Supplementary Material for

Cancer radiotheranostics targeting carbonic anhydrase-IX with ^{111}In- and ^{90}Y-labeled ureidosulfonamide scaffold for SPECT imaging and radionuclide-based therapy

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Methods

General

All reagents were obtained commercially and used without further purification unless otherwise indicated. $^{111}InCl_3$ and $^{90}YCl_3$ were purchased from Nihon Medi-Physics (Tokyo, Japan) and Eckert & Ziegler Radiopharma GmbH (Berlin, Germany), respectively. W-Prep 2XY (Yamazen, Osaka, Japan) was used for silica gel column chromatography on a Hi Flash silica gel column (40 μm, 60 Å, Yamazen). ^{1}H and ^{13}C NMR spectra were recorded on a JNM-ECS400 (JEOL, Tokyo, Japan) with tetramethylsilane as an internal standard. Coupling constants are reported in Hertz. Multiplicity was defined as singlet (s), doublet (d), or multiplet (m). High-resolution mass spectrometry (HRMS) was conducted with an LCMS-IT-TOF (SHIMAZDU, Kyoto, Japan). Reversed-phase high-performance liquid chromatography (RP-HPLC) was performed with a Shimadzu system (SHIMADZU, an LC-20AT pump with an SPD-20A UV detector, λ = 254 nm) with a Cosmosil C_{18} column (Nacalai Tesque, Kyoto, Japan, 5C_{18}-PAQ, 4.6 × 250 mm) delivered at a flow rate of 1.0 mL/min using a solvent of H_2O/MeCN/trifluoroacetic acid (TFA) [90:10:0.1 (0 min) to 60:40:0.1 (30 min)] as the mobile phase. The specific radioactivity was determined as the ratio of radioactivity collected at the retention time of a product during the RP-HPLC

purification to the mass corresponding to the area under the curve of the UV absorption.

Chemistry

Synthesis of 2,2'-(4,10-bis(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (**5**)

Compound **5** was prepared in five steps from cyclen according to a previous report [54].

Synthesis of 4-(3-(4-aminophenyl)ureido)benzenesulfonamide (**6**) [55]

A solution of sulfanilamide (172 mg, 1.0 mmol) and 1,1'-carbonyldiimidazole (195 mg, 1.2 mmol) in dimethyl sulfoxide (DMSO) (5 mL) was stirred at room temperature for 3 h. After confirming the completion of the reaction by thin-layer chromatography monitoring, the reaction mixture was lyophilized overnight. The residue was resolved in MeCN (10 mL), followed by the addition of 1,4-phenylenediamine (130 mg, 1.2 mmol). The reaction mixture was stirred at room temperature for 1 h. After being evaporated to dryness, the residue was purified by silica gel chromatography ($CHCl_3$/MeOH = 10:1) to give 71 mg of **6** (23%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s,1H), 9.86 (s, 1H), 7.83 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 8.8 Hz, 2H), 7.69 (d, J = 8.8 Hz, 2H), 7.35 (d, J =

8.8 Hz, 2H), 7.28 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.8, 143.5, 139.7, 137.0, 127.2 (2C), 126.0, 123.8 (2C), 119.6 (2C), 117.9 (2C). HRMS (ESI): *m/z* calculated for $C_{13}H_{15}N_4O_3S^+$ (MH^+), 307.0859; found, 307.0852.

Synthesis of 2,2',2''-(10-(2-oxo-2-((4-(3-(4-sulfamoylphenyl)ureido)phenyl)amino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (**7**)

To a solution of **5** (22 mg, 42 μmol) in dimethylformamide (DMF) (10 mL) were added **6** (13 mg, 42 μmol), 1-hydroxybenzotriazole (HOBT) hydrate (13 mg, 84 μmol), 1-ethyl-3-(dimethylaminopropyl)carbodiimide (EDC) hydrochloride (16 mg, 84 μmol), and triethylamine (12 μL, 84 μmol). The solution was stirred at room temperature for 48 h. After removing the solvents, ethyl acetate (50 mL) was added. The mixture was washed successively with 0.5 M NaOH (50 mL × 2), saturated $NaHCO_3$ water (50 mL), and brine (50 mL). After being evaporated to dryness, TFA (5 mL) was added to the residue and the solution was stirred at room temperature for 6 h. After concentration of the solution, the residue was purified by RP-HPLC using a solvent of H_2O/MeCN/TFA [90:10:0.1 (0 min) to 60:40:0.1 (30 min)] as the mobile phase to give 6 mg of **7** (21%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 9.76 (s, 1H), 9.51 (s, 1H), 7.71 (d, *J* =

8.8 Hz, 2H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.20 (s, 2H), 3.32 (s, broad, 8H), 3.22 (s, broad, 8H), 2.50–2.48 (m, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.7, 158.4, 152.5, 143.3, 136.6, 126.8 (4C), 118.75 (2C), 118.70 (2C), 117.9 (2C), 117.3 (2C), 40.2 (4C), 40.0 (8C). HRMS (ESI): *m*/*z* calculated for $C_{29}H_{41}N_8O_{10}S^+$ (MH^+), 693.2661; found, 693.2646.

