Synthesis of DOTA-conjugated Peptidomimetics

Solid-phase synthesis of HTK03026, HTK03029 and HTK03041 was modified from literature procedures for the synthesis of PSMA-617 derivatives (*16*). Fmoc-Lys(ivDde)-Wang resin (0.3 mmol, 0.61 mmol/g loading) was suspended in DMF for 30 min. Fmoc was then removed by treating the resin with 20% piperidine in DMF (3 × 8 min). The isocyanate derivative of di-*t*-butyl ester of glutamate (3 eq.) was prepared according to literature procedures (*13*), and added to the lysine-immobilized resin and reacted for 16 h. After washing the resin with DMF, the ivDde-protecting group was removed with 2% hydrazine in DMF (5 × 5 min). Fmoc-2-Aoc-OH (for HTK03026), Fmoc-Ala(1-Pyn)-OH (for HTK03029) or Fmoc-Ala(9-Anth)-OH (for HTK03041) was then coupled to the side chain of Lys using Fmoc-protected amino acid (3 eq.), HBTU (3 eq.), HOBT (3 eq.) and *N*,*N*-diisopropylethylamine (8 eq.). Afterwards, elongation was continued with the addition of Fmoc-tranexamic acid, and finally DOTA-tris(*t*-butyl)ester (tri-*t*-butyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate).

For the preparation of albumin-binder-conjugated derivatives, Fmoc-Lys(ivDde)-OH, Fmoc-Gly-OH and *p*-substituted 4-phenylbutyric acid were subsequently coupled to the sequence after Fmoc-tranexamic acid. After selective removal of the ivDde-protecting group with 2% hydrazine in DMF (5 × 5 min), DOTA-tris(*t*-butyl)ester was then coupled to the Lys side chain.

The peptides were deprotected and simultaneously cleaved from the resin by treating with 95/5 trifluoroacetic acid (TFA)/triisopropylsilane for 2 h at room temperature. After filtration, the peptides were precipitated by the addition of cold diethyl ether to the TFA solution. The crude peptides were purified by HPLC using the semi-preparative column. The eluates containing the desired peptides were collected, pooled, and lyophilized. The HPLC conditions, retention times, isolated yields and MS confirmations of these DOTA-conjugated peptidomimetics are provided in Supplemental Tables 1, 5 and 9.

Synthesis of Nonradioactive Ga-complexed Standards

To prepare nonradioactive Ga-complexed standards, a solution of the DOTA-conjugated precursor was incubated with $GaCl_3$ (5 eq.) in NaOAc buffer (0.1 M, 500 µL, pH 4.2) at 80 °C for 15 min. The reaction mixture was then purified by HPLC using the semi-preparative column, and the HPLC eluates containing the desired peptide were collected, pooled, and lyophilized. The HPLC conditions, retention times, isolated yields and MS confirmations of these nonradioactive Ga-complexed standards are provided in Supplemental Tables 2 and 6.

Synthesis of Nonradioactive Lu-complexed Standards

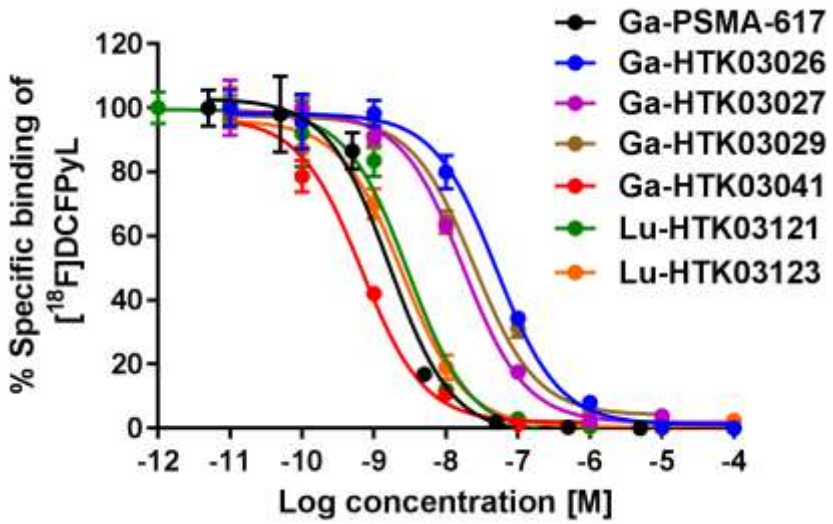
To prepare nonradioactive Lu-complexed standards, a solution of the DOTA-conjugated precursor was incubated with $LuCl_3$ (5 eq.) in NaOAc buffer (0.1 M, 500 µL, pH 4.2) at 95 °C for 15 min. The reaction mixture was then purified by HPLC using the semi-preparative column, and the HPLC eluates containing the desired peptide were collected, pooled, and lyophilized. The HPLC conditions, retention times, isolated yields and MS confirmations of these nonradioactive Lu-complexed standards are provided in the Supplemental Table 10.

Synthesis of ^{68}Ga-labeled Compounds

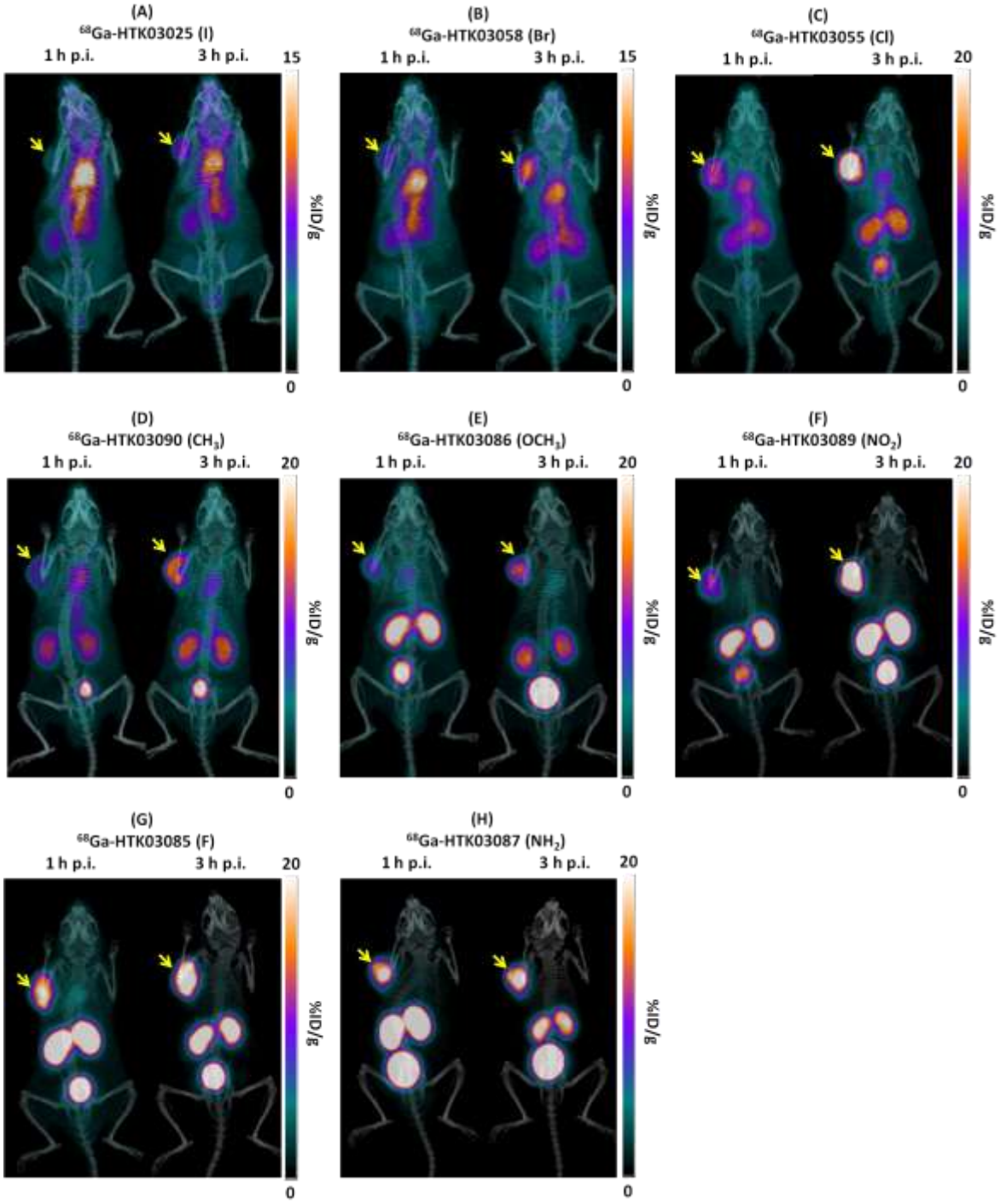
The radiolabeling experiments were performed following previously published procedures (*15,17-19*). Purified ^{68}Ga in 0.5 mL water was added into a 4-mL glass vial preloaded with 0.7 mL of HEPES buffer (2 M, pH 5.0) and 50 μg precursor. The radiolabeling reaction was carried out under microwave heating for 1 min. The reaction mixture was purified by HPLC using the semi-preparative column. The eluate fraction containing the radiolabeled product was collected, diluted with water (50 mL), and passed through a C18 Sep-Pak cartridge that was pre-washed with ethanol (10 mL) and water (10 mL). After washing the C18 Sep-Pak cartridge with water (10 mL), the ^{68}Ga-labeled product was eluted off the cartridge with ethanol (0.4 mL), and diluted with saline for imaging and biodistribution. Quality control was performed using the analytical column. The HPLC conditions and retention times are provided in Supplemental Tables 3 and 7. For tracers presented in the Supplemental Table 3, they were obtained in 40-72% decay-corrected radiochemical yields with >66 GB/μmol molar activity and >95% radiochemical purity. For tracers presented in the Supplemental Table 7, they were obtained in 18-69% decay-corrected radiochemical yields with >37 GB/μmol molar activity and >95% radiochemical purity.

