-butoxy)-5-oxopentanoic acid (6)**

To a solution of **5** (317 mg, 0.41 mmol) in anhydrous DMF (2 mL) was added 1-((1-(cyano-2-ethoxy-2-oxo-ethylideneamino)oxy)dimethylaminomorpholino))uranium hexafluorophosphate (COMU) (176 mg, 0.41 mmol) at 0 °C. The solution was stirred at 0 °C for 15 min. To the mixture was added *N,N*-diisopropylethylamine (DIPEA) (72 µL, 0.41 mmol) at 0 °C. The solution was stirred at 0 °C for 15 min. To the mixture was added **4** (163 mg, 0.34 mmol). After being stirred at room temperature for 12 h, the solution was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (20 × 250 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 7/3 (0 min) to 1/9 (40 min)], which was delivered at a flow rate of 5.0 mL/min, to give 229 mg of **6** (55% yield). HRMS (ESI) *m/z* calculated for C<sub>58</sub>H<sub>98</sub>IN<sub>6</sub>O<sub>14</sub><sup>+</sup>, 1229.6181 [M + H]<sup>+</sup>; found, 1229.6182

**Di-tert-butyl 2,2'-(4-(1-(tert-butoxy)-5-(((S)-1-(tert-butoxy)-6-(4-(4-iodophenyl)butanamido)-1-oxohexan-2-yl)amino)-1,5-dioxopentan-2-yl)-10-(1-(tert-butoxy)-5-((3-(11,12-didehydrodibenzo[b,f]azocin-5(6H)-yl)-3-oxopropyl)amino)-1,5-dioxopentan-2-yl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (7)**

To a solution of **6** (106 mg, 0.086 mmol) in anhydrous DMF (600 µL) was added COMU

(147 mg, 0.34 mmol) at 0 °C. The solution was stirred at 0 °C for 15 min. To the mixture was added DIPEA (120  $\mu$ L, 0.69 mmol) at 0 °C. The solution was stirred at 0 °C for 15 min. To the mixture was added dibenzocyclooctyne-amine (ADIBO-NH<sub>2</sub>) (36 mg, 0.22 mmol). After being stirred at room temperature for 12 h, the solution was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (20  $\times$  250 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 8/2 (0 min) to 1/9 (35 min)], which was delivered at a flow rate of 5.0 mL/min, to give 51 mg of **7** (40% yield). HRMS (ESI)  $m/z$  calculated for C<sub>76</sub>H<sub>112</sub>IN<sub>8</sub>O<sub>14</sub><sup>+</sup>, 1487.7338 [M + H]<sup>+</sup>; found, 1487.7351



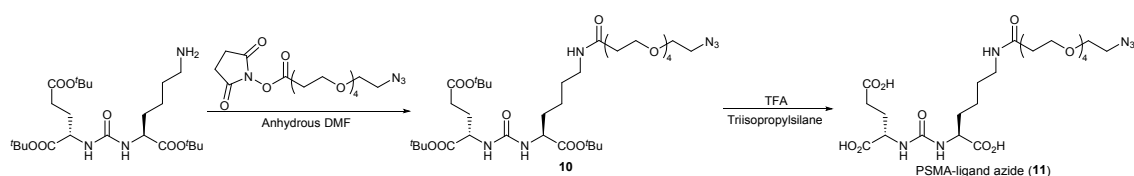
**2,2'-(4-(1-Carboxy-4-(((S)-1-carboxy-5-(4-(4-iodophenyl)butanamido)pentyl)amino)-4-oxobutyl)-10-(1-carboxy-4-((3-(11,12-didehydrodibenzo[b,f]azocin-5(6H)-yl)-3-oxopropyl)amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (ADIBO-DOTADG-ALB (ADA)) (8)**

To a solution of **6** (50 mg, 0.041 mmol) in anhydrous MeCN (400  $\mu$ L) was added COMU (70 mg, 0.16 mmol) at 0 °C. The solution was stirred at 0 °C for 15 min. To the mixture was added DIPEA (36  $\mu$ L, 0.21 mmol) at 0 °C. The solution was stirred at 0 °C for 15 min. To the mixture was added *N*-hydroxysuccinimide (19 mg, 0.16 mmol). After being stirred at room temperature for 24 h, the solution was washed with H<sub>2</sub>O (100 mL) and extracted with an EtOAc/hexane mixture (1/5, 100 mL  $\times$  2). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and filtrated. The filtrate was evaporated, and the residue was used for the next reaction without further purification. Half of the crude residue (ideally 0.021 mmol) was dissolved in a mixture of TFA/thioanisole/triisopropylsilane (95/3/2, 2 mL). After the mixture had been stirred at room temperature for 11 h, the solvent was removed. The residue (ideally 0.021 mmol) was dissolved in anhydrous DMF (400  $\mu$ L) and Et<sub>3</sub>N (10  $\mu$ L). To the solution was added ADIBO-NH<sub>2</sub> (6.2 mg, 0.023 mmol). After being stirred

at room temperature for 24 h, the solution was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (4.6 × 150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 1/9 (40 min)], which was delivered at a flow rate of 1.0 mL/min, to give 6.7 mg of **8** (27% yield, the total of three consecutive reactions). HRMS (ESI) *m/z* calculated for C<sub>56</sub>H<sub>72</sub>IN<sub>8</sub>O<sub>14</sub><sup>+</sup>, 1207.4208 [M + H]<sup>+</sup>; found, 1207.4213

**Indium(III)** *2,2'-(4-(1-carboxy-4-(((S)-1-carboxy-5-(4-(4-iodophenyl)butanamido)pentyl)amino)-4-oxobutyl)-10-(1-carboxy-4-((3-(11,12-didehydrodibenzo[b,f]azocin-5(6H)-yl)-3-oxopropyl)amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (ADIBO-[<sup>nat</sup>In]In-DOTADG-ALB ([<sup>nat</sup>In]In-ADA)) (9)*

Compound **8** (1 eq) was dissolved in acetate buffer (1.0 M, pH 5.0, 100 μL). To the solution was added anhydrous indium(III) chloride (10 eq). After being incubated at 90 °C for 5 min, the solution was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (4.6 × 150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 1/9 (40 min)], which was delivered at a flow rate of 1.0 mL/min, to give **9**. MS (ESI) *m/z* calculated for C<sub>56</sub>H<sub>69</sub>I<sup>115</sup>InN<sub>8</sub>O<sub>14</sub><sup>+</sup>, 1319.3 [M + H]<sup>+</sup>; found, 1319.3



**Tri-tert-butyl (21S, 25S)-1-azido-15,23-dioxo-3,6,9,12-tetraoxa-16,22,24-triazaheptacosane-21,25,27-tricarboxylate (10)**