Synthesis of 2,2'-(4,10-bis(2-oxo-2-((4-(3-(4-sulfamoylphenyl)ureido)pheny)amino)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (**8**)

A solution of **5** (59 mg, 0.12 mmol), **6** (71 mg, 0.23 mmol), HOBT hydrate (35 mg, 0.23 mmol), EDC hydrochloride (44 mg, 0.23 mmol), and triethylamine (32 μL, 0.23 mmol) in DMF (10 mL) was stirred at room temperature for 26 h. After removing the solvents, ethyl acetate (50 mL) was added. The mixture was washed successively with 0.5 M NaOH (50 mL × 2), saturated $NaHCO_3$ water (50 mL), and brine (50 mL). After being evaporated to dryness, TFA (5 mL) was added to the residue and the solution was stirred at room temperature for 4 h. After concentration of the solution, the residue was purified by RP-HPLC using a solvent of H_2O/MeCN/TFA [90:10:0.1 (0 min) to 60:40:0.1 (30 min)] as the mobile phase to give 19 mg of **8** (17%). ^{1}H NMR (400 MHz,

DMSO-d_6) δ 10.46 (s, 2H), 9.54 (s, 2H), 9.30 (s, 2H), 7.70 (d, J = 8.8 Hz, 4H), 7.60 (d, J = 8.8 Hz, 4H), 7.52 (d, J = 8.8 Hz, 4H), 7.44 (d, J = 8.8 Hz, 4H), 7.19 (s, 4H), 4.13 (s, broad, 4H), 3.78 (s, broad, 8H), 3.64 (s, broad, 4H), 3.45 (s, broad, 4H), 3.15 (s, broad, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.7, 158.4, 152.4 (2C), 143.2 (2C), 136.6 (2C), 135.7 (2C), 132.7 (2C), 126.8 (4C), 120.1 (4C), 118.8 (4C), 118.2, 117.4 (4C), 115.3, 40.2 (4C), 40.0 (8C). HRMS (ESI): m/z calculated for $C_{42}H_{53}N_{12}O_{12}S_2^+$ (MH^+), 981.3342; found, 981.3351.

Synthesis of [$^{113/115}$In]US1 (**9**)

To a solution of **7** (100 mg, 0.14 mmol) in DMSO (1 mL) were added $InCl_3$ anhydrous (250 mg, 1.13 mmol) and 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (0.1 M, pH 5.5, 10 mL). The solution was stirred at 60 °C for 6 h, and the mixture was purified by RP-HPLC using a solvent of H_2O/MeCN/TFA [95:5:0.1 (0 min) to 65:35:0.1 (30 min)] as the mobile phase to give 40 mg of **9** (35%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 9.36 (s, 1H), 9.14 (s, 1H), 7.65 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 8.8 Hz, 2H), 7.39 (d, J = 8.8 Hz, 2H), 7.15 (s, 2H), 3.05 (s, broad, 8H), 2.77 (s, broad, 8H), 2.46–2.43 (m, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.3 (2C), 158.6, 158.2, 152.4, 143.1, 136.7, 126.8 (4C), 120.1 (2C), 118.6 (2C), 117.4 (2C), 40.4

(8C), 40.2 (2C), 40.0 (2C). HRMS (ESI): *m/z* calculated for $C_{29}H_{38}InN_8O_{10}S^+$ (M^+), 805.1465; found, 805.1450.

Synthesis of [$^{113/115}$In]US2 (**10**)

To a solution of **8** (10 mg, 10 μmol) in DMSO (1 mL) were added $InCl_3$ anhydrous (23 mg, 0.10 mmol) and MES buffer (0.1 M, pH 5.5, 10 mL). The solution was stirred at 60 °C for 12 h, and the mixture was purified by RP-HPLC using a solvent of H_2O/MeCN/TFA [90:10:0.1 (0 min) to 60:40:0.1 (30 min)] as the mobile phase to give 9 mg of **10** (80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 2H), 9.33 (s, 2H), 9.11 (s, 2H), 7.71 (d, J = 8.8 Hz, 4H), 7.60 (d, J = 8.8 Hz, 4H), 7.49 (d, J = 8.8 Hz, 4H), 7.44 (d, J = 8.8 Hz, 4H), 7.20 (s, 4H), 2.83 (s, broad, 8H), 2.52–2.49 (m, 16H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.3 (2C), 143.0 (2C), 136.7 (2C), 136.4 (2C), 131.7 (2C), 126.8 (4C), 120.8 (4C), 118.5 (4C), 117.4 (4C), 40.4 (16C). HRMS (ESI): *m/z* calculated for $C_{42}H_{50}InN_{12}O_{12}S_2^+$ (M^+), 1093.2146; found, 1093.2151.

Radiolabeling

For ^{111}In-labeling, an $^{111}InCl_3$ (200 μL) solution was mixed with MES buffer (0.01 or 0.1 M, pH 5.5, 600 μL) or NaOAc buffer (0.01 or 0.1 M, pH 6.0, 600 μL) and

pre-incubated at room temperature for 15 min. To this solution was added 50 μL of the precursor (**7** or **8**) in H_2O (final 0.05 mM), and the mixture was incubated at room temperature, 60 °C, or 90 °C for 30 min. After cooling to room temperature, the mixture was purified by RP-HPLC. The ^{111}In-labeled compound was analyzed by analytical RP-HPLC on a Cosmosil C_{18} column ($5C_{18}$-PAQ, 4.6 × 250 mm) with a solvent of H_2O/MeCN/TFA [90:10:0.1 (0 min) to 60:40:0.1 (30 min)] as the mobile phase at a flow rate of 1.0 mL/min (**Table S1**).

