Supplementary Information for:

Dual pharmacokinetic modifier strategy for synergistically enhanced tumor accumulation of PSMA-targeting radioligand

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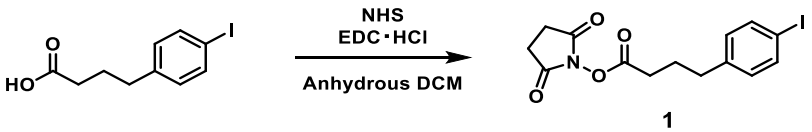
1. General remarks

All reagents were obtained commercially and used without further purification unless otherwise indicated. [^{111}In]$InCl_3$ was purchased from Nihon Medi-Physics (Tokyo, Japan). High-resolution mass spectrometry (HRMS) was conducted with LCMS-IT-TOF (Shimadzu, Kyoto, Japan). ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-ECA500 system (JEOL, Tokyo, Japan). Chemical shifts for ^{1}H NMR are reported in ppm units relative to the residual solvent signal (7.26 ppm in $CDCl_3$). Coupling constants are reported in Hertz. Multiplicity was defined as singlet (s), doublet (d), triplet (t), quintet (q), double of doublet (dd), or multiplet (m). Reversed-phase high-performance liquid chromatography (RP-HPLC) was performed using a Shimadzu system (LC-20AT or LC-20AD pump with SPD-20A UV detector, λ = 254 nm) with a Cosmosil C_{18} column (5C_{18}-AR-II, 4.6 × 150 or 10 × 250 mm; Nacalai Tesque, Kyoto, Japan). No unexpected or unusually high safety hazards were encountered.

2. Chemistry

Scheme S1. Synthetic routes for compound 1.

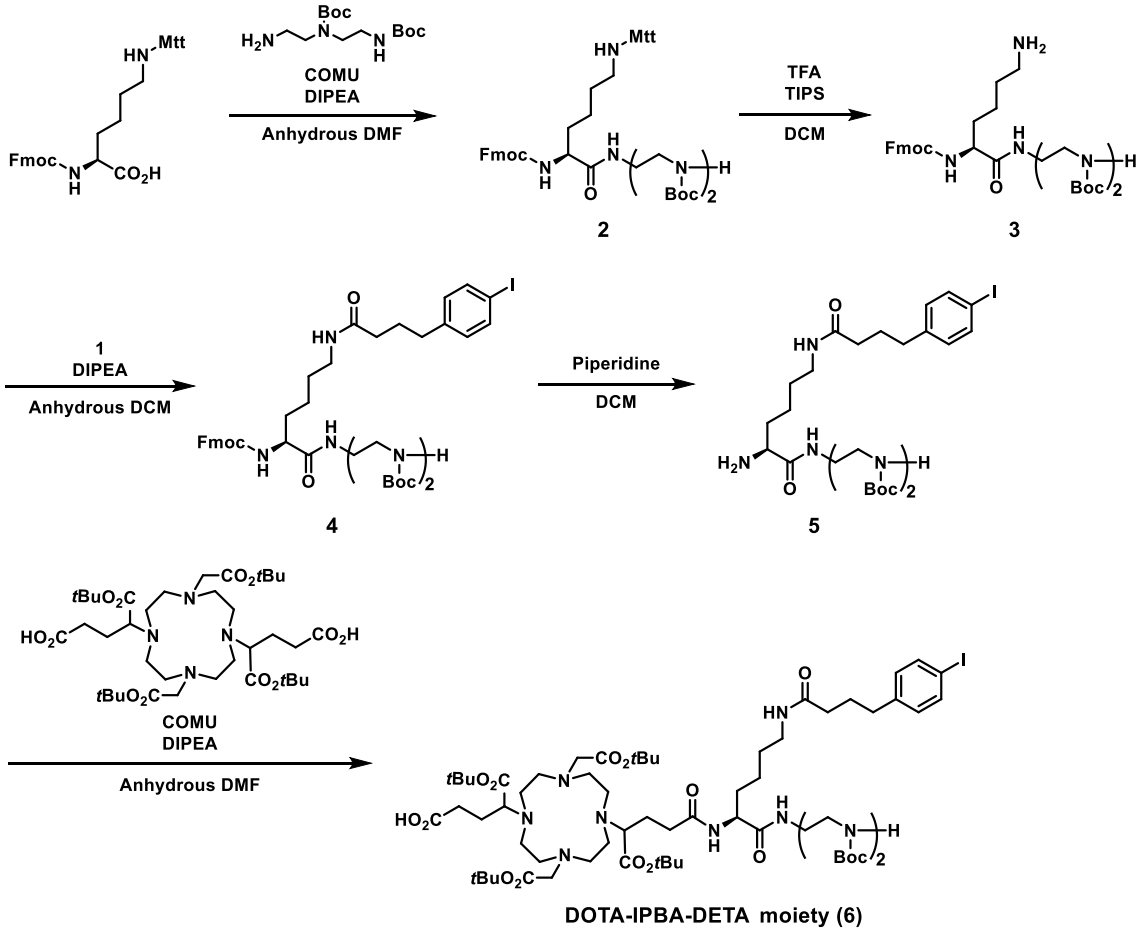


1

2,5-Dioxopyrrolidin-1-yl 4-(4-iodophenyl)butanoate (1)

A solution of 4-(4-iodophenyl)butanoic acid (4.0 g, 14 mmol) in anhydrous dichloromethane (DCM; 50 mL) was cooled to 0°C. It was stirred at 0°C for 15 min, and *N*-hydroxysuccinimide (NHS; 1.8 g, 15 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·hydrochloride (EDC·HCl; 2.7 g, 14 mmol) were added. After being stirred again at 0°C for 15 min, the solution was warmed to room temperature (r.t.). Then, after stirring at r.t. for 12 h, the solution was washed with H_2O (three times). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent with $CHCl_3$, isocratic flow) to give 5.0 g of **1** (92% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (d, J = 4.3 Hz, 2H), 6.84 (d, J = 4.3 Hz, 2H), 2.66 (s, 4H), 2.54 (t, J = 7.2 Hz, 2H), 2.46 (t, J = 7.2, 2H), 1.89 (q, J = 7.2 Hz, 2H). HRMS (ESI) *m/z* calcd for $C_{14}H_{14}INNaO_4^+$, 409.9869 $[M + Na]^+$; found, 409.9859.