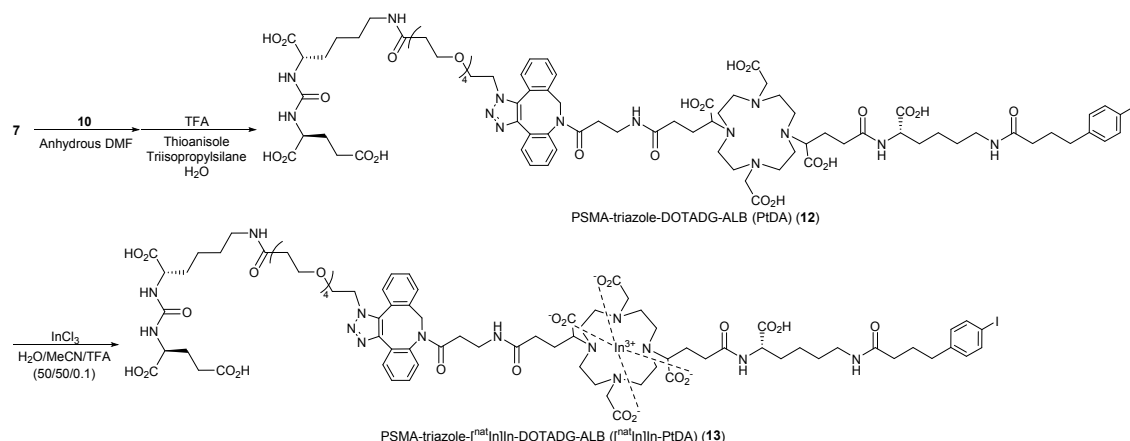
To a solution of (S)-di-tert-butyl 2-(3-((S)-6-amino-1-tert-butoxy-1-oxohexan-2-yl)ureido)pentanedioate (31 mg, 0.064 mmol) in anhydrous DMF (500 μL) was added 2,5-dioxopyrrolidin-1-yl 1-azido-3,6,9,12-tetraoxapentadecan-15-oate (azido-PEG<sub>4</sub>-NHS-ester) (25 mg, 0.064 mmol). After being stirred at room temperature for 12 h, the solution was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (10 × 250 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 7/3 (0 min) to 1/9 (30 min)], which was delivered at a flow rate of 4.0 mL/min, to give 35 mg of **10** (72% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.26-4.23 (m, 2H), 3.75 (t, *J* = 5.2 Hz, 2H), 3.69-3.61 (m,



14H), 3.41 (t,  $J = 5.2$  Hz, 2H), 3.38-3.16 (m, 2H), 2.59 (t,  $J = 5.2$  Hz, 2H), 2.34 (dt,  $J = 2.3, 7.5$  Hz, 2H), 2.11-1.60 (m, 4H), 1.54 (t,  $J = 7.0$  Hz, 2H), 1.48-1.40 (m, 27H), 1.39-1.26 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  174.3 (2C), 172.8 (2C), 158.4 (1C), 82.7 (1C), 82.4 (1C), 81.2 (1C), 70.4 (2C), 70.3 (1C), 70.1 (1C), 70.0 (2C), 69.9 (1C), 66.8 (1C), 53.7 (1C), 53.3 (1C), 50.5 (1C), 39.2 (1C), 35.8 (1C), 31.5 (1C), 31.3 (1C), 28.2 (1C), 27.9 (3C), 27.8 (7C), 21.9 (1C). HRMS (ESI)  $m/z$  calculated for  $\text{C}_{35}\text{H}_{65}\text{N}_6\text{O}_{12}^+$ , 761.4655  $[\text{M} + \text{H}]^+$ ; found, 761.4656

**(21S,25S)-1-Azido-15,23-dioxo-3,6,9,12-tetraoxa-16,22,24-triazaheptacosane-21,25,27-tricarboxylic acid (PSMA-ligand azide) (11)**

Compound **10** (23 mg, 0.030 mmol) was dissolved in TFA (950  $\mu\text{L}$ ) and triisopropylsilane (50  $\mu\text{L}$ ). The solution was stirred at room temperature for 4 h. The solvent was removed, and the residue was purified by RP-HPLC on a Cosmosil  $\text{C}_{18}$  column ( $10 \times 250$  mm) using a mobile phase [ $\text{H}_2\text{O}$  with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 6/4 (30 min)], which was delivered at a flow rate of 4.0 mL/min, to give 11 mg of **11** (63% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.52 (s, 1H), 6.37 (s, 2H), 4.39 (s, 1H), 4.31 (s, 1H), 3.87-3.53 (m, 16H), 3.40 (s, 2H), 2.53-1.23 (m, 14H). HRMS (ESI)  $m/z$  calculated for  $\text{C}_{23}\text{H}_{41}\text{N}_6\text{O}_{12}^+$ , 593.2777  $[\text{M} + \text{H}]^+$ ; found, 593.2779



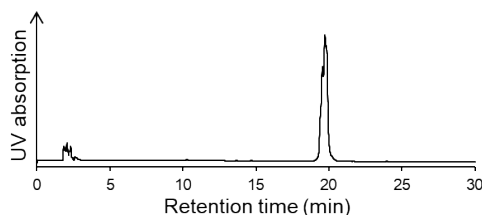
**(21S,25S)-1-(8-(3-(4-Carboxy-4-(7-(1-carboxy-4-(((S)-1-carboxy-5-(4-(4-iodophenyl)butanamido)pentyl)amino)-4-oxobutyl)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)butanamido)propanoyl)-8,9-dihydro-1H-**

***dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-1-yl)-15,23-dioxo-3,6,9,12-tetraoxa-16,22,24-triazaheptacosane-21,25,27-tricarboxylic acid (PSMA-triazole-DOTADG-ALB (PtDA)) (12)***