For ^{90}Y-labeling, a $^{90}YCl_3$ solution (100 μL) was mixed with 0.1 M MES buffer (pH 5.5, 300 μL) and pre-incubated at room temperature for 15 min to give a ^{90}Y-MES solution. Compound **8** in H_2O (final 0.04 mM, 50 μL) was added to the ^{90}Y-MES solution (400 μL), and then the mixture was incubated at 90 °C for 30 min. After cooling to room temperature, the mixture was purified by RP-HPLC. The ^{90}Y-labeled compound was analyzed by analytical RP-HPLC on a Cosmosil C_{18} column ($5C_{18}$-PAQ, 4.6 × 250 mm) with a solvent of H_2O/MeCN/TFA [90:10:0.1 (0 min) to 60:40:0.1 (30 min)] as the mobile phase at a flow rate of 1.0 mL/min.

Measurement of partition coefficient

The experimental determination of partition coefficients was performed in 1-octanol

and phosphate-buffered saline (PBS) (pH 7.4). The two phases were presaturated with each other. 1-Octanol (3 mL) and PBS (3 mL) were pipetted into a 15-mL test tube containing [^{111}In]US1 or [^{111}In]US2 (50 kBq, 130.7 GBq/μmol for [^{111}In]US1 and 59.5 GBq/μmol for [^{111}In]US2). The test tube was vortexed for 2 min and centrifuged (4,000 ×g, 5 min). Aliquots (0.5 mL) from the 1-octanol and PBS phases were transferred into two test tubes for counting. The remaining PBS phase (1 mL), newly prepared 1-octanol (3 mL), and PBS (2 mL) were pipetted into a new test tube. The vortexing, centrifuging, and counting were repeated until consistent partition coefficient values were obtained (usually the sixth partition). The amount of radioactivity in each tube was measured with a γ counter (Wallac 1470 Wizard; PerkinElmer, Massachusetts, U.S.A.). The partition coefficient was calculated using the equation: log P_{ow} = log[count$_{1\text{-octanol}}$/count$_{PBS}$].

Biodistribution study in normal mice

A saline solution (100 μL) of [^{111}In]US2 (40 kBq, 177.8 GBq/μmol) was directly injected into the tail vein of ddY mice (male, 5 weeks old). The mice were sacrificed at 1, 4, 8, and 24 h postinjection. The blood, spleen, pancreas, stomach, intestines, kidneys, liver, heart, lungs, brain, and muscle were collected. Each organ was weighed and the

radioactivity was measured using a γ counter (PerkinElmer). The % injected dose/g of samples was calculated by comparing the sample counts with the count of the initial dose.

Table S1. Radiochemical Yields of [^{111}In]US2 under the Given Conditions

Temperature	Solvent	Radiochemical yield (%)
Room temperature	0.01 M NaOAc (pH 6.0)	0.0
	0.1 M NaOAc (pH 6.0)	1.5
	0.01 M MES (pH 5.5)	0.5
	0.1 M MES (pH 5.5)	1.2
60 °C	0.01 M NaOAc (pH 6.0)	6.5
	0.1 M NaOAc (pH 6.0)	12.0
	0.01 M MES (pH 5.5)	0.7
	0.1 M MES (pH 5.5)	23.2
90 °C	0.01 M NaOAc (pH 6.0)	47.7
	0.1 M NaOAc (pH 6.0)	57.9
	0.01 M MES (pH 5.5)	7.0
	0.1 M MES (pH 5.5)	66.0

Table S2. RP-HPLC Retention Times and Partition Coefficients of ^{111}In, $^{113/115}$In, and ^{90}Y Complexes and Corresponding Precursors

Compound	Retention time (min)*	Log P_{ow}
Compound **7**	13.2	Not determined
[^{111}In]US1	10.5	–3.38 ± 0.09
[$^{113/115}$In]US1	10.3	Not determined
Compound **8**	19.1	Not determined
[^{111}In]US2	17.7	–2.81 ± 0.01
[$^{113/115}$In]US2	17.6	Not determined
[^{90}Y]US2	17.6	Not determined

*RP-HPLC on a Cosmosil C_{18} column ($5C_{18}$-PAQ, 4.6 × 250 mm) with a solvent of H_2O/MeCN/TFA [90:10:0.1 (0 min) to 60:40:0.1 (30 min)] as the mobile phase at a flow rate of 1.0 mL/min.

Table S3. Radioactivity of Extracted Organs after Intravenous Injection of [^{111}In]US1 in the HT-29 and MDA-MB-231 Tumor-Bearing Mice