Scheme S2. Synthetic routes for DOTA-IPBA-DETA moiety.



tert-Butyl (S)-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((diphenyl (p-tolyl)methyl)amino)hexanamido)ethyl)(2-((tert-butoxycarbonyl)amino)ethyl) carbamate (2)

A solution of N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^6-(diphenyl(*p*-tolyl)methyl)-L-lysine (2.5 g, 4.0 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF; 20 mL) was cooled to 0°C. It was stirred at 0°C for 15 min, and 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholino)] uronium hexafluorophosphate (COMU; 2.6 g, 6.0 mmol) and *N,N*-diisopropylethylamine (DIPEA; 1.1 mL, 6.0 mmol)

were added to it. After being stirred again at 0°C for 15 min, *tert*-butyl-(2-aminoethyl)(2-((*tert*-butoxycarbonyl)amino)ethyl)carbamate[1] (1.5 g, 4.8 mmol) was added to the solution. It was warmed to r.t. and stirred at this temperature for 48 h. The solution was mixed with H_2O and extracted using a mixture (hexane/ethyl acetate = 4/1, v/v) (two times), and the combined organic layers were washed with brine (three times). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent with $CHCl_3$/MeOH = 100/0 to 90/10, gradient flow) to give 2.6 g of **2** (72% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77–7.72 (m, 2H), 7.60 (d, J = 7.2 Hz, 2H), 7.49 (dd, J = 0.9 and 7.2 Hz, 4H), 7.41–7.33 (m, 4H), 7.31–7.23 (m, 6H), 7.18 (t, J = 7.2 Hz, 2H), 7.09 (d, J = 7.2 Hz, 2H), 4.44 (t, J = 9.5 Hz, 1H), 4.34 (t, J = 9.5 Hz, 1H), 4.21 (t, J = 7.2 Hz, 2H), 3.56–2.80 (m, 10H), 2.31 (s, 3H), 2.14 (t, J = 7.2 Hz, 2H), 1.57–1.33 (m, 22H). HRMS (ESI) *m/z* calcd for $C_{55}H_{68}N_5O_7^+$, 910.5114 $[M + H]^+$; found, 910.5115.

tert-Butyl (S)-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-aminohexanamido)ethyl)(2-((tert-butoxycarbonyl)amino)ethyl)carbamate (3)

Compound **2** (2.6 g, 2.9 mmol) was dissolved in a mixture of trifluoroacetic acid (TFA; 300 μL), triisopropylsilane (TIPS; 750 μL), and DCM (13.95 mL). After being stirred at

r.t. for 22 h, the solution was evaporated under reduced pressure. The residue was purified by silica gel chromatography (eluent with $CHCl_3$/MeOH = 73/27 to 50/50, gradient flow) to give 1.9 g of **3** (quantitative yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72–7.59 (m, 2H), 7.56–7.39 (m 2H), 7.34–7.26 (m, 2H), 7.25–7.17 (m, 2H), 4.37–4.00 (m, 3H), 3.37–2.74 (m, 10H), 1.77–1.46 (m, 2H), 1.42–1.00 (m, 22H). HRMS (ESI) *m/z* calcd for $C_{35}H_{52}N_5O_7^+$, 654.3862 $[M + H]^+$; found, 654.3859.

tert-Butyl (S)-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(4-(4-iodophenyl)butanamido)hexanamido)ethyl)(2-((tert-butoxycarbonyl)amino)ethyl) carbamate (4)

A solution of compound **3** (1.9 g, 2.9 mmol) in anhydrous DCM (12.7 mL) was cooled to 0°C. The solution was stirred at 0°C for 15 min, and compound **1** (1.3 g, 3.5 mmol) and DIPEA (0.6 mL, 3.5 mmol) were added to it. After being stirred again at 0°C for 15 min, the solution was warmed to r.t. and stirred at this temperature for 12 h. The solution was mixed with H_2O and extracted using $CHCl_3$ (two times), and the combined organic layers were washed with brine (three times). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent with $CHCl_3$/MeOH = 100/0 to 94/6,

gradient flow) to give 1.8 g of **4** (69% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.62 (d, J = 8.0 Hz, 2H), 7.51–7.37 (m, 4H), 7.26 (t, J = 7.4 Hz, 2H), 7.16 (t, J = 7.4 Hz, 2H), 6.79–6.71 (m, 2H), 4.27–4.02 (m, 4H), 3.37–3.03 (m, 10H), 2.39 (t, J = 7.7 Hz, 2H), 2.02 (t, J = 7.4 Hz, 2H), 1.81–1.55 (m, 4H), 1.45–1.15 (m, 22H). HRMS (ESI) *m/z* calcd for $C_{45}H_{61}IN_5O_8^+$, 926.3560 $[M + H]^+$; found, 926.3536.