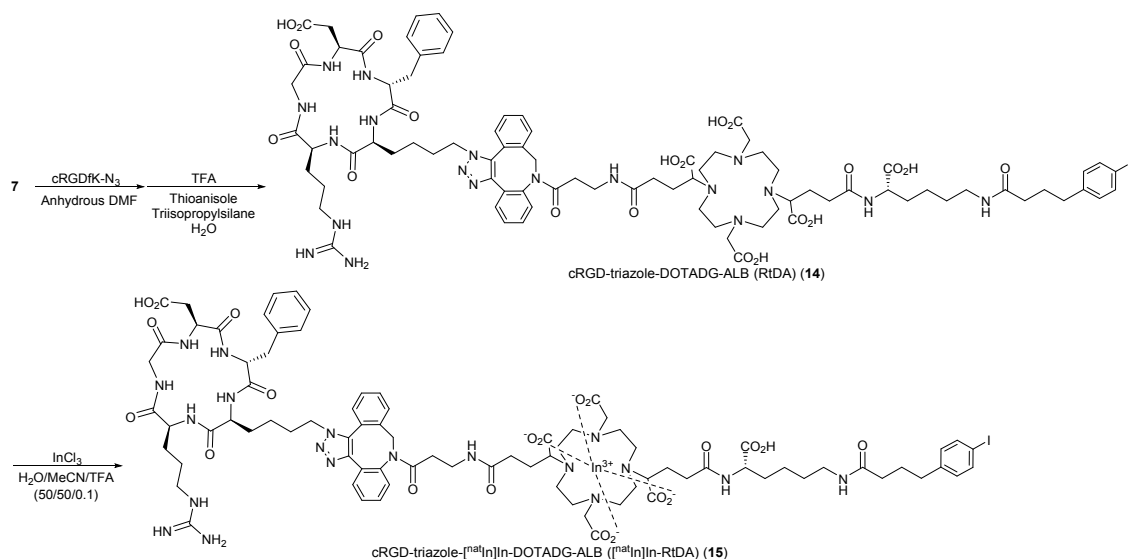
Compound **7** (20 mg, 0.013 mmol) was dissolved in anhydrous DMF (600  $\mu$ L). To the solution was added **10** (35 mg, 0.046 mmol). After the mixture had been stirred at room temperature for 12 h, the solvent was removed. To the residue was added TFA (1,900  $\mu$ L), thioanisole (60  $\mu$ L), triisopropylsilane (20  $\mu$ L), and H<sub>2</sub>O (20  $\mu$ L). After the mixture had been stirred at room temperature for 10 h, the solvent was removed, and the residue was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (4.6  $\times$  150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1.0 mL/min, to give 1.0 mg of **12** (4.2% yield). HRMS (ESI)  $m/z$  calculated for C<sub>79</sub>H<sub>113</sub>IN<sub>14</sub>O<sub>26</sub><sup>2+</sup>, 900.3492 [M + 2H]<sup>2+</sup>; found, 900.3488

***Indium(III) (21S,25S)-1-(8-(3-(4-carboxy-4-(7-(1-carboxy-4-(((S)-1-carboxy-5-(4-(4-iodophenyl)butanamido)pentyl)amino)-4-oxobutyl)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)butanamido)propanoyl)-8,9-dihydro-1H-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-1-yl)-15,23-dioxo-3,6,9,12-tetraoxa-16,22,24-triazaheptacosane-21,25,27-tricarboxylic acid (PSMA-triazole-[<sup>nat</sup>In]In-DOTADG-ALB ([<sup>nat</sup>In]In-PtDA)) (13)***

Compound **12** (0.5 mg, 0.28  $\mu$ mol) was dissolved in H<sub>2</sub>O/MeCN/TFA solution (49.95/49.95/0.1, 300  $\mu$ L). To the solution was added anhydrous indium(III) chloride (0.62 mg, 2.8  $\mu$ mol). After being stirred at room temperature for 18 h, the solution was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (4.6  $\times$  150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1.0 mL/min, to give 0.05 mg of **13** (9.4% yield). HRMS (ESI)  $m/z$  calculated for C<sub>79</sub>H<sub>110</sub>I<sup>115</sup>InN<sub>14</sub>O<sub>26</sub><sup>2+</sup>, 956.2894 [M + 2H]<sup>2+</sup>; found, 956.2893



**Fig. S1** The HPLC chromatogram of UV absorption for purified [<sup>nat</sup>In]In-PtDA.



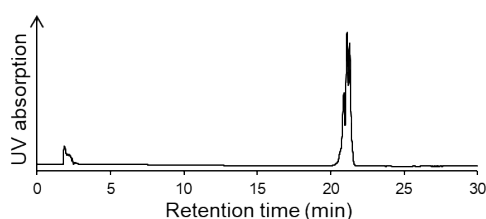
**2,2'-(4-(4-((3-(1-(4-((2*S*,5*S*,11*S*,14*R*)-14-Benzyl-11-(carboxymethyl)-5-(3-guanidinopropyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)butyl)-1,9-dihydro-8*H*-dibenzo[*b,f*][1,2,3]triazolo[4,5-*d*]azocin-8-yl)-3-oxopropyl)amino)-1-carboxy-4-oxobutyl)-10-(1-carboxy-4-(((*S*)-1-carboxy-5-(4-(4-iodophenyl)butanamido)pentyl)amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (cRGD-triazole-DOTADG-ALB (RtDA)) (**14**)**

Compound **7** (3.9 mg, 2.6  $\mu\text{mol}$ ) was dissolved in anhydrous DMF (400  $\mu\text{L}$ ). To the solution was added cyclo[Arg-Gly-Asp-*D*-Phe-Lys(azide)] (cRGD azide) (1.7 mg, 2.6  $\mu\text{mol}$ ). After the mixture had been stirred at room temperature for 12 h, the solvent was removed. To the residue was added TFA (1,900  $\mu\text{L}$ ), thioanisole (60  $\mu\text{L}$ ), triisopropylsilane (20  $\mu\text{L}$ ), and H<sub>2</sub>O (20  $\mu\text{L}$ ). After the mixture had been stirred at room temperature for 10 h, the solvent was removed, and the residue was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (4.6  $\times$  150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1.0 mL/min, to give 2.8 mg of **14** (58% yield). HRMS (ESI)  $m/z$  calculated for C<sub>83</sub>H<sub>112</sub>IN<sub>19</sub>O<sub>21</sub><sup>2+</sup>, 918.8657 [ $M + 2H$ ]<sup>2+</sup>; found, 918.8662