Organs	Time after injection (h)				
	1	4	8	24	24 + Block*
Blood	1.07 ± 0.25	0.12 ± 0.01	0.12 ± 0.01	0.03 ± 0.01	0.02 ± 0.00
Spleen	0.61 ± 0.08	0.36 ± 0.12	0.38 ± 0.03	0.56 ± 0.05	0.30 ± 0.04
Pancreas	0.41 ± 0.14	0.10 ± 0.03	0.10 ± 0.03	0.08 ± 0.01	0.04 ± 0.02
Stomach†	1.44 ± 0.47	0.31 ± 0.27	0.89 ± 0.61	0.15 ± 0.08	0.43 ± 0.27
Intestine	2.00 ± 1.16	8.22 ± 2.47	17.60 ± 7.39	2.69 ± 1.29	2.58 ± 1.03
Kidney	6.85 ± 1.36	3.71 ± 0.69	3.41 ± 0.55	3.25 ± 0.39	0.93 ± 0.11
Liver	1.25 ± 0.18	0.85 ± 0.17	0.87 ± 0.08	0.94 ± 0.09	0.45 ± 0.06
Heart	0.54 ± 0.04	0.09 ± 0.05	0.09 ± 0.03	0.12 ± 0.03	0.04 ± 0.03
Lung	1.55 ± 0.29	0.21 ± 0.07	0.22 ± 0.03	0.21 ± 0.08	0.08 ± 0.04
Brain	0.06 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
HT-29	2.12 ± 0.46	0.35 ± 0.09	0.31 ± 0.07	0.27 ± 0.04	0.18 ± 0.07
MDA-MB-231	1.48 ± 0.63	0.64 ± 0.28	0.27 ± 0.03	0.21 ± 0.02	0.11 ± 0.03
Muscle	0.39 ± 0.08	0.08 ± 0.03	0.09 ± 0.03	0.08 ± 0.02	0.04 ± 0.01
HT-29/Blood	2.07 ± 0.61	2.80 ± 0.60	2.65 ± 0.71	10.03 ± 3.01	9.45 ± 4.66
HT-29/Muscle	5.49 ± 1.07	4.95 ± 2.80	3.75 ± 1.14	3.74 ± 0.98	4.26 ± 1.69
HT-29/MDA-MB-231	1.53 ± 0.42	0.59 ± 0.19	1.14 ± 0.20	1.27 ± 0.22	1.59 ± 0.63

Values are expressed as % injected dose per gram of tissue. Each value is the mean ± standard deviation of five animals at each interval. *Coinjection of acetazolamide (10 mg/kg). †Values are expressed as % injected dose.

Table S4. Radioactivity of Extracted Organs after Intravenous Injection of [^{111}In]US2 in the HT-29 and MDA-MB-231 Tumor-Bearing Mice

Organs	Time after injection (h)				
	1	4	8	24	24 + Block*
Blood	4.17 ± 0.56	2.85 ± 0.50	1.09 ± 0.15	0.17 ± 0.05	0.09 ± 0.02
Spleen	2.82 ± 0.44	2.01 ± 0.21	1.89 ± 0.40	1.42 ± 0.23	0.80 ± 0.07
Pancreas	3.22 ± 0.54	1.80 ± 0.19	1.32 ± 0.23	0.43 ± 0.10	0.17 ± 0.04
Stomach†	8.86 ± 0.98	6.64 ± 0.86	4.90 ± 0.70	1.25 ± 0.13	0.30 ± 0.03
Intestine	6.86 ± 1.24	6.64 ± 0.79	5.76 ± 1.92	1.51 ± 0.31	0.43 ± 0.13
Kidney	18.59 ± 0.53	12.96 ± 1.27	12.20 ± 1.49	9.40 ± 1.42	2.37 ± 0.22
Liver	4.17 ± 0.46	3.90 ± 0.38	3.80 ± 0.34	3.93 ± 0.64	1.87 ± 0.43
Heart	2.78 ± 0.27	1.59 ± 0.16	1.07 ± 0.21	0.58 ± 0.10	0.20 ± 0.04
Lung	8.76 ± 0.53	4.99 ± 0.20	3.64 ± 0.91	0.99 ± 0.22	0.48 ± 0.10
Brain	0.17 ± 0.03	0.13 ± 0.01	0.11 ± 0.01	0.07 ± 0.01	0.02 ± 0.00
HT-29	4.57 ± 0.21‡	4.51 ± 0.62‡	3.78 ± 0.54‡	1.72 ± 0.20‡, §	0.63 ± 0.03
MDA-MB-231	1.64 ± 0.28	1.93 ± 0.38	2.13 ± 0.40	1.34 ± 0.17	0.63 ± 0.10
Muscle	1.62 ± 0.24	0.82 ± 0.12	0.59 ± 0.04	0.23 ± 0.06	0.07 ± 0.02
HT-29/Blood	1.11 ± 0.16	1.61 ± 0.30	3.47 ± 0.37	10.78 ± 2.80	7.06 ± 1.70
HT-29/Muscle	2.86 ± 0.43	5.62 ± 1.45	6.41 ± 1.16	7.75 ± 1.98	9.64 ± 4.15
HT-29/MDA-MB-231	2.84 ± 0.41	2.38 ± 0.34	1.81 ± 0.32	1.31 ± 0.30	1.01 ± 0.14