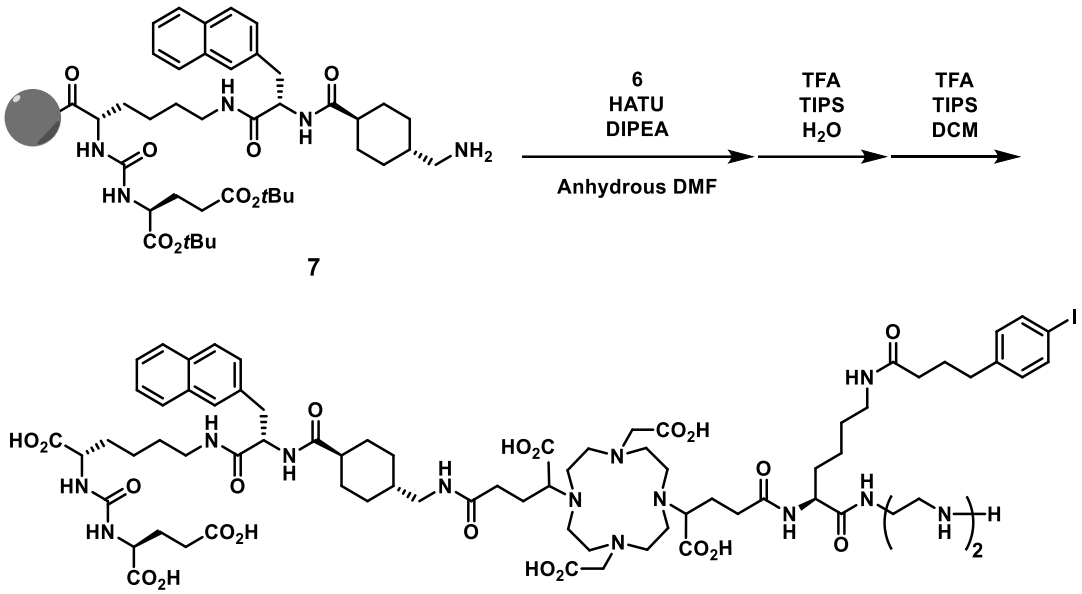
tert-Butyl (S)-(2-(2-amino-6-(4-(4-iodophenyl)butanamido)hexanamido)ethyl)(2-((tert-butoxycarbonyl)amino)ethyl)carbamate (5)

Compound **4** (1.8 g, 2.0 mmol) was dissolved in DCM (4.0 mL), and piperidine (1.0 mL) was added to the solution. After being stirred at r.t. for 3 h, the solution was evaporated under reduced pressure. The residue was purified by amino-functionalized silica gel chromatography (eluent with $CHCl_3$/MeOH = 100/0 to 94/6, gradient flow) to give 1.4 g of **5** (69% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.51 (d, J = 8.0 Hz, 2H), 6.86 (d, J = 8.0 Hz, 2H), 6.22 (s, 1H), 3.37–3.10 (m, 11H), 2.50 (t, J = 7.4 Hz, 2H), 2.09 (t, J = 7.4 Hz, 2H), 1.89–1.63 (m, 4H), 1.49–1.26 (m, 22H). HRMS (ESI) *m/z* calcd for $C_{30}H_{51}IN_5O_6^+$, 704.2876 $[M + H]^+$; found, 704.2866.

4-(4,10-Bis(2-(tert-butoxy)-2-oxoethyl)-7-((13S)-8-(tert-butoxycarbonyl)-13-(4-(4-(4-iodophenyl)butanamido)butyl)-2,2,21,21-tetramethyl-4,12,15,19-tetraoxo-3,20-dioxa-5,8,11,14-tetraazadocosan-18-yl)-1,4,7,10-tetraazacyclododecan-1-yl)-5-(tert-butoxy)-5-oxopentanoic acid (DOTA-IPBA-DETA moiety) (6)

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)[2] (0.91 g, 1.2 mmol) was dissolved in anhydrous DMF (5 mL), and the solution was cooled to 0°C. It was stirred at 0°C for 15 min, and COMU (2.6 g, 6.0 mmol) and DIPEA (250 μL, 6.0 mmol) were added to it. After the solution was stirred again at 0°C for 15 min, compound **5** (0.75 g, 1.1 mmol) was slowly added to it. After being stirred at r.t. for 3 h, the solution was purified by RP-HPLC performed with a Cosmosil C_{18} column (5C_{18}-AR-II, 10 × 250 mm) using a mobile phase [H_2O with 0.1% TFA/MeCN with 0.1% TFA = 70/30 (0 min) to 25/75 (30 min)], which was delivered at a flow rate of 4.0 mL, to give 0.22 g of **6** (14% yield). HRMS (ESI) *m/z* calcd for $C_{68}H_{118}IN_9O_{17}^{2+}$, 729.8840 $[M + 2H]^{2+}$; found, 729.8834.

Scheme S3. Synthetic routes for PDAI2.