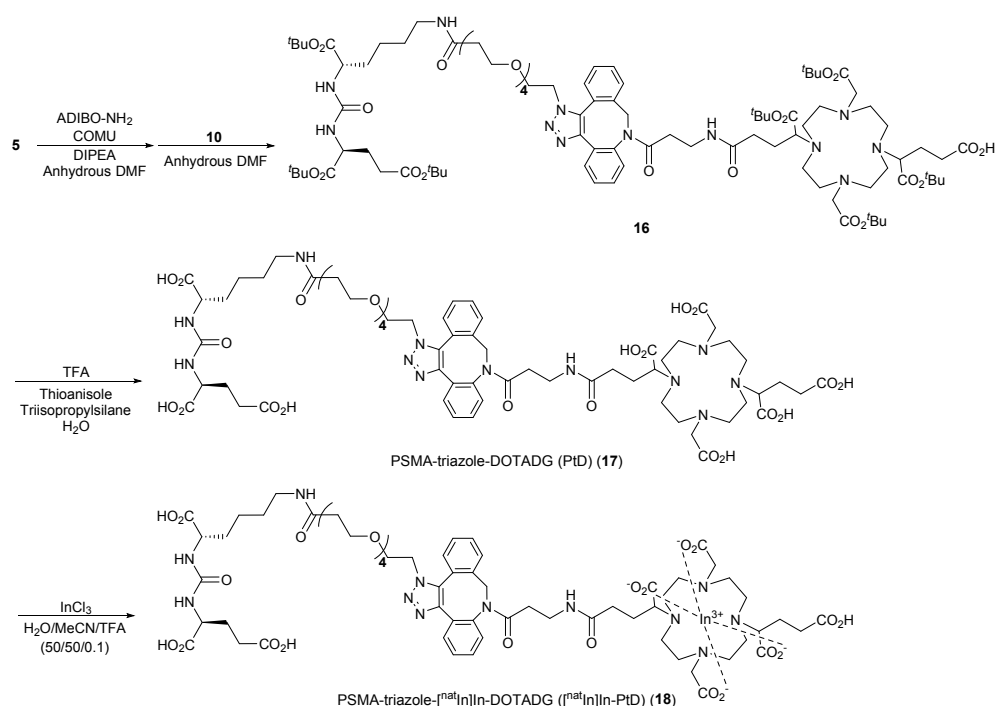
**Indium(III)** **2,2'-(4-(4-((3-(1-(4-((2*S*,5*S*,11*S*,14*R*)-14-benzyl-11-(carboxymethyl)-5-(3-guanidinopropyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)butyl)-1,9-dihydro-8*H*-dibenzo[*b,f*][1,2,3]triazolo[4,5-*d*]azocin-8-yl)-3-oxopropyl)amino)-1-carboxy-4-oxobutyl)-10-(1-carboxy-4-(((*S*)-1-carboxy-5-(4-(4-**

***iodophenyl)butanamido)pentyl)amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (cRGD-triazole-[<sup>nat</sup>In]In-DOTADG-ALB ([<sup>nat</sup>In]In-RtDA)) (15)***

Compound **14** (0.8 mg, 0.40  $\mu$ mol) was dissolved in H<sub>2</sub>O/MeCN/TFA solution (49.95/49.95/0.1, 300  $\mu$ L). To the solution was added anhydrous indium(III) chloride (1.0 mg, 4.0  $\mu$ mol). After being stirred at room temperature for 18 h, the solution was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (4.6  $\times$  150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1.0 mL/min, to give 0.06 mg of **15** (7.7% yield). HRMS (ESI)  $m/z$  calculated for C<sub>83</sub>H<sub>109</sub>I<sup>115</sup>InN<sub>19</sub>O<sub>21</sub><sup>2+</sup>, 974.8059 [M + 2H]<sup>2+</sup>; found, 974.8051



**Fig. S2** The HPLC chromatogram of UV absorption for purified [<sup>nat</sup>In]In-RtDA.



***4-(7-(5-((3-(1-((21S,25S)-21,25-Bis(tert-butoxycarbonyl)-30,30-dimethyl-15,23,28-trioxo-3,6,9,12,29-pentaoxa-16,22,24-triazahentriacontyl)-1,9-dihydro-8H-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8-yl)-3-oxopropyl)amino)-1-(tert-butoxy)-1,5-***

***dioxopentan-2-yl)-4,10-bis(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)-5-(tert-butoxy)-5-oxopentanoic acid (16)***

To a solution of **5** (80 mg, 0.10 mmol) in anhydrous DMF (400  $\mu$ L) was added COMU (23 mg, 0.053 mmol) at 0 °C. The solution was stirred at 0 °C for 15 min. To the mixture was added DIPEA (9.2  $\mu$ L, 0.053 mmol) at 0 °C. The solution was stirred at 0 °C for 15 min. To the mixture was added ADIBO-NH<sub>2</sub> (12 mg, 0.43 mmol). After the mixture had been stirred at room temperature for 10 h, **10** was added to the mixture. After being stirred at room temperature for 24 h, the solution was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (4.6  $\times$  150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1.0 mL/min, to give 7.8 mg of **16** (11% yield). HRMS (ESI)  $m/z$  calculated for C<sub>91</sub>H<sub>148</sub>N<sub>12</sub>O<sub>24</sub><sup>2+</sup>, 896.5359 [M + 2H]<sup>2+</sup>; found, 896.5318

***(21S,25S)-1-(8-(3-(4-(4,10-Bis(carboxymethyl)-7-(1,3-dicarboxypropyl)-1,4,7,10-tetraazacyclododecan-1-yl)-4-carboxybutanamido)propanoyl)-8,9-dihydro-1H-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-1-yl)-15,23-dioxo-3,6,9,12-tetraoxa-16,22,24-triazaheptacosane-21,25,27-tricarboxylic acid (PSMA-triazole-DOTADG (PtD)) (17)***

Compound **16** (2.4 mg, 1.3  $\mu$ mol) was dissolved in TFA (1,900  $\mu$ L), thioanisole (60  $\mu$ L), triisopropylsilane (20  $\mu$ L), and H<sub>2</sub>O (20  $\mu$ L). The solution was stirred at room temperature for 13 h. The solvent was removed, and the residue was purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (4.6  $\times$  150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1.0 mL/min, to give 0.46 mg of **17** (25% yield). HRMS (ESI)  $m/z$  calculated for C<sub>63</sub>H<sub>92</sub>N<sub>12</sub>O<sub>24</sub><sup>2+</sup>, 700.3168 [M + 2H]<sup>2+</sup>; found, 700.3143

***Indium(III) (21S,25S)-1-(8-(3-(4-(4,10-bis(carboxymethyl)-7-(1,3-dicarboxypropyl)-1,4,7,10-tetraazacyclododecan-1-yl)-4-carboxybutanamido)propanoyl)-8,9-dihydro-1H-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-1-yl)-15,23-dioxo-3,6,9,12-tetraoxa-16,22,24-triazaheptacosane-21,25,27-tricarboxylic acid (PSMA-triazole-[<sup>nat</sup>In]In-DOTADG ([<sup>nat</sup>In]In-PtD)) (18)***

Compound **17** (0.25 mg, 0.18  $\mu$ mol) was dissolved in H<sub>2</sub>O/MeCN/TFA solution (49.95/49.95/0.1, 150  $\mu$ L). To the solution was added anhydrous indium(III) chloride (0.40 mg, 1.8  $\mu$ mol). After being stirred at room temperature for 72 h, the solution was