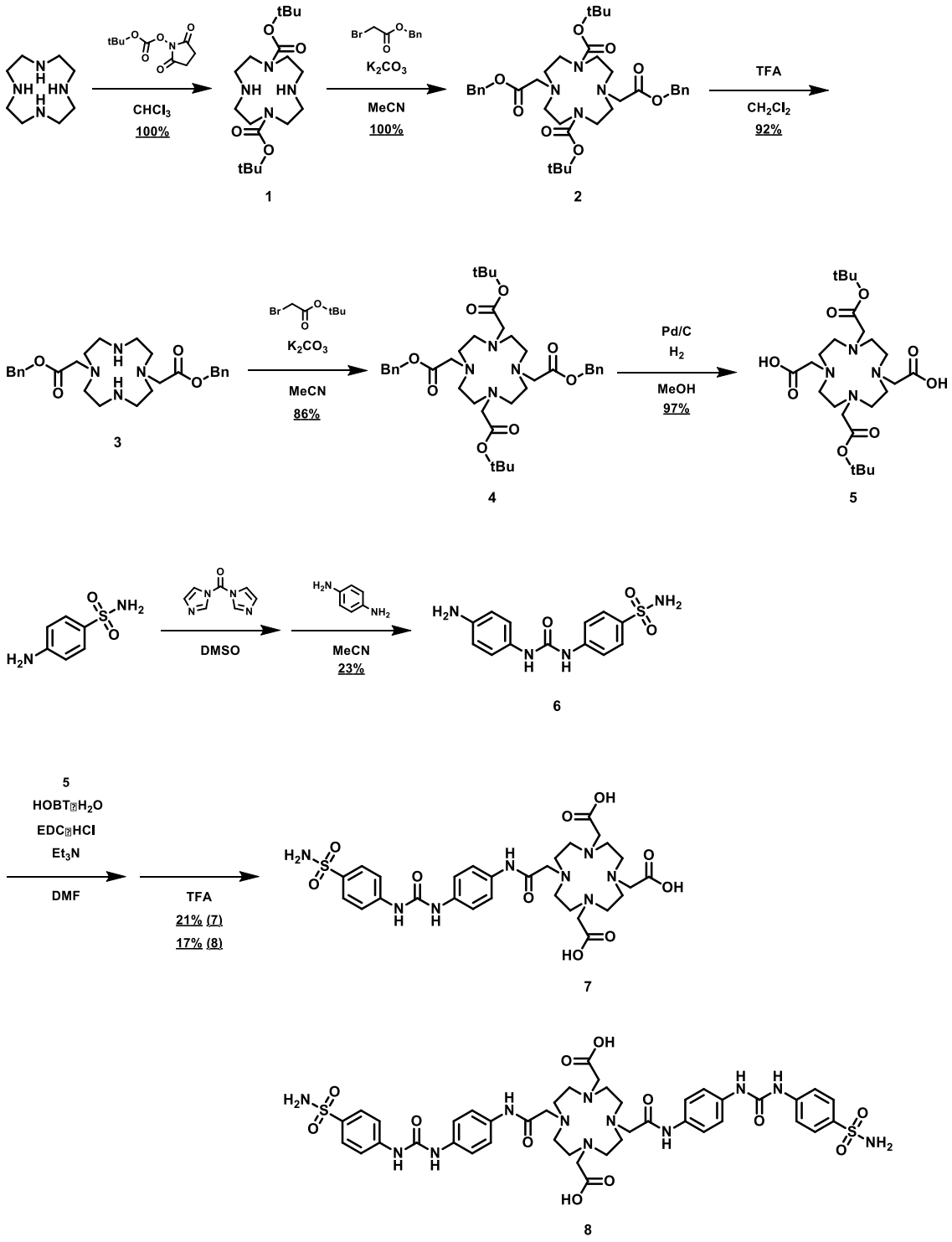
Values are expressed as % injected dose per gram of tissue. Each value is the mean ± standard deviation of five animals at each interval. *Coinjection of acetazolamide (10 mg/kg). †Values are expressed as % injected dose. ‡$P < 0.05$ compared with MDA-MB-231 each time. §$P < 0.001$ compared with 24 h + Block (Student's *t*-test).

Table S5. Radioactivity of Extracted Organs after Intravenous Injection of [^{111}In]US2 in Normal Mice

