SUPPLEMENTARY INFO

Tailoring PSMA-617-Based Inhibitors through Charged Linker Modifications: Insights into Structure–Activity Relationships

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Table of contents

S4. Supplementary Tables

S1. General Info

Chemicals: Chemicals (>95% purity; for radiolabeling, highly pure chemicals) and solvents (metal-free nanopure water; HPLC-grade solvents) used for synthesis and analysis were purchased commercially from Sigma-Aldrich (Taufkirchen, Germany), Carl Roth (Karlsruhe), Merck (Darmstadt, Germany), Iris Biotech (Marktredwitz, Germany), CheMatech (Dijon, France) and were used without further purification.

^{68}Gallium (^{68}Ga): ^{68}Gallium (^{68}Ga): The radiometal 68Ga (half-life 69 min; β^+ 89 %; Eβ^+ max. 1.9 MeV) used was obtained from a 3.7 GBq ^{68}Ge/^{68}Ga generator (GalliaPharm®) from Eckert & Ziegler (Berlin, Germany) in the form of [^{68}Ga]Ga^{3+} eluate ions in acidic solution.

^{177}Lutetium (^{177}Lu): The radiometal ^{177}Lu (half-life 6.65 days; β^- 79 %; 0.50 MeV) was purchased in form of a [^{177}Lu]$LuCl_3$ solution from ITM (Kampen, Netherlands).

S2. Supplementary Methods

Synthesis of the EuK binding motif: 2-Chlorotrityl resin (2-CT resin, 5.00 g, 5.70 mmol, 1.0 eq) was weighed into a dry round bottom flask and allowed to swell in dried dichloromethane (DCM; 100 ml) for 45 min at room temperature. Fmoc-Lys(Alloc)-OH (3.10 g, 6.84 mmol, 1.2 eq) was dissolved in DCM (50 ml) and added slowly to the swollen 2-CT resin alongside *N,N*-diisoprpylethylamine (DIPEA, 4.68 ml, 3.54 g, 27.4 mmol, 4.8 eq) and shaken slowly over 16 h at room temperature.

The DCM was removed the next day and the residue was washed 6× with DCM and unreacted chlorotrityl groups were blocked with a mixture of DCM/methanol/DIPEA in a ratio of 17:2:1 and the residue was washed a further 6× with DCM.

To remove the Fmoc protecting group, the lysine-coupled resin (ca. 350 mg, 0.3 eq) was allowed to swell in DCM (5 ml) for 30 min and rinsed 6× with dimethylformamide (DMF). The resin was then gently shaken for 2 min and 5 min in a 1:1 mixture of DMF and piperidine and then washed 6× with DMF and 6× with DCM.

To form the isocyanate group on glutamate, triphosgene (296.8 mg, 1.00 mmol, 1.0 eq) was first weighed into a round-necked flask and dissolved in DCM (10 ml). The bis(*t*Bu)-L-glutamate (890.4 mg, 3 mmol, 3 eq, 13) was dissolved in DIPEA (2.00 ml, 1.51 g, 11.7 mmol, 11.7 eq) and DCM (200 ml). This solution was slowly added to the dissolved phosgene over 4 h at 0 °C in an ice bath.

After the addition was complete, the deprotected lysine-coupled resin was slowly added dropwise to the triphosgene mixture and then stirred for 16 h at room temperature.

In a next step, the 2-CT resin was separated via a 5 ml syringe with a frit. To remove the Alloc protecting group, tetrakis(triphenylphosphine)-palladium(0) (100 mg, 0.1 mmol, 0.3 eq) was dissolved in DCM (2.7 ml) and morpholine (300 µl, 0.30 g, 3.44 mmol, 11 eq) was added. The mixture was added to the

protected product on the resin in the syringe and slowly rotated for 1 h under exclusion of light at room temperature. The procedure was carried out analogously a second time with fresh tetrakis(triphenylphosphine)-palladium(0) and morpholine and again allowed to rotate slowly for 1 h. The eluate was removed and the mixture was removed from the syringe and washed 6× with DCM and 6× with DMF.

The residue was further rinsed 8× with DIPEA (1 vol% in DMF, 5 ml each) and 8× with sodium diethyl dithiocarbamate trihydrate (90 mM in DMF, 5 ml each) and washed 6× with DCM and 6× with DMF. The residue was dried over diethyl ether and aliquoted in 3 parts of 0.1 mmol each. Representation of the synthesis steps is displayed in Sup. Fig. 1.

Further synthesis steps were carried out utilizing solid phase synthesis via Fmoc strategy (Figure S2).

Analytical HPLC: Analytical high performance liquid chromatography was carried out using an Agilent 1100 series system (Agilent Technologies, USA) equipped with a dual UV detector (1200 Series DAD; monitored wavelength: 220/280 nm; Agilent Technologies, USA) and a Chromolith RP-18e (100 × 4.6 mm, 2 µm, 130Å; Merck, USA) column. A linear gradient (95 % A (0.1 % TFA in H_2O) to 95 % B (0.1 % TFA in CH_3CN)) in 10 min at 1 mL/min was used.

Analytical radio HPLC: Analytical radio high performance liquid chromatography was carried out using an Agilent 1100 series system (Agilent Technologies, USA) equipped with a gamma counter (RAMONA; Elysia-Raytest, Straubenhardt, Germany) and a Chromolith RP-18e (100 × 4.6 mm, 2 µm, 130Å; Merck, USA) column. A linear gradient (95 % A (0.1 % TFA in H_2O) to 95 % B (0.1 % TFA in CH_3CN)) in 14 min at 1 mL/min was used.

Thin-Layer Chromatography (TLC): Radiochemical purity of the radiolabeled PSMA compounds was assessed using thin-layer chromatography (TLC) on cellulose-based strips. The mobile phase consisted of a 1:1 mixture of 10 % ammonium acetate (NH_4OAc) and dimethylformamide. The strips were analyzed using a radio-TLC scanner.

Purification: Purifications were performed utilizing the automated reverse phase flash chromatography Isolera Prime System (Biotage, Sweden), equipped with a Sfär Bio C18 column (Duo 300 Å, 20 µm; Biotage, Sweden). Different gradients (70 - 95 % A (0.1 % TFA in H_2O) to 95 % B (0.1 % TFA in CH_3CN)) in 10 - 30 min at 25 ml/min were used.

Mass spectrometry: For mass spectrometry, a Flexar SQ 300 MS ESI system (PerkinElmer, USA) was used. Tracer amounts of substance were dissolved in ultrapure water and directly injected into the ESI.

S3. Supplementary Figures

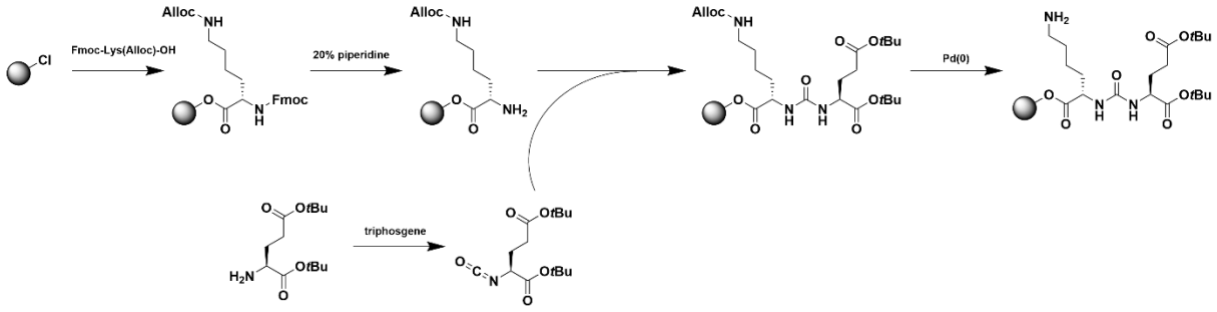


Figure S1: Schematic representation of the synthesis of the PSMA binding motif consisting of glutamic acid, urea and lysine (EuK).

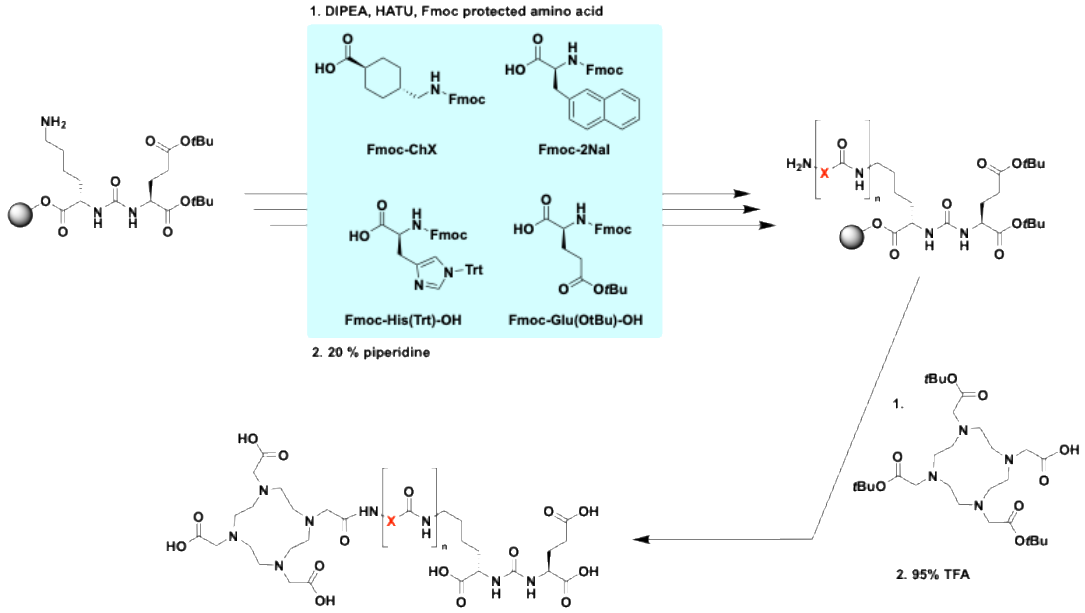
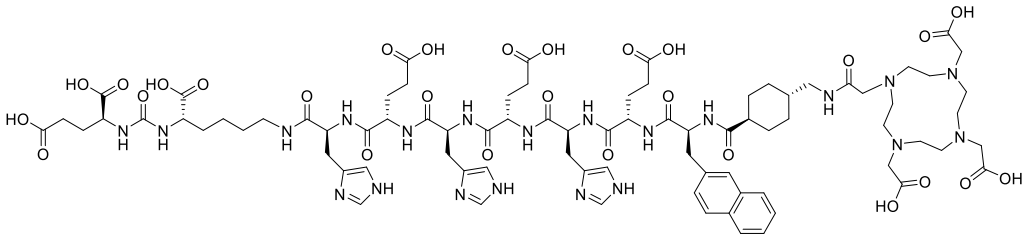


Figure S2: Schematic representation of the synthesis of the linker modified PSMA inhibitors PS1 - PS11 starting from the the EuK binding-motif and utilizing solid phase peptide synthesis with a Fmoc-strategy.