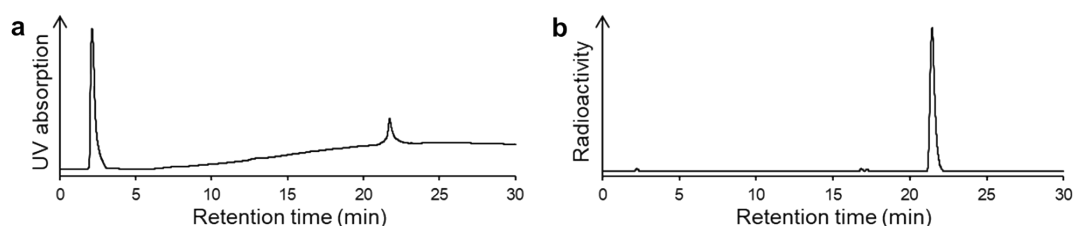
purified by RP-HPLC on a Cosmosil C<sub>18</sub> column (4.6 × 150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1.0 mL/min, to give 0.27 mg of **18** (100% yield). HRMS (ESI) *m/z* calculated for C<sub>63</sub>H<sub>89</sub><sup>115</sup>InN<sub>12</sub>O<sub>24</sub><sup>2+</sup>, 756.2570 [M + 2H]<sup>2+</sup>; found, 756.2541

### 3. Radiolabeling

#### 3.1 Route A

##### *[<sup>111</sup>In]In-ADA*

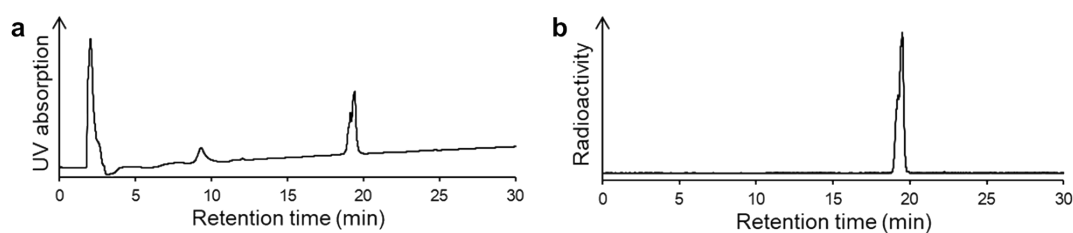
To a mixture of 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (0.1 M, pH 5.7, 100 μL) and [<sup>111</sup>In]InCl<sub>3</sub> solution (100 μL, 9.2 MBq) was added precursor **8** (0.60 mM in dimethyl sulfoxide (DMSO), 7 μL). The mixture was incubated at 90 °C for 5 min and then purified by RP-HPLC with a Cosmosil C<sub>18</sub> column (5C<sub>18</sub>-AR-II, 4.6 × 150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1 mL/min.



**Fig. S3** The HPLC chromatograms of UV absorption (a) and radioactivity (b) for a mixture of [<sup>nat</sup>In]In-ADA and [<sup>111</sup>In]In-ADA.

##### *[<sup>111</sup>In]In-PtDA*

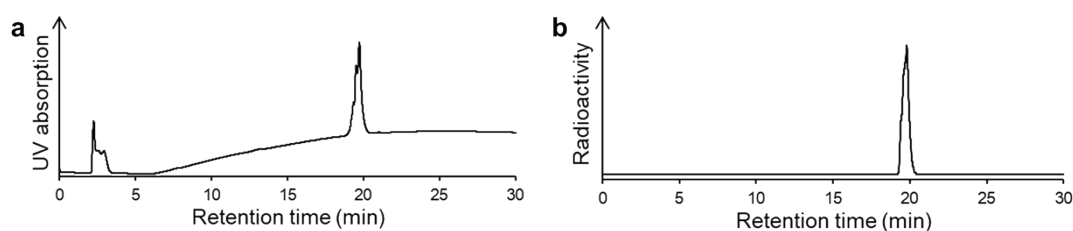
[<sup>111</sup>In]In-ADA (0.80 MBq) was added to phosphate-buffered saline (PBS)/DMSO (9/1, 200 μL) or DMSO (200 μL). To the mixture was added **11** (0.2 mg). The mixture was incubated at 37 °C for 10 or 30 min (30 min for DMSO solutions) and purified by RP-HPLC with a Cosmosil C<sub>18</sub> column (5C<sub>18</sub>-AR-II, 4.6 × 150 mm) using a mobile phase [H<sub>2</sub>O with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1 mL/min.



**Fig. S4** The HPLC chromatograms of UV absorption (a) and radioactivity (b) for a mixture of [ $^{nat}\text{In}$ ]In-PtDA and [ $^{111}\text{In}$ ]In-PtDA that was radiosynthesized using Route A.

### **[ $^{111}\text{In}$ ]In-RtDA**

To a mixture of PBS (180  $\mu\text{L}$ ) and DMSO (20  $\mu\text{L}$ ) was added [ $^{111}\text{In}$ ]In-ADA (1.5 MBq). To the mixture was added cRGD-azide (0.2 mg). The mixture was incubated at 37  $^{\circ}\text{C}$  for 10 min and purified by RP-HPLC with a Cosmosil  $\text{C}_{18}$  column (5C<sub>18</sub>-AR-II, 4.6  $\times$  150 mm) using a mobile phase [ $\text{H}_2\text{O}$  with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1 mL/min.

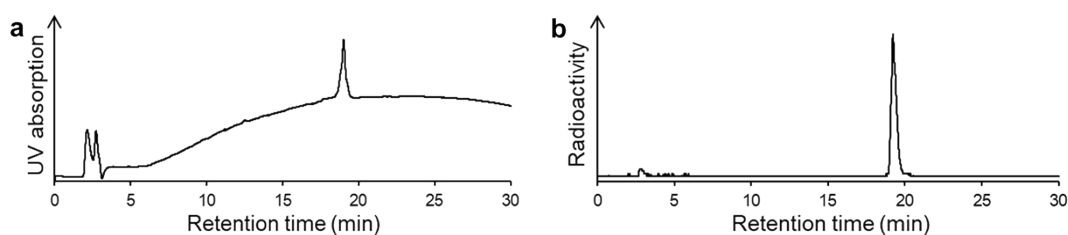


**Fig. S5** The HPLC chromatograms of UV absorption (a) and radioactivity (b) for a mixture of [ $^{nat}\text{In}$ ]In-RtDA and [ $^{111}\text{In}$ ]In-RtDA that was radiosynthesized using Route A.