Synthesis of ^{177}Lu-labeled Compounds

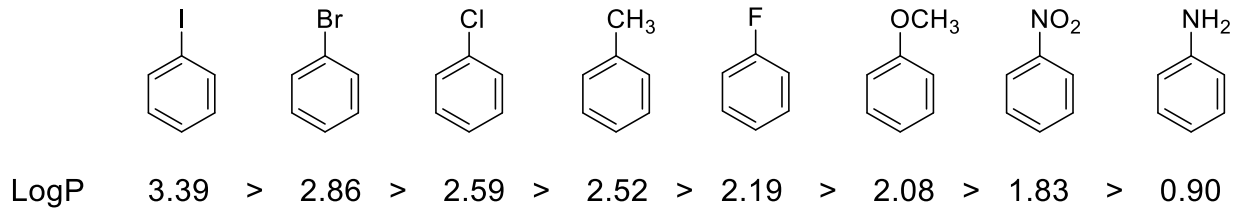
$^{177}LuCl_3$ (548–895 MBq in 10–20 μL) was added to a solution of HTK03121 or HTK03123 (25 μg) in NaOAc buffer (0.5 mL, 0.1 M, pH 4.5). The mixture was incubated at 90 °C for 15 min, and then purified by HPLC using the semi-preparative column. The eluate fraction containing the radiolabeled product was collected, diluted with water (50 mL), and passed through a C18 Sep-Pak cartridge that was pre-washed with ethanol (10 mL) and water (10 mL). After washing the C18 Sep-Pak cartridge with water (10 mL), the ^{177}Lu-labeled product was eluted off the cartridge with ethanol (0.4 mL), and diluted with saline for imaging and biodistribution. Quality control was performed using the analytical column. The HPLC conditions and retention times are provided in the Supplemental Table 11. These two ^{177}Lu-labeled compounds were obtained in 55-67% decay-corrected radiochemical yields with >144 GB/μmol molar activity and >95% radiochemical purity.



Supplemental Figure 1: Representative displacement curves of ^{18}F-DCFPyL by Ga-HTK03026, Ga-HTK03027, Ga-HTK03029, Ga-HTK03041, Lu-HTK03121 and Lu-HTK03123.

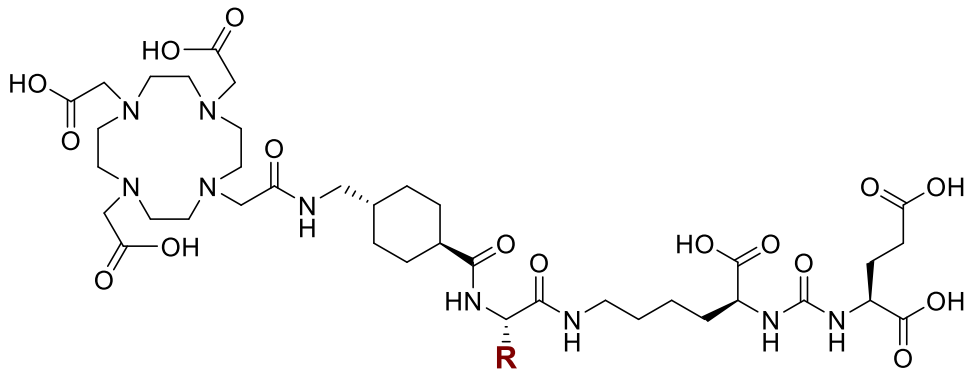


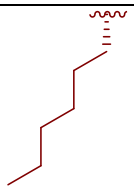
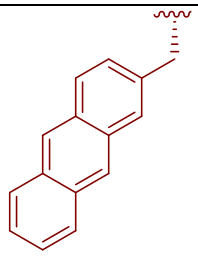
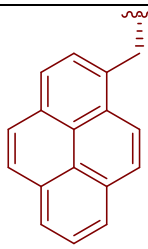
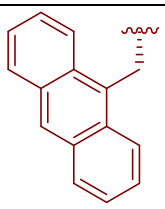
Supplemental Figure 2: Representative PET/CT images of (A) ^{68}Ga-HTK03025, (B) ^{68}Ga-HTK03058, (C) ^{68}Ga-HTK03055, (D) ^{68}Ga-HTK03090, (E) ^{68}Ga-HTK03086, (F) ^{68}Ga-HTK03089, (G) ^{68}Ga-HTK03085, and (H) ^{68}Ga-HTK03087 acquired at 1 h and 3 h post-injection in mice bearing LNCaP tumors (arrows).



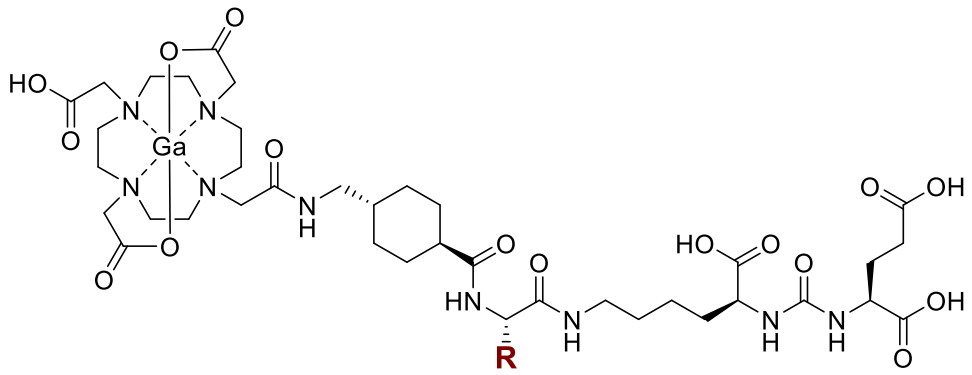
Supplemental Figure 3: Comparison of lipophilicity of various substituted benzene derivatives. The LogP values were obtained using the Cambridgesoft (Cambridge, MA) ChemDraw Pro 12.0 software.

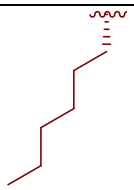
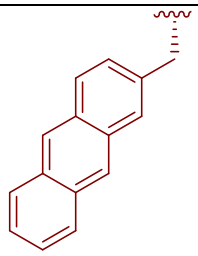
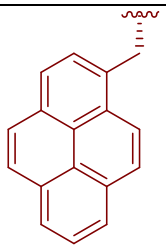
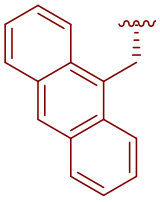
Supplemental Table 1: HPLC purification conditions and MS characterizations of HTK03026, HTK03029 and HTK03041.