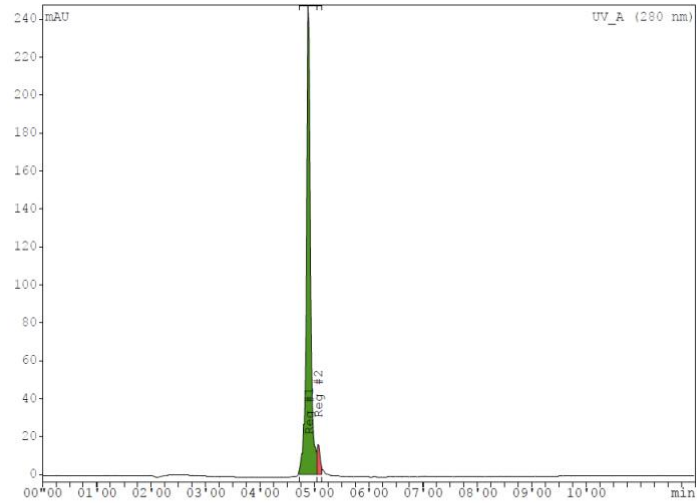
PS1 (EuK-**HEHEHE**-2NaI-ChX-DOTA)

A



Exact Mass: 1839,81
Molecular Weight: 1840,93

B



Integration UV_A (280 nm)

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Summe in ROI			1328,730	

C

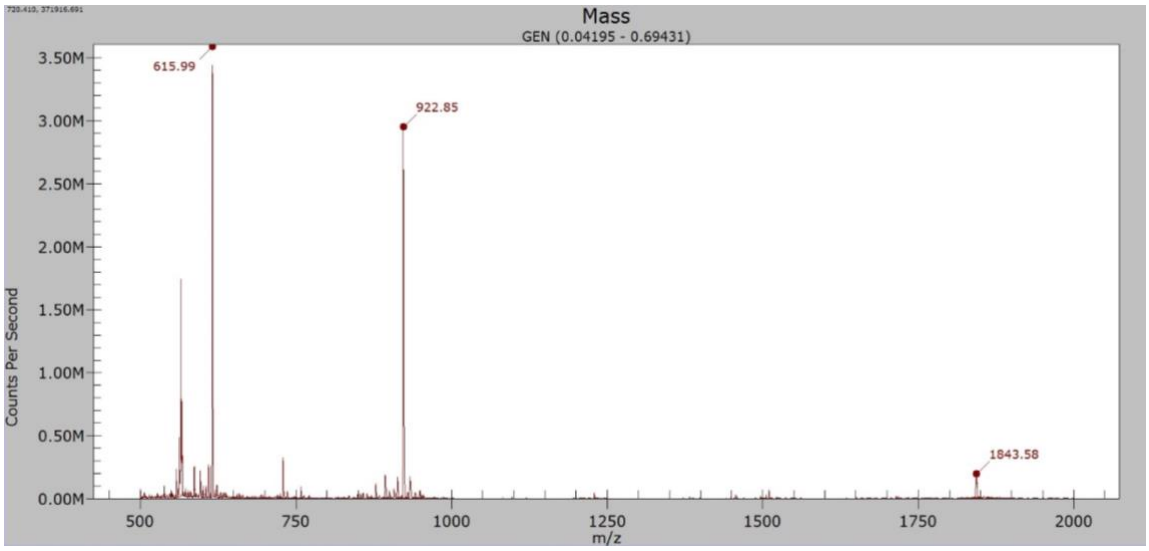
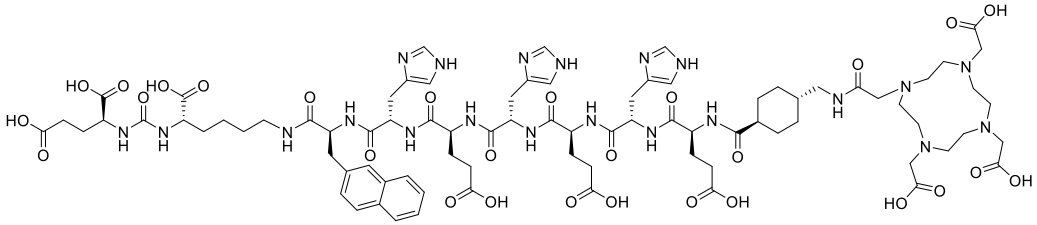


Figure S3: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS1 (EuK-**HEHEHE**-2NaI-ChX-DOTA) according to the methods described in S2. Supplementary Methods.

PS2 (EuK-2NaI-**HEHEHE**-ChX-DOTA)

A



Molecular Weight: 1840,93

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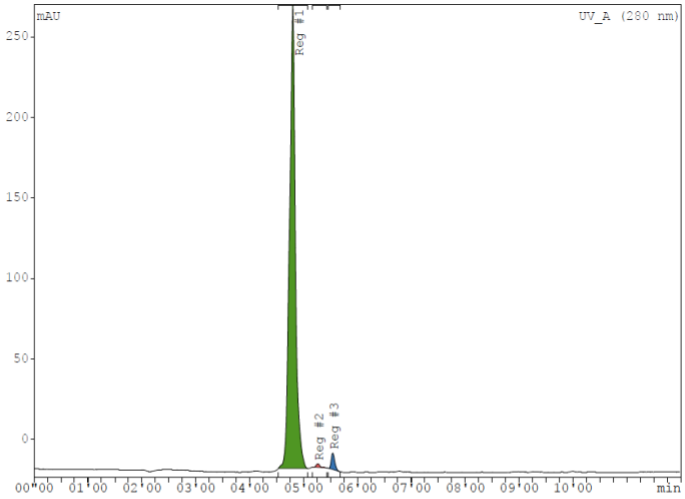

Integration UV_A (280 nm)

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Reg #3	05'33	BB(M	45,676	2,04
Summe in ROI			2238,228	

C

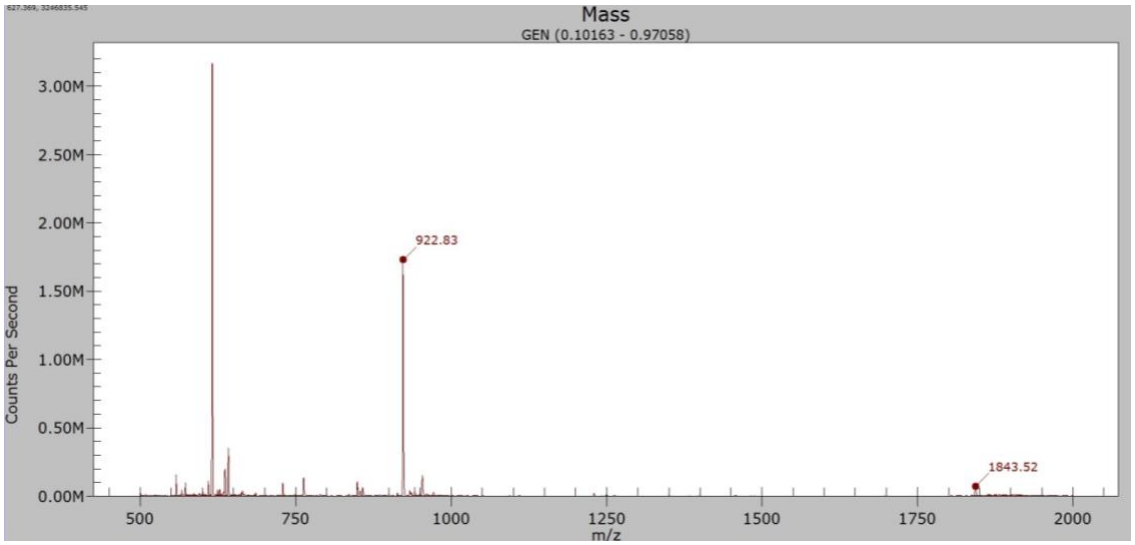
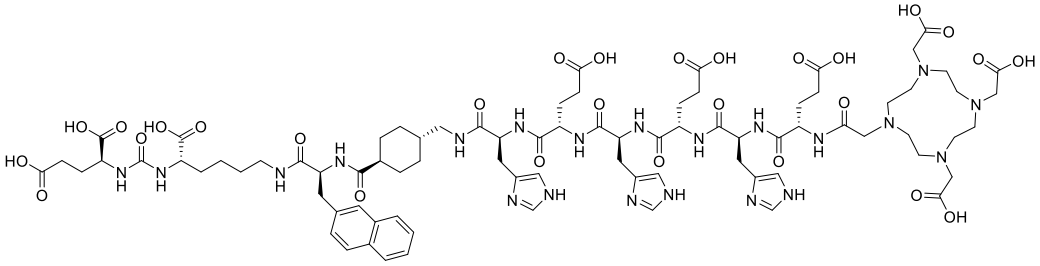


Figure S4: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS2 (EuK-2NaI-**HEHEHE**-ChX-DOTA) according to the methods described in S2. Supplementary Methods.

PS3 (EuK-2NaI-ChX-**HEHEHE**-DOTA)

A



Molecular Weight: 1840,93

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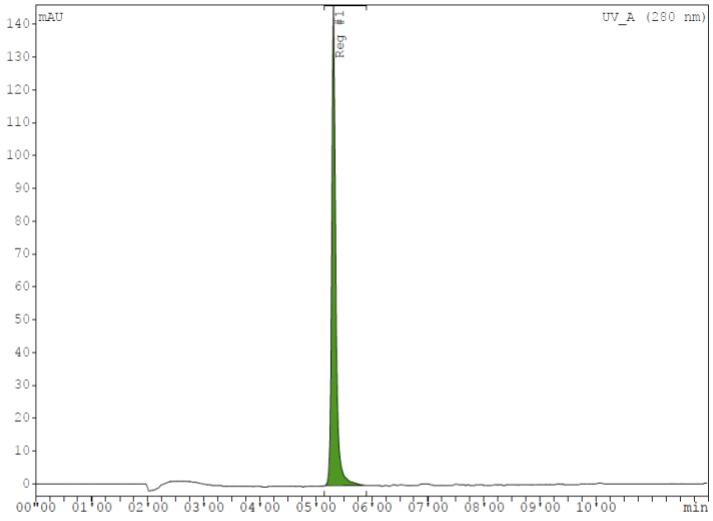

Integration UV_A (280 nm)

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Summe in ROI			750,5672	

C

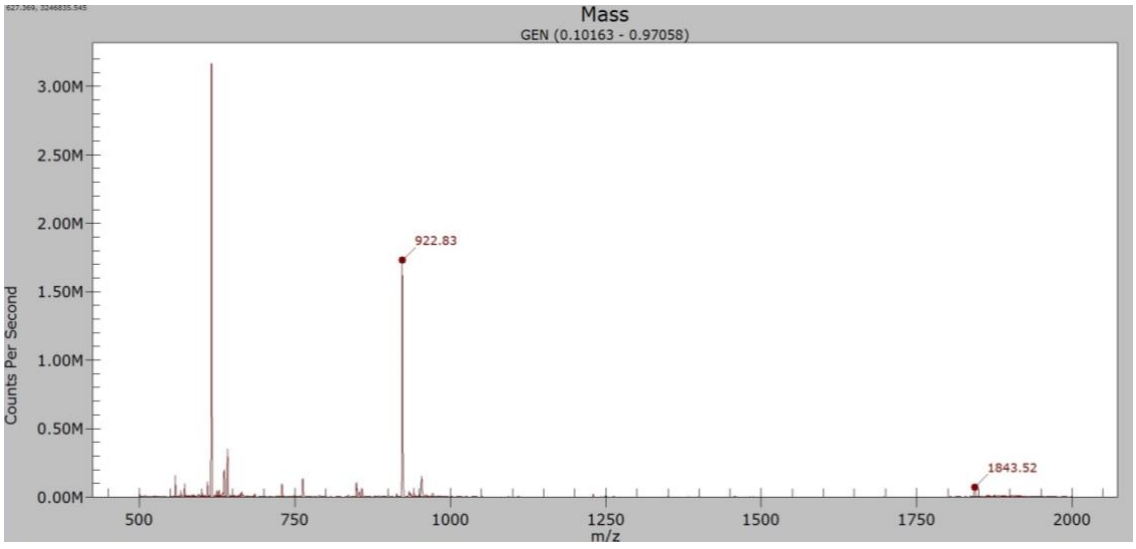
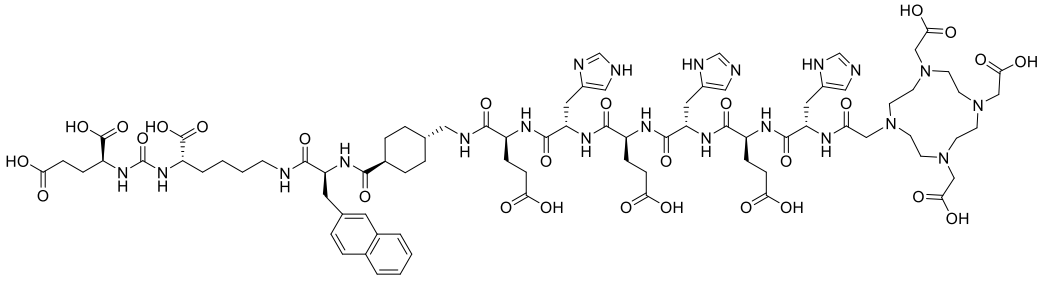


Figure S5: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS3 (EuK-2NaI-ChX-**HEHEHE**-DOTA) according to the methods described in the S2. Supplementary Methods.