## **3.2 Route B**

### **[ $^{111}\text{In}$ ]In-PtDA**

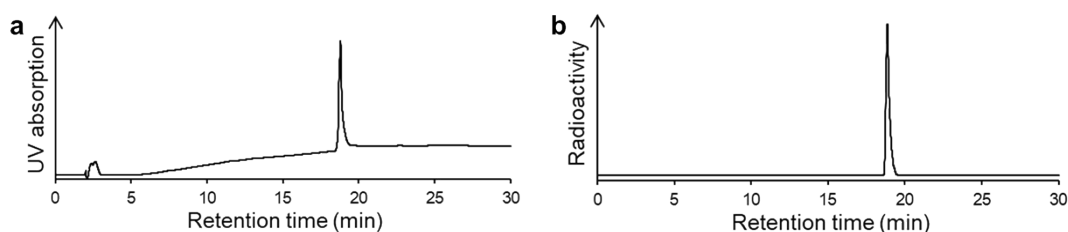
To a mixture of MES buffer (0.1 M, pH 5.7, 150  $\mu\text{L}$ ) and [ $^{111}\text{In}$ ]InCl<sub>3</sub> solution (100  $\mu\text{L}$ , 2.1 MBq) was added precursor **12** (0.56 mM in DMSO, 2  $\mu\text{L}$ ). The mixture was incubated at 90  $^{\circ}\text{C}$  for 5 min and purified by RP-HPLC with a Cosmosil  $\text{C}_{18}$  column (5C<sub>18</sub>-AR-II, 4.6  $\times$  150 mm) using a mobile phase [ $\text{H}_2\text{O}$  with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1 mL/min.



**Fig. S6** The HPLC chromatograms of UV absorption (a) and radioactivity (b) for a mixture of  $[\text{natIn}]\text{In-PtDA}$  and  $[^{111}\text{In}]\text{In-PtDA}$  that was radiosynthesized using Route B.

### ***$[^{111}\text{In}]\text{In-RtDA}$***

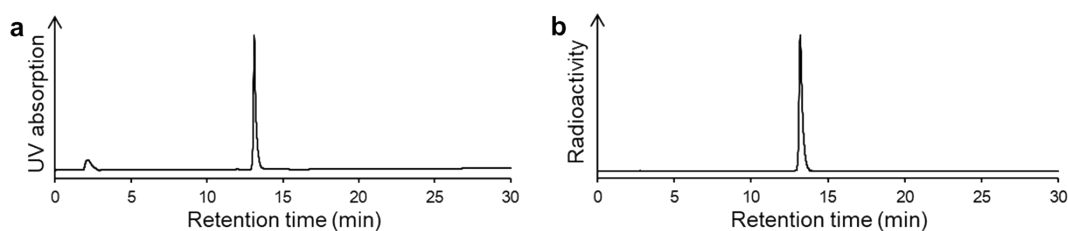
To a mixture of MES buffer (0.1 M, pH 5.7, 100  $\mu\text{L}$ ) and  $[^{111}\text{In}]\text{InCl}_3$  solution (80  $\mu\text{L}$ , 2.1 MBq) was added precursor **14** (0.27 mM in DMSO, 2  $\mu\text{L}$ ). The mixture was incubated at 90  $^\circ\text{C}$  for 5 min and purified by RP-HPLC with a Cosmosil  $\text{C}_{18}$  column (5 $\text{C}_{18}$ -AR-II, 4.6  $\times$  150 mm) using a mobile phase [ $\text{H}_2\text{O}$  with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1 mL/min.



**Fig. S7** The HPLC chromatograms of UV absorption (a) and radioactivity (b) for a mixture of  $[\text{natIn}]\text{In-RtDA}$  and  $[^{111}\text{In}]\text{In-RtDA}$  that was radiosynthesized using Route B.

### ***$[^{111}\text{In}]\text{In-PtD}$***

To a mixture of MES buffer (0.1 M, pH 5.7, 150  $\mu\text{L}$ ) and  $[^{111}\text{In}]\text{InCl}_3$  solution (50  $\mu\text{L}$ , 3.8 MBq) was added precursor **17** (0.71 mM in DMSO, 2  $\mu\text{L}$ ). The mixture was incubated at 90  $^\circ\text{C}$  for 10 min and purified by RP-HPLC with a Cosmosil  $\text{C}_{18}$  column (5 $\text{C}_{18}$ -AR-II, 4.6  $\times$  150 mm) using a mobile phase [ $\text{H}_2\text{O}$  with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1 mL/min.





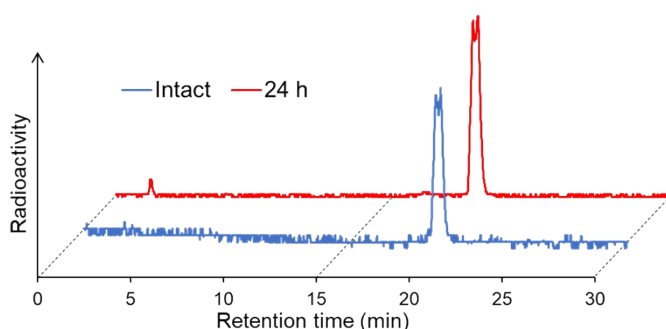
**Fig. S8** The HPLC chromatograms of UV absorption (a) and radioactivity (b) for a mixture of [ $^{nat}\text{In}$ ]In-PtD and [ $^{111}\text{In}$ ]In-PtD.

#### 4. Animals

All animal experiments were conducted in accordance with our institutional guidelines and approved by Kyoto University Animal Care Committee. Male CB17/IcrJcl-*Prkdc<sup>scid</sup>* mice (5 weeks old) and male ddY mice (5 weeks old) were purchased from CLEA Japan (Tokyo, Japan) and Japan SLC (Shizuoka, Japan), respectively, and housed in a sterile environment under a 12-h light-dark cycle.

#### 5. *In vitro* stability assay using mouse plasma

Fresh ddY mouse blood was collected in venous blood collection tubes (Becton, Dickinson and Company, New Jersey, U.S.A.). The blood was centrifuged at  $1,200 \times g$  for 10 min to obtain the plasma. To the fresh plasma (200  $\mu\text{L}$ ) was added a solution of [ $^{111}\text{In}$ ]In-PtDA (259 kBq) in saline (20  $\mu\text{L}$ ). The mixture was incubated at 37 °C for 24 h ( $n = 3$ ). After the mixture had been incubated, MeCN (400  $\mu\text{L}$ ) was added to it. The mixture was then centrifuged at  $10,000 \times g$  for 5 min. The supernatant was filtered with a Cosmonice filter S (0.45  $\mu\text{m}$ , 4 mm) (Nacalai Tesque), and the filtrate was analyzed by RP-HPLC.