(((1S)-5-((2S)-2-((1R,4S)-4-((4-(7-(4-(((S)-1-((2-((2-Aminoethyl)amino)ethyl)amino)-6-(4-(4-iodophenyl)butanamido)-1-oxohexan-2-yl)amino)-1-carboxy-4-oxobutyl)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)-4-carboxybutanamido)methyl)cyclohexane-1-carboxamido)-3-(naphthalen-2-yl)propanamido)-1-carboxypentyl)carbamoyl)-L-glutamic acid (PDAI2) (8)

Compound **7** on Cl-Trt(2-Cl) resin (45 μmol) was prepared according to our previous report.[3] To the resin with **7** was added an anhydrous DMF solution (500 μL) containing compound **6** (0.22 g, 0.15 mmol) activated by *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU; 75 mg, 0.20 mmol) and DIPEA (35 μL, 0.20 mmol), and the mixture was agitated at r.t. for 15 h. After the resin was washed with DMF (500 μL, three times) and subsequent DCM (500 μL, three times), a mixture of TFA

(950 μL), TIPS (25 μL), and H_2O (25 μL) was added to it. After the mixture was agitated at r.t. for 3 h, the solution was collected and evaporated under Ar-gas flow. To the residue were added a mixture of TFA (1.6 mL), TIPS (100 μL), and DCM (300 μL). After being stirred at r.t. overnight to complete the deprotection reaction, the solution was concentrated under Ar-gas flow. The residue was purified by RP-HPLC performed with a Cosmosil C_{18} column ($5C_{18}$-AR-II, 10 × 250 mm) using a mobile phase [H_2O with 0.1% TFA/MeCN with 0.1% TFA = 70/30 (0 min) to 40/60 (30 min)], which was delivered at a flow rate of 4.0 mL, to give 11 mg of **8** (14% yield). HRMS (ESI) *m/z* calcd for $C_{75}H_{111}IN_{14}O_{21}^{2+}$, 836.3619 $[M + 2H]^{2+}$; found, 836.3605.

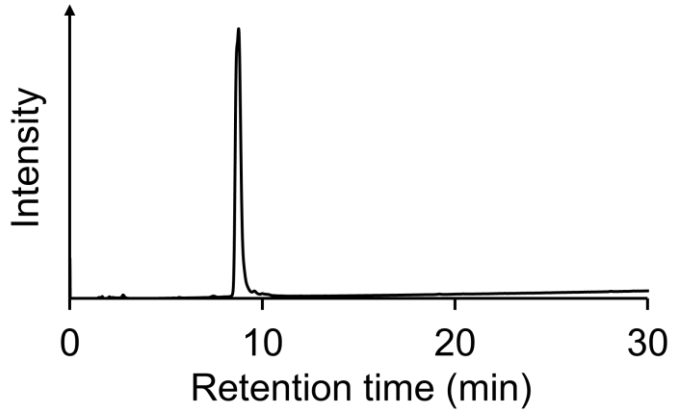
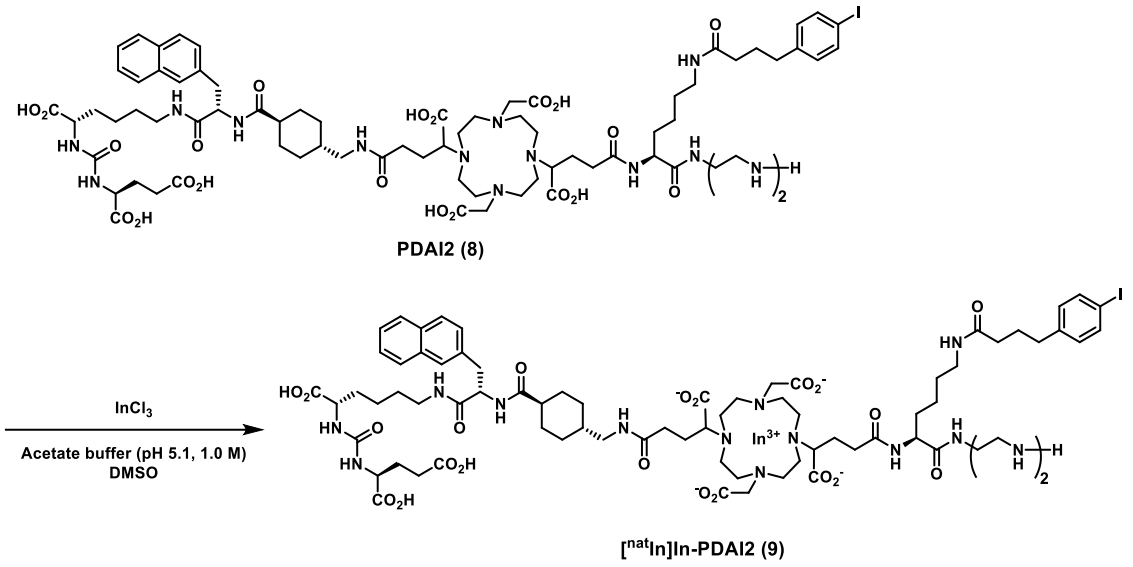


Fig. S1 HPLC chromatograms of UV absorption at 254 nm for PDAI2.

Scheme S4. Synthetic routes for [^{nat}In]In-PDAI2.



Indium (III) (((1S)-5-((2S)-2-((1r,4S)-4-((4-(7-(4-(((S)-1-((2-((2-aminoethyl)amino)ethyl)amino)-6-(4-(4-iodophenyl)butanamido)-1-oxohexan-2-yl)amino)-1-carboxy-4-oxobutyl)-4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)-4-carboxybutanamido)methyl)cyclohexane-1-carboxamido)-3-(naphthalen-2-yl)propanamido)-1-carboxypentyl)carbamoyl)-L-glutamic acid ([^{nat}In]In-PDAI2) (9)

A solution of compound **8** (2.8 mg, 1.7 μmol) in dimethyl sulfoxide (DMSO) (50 μL) was added to acetate buffer (1.0 M, pH 5.1, 200 μL). Anhydrous indium(III) chloride (3.7 mg, 17 μmol) was then added to the solution. It was incubated at 90°C for 20 min. After the suspension was centrifuged, the supernatant was filtered with Cosmonice Filter S (0.45 μm, 4 mm). The filtrate was purified by RP-HPLC performed with a Cosmosil C_{18} column ($5C_{18}$-AR-II, 4.6 × 150 mm) using a mobile phase [H_2O with 0.1% TFA/MeCN

with 0.1% TFA = 80/20 (0 min) to 50/50 (30 min)], which was delivered at a flow rate of 1.0 mL/min, to give 0.53 mg of **9** (18% yield). HRMS (ESI) *m/z* calcd for $C_{75}H_{110}IInN_{14}O_{21}^{2+}$, 892.3021 $[M + 3H]^{2+}$; found, 892.3023.