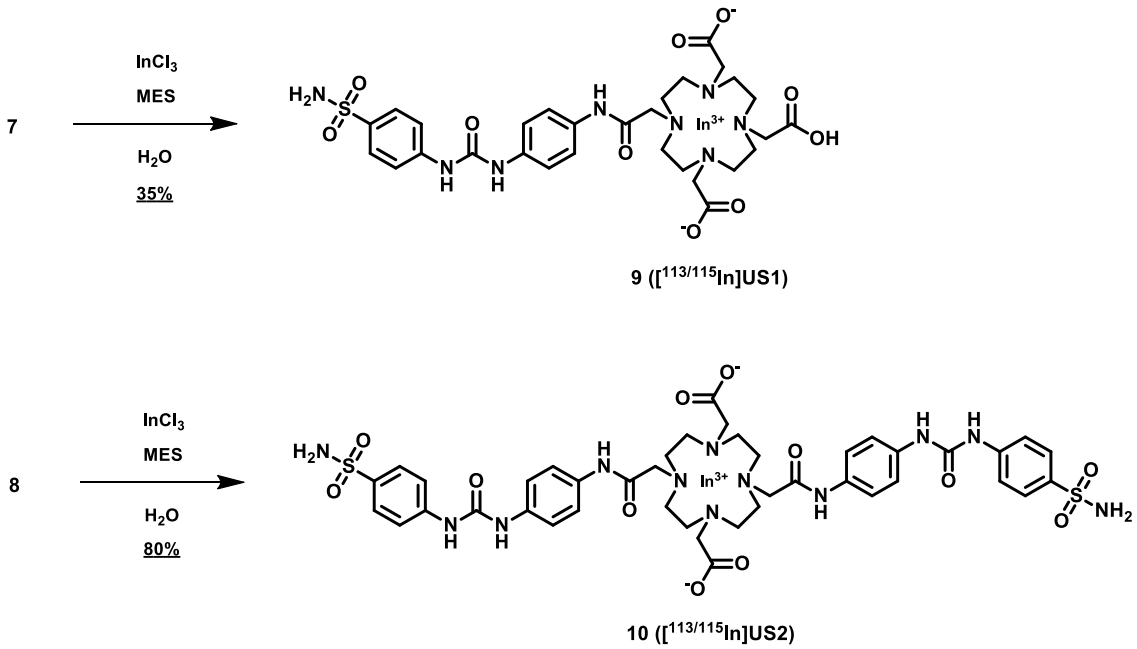
Organs	Time after injection (h)			
	1	4	8	24
Blood	2.34 ± 0.46	1.36 ± 0.16	1.02 ± 0.19	0.10 ± 0.01
Spleen	1.46 ± 0.31	1.00 ± 0.15	0.88 ± 0.15	0.45 ± 0.08
Pancreas	3.07 ± 1.05	2.20 ± 0.57	1.38 ± 0.31	0.47 ± 0.17
Stomach*	8.05 ± 4.22	7.00 ± 1.85	3.92 ± 1.37	1.18 ± 0.27
Intestine	2.26 ± 0.63	2.05 ± 0.73	2.57 ± 0.78	0.69 ± 0.15
Kidney	11.39 ± 2.00	11.11 ± 1.34	9.88 ± 1.49	7.11 ± 1.40
Liver	2.04 ± 0.26	2.17 ± 0.08	3.16 ± 0.57	1.77 ± 0.24
Heart	2.02 ± 0.43	1.32 ± 0.16	1.04 ± 0.21	0.39 ± 0.04
Lung	6.35 ± 1.76	4.17 ± 0.64	2.85 ± 0.77	0.68 ± 0.16
Brain	0.13 ± 0.02	0.11 ± 0.02	0.10 ± 0.01	0.07 ± 0.02
Muscle	1.14 ± 0.17	0.76 ± 0.10	0.58 ± 0.08	0.22 ± 0.06

Values are expressed as % injected dose per gram of tissue. Each value is the mean ± standard deviation of five animals at each interval. *Values are expressed as % injected dose.

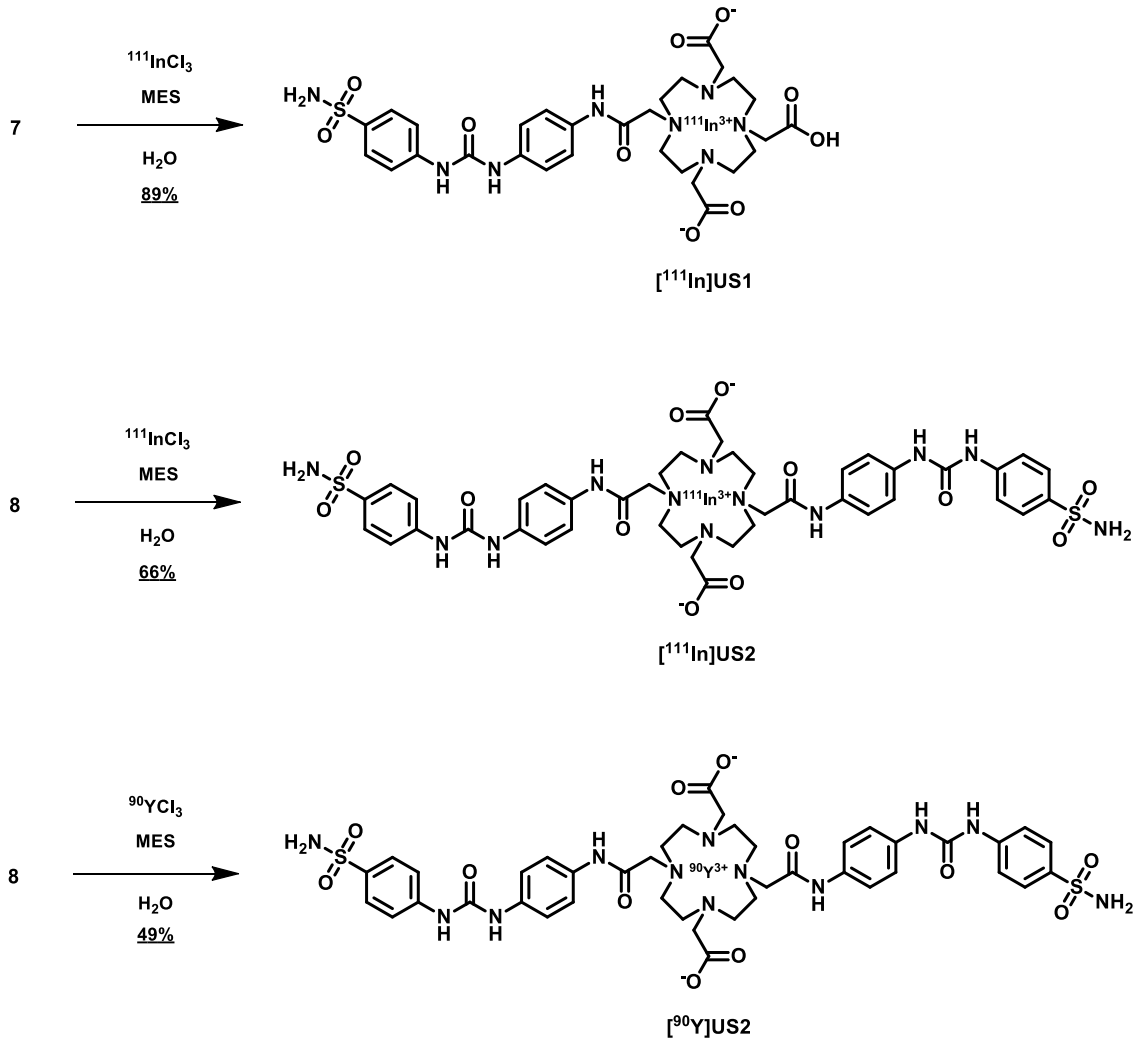
Scheme S1. Synthetic Route of the Precursor for Radiolabeling of the Ureidosulfonamide Derivatives