**Fig. S9** *In vitro* stability of [ $^{111}\text{In}$ ]In-PtDA after it was incubated in mouse plasma for 24 h. The radiochemical purity of [ $^{111}\text{In}$ ]In-PtDA was >95%, according to RP-HPLC performed with a Cosmosil C<sub>18</sub> column (5C<sub>18</sub>-AR-II, 4.6  $\times$  150 mm) using a mobile phase [ $\text{H}_2\text{O}$  with 0.1% TFA/MeCN with 0.1% TFA = 9/1 (0 min) to 3/7 (30 min)], which was delivered at a flow rate of 1 mL/min.

## 6. Determination of the partition coefficient

[<sup>111</sup>In]In-PtDA (111 kBq) was added to a mixture of 1-octanol (3 mL) and PBS (pH 7.4, 3 mL). After being vortexed for 2 min, the phases were separated by centrifugation at  $4,000 \times g$  for 5 min. 1 mL aliquots were taken from the PBS and 1-octanol phases. The radioactivity in each phase was measured with a  $\gamma$ -counter (Wallac 2470 Wizard; PerkinElmer, Massachusetts, U.S.A.). To the remaining PBS phase (1 mL) were added newly prepared 1-octanol (3 mL) and PBS (2 mL). The vortexing, centrifuging, and counting were repeated twice. The partition coefficient ( $\log P_{OW}$ ) was calculated as the average log value of the ratio between the radioactivity in the 1-octanol and PBS phases from three samples.

## 7. Cell culture

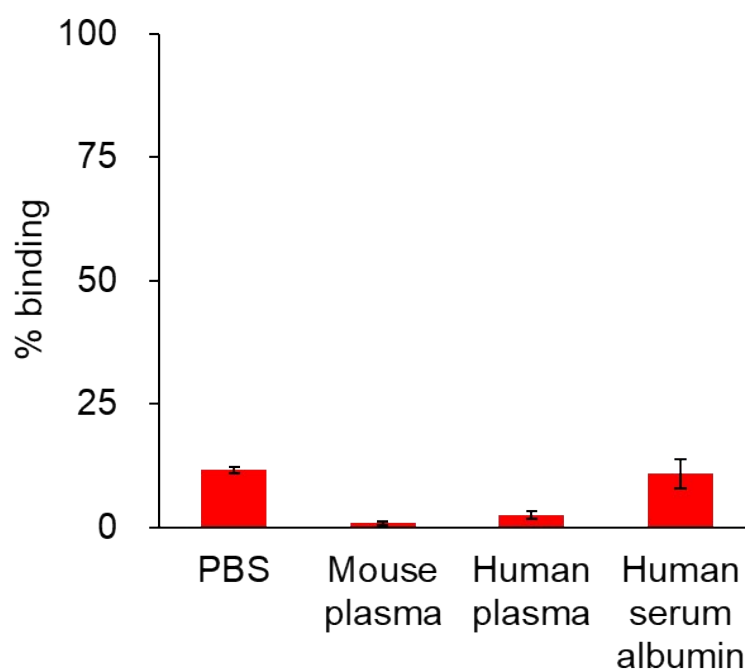
Prostate-specific membrane antigen (PSMA)-positive LNCaP and PSMA-negative PC-3 human prostate cancer cells were purchased from the American Type Culture Collection (Virginia, U.S.A.) and DS Pharma Biomedical (Osaka, Japan), respectively. All cells were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (Nacalai Tesque) containing 10% fetal bovine serum (FBS) and 100 U/mL of penicillin and streptomycin at 37 °C in an atmosphere containing 5% CO<sub>2</sub>.

## 8. *In vitro* cell-binding assay

LNCaP and PC-3 cells were seeded onto 12-well plates ( $4.0 \times 10^5$  cells/well) and incubated at 37 °C in an atmosphere containing 5% CO<sub>2</sub> for 48 h. Subsequently, the culture medium was replaced with medium containing 0.5% FBS and [<sup>111</sup>In]In-PtDA (37 kBq) with or without 2-(phosphonomethyl)pentanedioic acid (2-PMPA) (100  $\mu$ M) and incubated at 37 °C in an atmosphere containing 5% CO<sub>2</sub> for 1 h. After removing the medium, the cells were washed with RPMI-1640 medium containing 0.5% FBS (500  $\mu$ L  $\times$  2) and then were lysed with 1 N NaOH aqueous solution (200  $\mu$ L  $\times$  2). The radioactivity in the cell solution was measured with a  $\gamma$ -counter (PerkinElmer). The total protein concentration was determined by a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Massachusetts, U.S.A.).

## 9. Protein-binding assay

The protein-binding assay was performed according to a previously described method.<sup>3</sup> Mouse plasma was obtained by centrifuging mouse blood (ddY mouse, male, 5 weeks old) in venous blood collection tubes (Becton, Dickinson and Company). Human plasma was purchased from Cosmo Bio (Tokyo, Japan). A solution of [<sup>111</sup>In]In-PtDA or [<sup>111</sup>In]In-PtD (37 kBq) in PBS (50  $\mu$ L) was added to 200  $\mu$ L of PBS, human plasma, mouse plasma, or human serum albumin solution (45 mg in 1 mL of PBS). After being vortexed, the solution was incubated at 37 °C for 10 min. Then, 100  $\mu$ L of the solution was loaded onto a gel filtration column (Sephadex G-50 Fine; Cytiva, Tokyo, Japan) with 0.1 M acetate buffer (pH 6.0), before being centrifuged (1,500  $\times$  g, 2 min). The radioactivity of the column and eluate were then measured with a  $\gamma$ -counter (PerkinElmer).



**Fig. S10** Protein binding of [<sup>111</sup>In]In-PtD in PBS, mouse plasma, human plasma, and human serum albumin solution. Values are expressed as the mean  $\pm$  standard deviation of three independent experiments.