3. Radiolabeling

An [^{111}In]$InCl_3$ solution (200 µL) was added to a mixture of acetate buffer (0.1 M, pH 5.5, 200−250 µL) and PDAI2 solution in DMSO (0.2 µg/µL, 5 µL). The mixture was incubated at 90°C for 20 min. After being cooled to room temperature, the mixture was purified by RP-HPLC performed with a Cosmosil C_{18} column (5C_{18}-AR-II, 4.6 × 150 mm) using a mobile phase [H_2O with 0.1% TFA/MeCN with 0.1% TFA = 70/30 (0 min) to 50/50 (20 min)], which was delivered at a flow rate of 1.0 mL/min. [^{111}In]In-PSMA-617 and [^{111}In]In-PDI2 were prepared according to our previous reports.[4, 5]

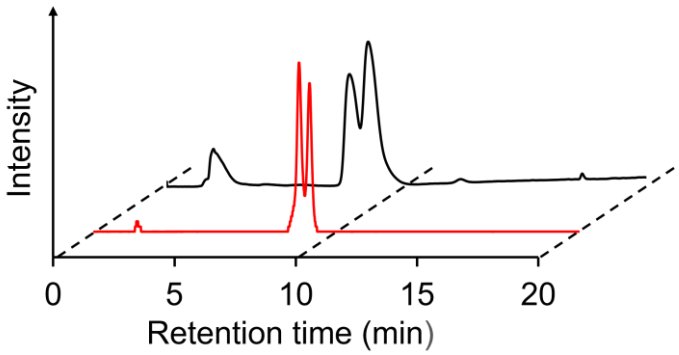


Fig. S2 HPLC chromatograms of UV absorption at 254 nm (black line) and radioactivity (red line) showing the co-injection of purified [^{111}In]In-PDAI2 and [^{nat}In]In-PDAI2.

4. Cellulose Acetate Electrophoresis (CAE) Assay

CAE analysis was performed according to our previous report with some modifications.[4] A cellulose acetate (CA) membrane (6 × 13 cm) (SELECA-VSP, JOKOH Co., Ltd., Tokyo, Japan) and two chromatography papers (5 × 8 cm) (Whatman 3MM CHR, Cytiva, Tokyo, Japan) were moistened with phosphate buffer (20 mM, pH 7.4). After the membrane and papers were set on the electrophoresis equipment (NIHON EIDO CO., Ltd., Tokyo, Japan), an aliquot of the solution containing [^{111}In]In-PDAI2 and [^{111}In]In-PDI2 (370 Bq, 1 μL) was spotted on the CA membrane. Electrophoresis was performed at a constant current of 2 mA for 30 min. The membrane was cut into fragments at a length of 4 mm, and the radioactivity of these fragments was measured with a γ-counter (2470 WIZARD^2; PerkinElmer, Massachusetts, U.S.A.).

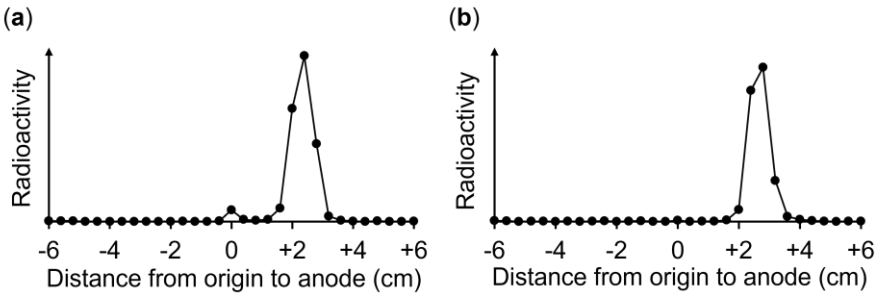


Fig. S3 CAE radioactivity profiles of [^{111}In]In-PDAI2 (a) and [^{111}In]In-PDI2 (b). Positive or negative distance from the origin means migration to the anode or cathode, respectively. Distances from the origin to anode of [^{111}In]In-PDAI2 and [^{111}In]In-PDI2 were approximately 2.4 and 2.8 cm, respectively.

5. Cell Culture

LNCaP cells, which are human prostate carcinoma cell lines, were purchased from the American Type Culture Collection (Virginia, U.S.A.). The cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium containing 10% heat-inactivated fetal bovine serum (FBS) and 100 U/mL of penicillin and streptomycin at 37°C in an atmosphere containing 5% CO_2.