PS4 (EuK-2NaI-ChX-**EHEHEH**-DOTA)

A



Molecular Weight: 1840,93

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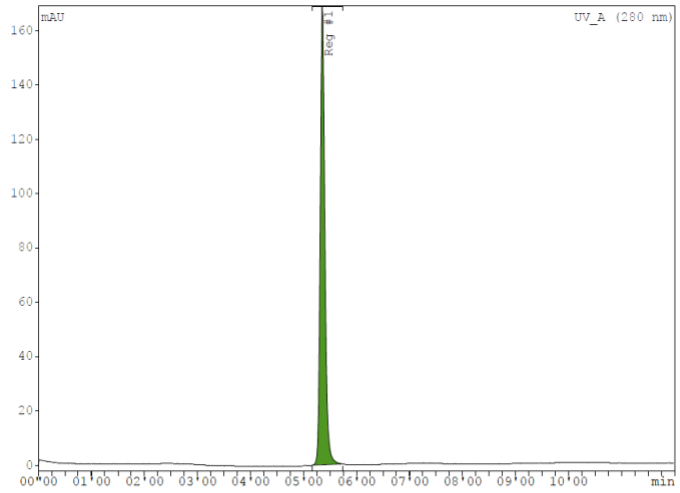

Integration UV_A (280 nm)

Substanz	Ret s	Typ	Fläche mAU*s	%Fläche %
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Summe in ROI			1019,780	

C

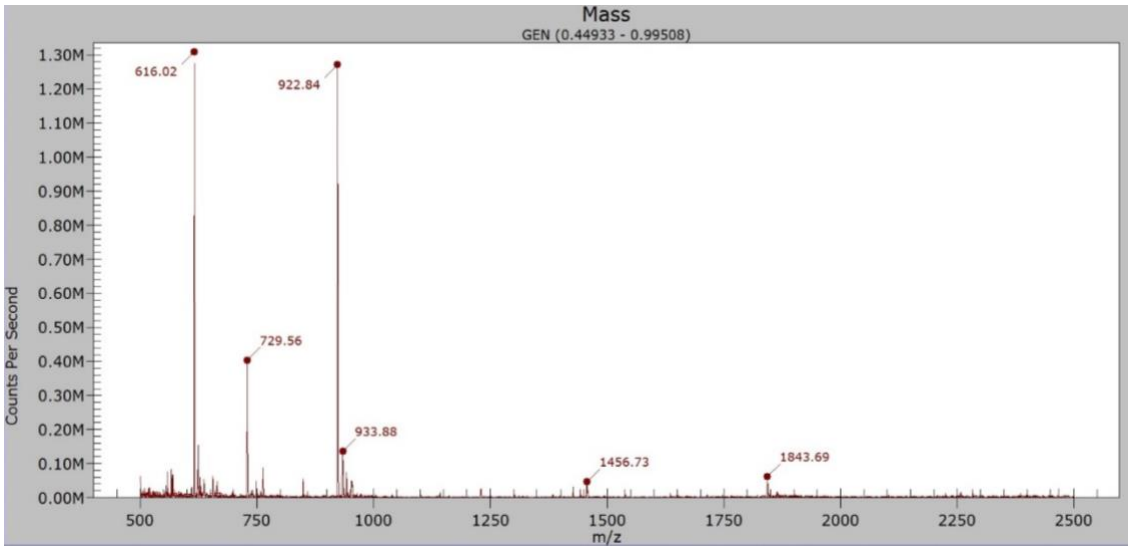
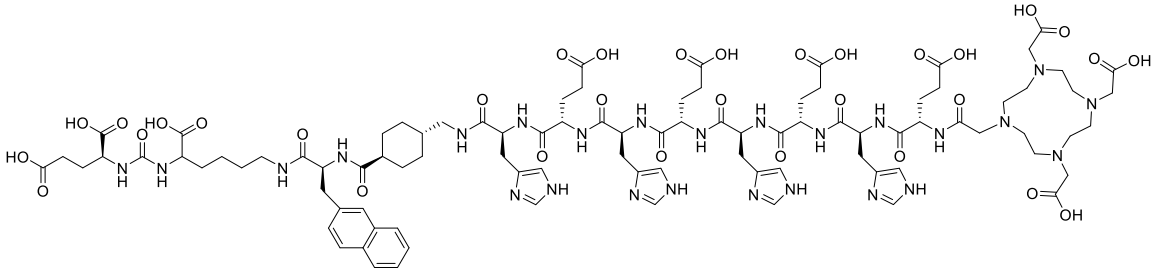


Figure S6: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS4 (EuK-2NaI-ChX-**EHEHEH**-DOTA) according to the methods described in S2. Supplementary Methods.

PS5 (EuK-2NaI-ChX-**HEHEHEHE**-DOTA)

A



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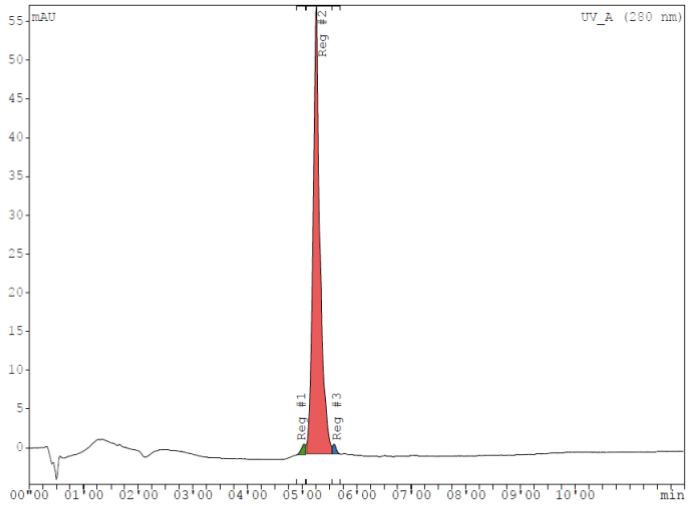

Integration UV_A (280 nm)

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Reg #2	05'15	DD(M	508,9305	97,54
Reg #3	05'37	DB(M	6,2146	1,19
Summe in ROI			521,7579	

C

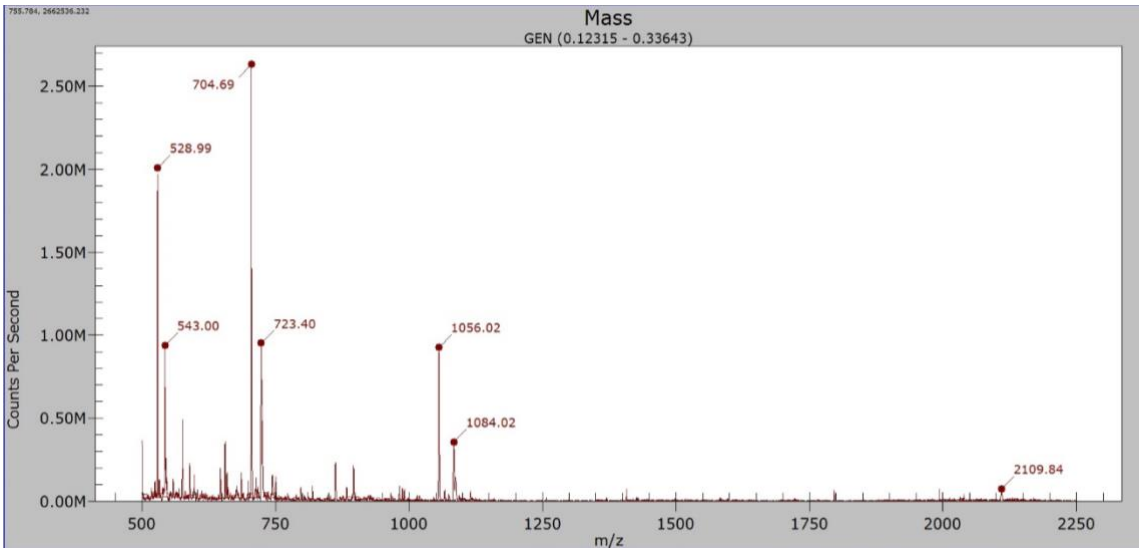
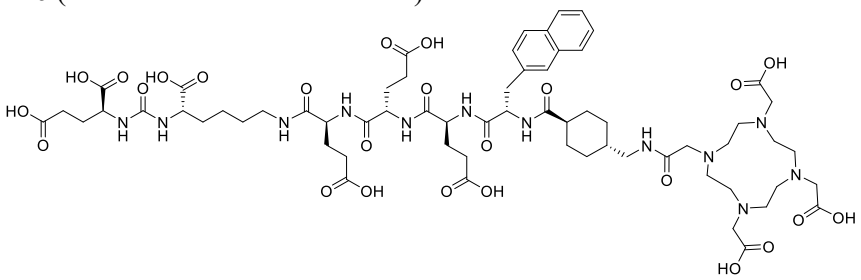


Figure S7: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS5 (EuK-2NaI-ChX-**HEHEHEHE**-DOTA) according to the methods described in S2. Supplementary Methods.

PS6 (EuK-**EEE**-2NaI-ChX-DOTA)

A



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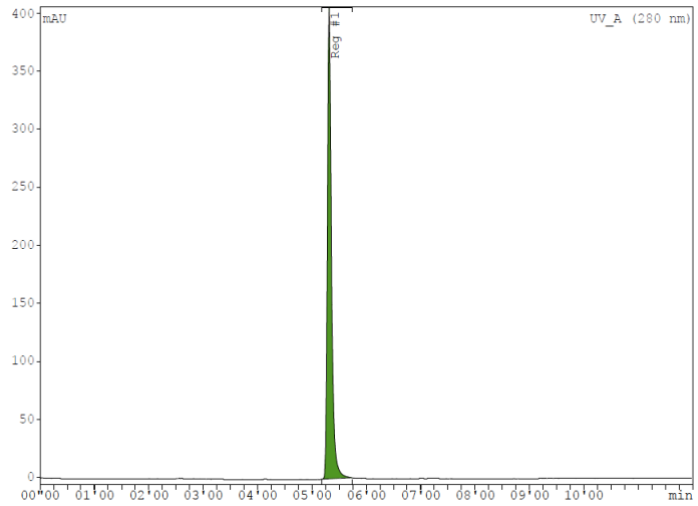

Integration UV_A (280 nm)

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Summe in ROI			2093,906	

C

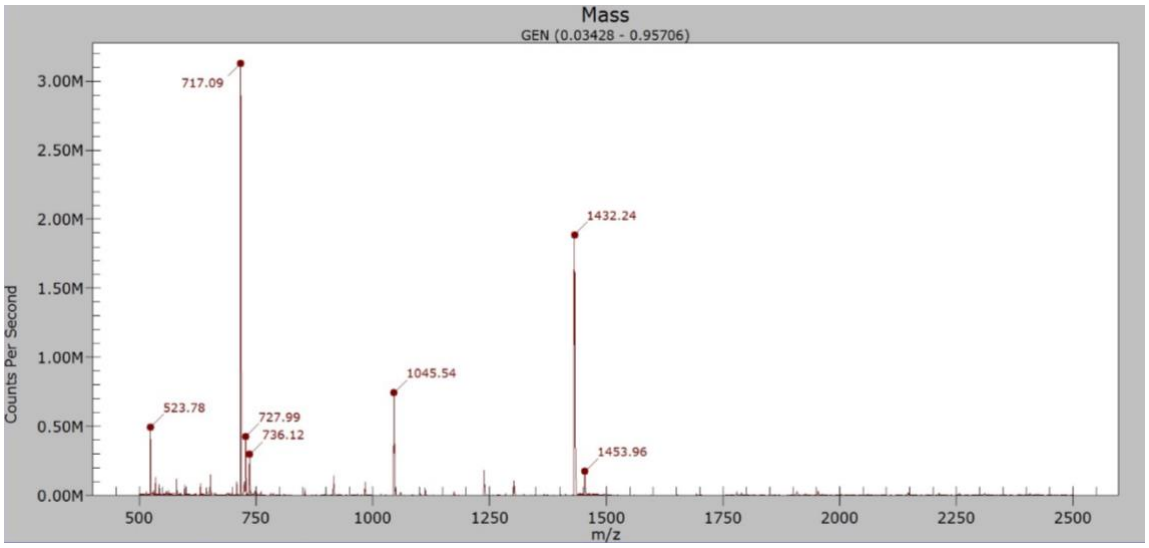
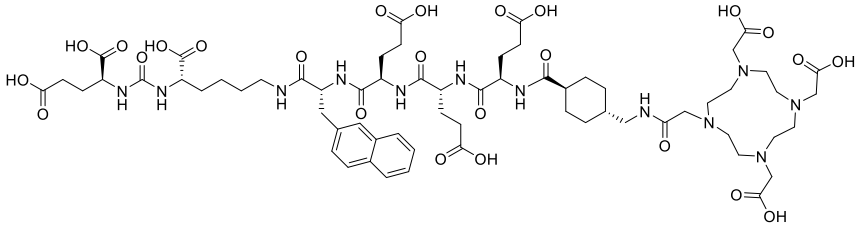


Figure S8: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS6 (EuK-**EEE**-2NaI-ChX-DOTA) according to the methods described in S2. Supplementary Methods.