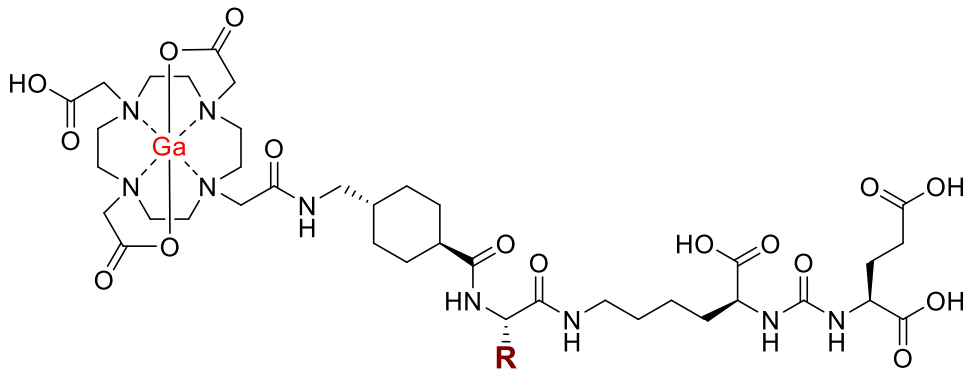
Compound name	R	HPLC conditions	Retention time (min)	Yield (%)	Calculated mass	Found
HTK03026		27% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	10.7	39	$[M+H]^+$ 986.5	$[M+H]^+$ 986.6
HTK03027		32% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	7.1	33	$[M+H]^+$ 1092.5	$[M+H]^+$ 1092.6
HTK03029		33% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	7.3	51	$[M+H]^+$ 1116.5	$[M+H]^+$ 1116.6
HTK03041		31% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	7.2	27	$[M+H]^+$ 1092.5	$[M+H]^+$ 1092.6

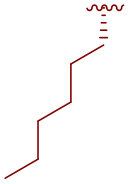
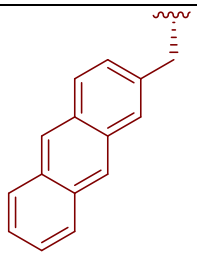
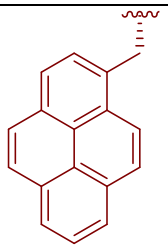
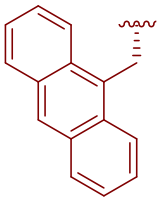
Supplemental Table 2: HPLC purification conditions and MS characterizations of nonradioactive Ga-complexed HTK03026, HTK03029 and HTK03041 standards.



Compound name	R	HPLC conditions	Retention time (min)	Yield (%)	Calculated mass	Found
Ga-HTK03026		27% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	9.4	76	$[M+H]^+$ 1052.4	$[M+H]^+$ 1052.5
Ga-HTK03027		32% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	9.5	37	$[M+H]^+$ 1159.4	$[M+H]^+$ 1159.3
Ga-HTK03029		33% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	10.3	89	$[M+H]^+$ 1183.4	$[M+H]^+$ 1183.4
Ga-HTK03041		31% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	9.3	89	$[M+H]^+$ 1159.4	$[M+H]^+$ 1159.4

Supplemental Table 3: HPLC conditions for the purification and quality control of ^{68}Ga-labeled HTK03026, HTK03029 and HTK03041.

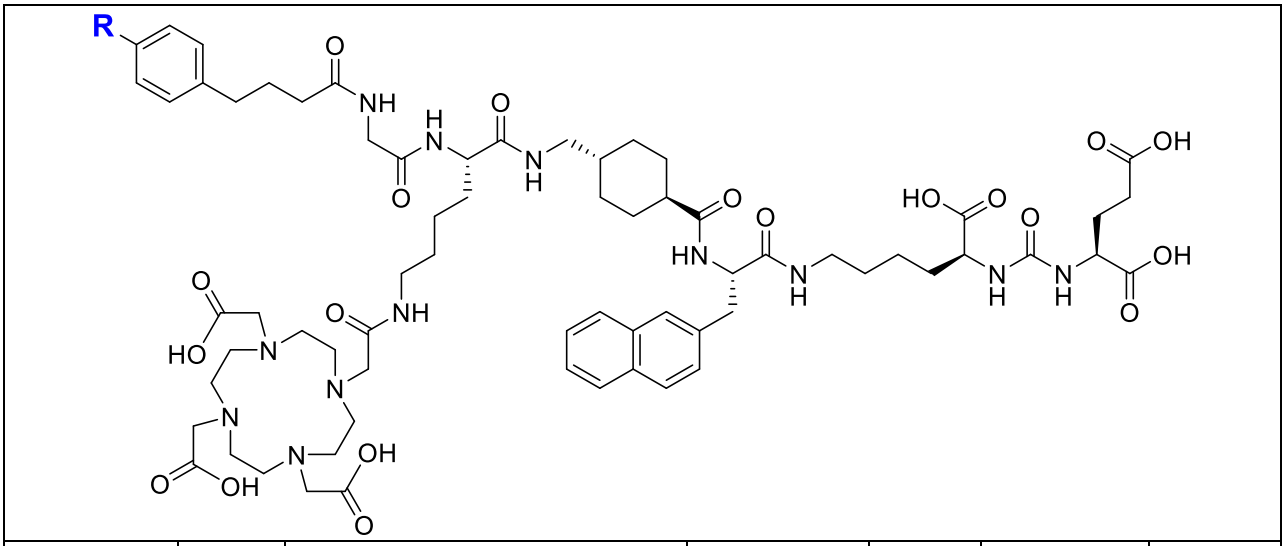


Compound name	R	HPLC conditions		Retention time (min)
^{68}Ga-HTK03026		Semi-Prep	25% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	15.5
		QC	26% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	6.2
^{68}Ga-HTK03027		Semi	31% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	14.0
		Semi	31% CH_3CN and 0.1% TFA in H_2O; flow rate 2 mL/min	5.5
^{68}Ga-HTK03029		Semi-Prep	28% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	25.1
		QC	31% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	7.2
^{68}Ga-HTK03041		Semi-Prep	28% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	18.8
		QC	28% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	8.0

Supplemental Table 4: Biodistribution (mean ± SD) and uptake ratios of ^{68}Ga-labeled PSMA-targeting tracers in LNCaP tumor-bearing mice. The mice in the blocked group were co-injected with DCFPyL (0.5 mg).