6. Cell Saturation Binding Assay

The cell saturation binding assay was performed according to our previous method with some modifications.[4] LNCaP cells were incubated in 12-well plates (2×10^5 cells/well) at 37°C in an atmosphere containing 5% CO_2 for 48 h. After removing the medium, the cells were washed with the assay medium (RPMI-1640 medium supplemented with 0.5% FBS, 500 μL) and incubated for 30 min on ice. Subsequently, the cells were incubated with increasing concentrations of radioligands and their corresponding precursors (7.4 MBq/nmol, 0.195−50 nM) in the fresh assay medium with or without 2-(phosphonomethyl)pentane-1,5-dioic acid (2-PMPA, 100 μM) for 2 h on ice. After the medium was removed, the cells were washed with fresh assay medium (500 μL, two times) and lysed with 1 N NaOH aqueous solution (200 μL, two times). Radioactivity in

the cell lysate was measured with a γ-counter (2470 WIZARD^2). Total protein was determined using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Massachusetts, U.S.A.).

7. Albumin-binding Assay

The albumin-binding assay was performed according to our previous method with some modifications.[6] A saline solution containing 10% ethanol (15 μL) of [^{111}In]In-PDAI2 or [^{111}In]In-PDI2 (5.3 MBq/nmol, 50 kBq) was incubated with 0.3−3000 μM human serum albumin (HSA) in PBS (150 μL) at 37°C for 30 min. After the incubation, to the HSA solution was added PBS (300 μL) followed by centrifugation at 14,000 *g* for 30 min on Amicon Ultra-0.5 mL (30 kDa, Merck Millipore, Massachusetts, U.S.A.). Inserts of filter devices were inverted and centrifuged at 200 *g* for 3 min. Radioactivity of the high-molecular-weight, filtrate, and filter fractions was measured using a γ-counter (2470 WIZARD^2), and the amount of the high-molecular-weight fraction (albumin-bound fraction) as a percentage of the sum of all fractions was calculated. The albumin-bound fraction [%] was plotted against HSA-to-ligand concentration ratios and fitted with a nonlinear regression curve (specific binding with Hill slope, B_{max} set to 100%) using GraphPad Prism 6.0.

8. Cell Internalization Assay

The cell internalization assay was performed according to our previous method with some modifications.[4] LNCaP cells were incubated in 6-well plates (3×10^5 cells/well) at 37°C in an atmosphere containing 5% CO_2 for 48 h. After the medium was removed, the cells were incubated with fresh assay medium (1 mL) for 30 min on ice. On removing the medium, [^{111}In]In-PDAI2 or [^{111}In]In-PDI2 (5.3 GBq/nmol, 37 kBq) in fresh assay medium (1 mL) were added and incubated on ice for 2 h. After the medium was removed again, the cells were washed with fresh assay medium containing 1% bovine serum albumin (BSA; 1 mL, five times), and replenished by fresh RPMI-1640 medium containing 10% FBS and 100 U/mL of penicillin and streptomycin. The cells were incubated at 37°C in an atmosphere containing 5% CO_2 for 0 h, 1 h, 2 h, and 4 h. After the medium was collected, the cells were washed with fresh assay medium containing 1% BSA (1 mL, five times) and the medium was also collected and merged as a medium fraction. To dissociate the radioactivity on the cell surfaces, the cells were washed with PBS containing glycine-HCl (50 mM, pH 2.8, 1 mL) with incubation for 2 min on ice (three times). After collecting the wash solutions, the cells were washed with fresh assay medium containing 1% BSA (1 mL, five times), and the wash solutions were also collected and merged as a surface-bound fraction. The cells were lysed with 1 N NaOH

aqueous solution (1 mL, two times), and the cell lysates were collected as an internalized fraction. The radioactivity in all fractions was measured with a γ-counter (2470 WIZARD^2), and the amount of each fraction as a percentage of the sum of medium, surface-bound, and internalized fractions was calculated.

Table S1 Internalization assay of ^{111}In-labeled radioligands using LNCaP cells[a]

[^{111}In]In-PDAI2	Time after incubation (h)			
	0	1	2	4
Medium	8.64 ± 0.31	33.32 ± 2.24	32.48 ± 0.29	35.07 ± 2.95
Surface	73.22 ± 1.15	23.59 ± 1.63	19.63 ± 2.46	15.56 ± 1.03
Internalized	18.13 ± 0.91	43.10 ± 3.59	47.89 ± 2.52	49.36 ± 1.92

[^{111}In]In-PDI2	Time after incubation (h)			
	0	1	2	4
Medium	7.94 ± 1.15	34.44 ± 1.78	39.11 ± 2.25	46.02 ± 3.28
Surface	87.07 ± 1.38	25.62 ± 0.57	17.53 ± 0.82	13.91 ± 0.66
Internalized	4.98 ± 0.24	39.94 ± 1.26	43.36 ± 1.54	40.07 ± 2.64

[a]Each value represents the mean ± standard deviation.

9. Cell Efflux Assay

The cell efflux assay was performed according to our previous method with some modifications.[4] LNCaP cells were incubated in 6-well plates (3×10^5 cells/well) at 37°C in an atmosphere containing 5% CO_2 for 48 h. After removing the medium, [^{111}In]In-PDAI2 or [^{111}In]In-PDI2 (5.3 MBq/nmol, 37 kBq) in the assay medium (1 mL) were

added and incubated at 37°C in an atmosphere containing 5% CO_2 for 2 h. On removing the medium, cells were washed with PBS containing glycine-HCl (50 mM, pH 2.8, 1 mL) with incubation for 2 min on ice (three times) to remove the radioactivity on the cell surface. After the acid washing, the cells were washed with fresh assay medium containing 1% BSA (1 mL, five times), and replaced with fresh RPMI-1640 medium containing 10% FBS and 100 U/mL of penicillin and streptomycin. The cells were incubated at 37°C in an atmosphere containing 5% CO_2 for 0 h, 1 h, 2 h, 4 h, and 24 h. After the medium was collected, the cells were washed with fresh assay medium containing 1% BSA (1 mL, five times) and the wash solutions were also collected and merged as an efflux fraction. The cells were lysed with 1 N NaOH aqueous solution (1 mL, two times) and the cell lysates were collected as an internalized fraction. Radioactivity in both fractions was measured with a γ-counter (2470 WIZARD^2), and the amount of the efflux fraction as a percentage of the sum of both fractions was calculated.