PS7 (EuK-2NaI-**EEE**-ChX-DOTA)

A



Molecular Weight: 1429,50

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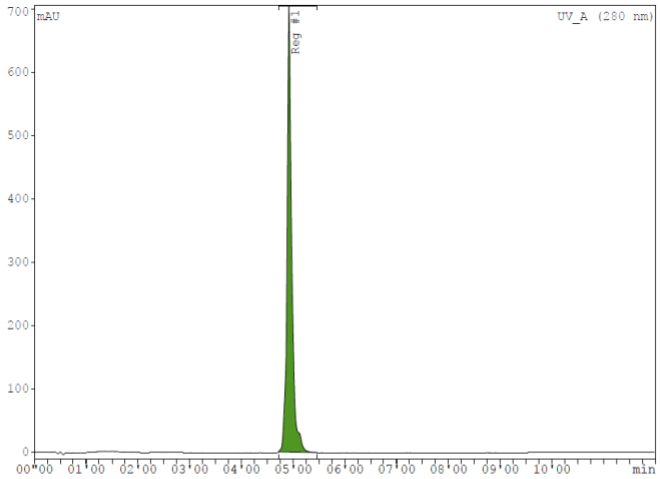

Integration UV_A (280 nm)

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Summe in ROI			4376,473	

C

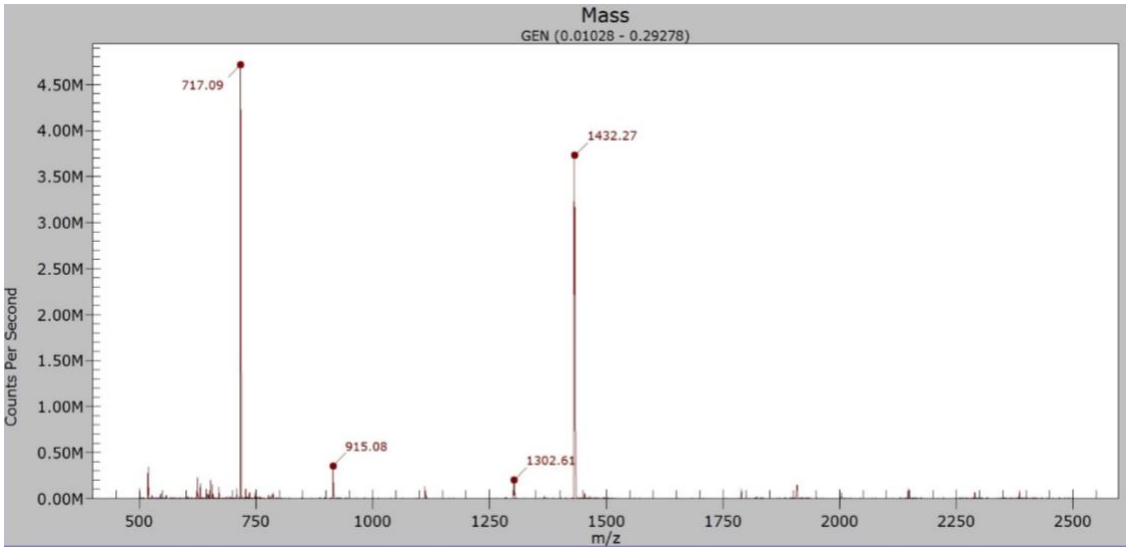
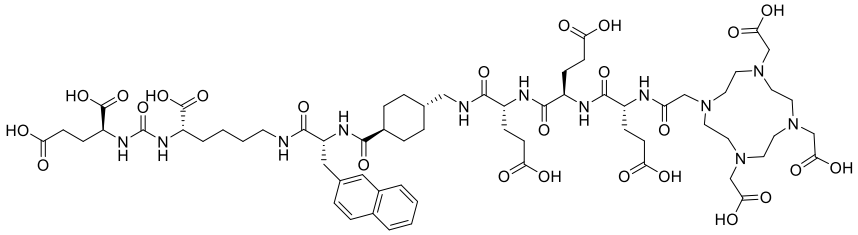


Figure S9 Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS7 (EuK-2NaI-**EEE**-ChX-DOTA) according to the methods described in S2. Supplementary Methods.

PS8 (EuK-2NaI-ChX-**EEE**-DOTA)

A



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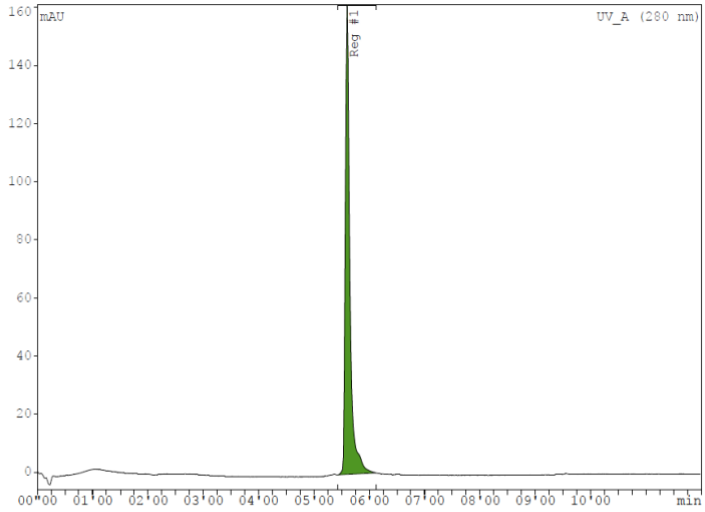

Integration UV_A (280 nm)

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Summe in ROI			920,8553	

C

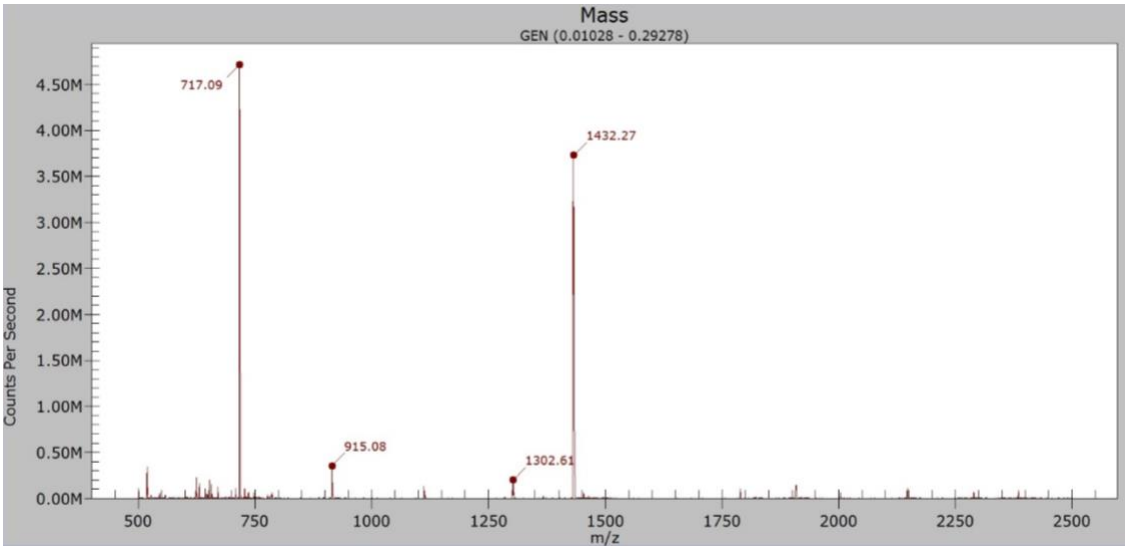
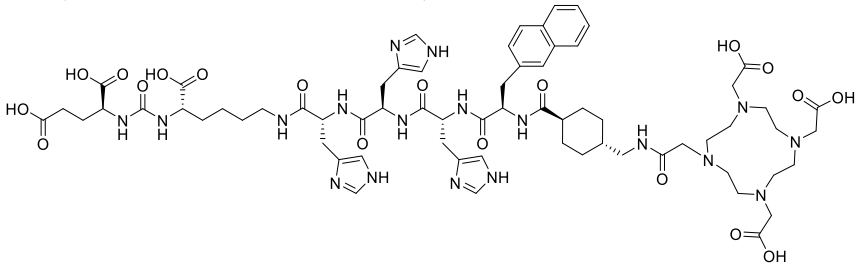


Figure S10: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) spectrometry of PS8 (EuK-2NaI-ChX-**EEE**-DOTA) according to the methods described in S2. Supplementary Methods.

PS9 (EuK-**HHH**-2NaI-ChX-DOTA)

A



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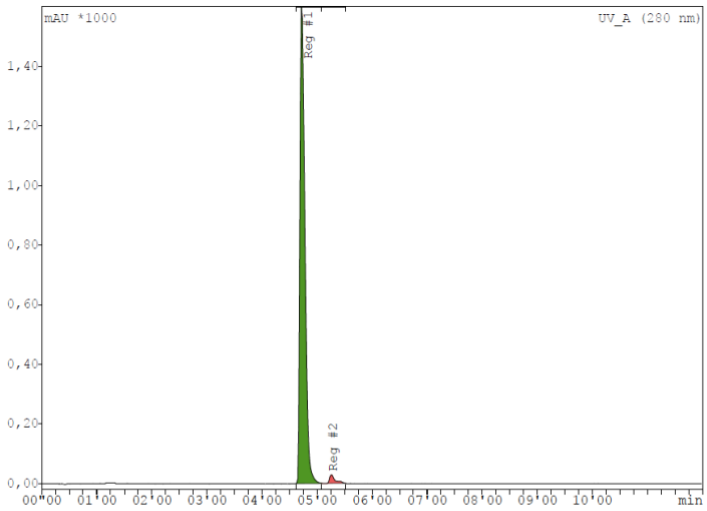

Integration UV_A (280 nm)