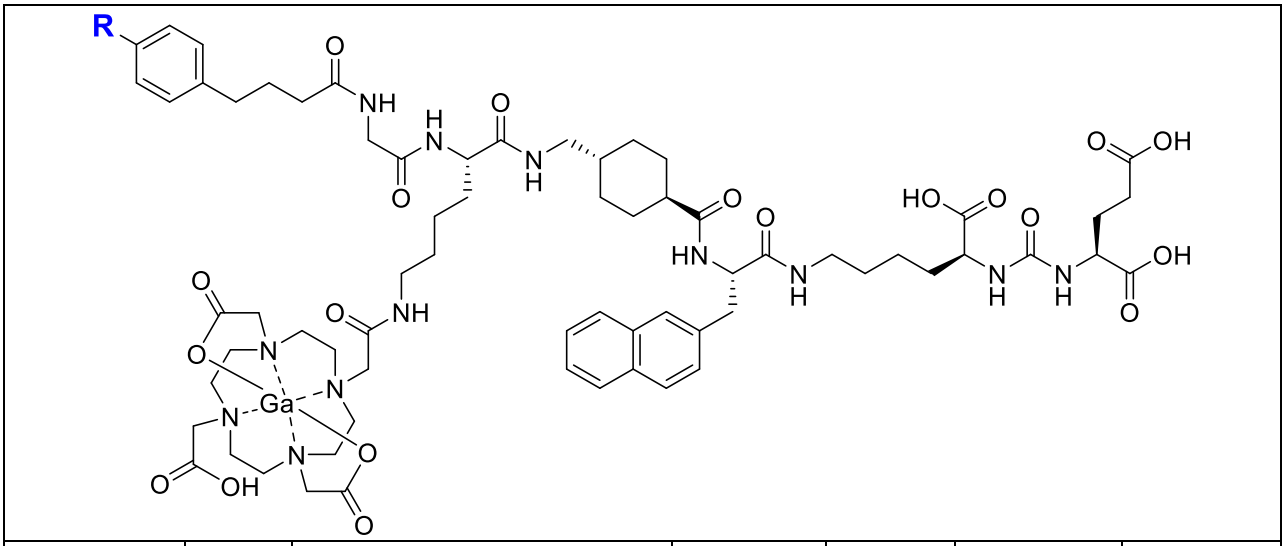
Tissue (%ID/g)	^{68}Ga-HTK03026	^{68}Ga-HTK03027	^{68}Ga-HTK03029	^{68}Ga-HTK03041		
	1 h (n = 7)	1 h (n = 5)	1 h (n = 5)	1 h (n = 6)	1 h blocked (n = 5)	3 h (n = 6)
Blood	0.70 ± 0.19	1.56 ± 0.57	3.93 ± 0.91	1.43 ± 0.30	1.36 ± 0.39	0.83 ± 0.27
Fat	0.45 ± 0.11	1.03 ± 0.67	1.57 ± 0.42	2.06 ± 0.59	0.29 ± 0.21	1.06 ± 0.20
Testes	0.35 ± 0.22	0.56 ± 0.18	1.02 ± 0.13	1.34 ± 0.22	0.33 ± 0.10	0.89 ± 0.12
Intestine	0.29 ± 0.06	0.44 ± 0.12	0.77 ± 0.11	1.14 ± 0.18	0.41 ± 0.09	0.67 ± 0.09
Stomach	0.10 ± 0.03	0.17 ± 0.10	0.31 ± 0.08	0.41 ± 0.11	0.20 ± 0.09	0.26 ± 0.06
Spleen	1.34 ± 0.54	3.36 ± 2.54	7.08 ± 5.08	8.95 ± 3.22	0.50 ± 0.04	4.19 ± 1.58
Liver	0.24 ± 0.06	0.46 ± 0.10	1.32 ± 0.63	1.38 ± 0.25	0.46 ± 0.11	0.82 ± 0.15
Pancreas	0.32 ± 0.06	0.57 ± 0.20	0.94 ± 0.18	1.47 ± 0.16	0.26 ± 0.05	0.71 ± 0.12
Kidneys	68.5 ± 28.2	85.6 ± 73.5	198 ± 49.3	170 ± 26.4	3.21 ± 0.72	121 ± 37.6
Lungs	0.82 ± 0.26	1.66 ± 0.67	3.59 ± 0.69	4.32 ± 0.62	1.04 ± 0.30	2.14 ± 0.39
Heart	0.30 ± 0.06	0.56 ± 0.24	1.25 ± 0.27	1.82 ± 0.21	0.49 ± 0.11	0.93 ± 0.17
Tumor	12.5 ± 2.90	13.3 ± 5.44	13.9 ± 6.58	23.1 ± 6.11	0.99 ± 0.36	28.2 ± 9.17
Muscle	0.28 ± 0.13	0.48 ± 0.37	0.63 ± 0.10	0.75 ± 0.09	0.19 ± 0.06	0.40 ± 0.06
Bone	0.54 ± 0.20	0.70 ± 0.33	0.92 ± 0.14	1.29 ± 0.45	0.35 ± 0.06	0.98 ± 0.40
Brain	0.06 ± 0.02	0.06 ± 0.03	0.09 ± 0.02	0.10 ± 0.05	0.04 ± 0.01	0.06 ± 0.02
Thyroid	0.35 ± 0.08	0.76 ± 0.33	1.57 ± 0.32	2.48 ± 0.44	0.40 ± 0.10	1.19 ± 0.23
Tumor/Muscle	52.8 ± 24.9	35.0 ± 18.7	21.3 ± 7.50	31.6 ± 12.1	5.06 ± 0.79	73.8 ± 30.9
Tumor/Blood	18.6 ± 5.54	8.57 ± 2.83	3.39 ± 1.01	17.3 ± 7.24	0.72 ± 0.10	36.6 ± 15.4
Tumor/Kidney	0.22 ± 0.15	0.27 ± 0.20	0.07 ± 0.02	0.14 ± 0.04	0.30 ± 0.05	0.26 ± 0.13

Supplemental Table 5: HPLC purification conditions and MS characterizations of albumin-binder-conjugated PSMA-targeting compounds.



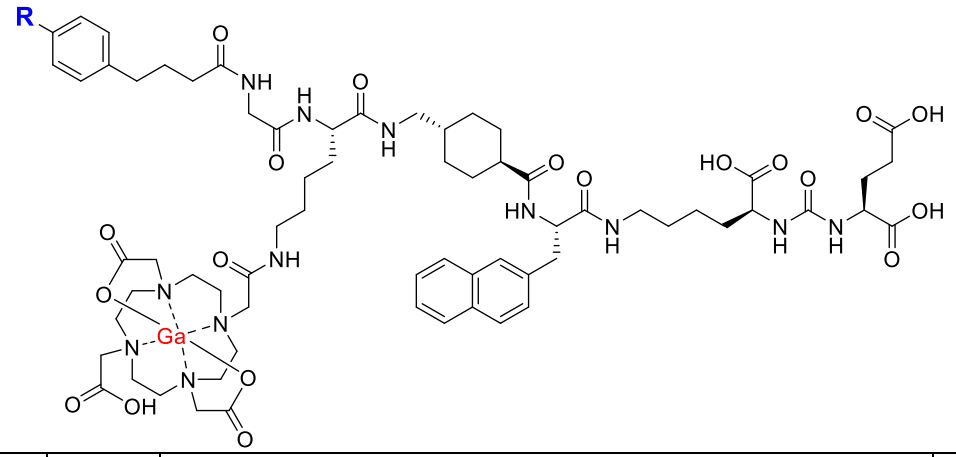
Compound name	R	HPLC conditions	Retention time (min)	Yield (%)	Calculated mass	Found
HTK03024	I	37% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	8.8	23	$[M+H]^+$ 1499.6	$[M+H]^+$ 1499.6
HTK03058	Br	0-80% CH_3CN in H_2O (containing 0.1% TFA) in 20 min; flow rate 4.5 mL/min	13.4	18	$[M+H]^+$ 1451.6	$[M+H]^+$ 1451.6
HTK03055	Cl	35% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	9.7	46	$[M+H]^+$ 1407.7	$[M+H]^+$ 1407.7
HTK03085	F	34% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	9.0	27	$[M+H]^+$ 1391.7	$[M+H]^+$ 1391.9
HTK03089	NO_2	33% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	10.6	23	$[M+H]^+$ 1418.7	$[M+H]^+$ 1419.0
HTK03090	CH_3	35% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	9.1	34	$[M+H]^+$ 1387.7	$[M+H]^+$ 1387.9
HTK03086	OCH_3	33% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	9.1	15	$[M+H]^+$ 1403.7	$[M+H]^+$ 1404.1
HTK03087	NH_2	23% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	13.9	32	$[M+H]^+$ 1388.7	$[M+H]^+$ 1389.0

Supplemental Table 6: HPLC purification conditions and MS characterizations of nonradioactive Ga-complexed albumin-binder-conjugated PSMA-targeting compounds.



Compound name	R	HPLC conditions	Retention time	Yield (%)	Calculated mass	Found
Ga-HTK03024	I	39% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	8.0 min	88	$[M+H]^+$ 1565.5	$[M+H]^+$ 1565.5
Ga-HTK03058	Br	34% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	10.3 min	37	$[M+H]^+$ 1517.5	$[M+H]^+$ 1518.0
Ga-HTK03055	Cl	35% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	12.7 min	99	$[M+2H]^{2+}$ 737.8	$[M+2H]^{2+}$ 738.6
Ga-HTK03085	F	34% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	9.0 min	51	$[M+H]^+$ 1458.6	$[M+H]^+$ 1458.6
Ga-HTK03089	NO_2	33% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	12.0 min	66	$[M+H]^+$ 1485.6	$[M+H]^+$ 1485.9
Ga-HTK03090	CH_3	35% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	11.3 min	48	$[M+H]^+$ 1454.6	$[M+H]^+$ 1454.6
Ga-HTK03086	OCH_3	33% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	10.7 min	65	$[M+H]^+$ 1469.6	$[M+H]^+$ 1469.8
Ga-HTK03087	NH_2	23% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	14.8 min	86	$[M+H]^+$ 1455.6	$[M+H]^+$ 1455.8

Supplemental Table 7: HPLC conditions for the purification and quality control of ^{68}Ga-labeled albumin-binder-conjugated PSMA-targeting compounds.