Scheme S2. Synthetic Route of the Indium-113/115 Complex with One or Two Scaffolds of Ureidosulfonamide



Scheme S3. Radiolabeling of the Indium-111 and Yttrium-90 Complex with One or Two Scaffolds of Ureidosulfonamide



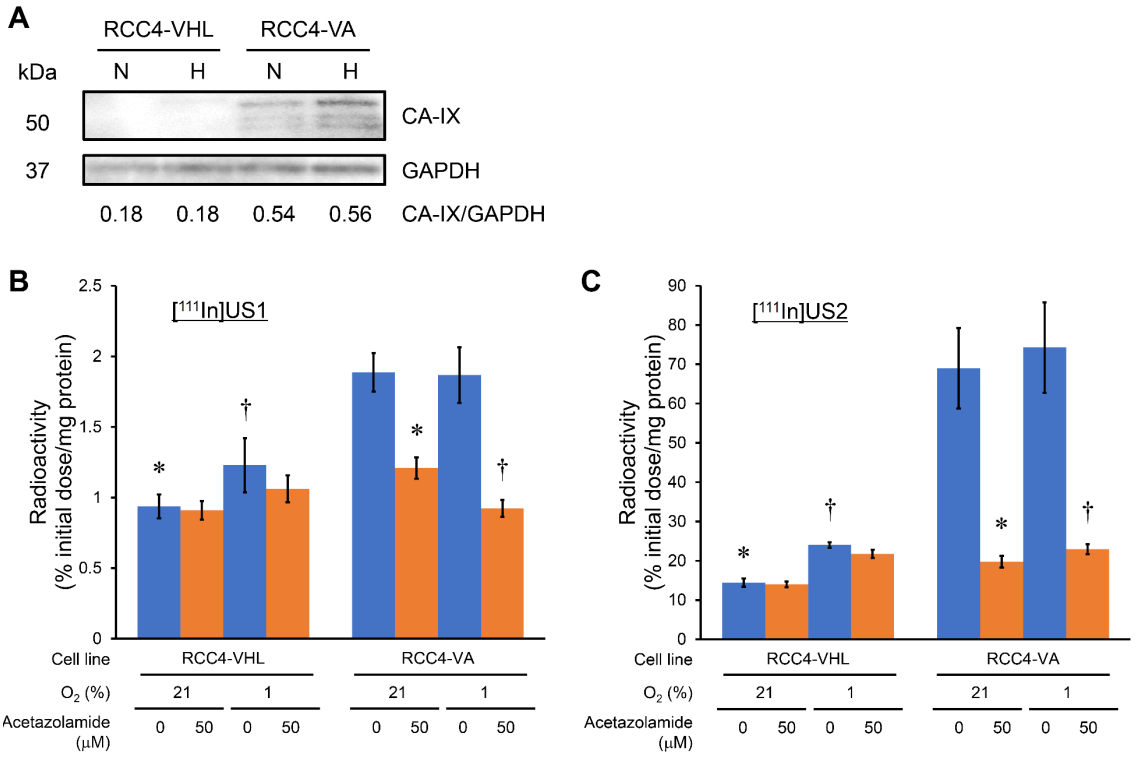


Figure S1. Cell binding assay with [^{111}In]US1 and [^{111}In]US2. (A) Western blotting analysis of RCC4-VHL and RCC4-VA cells under normoxic (N) and hypoxic (H) conditions. GAPDH was used as a loading control. **(B)** In vitro uptake of [^{111}In]US1 into cells. **(C)** In vitro uptake of [^{111}In]US2 into cells. Values are expressed as the mean ± standard error of six independent experiments. *$P < 0.005$ as compared with uptake into RCC4-VA cells under normoxic conditions, $^{\dagger}P < 0.05$ as compared with uptake into RCC4-VA cells under hypoxic conditions (Student's *t*-test).