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Summe in ROI			10263,94	

C

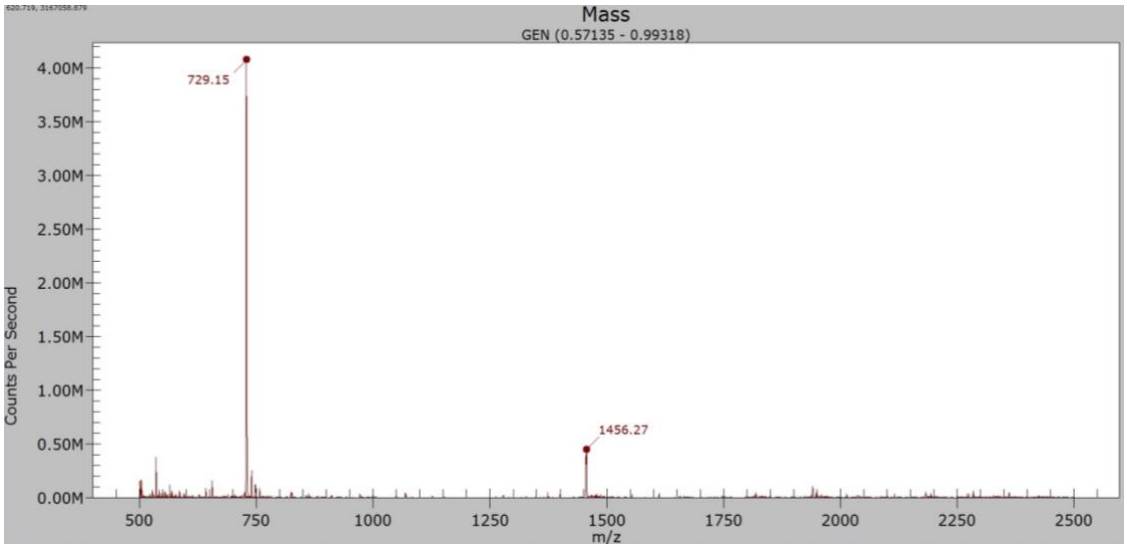
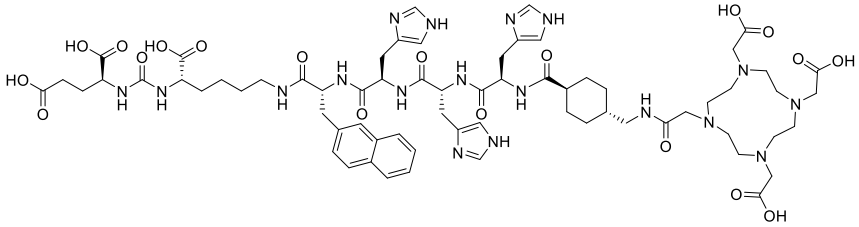


Figure S11: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS9 (EuK-**HHH**-2NaI-ChX-DOTA) according to the methods described in S2. Supplementary Methods.

PS10 (EuK-2NaI-**HHH**-ChX-DOTA)

A



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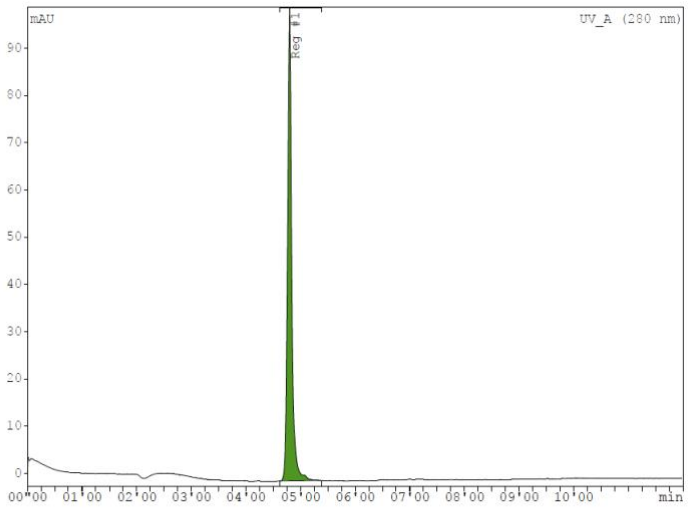

Integration UV_A (280 nm)

Substanz	Ret s	Typ	Fläche mAU*s	%Fläche %
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Summe in ROI			523,7071	

C

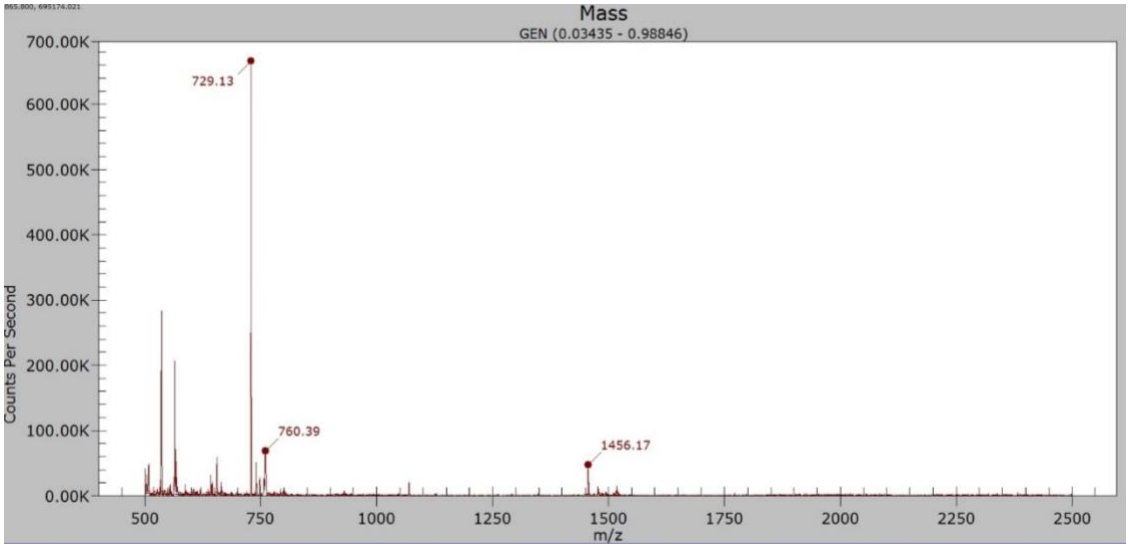
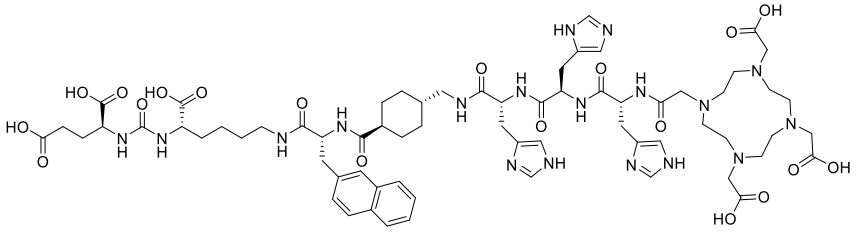


Figure S12: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS10 (EuK-2NaI-**HHH**-ChX-DOTA) according to the methods described in S2. Supplementary Methods.

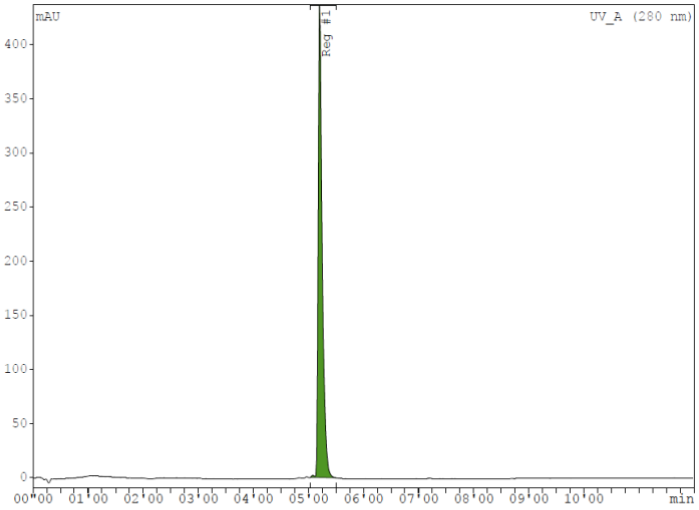
PS11 (EuK-2NaI-ChX-**HHH**-DOTA)

A



Molecular Weight: 1453,58

B



Integration UV_A (280 nm)

Substanz	Ret s	Typ	Fläche mAU*s	%Fläche %
Reg #1	05'13	BB(M	2192,076	100,00
Summe in ROI			2192,076	

C

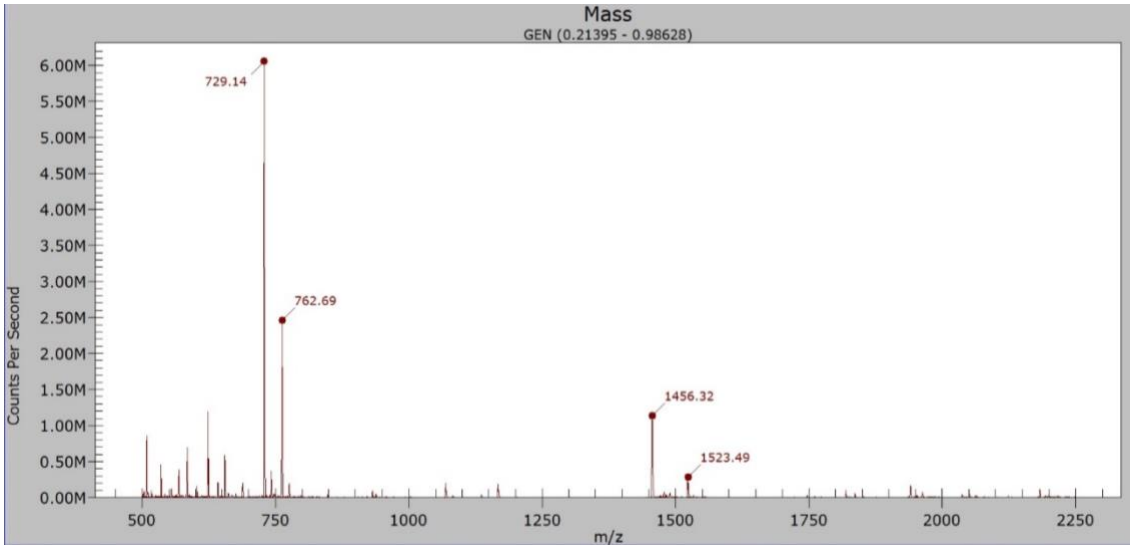


Figure S13: Chemical structure (A), analytical HPLC (B) and mass spectrometry (C) of PS11 (EuK-2NaI-ChX-**HHH**-DOTA) according to the methods described in S2. Supplementary Methods.

Supp. Fig. 14

PS – 1 (EuK-HEHEHE-2NaI-ChX-DOTA)

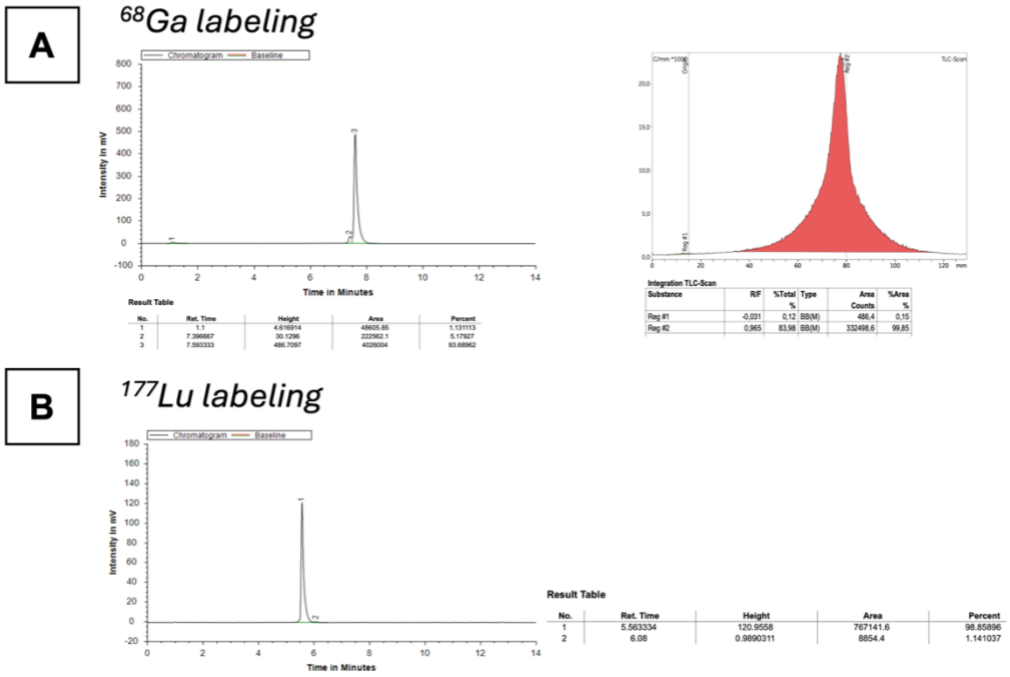


Figure S14: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS1; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS1.