Compound name	R	HPLC conditions		Retention time (min)
^{68}Ga-HTK03025	I	Semi-Prep	33% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	16.6
		QC	33% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	7.2
^{68}Ga-HTK03058	Br	Semi-Prep	34% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	19.1
		QC	35% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	7.3
^{68}Ga-HTK03055	Cl	Semi-Prep	32% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	19.0
		QC	34% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	12.1
^{68}Ga-HTK03085	F	Semi-Prep	31% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	23.7
		QC	32% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	9.6
^{68}Ga-HTK03089	NO_2	Semi-Prep	31% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	21.2
		QC	32% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	8.5
^{68}Ga-HTK03090	CH_3	Semi-Prep	33% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	18.5
		QC	34% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	7.8
^{68}Ga-HTK03086	OCH_3	Semi-Prep	31% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	18.3
		QC	32% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	7.5
^{68}Ga-HTK03087	NH_2	Semi-Prep	22% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	24.5
		QC	23% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	9.8

Supplemental Table 8: Biodistribution (mean ± SD) and uptake ratios of ^{68}Ga-labeled HTK01169 derivatives with various R-substituted albumin binders. The studies were conducted in LNCaP tumor-bearing mice.

Tissue (%ID/g)	^{68}Ga-HTK03025 (R = I)		^{68}Ga-HTK03058 (R = Br)		^{68}Ga-HTK03055 (R = Cl)	
	1 h (n = 7)	3 h (n = 6)	1 h (n = 4)	3 h (n = 5)	1 h (n = 5)	3 h (n = 5)
Blood	27.0 ± 5.72	21.6 ± 3.59	23.1 ± 3.53	18.5 ± 0.86	22.1 ± 2.04	17.4 ± 1.15
Fat	1.60 ± 0.43	3.08 ± 1.44	1.95 ± 0.56	1.77 ± 0.54	2.20 ± 0.41	2.12 ± 0.69
Testes	3.04 ± 0.34	3.84 ± 1.32	2.63 ± 0.40	3.38 ± 0.22	2.54 ± 0.34	2.63 ± 0.49
Intestine	1.84 ± 0.33	1.58 ± 0.19	1.52 ± 0.18	1.47 ± 0.24	1.52 ± 0.21	1.29 ± 0.18
Stomach	0.59 ± 0.11	0.74 ± 0.12	0.55 ± 0.09	0.69 ± 0.13	0.62 ± 0.09	0.59 ± 0.13
Spleen	2.45 ± 0.53	2.68 ± 0.91	3.71 ± 0.76	2.90 ± 0.75	3.05 ± 0.63	3.07 ± 0.78
Liver	4.16 ± 0.65	3.60 ± 0.68	4.59 ± 2.14	3.13 ± 0.42	3.35 ± 1.08	2.96 ± 0.56
Pancreas	2.04 ± 0.30	1.76 ± 0.23	2.07 ± 0.36	1.55 ± 0.12	2.02 ± 0.32	1.74 ± 0.30
Kidneys	8.48 ± 1.35	11.5 ± 1.43	13.9 ± 1.64	17.1 ± 1.69	31.1 ± 4.40	35.6 ± 7.23
Lungs	10.2 ± 2.87	9.90 ± 2.67	10.6 ± 3.67	8.58 ± 0.27	8.37 ± 1.10	7.50 ± 1.05
Heart	5.87 ± 0.92	5.33 ± 0.77	5.61 ±0.90	4.21 ± 0.17	5.20 ±0.59	3.89 ± 0.40
Tumor	4.34 ± 2.06	12.6 ± 4.05	6.36 ± 0.43	11.6 ± 3.12	17.2 ± 1.67	30.1 ± 3.12
Muscle	0.95 ± 0.18	1.43 ± 0.23	1.31 ± 0.14	1.29 ± 0.19	1.60 ± 0.19	1.26 ± 0.18
Bone	1.04 ± 0.29	1.38 ± 0.55	1.10 ± 0.20	1.05 ± 0.15	1.59 ± 0.44	2.11 ± 0.62
Brain	0.31 ± 0.04	0.30 ± 0.06	0.37 ± 0.05	0.26 ± 0.04	0.29 ± 0.04	0.28 ± 0.06
Thyroid	4.50 ± 0.47	4.00 ± 0.51	4.34 ± 0.52	3.53 ± 0.35	4.20 ± 0.53	3.40 ± 0.56
Tumor:Blood	0.17 ± 0.08	0.57 ± 0.13	0.28 ± 0.05	0.63 ± 0.15	0.78 ± 0.04	1.75 ± 0.28
Tumor:Muscle	4.54 ± 1.72	8.67 ± 1.97	4.89 ± 0.52	8.95 ± 2.03	10.8 ± 1.15	24.5 ± 5.08
Tumor:kidney	0.51 ± 0.22	1.07 ± 0.23	0.46 ± 0.08	0.67 ± 0.15	0.56 ± 0.06	0.88 ± 0.22

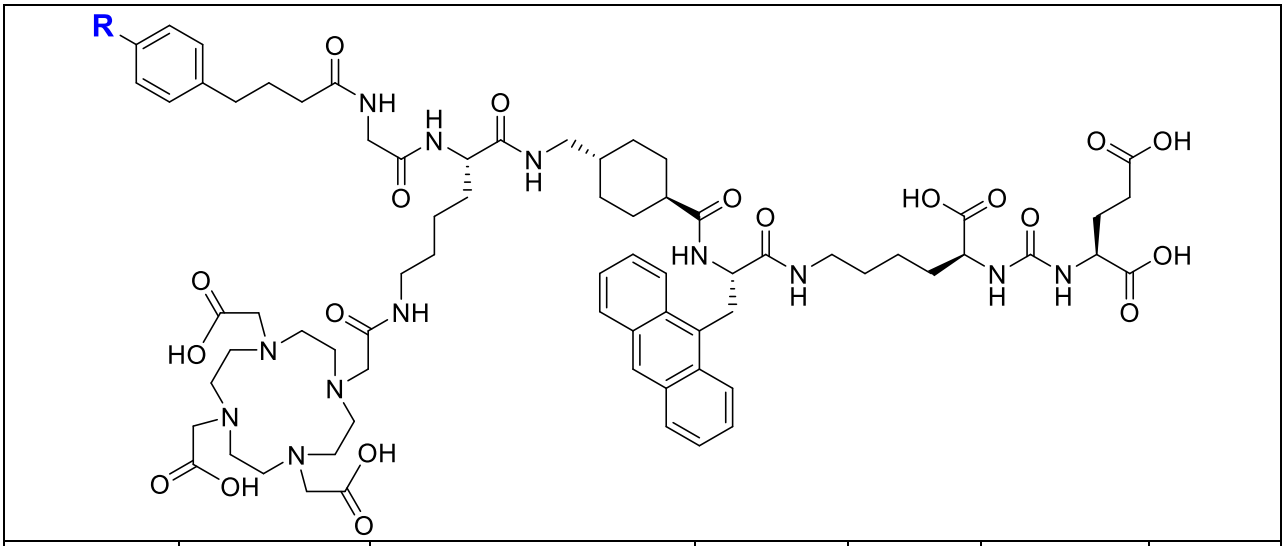
Supplemental Table 8 (Continued): Biodistribution (mean ± SD) and uptake ratios of ^{68}Ga-labeled HTK01169 derivatives with various R-substituted albumin binders. The studies were conducted in LNCaP tumor-bearing mice.