Table S2 Efflux assay of ^{111}In-labeled radioligands using LNCaP cells[a]

Radioligand	Time after incubation (h)			
	0	1	2	4
[^{111}In]In-PDAI2	6.48 ± 0.77	20.98 ± 0.27	26.43 ± 1.54	31.57 ± 0.65
[^{111}In]In-PDI2	2.98 ± 0.22	20.22 ± 0.57	28.58 ± 0.45	35.66 ± 0.83

[a]Each value represents the mean ± standard deviation.

10. Animals

All animal use and study protocols were approved by the Kyoto University Animal Experimentation Committee (Approval Numbers: 20-20-4 and 20-20-5), and all experiments were conducted in accordance with the ethical guidelines of the Committee. Male BALB/c nu/nu mice (5 weeks old) were purchased from Japan SLC (Shizuoka, Japan). The animals were housed in a sterile environment under a 12 h light−dark cycle, fed standard chow, and had free access to water.

11. Tumor Model

Under anesthesia (induced with 2% isoflurane), BALB/c *nu*/*nu* mice (male, 5−6 weeks old) were subcutaneously inoculated with LNCaP cells (5×10^6 cells/mouse) in a mixture (150 μL) of RPMI-1640 medium and Matrigel (Corning, Arizona, U.S.A.) at a ratio of 1:1, in the right flank. LNCaP tumors were grown for 4−5 weeks to enable them to reach approximately > 5 mm in diameter.

12. Biodistribution Study Using Model Mice

A saline solution containing 10% ethanol (100 μL) of [^{111}In]In-PDAI2 (5.3 MBq/nmol, 111 kBq) was injected into the tail vein of LNCaP tumor-bearing mice. The mice were

euthanized at 4 h, 24 h, or 96 h postinjection. The blood, organs, and tissues of interest were collected and weighed, and then the radioactivity of collected samples was measured with a γ-counter (2470 WIZARD^2). The % injected dose (ID)/g of samples was calculated using the following equation:

$$\%\ \text{ID/g} = \frac{\text{Radioactivity of each organ [Bq]}}{\text{Injected radioactivity [Bq]}} \times 100 \times \frac{1}{\text{Weight of each organ [g]}}$$

Table S3 Radioactivity of extracted organs and tissues after intravenous injection of [^{111}In]In-PDAI2 into LNCaP tumor-bearing mice[a]

[^{111}In]In-PDAI2	Organs or tissues	Time after injection (h)		
		4	24	96
% ID/g of organ tissue	Blood	16.05 ± 2.59	5.48 ± 1.37	0.67 ± 0.13
	Spleen	9.92 ± 6.66	6.02 ± 2.34	3.30 ± 0.62
	Pancreas	1.84 ± 0.33	0.96 ± 0.14	0.28 ± 0.06
	Stomach*	0.62 ± 0.11	0.23 ± 0.04	0.14 ± 0.03
	Intestine	4.26 ± 0.62	0.63 ± 0.10	0.59 ± 0.09
	Kidney	89.23 ± 7.42	74.56 ± 30.62	15.68 ± 2.04
	Liver	2.38 ± 0.03	1.58 ± 0.33	1.69 ± 0.23
	Heart	2.51 ± 0.39	1.70 ± 0.34	0.60 ± 0.02
	Lung	7.84 ± 0.78	3.80 ± 0.83	1.23 ± 0.25
	Brain	0.29 ± 0.01	0.13 ± 0.03	0.06 ± 0.01
	Salivary gland	3.24 ± 0.19	2.59 ± 0.60	1.33 ± 0.20
	Tumor	40.86 ± 9.37	99.53 ± 17.06	73.59 ± 16.14
	Muscle	0.90 ± 0.12	0.71 ± 0.25	0.20 ± 0.07
	Bone	1.20 ± 0.27	0.66 ± 0.33	0.22 ± 0.09
Ratio	Tumor/Blood	2.56 ± 0.46	18.54 ± 2.83	110.03 ± 18.68
	Tumor/Kidney	0.47 ± 0.15	1.44 ± 0.38	4.66 ± 0.43

[a]Each value represents the mean ± standard deviation. *Values are expressed as % ID.

Table S4 Radioactivity of extracted organs and tissues after intravenous injection of [^{111}In]In-PDI2 into LNCaP tumor-bearing mice[a]

[^{111}In]In-PDI2	Organs or tissues	Time after injection (h)		
		4	24	96
% ID/g of organ tissue	Blood	0.58 ± 0.13	0.12 ± 0.09	0.03 ± 0.01
	Spleen	0.90 ± 0.57	0.63 ± 0.42	0.48 ± 0.11
	Pancreas	0.25 ± 0.03	0.20 ± 0.07	0.20 ± 0.02
	Stomach*	0.05 ± 0.04	0.34 ± 0.41	0.12 ± 0.13
	Intestine	0.38 ± 0.29	0.46 ± 0.38	0.27 ± 0.08
	Kidney	52.31 ± 48.80	4.85 ± 3.45	1.21 ± 0.13
	Liver	0.37 ± 0.06	0.40 ± 0.08	0.40 ± 0.03
	Heart	0.27 ± 0.09	0.14 ± 0.12	0.10 ± 0.02
	Lung	0.54 ± 0.19	0.27 ± 0.13	0.15 ± 0.06
	Brain	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	Salivary gland	0.62 ± 0.46	0.31 ± 0.18	0.30 ± 0.06
	Tumor	20.74 ± 3.46	25.47 ± 6.13	13.54 ± 5.03
	Muscle	0.08 ± 0.04	0.09 ± 0.06	0.09 ± 0.03
	Bone	0.33 ± 0.09	0.36 ± 0.15	0.33 ± 0.09
Ratio	Tumor/Blood	35.79 ± 2.66	264.42 ± 134.10	414.96 ± 90.99
	Tumor/Kidney	0.60 ± 0.33	7.29 ± 5.42	11.06 ± 3.27