Supp. Fig. 15

PS – 2 (EuK-2NaI-HEHEHE-ChX-DOTA)

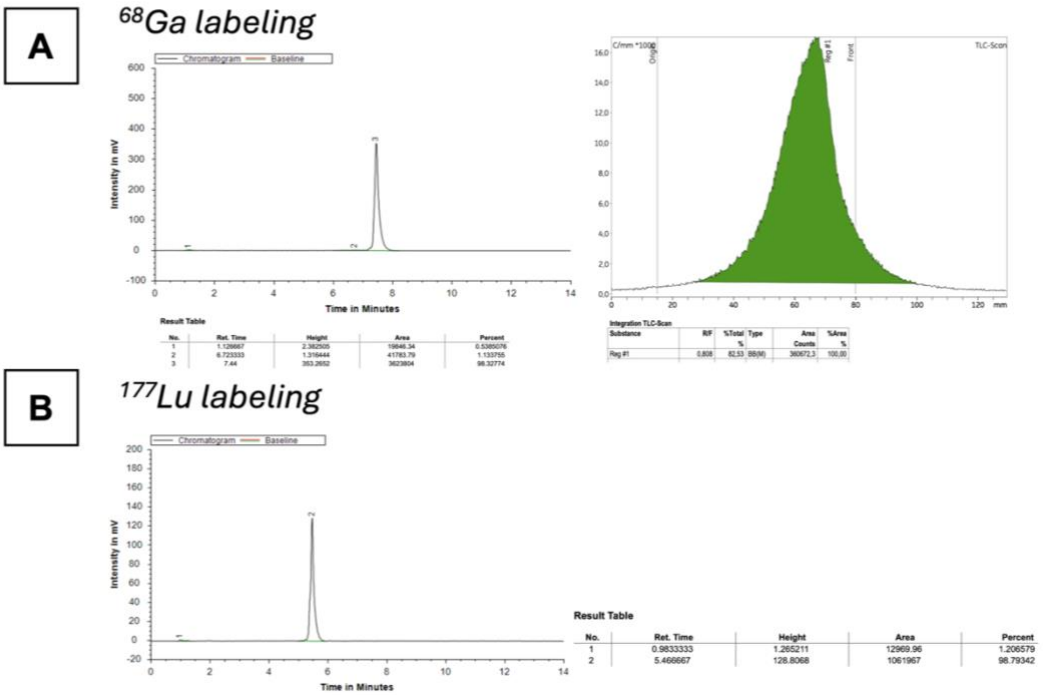


Figure S15: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS2; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS2.

Supp. Fig. 16

PS – 3 (EuK-2Nal-ChX-HEHEHE-DOTA)

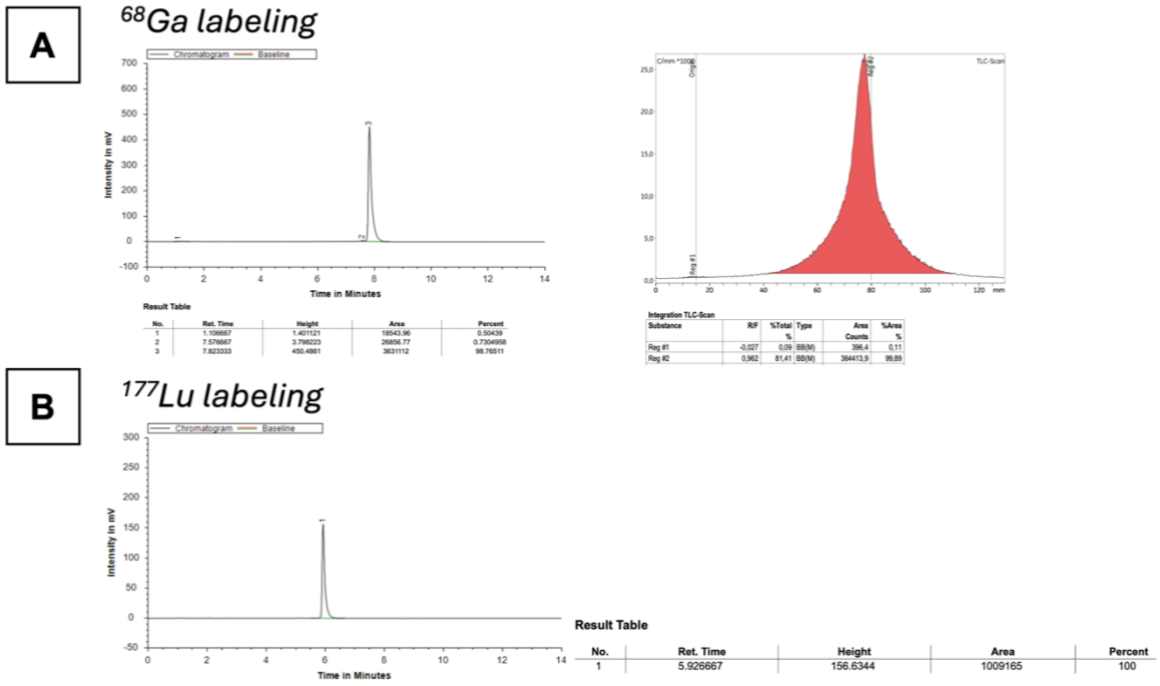


Figure S16: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS3; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS3.

Supp. Fig. 17

PS – 4 (EuK-2Nal-ChX-EHEHEH-DOTA)

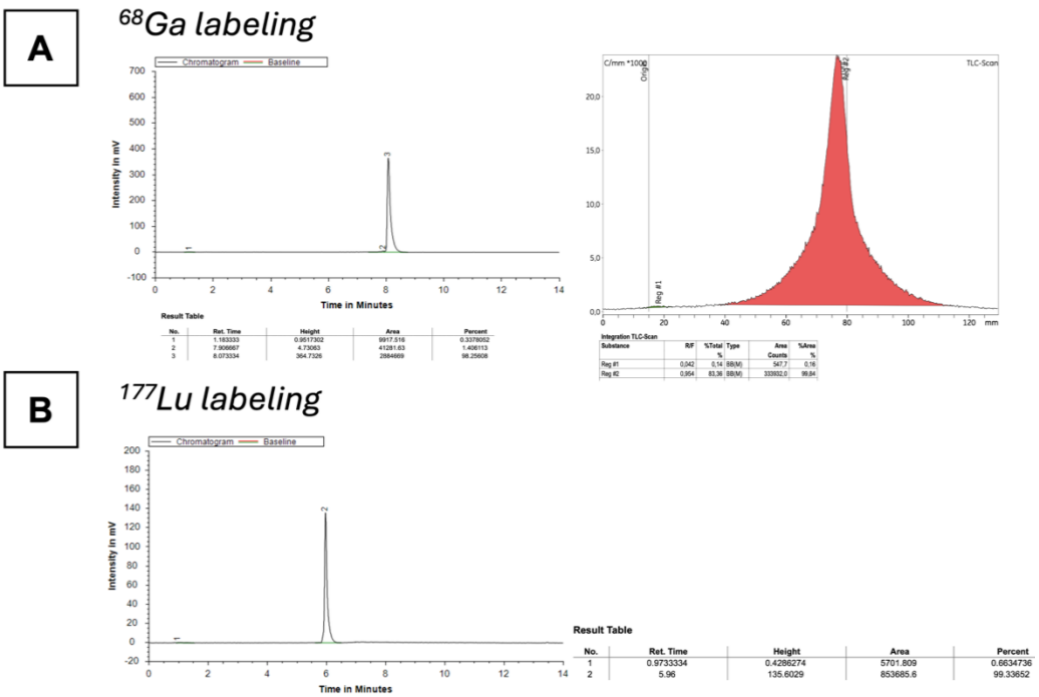


Figure S17: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS4; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS4.

Supp. Fig. 18

PS – 5 (EuK-2Nal-ChX-HEHEHEHE-DOTA)

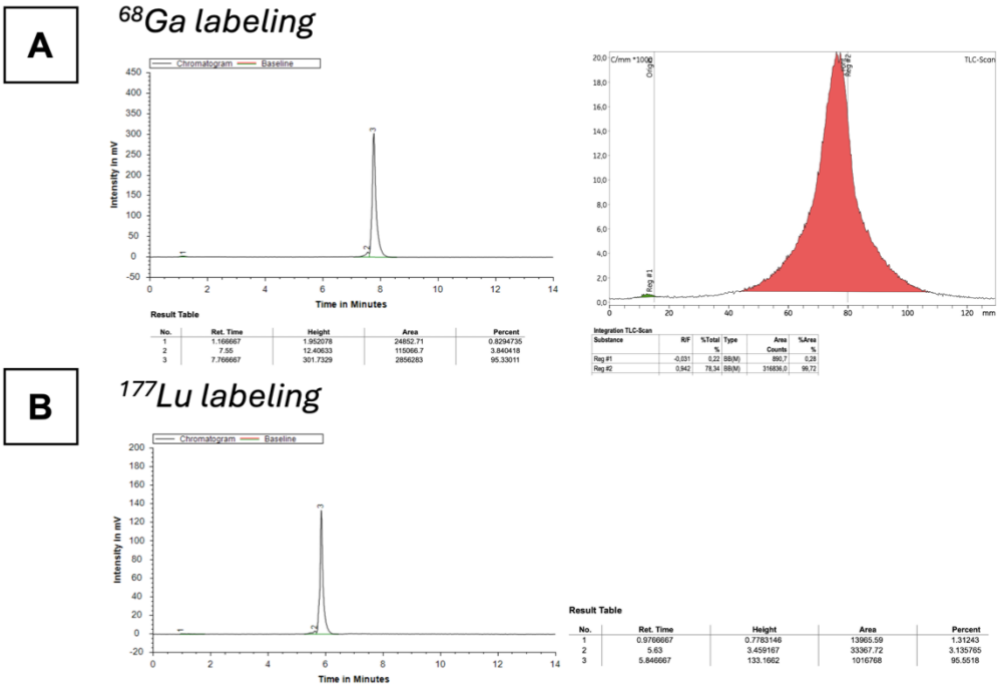


Figure S18: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS5; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS5.

Supp. Fig. 19

PS – 6 (EuK-EEE-2Nal-ChX-DOTA)

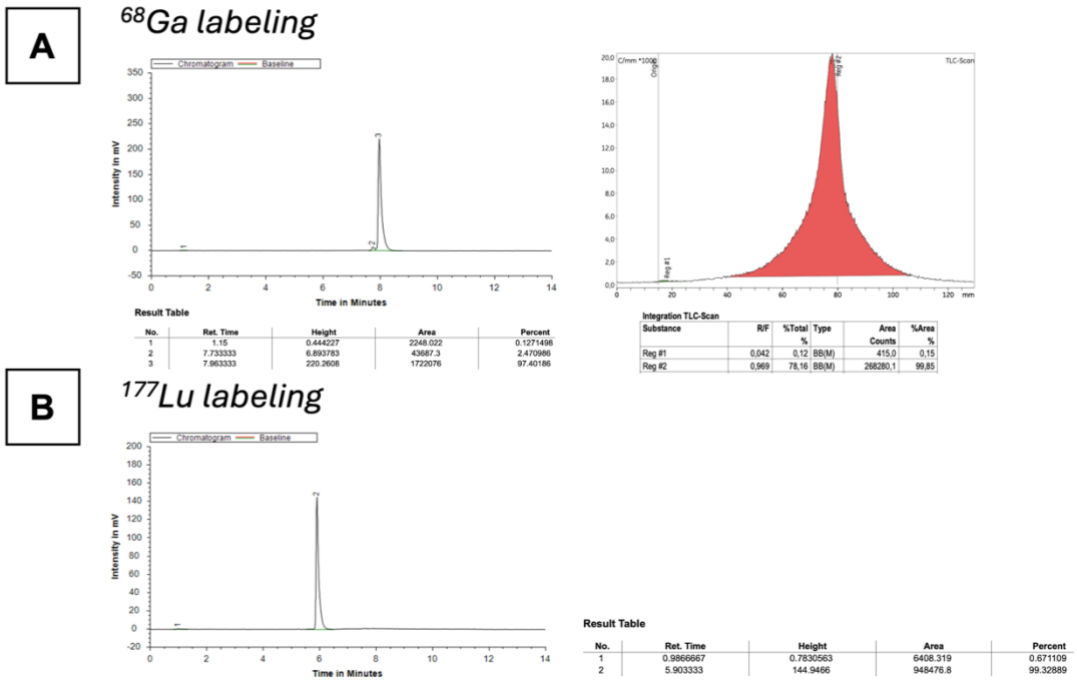


Figure S19: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS6; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS6.