Tissue (%ID/g)	^{68}Ga-HTK03090 (R = CH_3)		^{68}Ga-HTK03086 (R = OCH_3)		^{68}Ga-HTK03089 (R = NO_2)	
	1 h (n = 4)	3 h (n = 4)	1 h (n = 5)	3 h (n = 5)	1 h (n = 5)	3 h (n = 5)
Blood	17.0 ± 1.81	13.0 ± 0.63	12.1 ± 0.60	6.60 ± 0.84	6.69 ± 0.27	2.52 ± 0.69
Fat	1.63 ± 0.47	1.46 ± 0.31	1.37 ± 0.17	0.95 ± 0.12	2.14 ± 0.29	1.28 ± 0.25
Testes	2.08 ± 0.21	2.40 ± 0.17	1.54 ± 0.18	1.30 ± 0.30	1.35 ± 0.14	0.73 ± 0.16
Intestine	1.31 ± 0.22	1.06 ± 0.05	0.91 ± 0.07	0.71 ± 0.07	0.76 ± 0.05	0.45 ± 0.10
Stomach	0.44 ± 0.08	0.42 ± 0.07	0.34 ± 0.04	0.27 ± 0.08	0.34 ± 0.07	0.19 ± 0.04
Spleen	3.30 ± 0.46	2.42 ± 0.77	2.18 ± 0.51	1.58 ± 0.37	8.56 ± 3.07	2.56 ± 0.89
Liver	3.08 ± 0.58	2.50 ± 0.54	1.77 ± 0.27	1.40 ± 0.35	1.33 ± 0.27	0.73 ± 0.41
Pancreas	1.56 ± 0.10	1.13 ± 0.11	1.16 ± 0.11	0.70 ± 0.11	1.00 ± 0.07	0.47 ± 0.07
Kidneys	27.0 ± 3.42	33.7 ± 8.04	54.7 ± 4.84	48.5 ± 10.7	91.1 ± 7.42	96.4 ± 21.9
Lungs	7.70 ± 0.66	5.54 ± 0.22	5.43 ± 0.65	3.49 ± 0.64	4.68 ± 0.35	2.14 ± 0.42
Heart	4.04 ± 0.12	3.11 ± 0.13	2.76 ± 0.25	1.53 ± 0.21	1.73 ± 0.14	0.68 ± 0.16
Tumor	12.9 ± 4.26	22.6 ± 4.71	17.8 ± 2.89	28.2 ± 5.44	21.7 ± 2.48	31.2 ± 2.11
Muscle	1.13 ± 0.08	0.89 ± 0.07	0.94 ± 0.02	0.57 ± 0.09	0.79 ± 0.06	0.29 ± 0.04
Bone	0.80 ± 0.14	0.70 ± 0.03	0.72 ± 0.19	0.33 ± 0.16	0.41 ± 0.08	0.15 ± 0.05
Brain	0.24 ± 0.01	0.22 ± 0.03	0.15 ± 0.01	0.09 ± 0.02	0.09 ± 0.01	0.04 ± 0.02
Thyroid	3.44 ± 0.28	2.55 ± 0.19	2.24 ± 0.15	1.39 ± 0.16	2.32 ± 0.10	0.98 ± 0.25
Tumor:Blood	0.76 ± 0.25	1.73 ± 0.31	1.47 ± 0.28	4.33 ± 1.06	3.25 ± 0.43	13.1 ± 3.76
Tumor:Muscle	11.3 ± 3.40	25.3 ± 4.78	18.8 ± 2.96	50.5 ± 13.5	27.4 ± 4.01	108 ± 13.6
Tumor:kidney	0.49 ± 0.18	0.68 ± 0.11	0.32 ± 0.04	0.59 ± 0.10	0.24 ± 0.03	0.34 ± 0.09

Supplemental Table 8 (Continued): Biodistribution (mean ± SD) and uptake ratios of ^{68}Ga-labeled HTK01169 derivatives with various R-substituted albumin binders. The studies were conducted in LNCaP tumor-bearing mice.

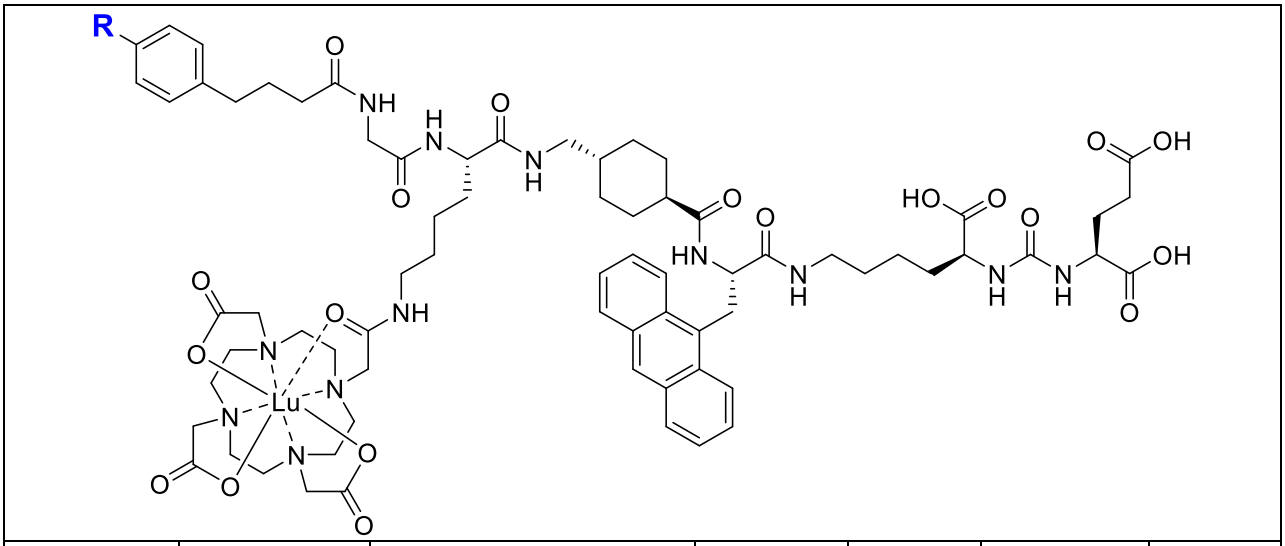
Tissue (%ID/g)	^{68}Ga-HTK03085 (R = F)		^{68}Ga-HTK03087 (R = NH_2)	
	1 h (n = 5)	3 h (n = 4)	1 h (n = 5)	3 h (n = 5)
Blood	5.73 ± 0.93	2.10 ± 0.48	1.74 ± 0.11	0.52 ± 0.08
Fat	1.36 ± 0.28	0.70 ± 0.13	0.77 ± 0.14	0.40 ± 0.11
Testes	1.03 ± 0.12	0.60 ± 0.09	0.48 ± 0.06	0.17 ± 0.05
Intestine	0.61 ± 0.10	0.39 ± 0.08	0.32 ± 0.06	0.23 ± 0.10
Stomach	0.24 ± 0.05	0.16 ± 0.09	0.14 ± 0.05	0.07 ± 0.04
Spleen	3.90 ± 1.57	1.05 ± 0.09	2.42 ± 0.79	0.80 ± 0.31
Liver	1.01 ± 0.29	0.61 ± 0.08	0.44 ± 0.06	0.31 ± 0.19
Pancreas	0.86 ± 0.09	0.35 ± 0.09	0.45 ± 0.02	0.14 ± 0.03
Kidneys	85.8 ± 8.36	63.4 ± 16.6	102 ± 8.91	56.1 ± 20.3
Lungs	3.67 ± 0.72	1.50 ± 0.26	1.75 ± 0.09	0.53 ± 0.12
Heart	1.46 ± 0.27	0.52 ± 0.09	0.52 ± 0.07	0.16 ± 0.03
Tumor	18.8 ± 3.35	28.9 ± 4.27	20.7 ± 3.79	20.4 ± 5.92
Muscle	0.58 ± 0.08	0.31 ± 0.08	0.31 ± 0.05	0.08 ± 0.02
Bone	0.34 ± 0.08	0.13 ± 0.04	0.16 ± 0.02	0.03 ± 0.03
Brain	0.08 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.01 ± 0.00
Thyroid	1.70 ± 0.25	0.64 ± 0.10	0.74 ± 0.04	0.24 ± 0.05
Tumor:Blood	3.33 ± 0.75	14.2 ± 2.77	11.9 ± 1.92	39.2 ± 8.42
Tumor:Muscle	33.2 ± 7.91	95.8 ± 20.9	66.6 ± 9.63	254 ± 88.9
Tumor:kidney	0.22 ± 0.03	0.48 ± 0.11	0.20 ± 0.02	0.40 ± 0.16

Supplemental Table 9: HPLC purification conditions and MS characterizations of HTK03121 and HTK03123.