[a]Each value represents the mean ± standard deviation. *Values are expressed as % ID. Data on [^{111}In]In-PDI2 were reproduced with permission from ref. 4, Copyright 2025 American Chemical Society.

Table S5 Results of statistical analysis on temporal changes in blood, tumor, and kidney accumulation of ^{111}In-labeled radioligands in the biodistribution assay

Comparison between ^{111}In-labeled radioligands	Blood			Tumor			Kidney		
	4 h	24 h	96 h	4 h	24 h	96 h	4 h	24 h	96 h
PDAI2 vs PDI2	**	**	ns	ns	**	**	ns	*	ns

*P < 0.01, **P < 0.0001 (two-way analysis of variance (ANOVA) with *post hoc* Bonferroni's test); ns means not significant.

13. *In Vivo* Blocking Study Using Model Mice

A saline solution containing 10% ethanol (100 μL) of [^{111}In]In-PDAI2 (5.3 MBq/nmol, 111 kBq) with 2-PMPA (200 μg) was injected into the tail vein of LNCaP tumor-bearing mice. The mice were euthanized at 4 h postinjection. The blood, organs, and tissues of interest were collected and weighed, and then the radioactivity of collected samples was measured with a γ-counter (2470 WIZARD^2). The % ID/g of samples was calculated by comparing the sample counts with injected activity counts.

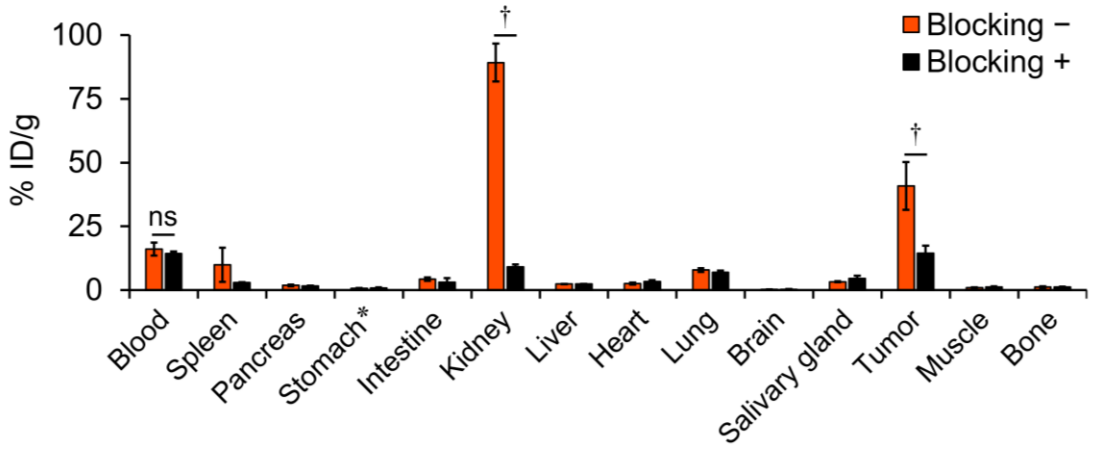


Fig. S4 Biodistribution of radioactivity among organs and tissues after the intravenous injection of [^{111}In]In-PDAI2 into LNCaP tumor-bearing mice in the absence (red; identical to Fig. 4 and Table S3) or presence (black) of the PSMA-inhibitor 2-PMPA. *Values are expressed as % ID. †$P < 0.05$ (Student's *t*-test); ns means not significant.

Table S6 Radioactivity of extracted organs and tissues after intravenous co-injection of [^{111}In]In-PDAI2 and PSMA-inhibitor 2-PMPA into LNCaP tumor-bearing mice[a]

[^{111}In]In-PDAI2 + Blocking	Organs or tissues	Time after injection (h)
		4
% ID/g of organ tissue	Blood	14.37 ± 0.78
	Spleen	2.94 ± 0.09
	Pancreas	1.57 ± 0.18
	Stomach*	0.74 ± 0.26
	Intestine	3.05 ± 1.64
	Kidney	9.03 ± 1.04
	Liver	2.39 ± 0.05
	Heart	3.33 ± 0.48
	Lung	6.90 ± 0.67
	Brain	0.30 ± 0.06
	Salivary gland	4.48 ± 1.11
	Tumor	14.41 ± 2.94
	Muscle	1.15 ± 0.35
	Bone	1.20 ± 0.17
Ratio	Tumor/Blood	1.01 ± 0.24
	Tumor/Kidney	1.62 ± 0.42

[a]Each value represents the mean ± standard deviation. *Values are expressed as % ID.

14. 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 two-way ANOVA with *post hoc* Bonferroni's test for albumin-binding, cell internalization, cell efflux, and biodistribution assays, and Student's *t*-test for the *in vivo* blocking study.

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