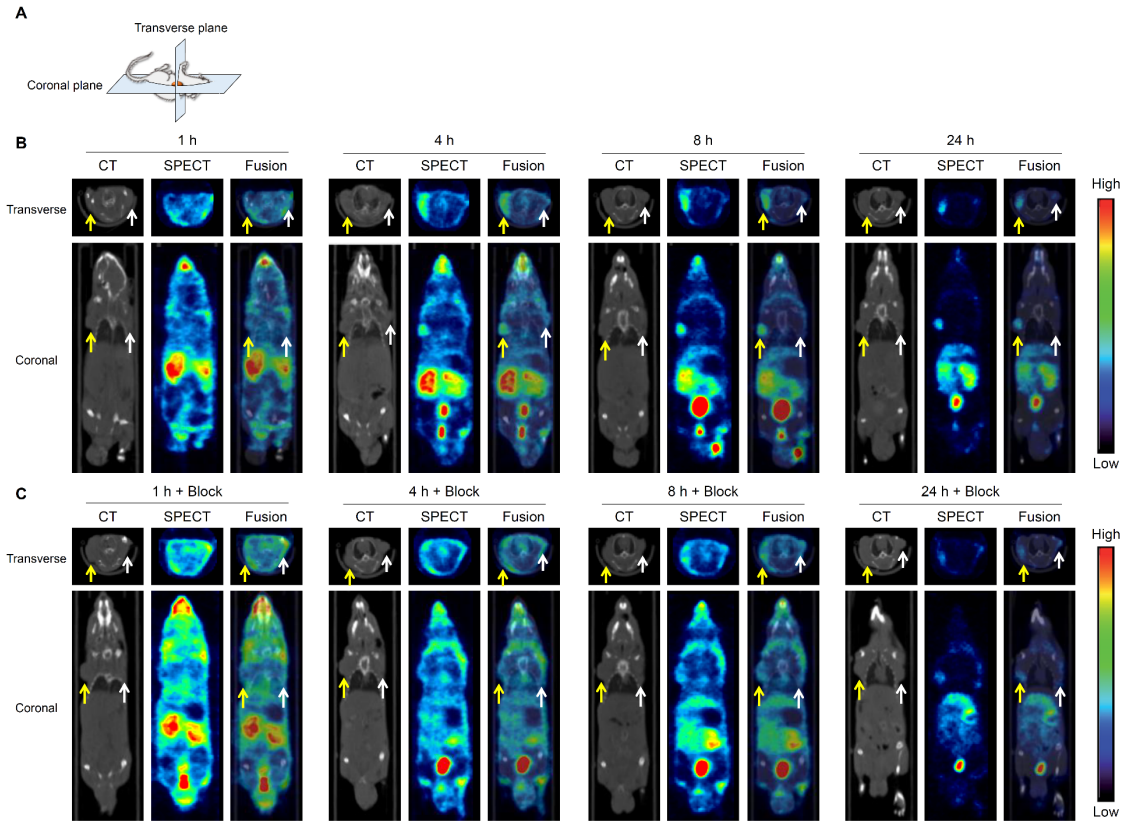


Figure S2. SPECT/CT images of the HT-29 and MDA-MB-231 tumor-bearing mice after [^{111}In]US2 administration. **(A)** Planes of collected images from mice. **(B)** CT-only, SPECT-only, and SPECT/CT fusion images after [^{111}In]US2 administration. **(C)** CT-only, SPECT-only, and SPECT/CT fusion images after [^{111}In]US2 administration with acetazolamide (10 mg/kg). Yellow and white arrows indicate the HT-29 and MDA-MB-231 tumors, respectively.

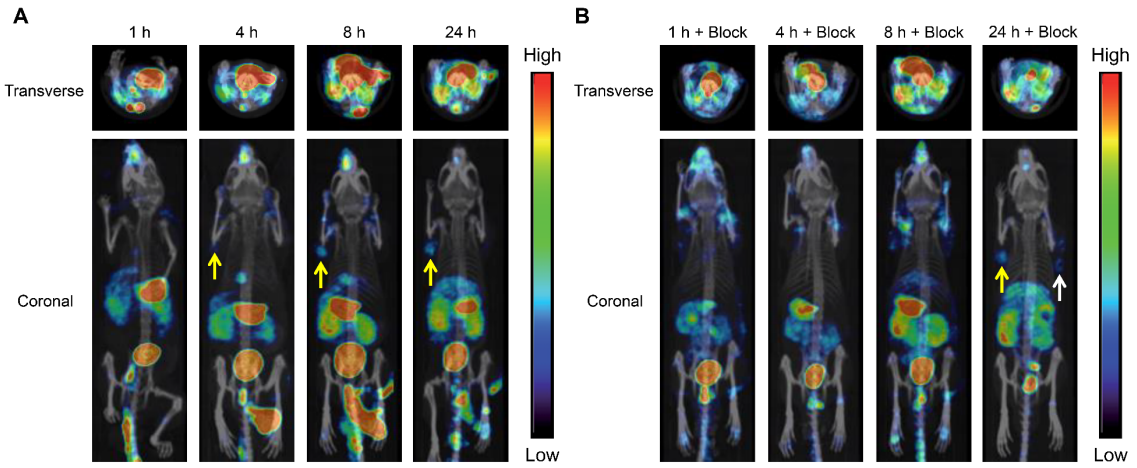


Figure S3. Maximum intensity projection of SPECT/CT images of the HT-29 and MDA-MB-231 tumor-bearing mice after [^{111}In]US2 administration. **(A)** Maximum intensity projection of SPECT/CT images after [^{111}In]US2 administration. **(B)** Maximum intensity projection of SPECT/CT images after [^{111}In]US2 administration with acetazolamide (10 mg/kg). Yellow and white arrows indicate the HT-29 and MDA-MB-231 tumors, respectively.