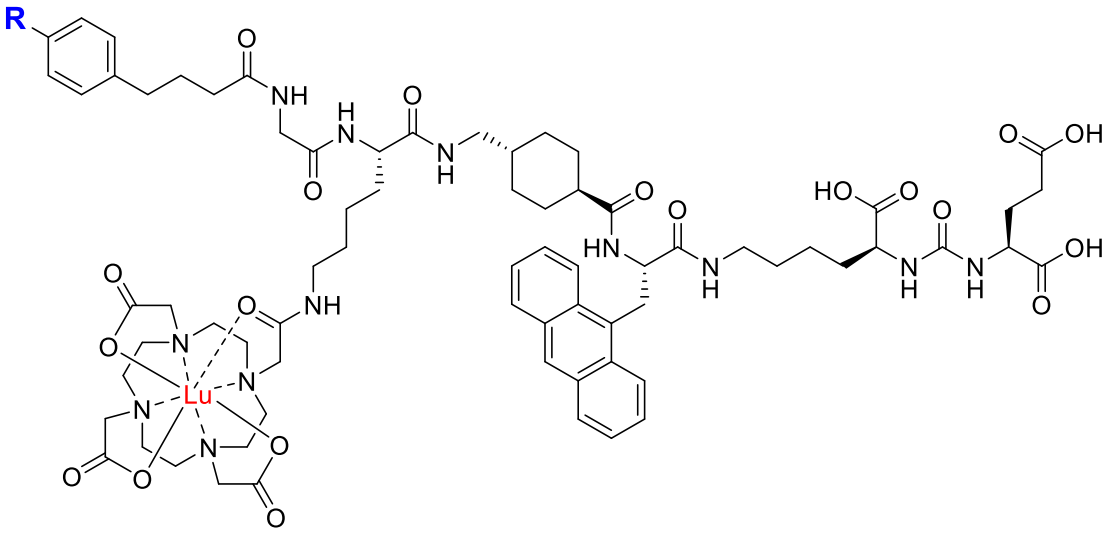
Compound name	**R**	HPLC conditions	Retention time (min)	Yield (%)	Calculated mass	Found
HTK03121	Cl	37% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	10.6	25	$[M+H]^+$ 1457.7	$[M+H]^+$ 1457.9
HTK03123	OCH_3	35% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	9.9	22	$[M+H]^+$ 1453.7	$[M+H]^+$ 1453.9

Supplemental Table 10: HPLC purification conditions and MS characterizations of Lu-HTK03121 and Lu-HTK03123.



Compound name	**R**	HPLC conditions	Retention time (min)	Yield (%)	Calculated mass	Found
Lu-HTK03121	Cl	38% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	10.5	98	$[M+H]^+$ 1629.6	$[M+H]^+$ 1629.6
Lu-HTK03123	OCH_3	35% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	14.3	77	$[M+H]^+$ 1625.6	$[M+H]^+$ 1625.7

Supplemental Table 11: HPLC conditions for the purification and quality control of ^{177}Lu-HTK03121 and ^{177}Lu-HTK03123.



Name	R	HPLC conditions		Retention time
^{177}Lu-HTK03121	Cl	Semi-prep	36% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	17.7
		QC	37% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	5.8
^{177}Lu-HTK03123	OCH_3	Semi-prep	33% CH_3CN and 0.1% TFA in H_2O; flow rate 4.5 mL/min	14.4
		QC	35% CH_3CN and 0.1% TFA in H_2O; flow rate 2.0 mL/min	6.4

Supplemental Table 12. Biodistribution (mean ± SD) and uptake ratios of ^{177}Lu-HTK03121 in LNCaP tumor-bearing mice.

Tissue (%ID/g)	1 h (n = 4)	4 h (n = 4)	24 h (n = 4)	72 h (n = 4	120 h (n = 4)
Blood	22.6 ± 5.35	15.3 ± 2.07	4.33 ± 0.88	0.75 ± 0.19	0.23 ± 0.05
Urine	17.6 ± 0.61	11.1 ± 4.81	19.8 ± 2.55	12.6 ± 5.46	5.92 ± 1.64
Fat	1.80 ± 0.34	2.16 ± 0.21	0.90 ± 0.24	0.36 ± 0.12	0.24 ± 0.05
Seminal	1.36 ± 0.33	1.20 ± 0.42	0.47 ± 0.12	0.20 ± 0.05	0.10 ± 0.04
Testes	2.31 ± 0.82	2.97 ± 0.24	1.57 ± 0.25	0.86 ± 0.15	0.51 ± 0.08
Intestine	1.59 ± 0.38	1.33 ± 0.21	0.62 ± 0.13	0.22 ± 0.07	0.11 ± 0.03
Stomach	0.64 ± 0.23	0.63 ± 0.09	0.45 ± 0.12	0.18 ± 0.04	0.10 ± 0.04
Spleen	6.05 ± 3.84	5.03 ± 1.30	2.60 ± 0.87	1.44 ± 0.77	1.25 ± 0.59
Liver	3.60 ± 1.08	3.50 ± 0.66	1.38 ± 0.07	0.63 ± 0.09	0.50 ± 0.11
Pancreas	2.22 ± 0.59	1.78 ± 0.38	0.71 ± 0.05	0.24 ± 0.09	0.12 ± 0.02
Adrenal glands	5.65 ± 1.22	6.06 ± 1.11	2.67 ± 0.80	2.52 ± 1.03	0.73 ± 0.34
Kidneys	20.6 ± 6.13	37.6 ± 10.5	34.3 ± 6.35	10.8 ± 2.71	4.30 ± 0.62
Lung	9.89 ± 2.25	8.85 ± 1.58	3.23 ± 0.48	0.93 ± 0.25	0.36 ± 0.05
Heart	5.45 ± 1.46	4.35 ± 0.58	1.38 ± 0.32	0.48 ± 0.10	0.20 ± 0.03
Tumor	14.0 ± 5.96	38.7 ± 12.6	104 ± 20.3	107 ± 29.0	85.9 ± 11.9
Muscle	1.35 ± 0.38	1.17 ± 0.14	0.40 ± 0.04	0.11 ± 0.05	0.05 ± 0.01
Bone	1.02 ± 0.15	0.82 ± 0.15	0.36 ± 0.09	0.30 ± 0.23	0.07 ± 0.03
Brain	0.32 ± 0.07	0.22 ± 0.05	0.10 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
Thyroid	4.41 ± 0.75	3.67 ± 0.51	1.72 ± 0.31	0.70 ± 0.26	0.38 ± 0.10
Salivary	4.41 ± 0.87	3.52 ± 0.55	1.45 ± 0.29	0.88 ± 0.25	0.30 ± 0.02
Lacrimal	0.37 ± 0.05	0.25 ± 0.05	0.14 ± 0.03	0.10 ± 0.12	0.08 ± 0.01
Tumor/muscle	10.9 ± 5.14	33.6 ± 12.6	261 ± 29.9	981 ± 222	1800 ± 184
Tumor/blood	0.63 ± 0.26	2.61 ± 1.08	24.3 ± 4.11	142 ± 3.34	378 ± 40.8
Tumor/kidney	0.70 ± 0.25	1.11 ± 0.49	3.06 ± 0.52	10.0 ± 1.98	20.0 ± 1.56

Supplemental Table 13: Biodistribution (mean ± SD) and uptake ratios of ^{177}Lu-HTK03123 in LNCaP tumor-bearing mice.