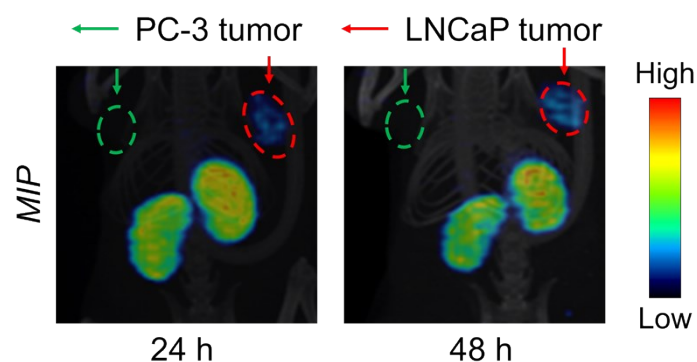
## 10. Tumor model

Under anesthesia (induced with 2% isoflurane), CB17/IcrJcl-*Prkdc*<sup>scid</sup> mice (male, 5

weeks old) were subcutaneously injected with LNCaP and PC-3 cells ( $1 \times 10^7$  cells/mouse), in 150  $\mu$ L of RPMI-1640 medium and Matrigel (Corning, Arizona, U.S.A.) at a ratio of 1:1, in the right and left shoulder, respectively, for the single photon emission computed tomography (SPECT)/CT experiment. For the biodistribution assay, LNCaP cells ( $1 \times 10^7$  cells/mouse) were subcutaneously injected into the right shoulder. The LNCaP and PC-3 tumors were allowed to grow until they reached 0.8 to 1.2 cm in diameter.

### 11. SPECT/CT of LNCaP and PC-3 tumor-bearing mice

A saline solution (100  $\mu$ L) of [ $^{111}\text{In}$ ]In-PtDA (2.7 MBq) was injected into the tail veins of the LNCaP and PC-3 tumor-bearing CB17/IcrJcl-*Prkdc*<sup>scid</sup> mice. The mice were anesthetized with isoflurane (2% in an air mixture). SPECT and CT images of the mice were collected at 24 and 48 h postinjection using Triumph combined positron emission tomography/SPECT/CT systems (TriFoil Imaging, California, U.S.A.) with 1.0-mm pinhole collimators, and the images were reconstructed using the ordered subset expectation maximization method, according to a previously described method.<sup>4</sup> The acquired SPECT and CT data were analyzed using the PMOD software (version 3.3; PMOD Technologies, Zürich, Switzerland).



**Fig. S11** Maximal intensity projections (MIP) of SPECT/CT images of LNCaP and PC-3 tumor-bearing mice at 24 and 48 h after the injection of [ $^{111}\text{In}$ ]In-PtDA.

### 12. Biodistribution assay using model mice

A saline solution (100  $\mu$ L) of [ $^{111}\text{In}$ ]In-PtDA or [ $^{111}\text{In}$ ]In-PtD (241 kBq) was injected

into the tail veins of LNCaP tumor-bearing CB17/IcrJcl-*Prkdc<sup>scid</sup>* mice. At 1, 24, and 48 h postinjection, some of the mice ( $n = 3$  at each timepoint) were euthanized. The blood and organs of interest were collected and weighed, and the radioactivity of the collected samples was measured with a  $\gamma$ -counter (PerkinElmer). The percentage of the injected dose per gram of organ tissue was calculated for each sample.

**Table S1.** Biodistribution of radioactivity among organs and tissues after the intravenous injection of [ $^{111}\text{In}$ ]In-PtDA into LNCaP tumor-bearing mice<sup>a</sup>

[ $^{111}\text{In}$ ]In-PtDA	Tissue	Time since injection (h)		
		1	24	48
% injected dose per gram of organ tissue	Blood	19.88 (2.74)	12.52 (1.47)	6.33 (1.53)
	Spleen	15.28 (4.89)	9.69 (0.92)	10.49 (5.62)
	Pancreas	2.31 (0.14)	2.03 (0.48)	1.12 (0.39)
	Stomach <sup>b</sup>	0.79 (0.19)	0.46 (0.23)	0.29 (0.20)
	Intestines	2.66 (0.28)	1.52 (0.41)	0.74 (0.28)
	Kidneys	37.18 (6.85)	67.98 (2.82)	55.89 (6.39)
	Liver	3.51 (0.38)	3.36 (0.77)	2.28 (0.75)
	Heart	5.49 (0.77)	3.95 (0.87)	1.98 (0.41)
	Lungs	11.87 (1.57)	8.63 (1.31)	6.16 (1.56)
	Brain	0.36 (0.05)	0.30 (0.05)	0.20 (0.07)
	Tumor	2.18 (0.17)	16.03 (9.49)	18.68 (5.21)
	Muscle	1.95 (0.30)	1.28 (0.34)	0.81 (0.40)
Ratio	Tumor/Blood	0.11 (0.02)	1.35 (0.86)	2.93 (0.18)
	Tumor/Muscle	1.13 (0.13)	13.52 (11.42)	24.48 (4.76)

<sup>a</sup>Each value represents the mean (standard deviation) of three mice.

<sup>b</sup>Expressed as % injected dose.

**Table S2.** Biodistribution of radioactivity among organs and tissues after the intravenous injection of [ $^{111}\text{In}$ ]In-PtD into LNCaP tumor-bearing mice<sup>a</sup>

$[^{111}\text{In}]\text{In-PtD}$	Tissue	Time since injection (h)		
		1	24	48
% injected dose per gram of organ tissue	Blood	0.50 (0.12)	0.10 (0.01)	0.04 (0.00)
	Spleen	7.92 (3.47)	0.64 (0.22)	0.69 (0.31)
	Pancreas	0.27 (0.10)	0.19 (0.06)	0.15 (0.09)
	Stomach <sup>b</sup>	0.06 (0.04)	0.03 (0.02)	0.03 (0.01)
	Intestines	0.15 (0.04)	0.15 (0.03)	0.20 (0.07)
	Kidneys	106.13 (43.24)	5.21 (6.22)	2.35 (0.91)
	Liver	0.21 (0.04)	0.28 (0.04)	0.38 (0.11)
	Heart	0.27 (0.12)	0.12 (0.07)	0.09 (0.03)
	Lungs	0.63 (0.18)	0.23 (0.11)	0.16 (0.07)
	Brain	0.02 (0.02)	0.01 (0.01)	0.03 (0.02)
	Tumor	2.71 (0.51)	1.04 (0.45)	1.04 (0.03)
	Muscle	0.24 (0.16)	0.08 (0.09)	0.07 (0.05)
	Tumor/Blood	5.52 (0.39)	10.55 (3.42)	28.97 (3.74)
Ratio	Tumor/Muscle	20.99 (23.12)	9.47 (12.58)	25.38 (22.56)

<sup>a</sup>Each value represents the mean (standard deviation) of three mice.

<sup>b</sup>Expressed as % injected dose.

### 13. Statistical Analysis

All data were analyzed with GraphPad Prism 6.0 or Microsoft Excel. Differences at the 95% confidence level ( $P < 0.05$ ) were determined using Kruskal-Wallis test with Dunn's post hoc test for the cell-binding assay and one-way analysis of variance (ANOVA) with Dunnett's post hoc test for the protein-binding assay.

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