Supp. Fig. 20

PS – 7 (EuK-2Nal-EEE-ChX-DOTA)

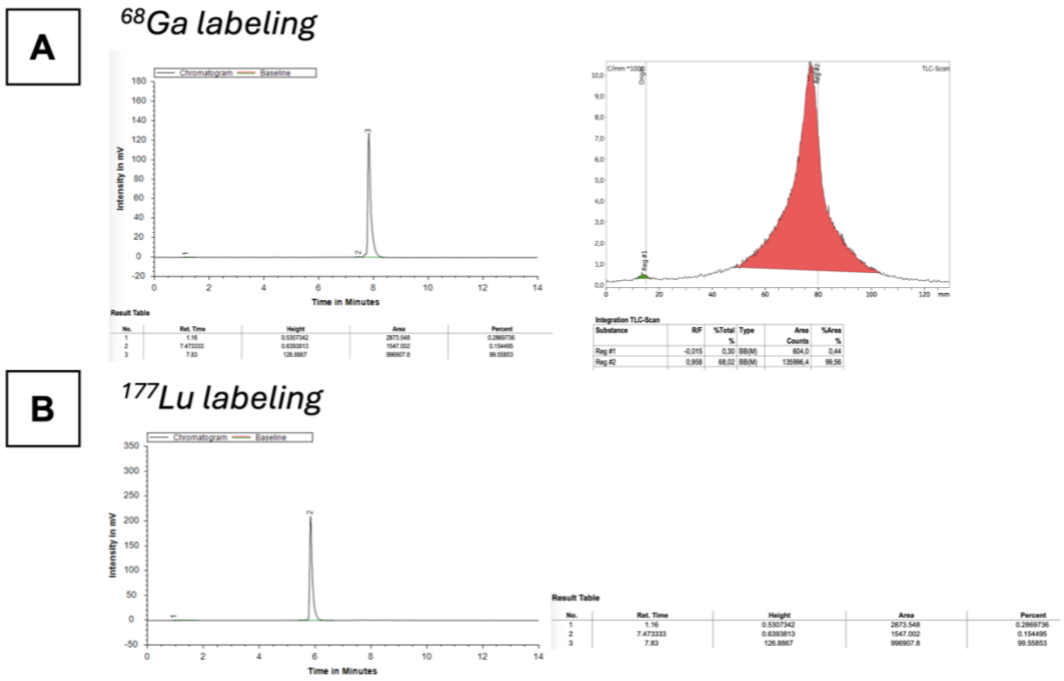


Figure S20: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS7; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS7.

Supp. Fig. 21

PS – 8 (EuK-2Nal-ChX-EEE-DOTA)

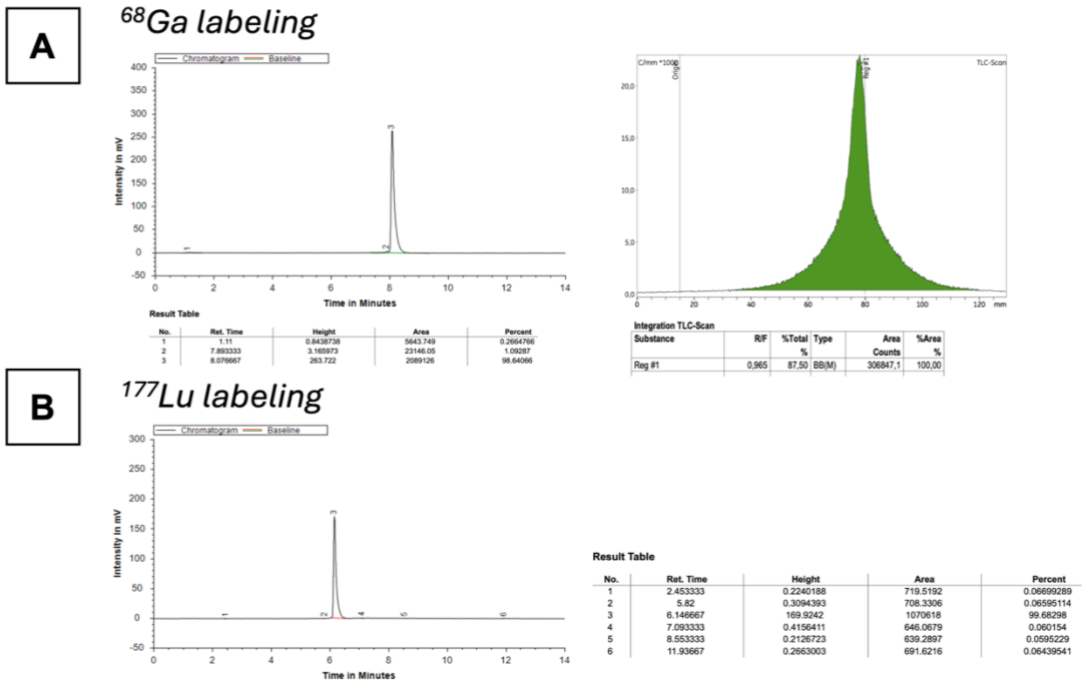


Figure S21: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS8; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS8.

Supp. Fig. 22

PS – 9 (EuK-HHH-2Nal-ChX-DOTA)

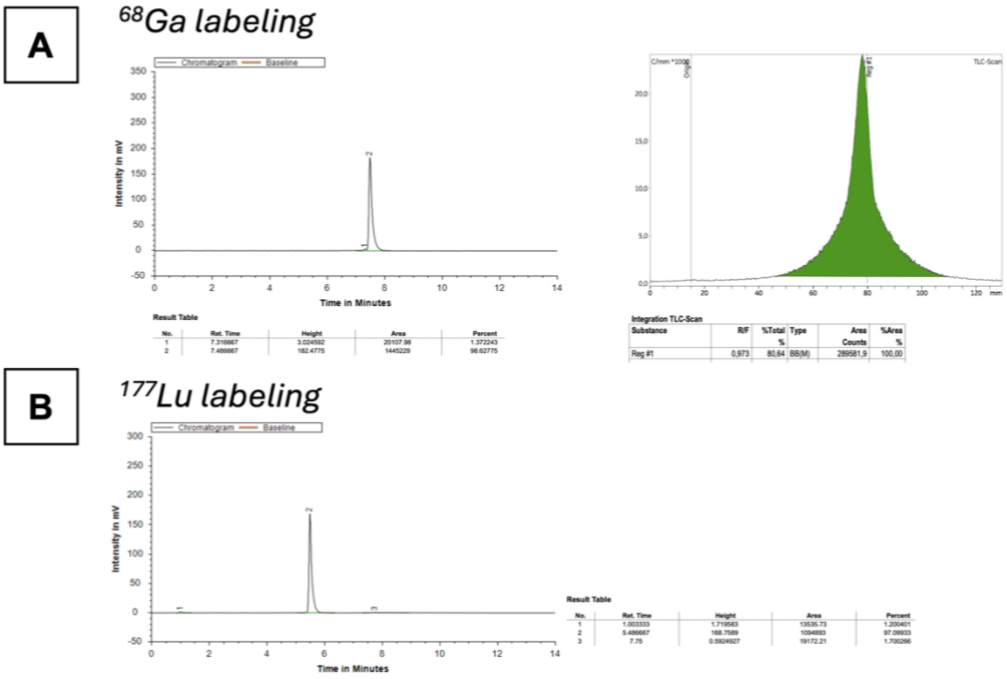


Figure S22: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS9; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS9.

Supp. Fig. 23

PS – 10 (EuK-2Nal-HHH-ChX-DOTA)

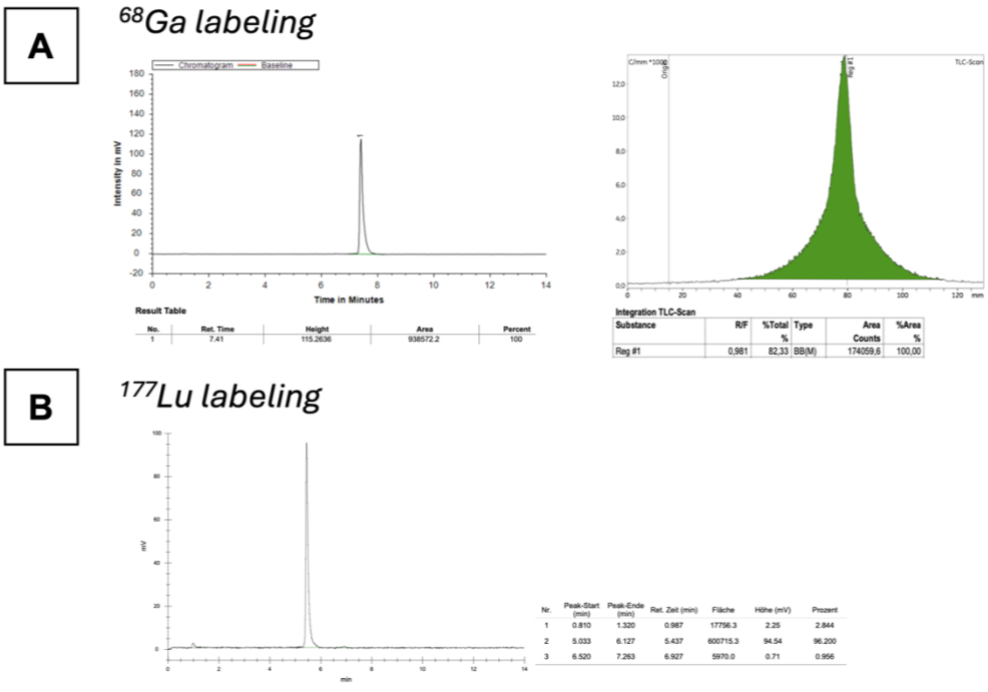


Figure S23: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS10; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS10.