Tissue (%ID/g)	1 h (n = 5)	4 h (n = 5)	24 h (n = 5)	72 h (n = 5)	120 h (n = 4)
Blood	14.1 ± 1.01	7.30 ± 1.39	0.71 ± 0.29	0.16 ± 0.01	0.09 ± 0.06
Urine	79.9 ± 30.4	46.0 ± 19.3	19.4 ± 3.45	4.28 ± 2.14	1.95 ± 0.46
Fat	1.85 ± 0.37	1.66 ± 0.24	0.50 ± 0.35	0.20 ± 0.07	0.39 ± 0.28
Seminal	1.02 ± 0.11	0.76 ± 0.12	0.37 ± 0.37	0.12 ± 0.09	0.06 ± 0.04
Testes	1.83 ± 0.35	2.03 ± 0.28	0.52 ± 0.13	0.33 ± 0.09	0.20 ± 0.02
Intestine	1.30 ± 0.13	1.08 ± 0.09	0.47 ± 0.28	0.37 ± 0.32	0.08 ± 0.01
Stomach	0.56 ± 0.11	0.50 ± 0.21	0.45 ± 0.16	0.77 ± 0.72	0.11 ± 0.02
Spleen	8.04 ± 0.90	5.67 ± 2.02	1.01 ± 0.58	0.59 ± 0.08	0.50 ± 0.17
Liver	2.40 ± 0.25	2.11 ± 0.61	0.49 ± 0.17	0.28 ± 0.04	0.21 ± 0.04
Pancreas	1.84 ± 0.25	1.29 ± 0.16	0.23 ± 0.09	0.09 ± 0.01	0.05 ± 0.01
Adrenal glands	5.47 ± 0.91	5.06 ± 0.31	1.28 ± 0.51	0.55 ± 0.20	0.52 ± 0.18
Kidneys	50.8 ± 5.61	74.0 ± 6.84	20.9 ± 8.09	5.52 ± 1.74	3.99 ± 2.43
Lung	7.72 ± 1.15	5.54 ± 0.88	0.96 ± 0.44	0.31 ± 0.07	0.15 ± 0.06
Heart	3.63 ± 0.22	2.42 ± 0.44	0.42 ± 0.17	0.15 ± 0.02	0.09 ± 0.00
Tumor	21.7 ± 6.16	52.9 ± 11.4	70.8 ± 23.7	75.8 ± 8.73	89.2 ± 24.0
Muscle	1.17 ± 0.16	0.79 ± 0.08	0.16 ± 0.05	0.06 ± 0.03	0.03 ± 0.01
Bone	0.81 ± 0.09	0.54 ± 0.15	0.09 ± 0.03	0.06 ± 0.01	0.04 ± 0.02
Brain	0.20 ± 0.02	0.11 ± 0.02	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
Thyroid	3.95 ± 0.58	2.61 ± 0.35	0.63 ± 0.21	0.30 ± 0.04	0.17 ± 0.03
Salivary	5.13 ± 0.72	2.78 ± 0.54	0.55 ± 0.10	0.24 ± 0.02	0.18 ± 0.07
Lacrimal	0.25 ± 0.08	0.12 ± 0.04	0.05 ± 0.03	0.03 ± 0.03	0.02 ± 0.01
Tumor/muscle	18.5 ± 4.02	68.2 ± 19.8	468 ± 184	1476 ± 556	2582 ± 620
Tumor/blood	1.54 ± 0.41	7.65 ± 2.70	108 ± 34.3	481 ± 62.1	1092 ± 292
Tumor/kidney	0.43 ± 0.10	0.72 ± 0.19	3.66 ± 1.40	14.8 ± 4.99	25.1 ± 6.65

Supplemental Table 14: Radiation doses (mGy/MBq) calculated for the major organs of 25-g mice using the OLINDA software.

Kinetics value [MBq-h/MBq]			Organ doses [mGy/MBq]		
Source organ	^{177}Lu-HTK03121	^{177}Lu-HTK3123	Target organ	^{177}Lu-HTK03121	^{177}Lu-HTK3123
Brain	0.05	0.02	Brain	8.83E-01	3.75E-01
Large intestine contents	0.34	0.13	Large intestine	2.01E+00	1.12E+00
Small intestine	1.02	0.38	Small intestine	1.90E+00	1.00E+00
Stomach contents	0.02	0.03	Stomach wall	1.89E+00	1.23E+00
Heart contents	0.32	0.12	Heart	2.21E+00	9.11E-01
Kidneys	6.99	7.07	Kidneys	1.81E+01	1.77E+01
Liver	2.73	1.01	Liver	2.64E+00	1.22E+00
Lungs	0.25	0.09	Lungs	3.09E+00	1.24E+00
Pancreas	0.21	0.08	Pancreas	2.47E+00	1.50E+00
Cortical bone	0.73	0.28	Skeleton	5.89E+01	2.42E+01
Spleen	0.44	0.18	Spleen	4.48E+00	2.26E+00
Testes	0.29	0.10	Testes	2.41E+00	1.02E+00
Thyroid	0.02	0.01	Thyroid	2.21E+00	9.19E-01
Urinary bladder contents	0.90	0.93	Urinary bladder wall	1.15E+01	1.11E+01
Remainder of the body	16.44	6.73	Remainder of the body	1.60E+00	9.22E-01

Supplemental Table 15: Radiation doses (mGy/MBq) calculated for the major organs of humans (male) using the OLINDA software.

Kinetics value [MBq-h/MBq]			Organ doses [mGy/MBq]		
Source organ	^{177}Lu-HTK03121	^{177}Lu-HTK3123	Target organ	^{177}Lu-HTK03121	^{177}Lu-HTK3123
Adrenals	0.02	0.01	Adrenals	1.08E-01	5.35E-02
Brain	0.06	0.02	Brain	3.80E-03	1.57E-03
Esophagus	-	-	Esophagus	1.28E-02	6.22E-03
Eyes	-	-	Eyes	1.06E-02	5.26E-03
Gallbladder contents	-	-	Gallbladder wall	1.36E-02	6.89E-03
Left colon	0.03	0.01	Left colon	3.01E-02	1.33E-02
Small Intestine	0.13	0.05	Small intestine	2.84E-02	1.24E-02
Stomach contents	0.02	0.03	Stomach Wall	1.62E-02	1.12E-02
Right colon	0.03	0.01	Right colon	2.11E-02	9.67E-03
Rectum	0.01	0.01	Rectum	1.98E-02	9.01E-03
Heart contents	0.63	0.20	-	-	-
Heart wall	0.15	0.06	Heart wall	9.71E-02	3.27E-02
Kidneys	2.46	2.49	Kidneys	6.88E-01	6.95E-01
Liver	0.97	0.36	Liver	5.01E-02	1.93E-02
Lungs	1.18	0.43	Lungs	8.64E-02	3.15E-02
Pancreas	0.03	0.01	Pancreas	2.31E-02	9.77E-03
Prostate	-	-	Prostate	1.19E-02	6.18E-03
Salivary glands	0.04	0.02	Salivary glands	4.47E-02	1.99E-02
Red marrow	0.49	0.15	Red marrow	2.93E-02	1.09E-02
Cortical bone	0.01	0.01	Osteogenic Cells	-	-
Trabecular bone	-	-	-	-	-
Spleen	0.21	0.08	Spleen	1.22E-01	5.16E-02
Testes	0.02	0.01	Testes	5.42E-02	1.94E-02
Thymus	-	-	Thymus	1.26E-02	5.98E-03
Thyroid	0.01	0.005	Thyroid	5.34E-02	2.13E-02
Urinary bladder contents	0.26	0.26	Urinary bladder wall	6.46E-02	6.06E-02
Remainder of the body	8.42	4.17	Remainder of the body	1.92E-02	1.06E-02

Supplemental Table 16: Radiation dose (mGy/MBq) calculated from unit density sphere models for the LNCaP tumors.

Sphere/Tumor volume (ml)	^{177}Lu-HTK03121	^{177}Lu-HTK3123
0.01	525000	355000
0.1	54900	37200
0.5	11100	7540
1	5620	3810
2	2820	1910
4	1420	959
6	946	641
8	711	481
10	570	386
20	286	193
40	143	97.1
60	95.8	64.8
80	72	48.8
100	57.7	39.1
300	19.4	13.1
400	14.6	9.89
500	11.7	7.93
600	9.79	6.62
1000	5.91	4
2000	2.99	2.03
3000	2.01	1.36
4000	1.52	1.03
5000	1.22	0.825
6000	1.02	0.69

Supplemental Table 17: Effective doses (mSv/MBq) calculated for the major organs of humans (male) using the OLINDA software with ICRP-103ED.

Target organ	^{177}Lu-HTK03121	^{177}Lu-HTK3123
Adrenals	1.00E-03	4.94E-04
Brain	3.80E-05	1.57E-05
Esophagus	5.12E-04	2.49E-04
Eyes	0.00E+00	0.00E+00
Gallbladder wall	1.25E-04	6.36E-05
Left colon	1.46E-03	6.44E-04
Small intestine	2.62E-04	1.14E-04
Stomach Wall	1.95E-03	1.34E-03
Right colon	1.02E-03	4.69E-04
Rectum	4.55E-04	2.07E-04
Heart wall	8.96E-04	3.02E-04
Kidneys	6.35E-03	6.42E-03
Liver	2.00E-03	7.73E-04
Lungs	1.04E-02	3.78E-03
Pancreas	2.14E-04	9.02E-05
Prostate	5.48E-05	2.85E-05
Salivary glands	4.47E-04	1.99E-04
Red marrow	3.51E-03	1.30E-03
Spleen	1.12E-03	4.76E-04
Testes	2.17E-03	7.74E-04
Thymus	1.16E-04	5.52E-05
Thyroid	2.14E-03	8.51E-04
Urinary bladder wall	2.58E-03	2.42E-03
Remainder of the body	0.00E+00	0.00E+00