Supp. Fig. 24

PS – 11 (EuK-2NaI-ChX-HHH-DOTA)

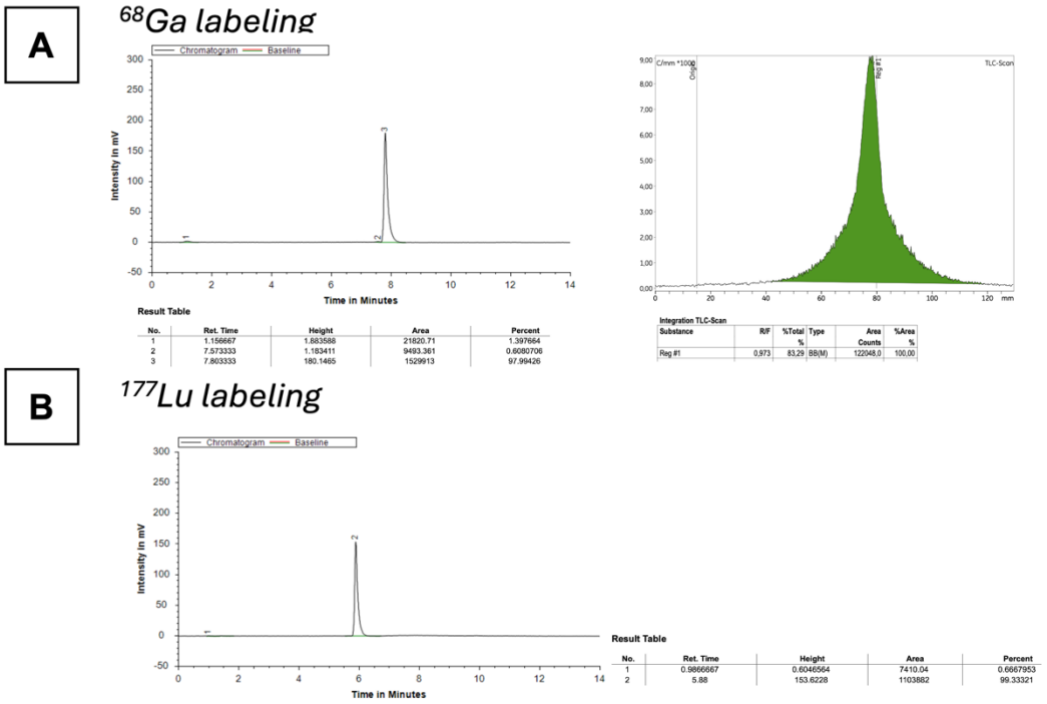
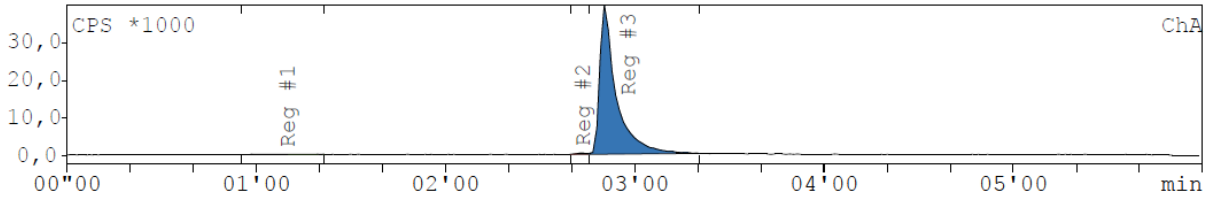


Figure S24: **A**: Analytical radio HPLC and TLC of [^{68}Ga]Ga-PS11; **B**: Analytical radio HPLC [^{177}Lu]Lu-PS11.

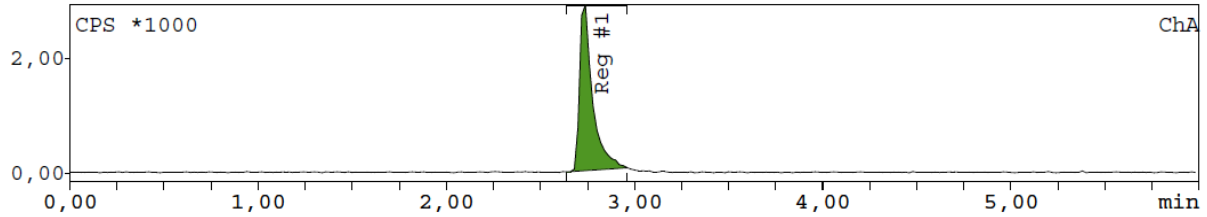
[^{68}Ga]Ga-PSMA-617



Integration ChA

Substanz	Ret s	Typ	Fläche Counts	%Fläche %
Reg #1	01'08	BB(M	1102,1	0,46
Reg #2	02'42	BD(M	913,8	0,38
Reg #3	02'52	DB(M	236028,6	99,15

[^{177}Lu]Lu-PSMA-617



Integration ChA

Substanz	Ret min	Typ	Fläche Counts	%Fläche %
Reg #1	2,74	BB(M	13679,01	100,00

Figure S25: Analytical radio HPLCs of [^{68}Ga]Ga-PSMA-617 and [^{177}Lu]Lu-PSMA-617.

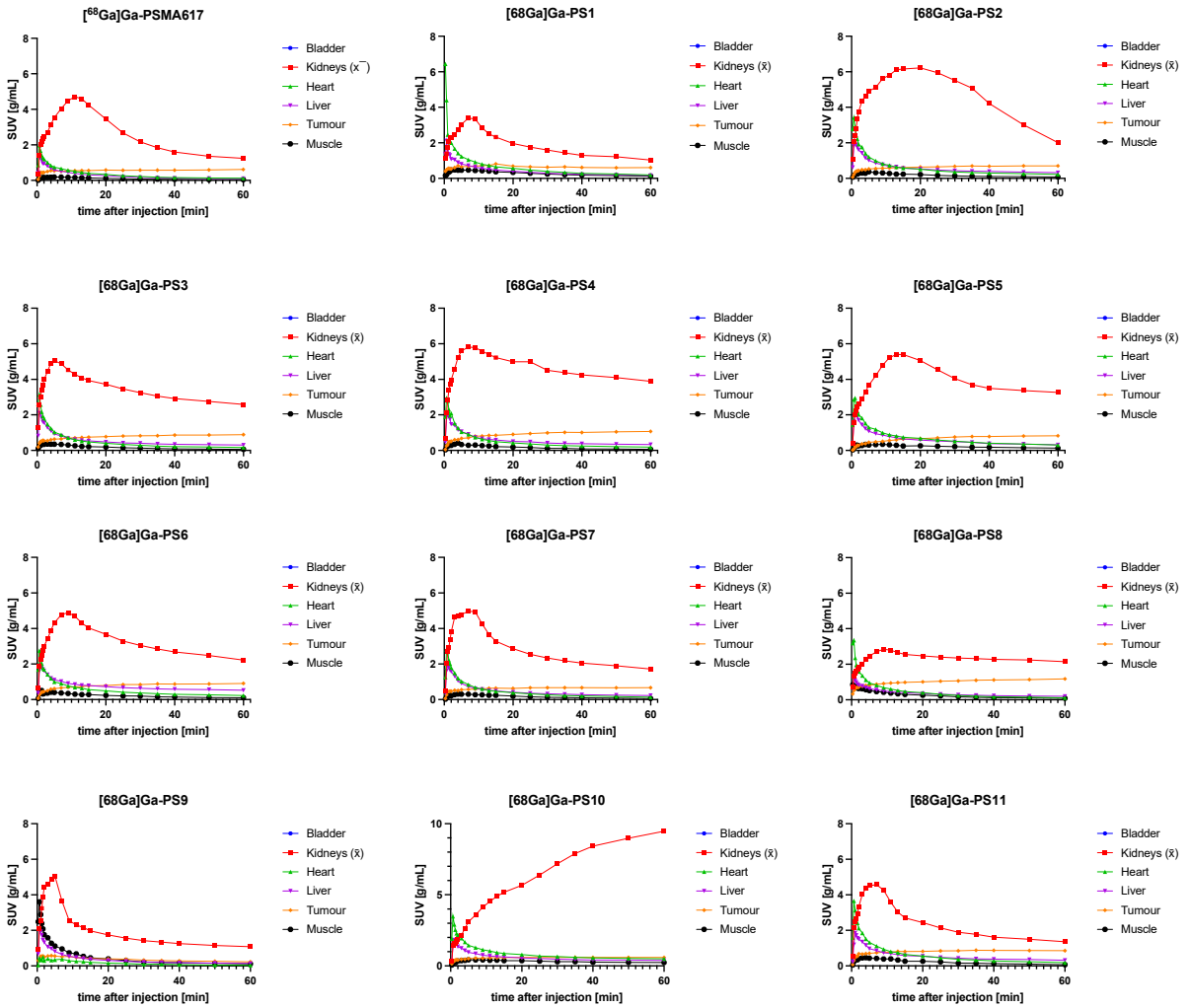


Figure S26: Time-activity curves (0 - 60 min) of PET scans from ^{68}Ga-labeled compounds showing accumulations in organs of interest in LNCaP tumor-bearing BALB/c nu/nu mice. SUV values of the two kidneys are displayed as an average.

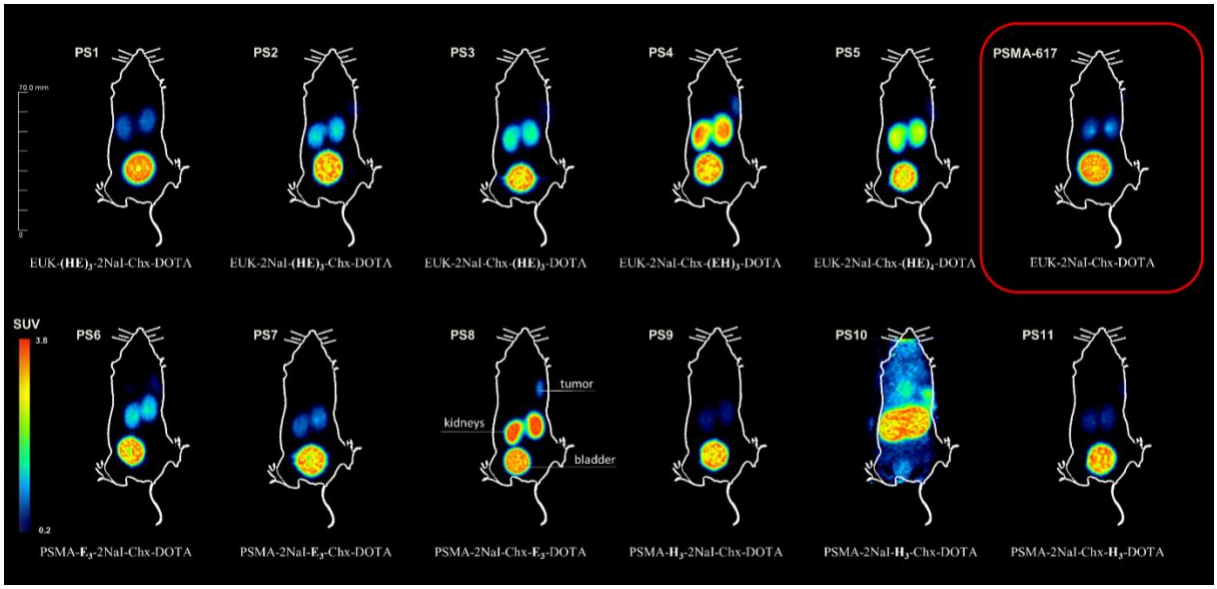


Figure S27: Maximum intensity projection from ^{68}Ga-labeled compounds 1 h p.i. of 0.5 nmol compound in LNCaP tumor-bearing BALB/c nu/nu mice.

S4. Supplementary Tables

Table S1: *p*-values of the Partition Coefficient (logD) of the ^{68}Ga-labeled compounds.

	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PSMA-617
PS1	-	0.614	0.701	0.192	0.073	0.313	0.230	0.016	0.156	0.613	0.279	0.013
PS2	-	-	0.514	0.119	0.193	0.514	0.666	0.058	0.509	0.598	0.413	0.016
PS3	-	-	-	0.024	0.438	0.244	0.286	0.061	0.268	0.713	0.798	0.024
PS4	-	-	-	-	0.532	0.140	0.105	0.024	0.117	0.810	0.463	0.010
PS5	-	-	-	-	-	0.094	0.006	0.009	0.017	0.982	0.343	0.009
PS6	-	-	-	-	-	-	0.383	0.699	0.565	0.072	0.094	0.302
PS7	-	-	-	-	-	-	-	0.091	0.205	0.336	0.027	0.043
PS8	-	-	-	-	-	-	-	-	0.224	0.229	0.043	0.011
PS9	-	-	-	-	-	-	-	-	-	0.271	0.001	0.087
PS10	-	-	-	-	-	-	-	-	-	-	0.798	0.152
PS11	-	-	-	-	-	-	-	-	-	-	-	0.031
PSMA-617	-	-	-	-	-	-	-	-	-	-	-	-

Table S2: Stability in mouse serum of the ^{177}Lu-labeled compounds.

Time [h]	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11
	[% intact substrate]										
0	100	100	100	100	100	100	100	100	100	100	100
6	97	100	100	100	100	100	100	100	81	97	100
24	95	98	100	100	100	96	100	100	61	91	100
48	91	96	100	100	100	94	100	100	39	64	100
72	85	93	98	100	97	94	100	100	35	63	100

Table S3: Stability in human serum of the ^{177}Lu-labeled compounds.

Time [h]	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11
	[% intact substrate]										
0	100	100	100	100	100	100	100	100	100	100	100
6	97	99	100	100	100	100	100	100	98	100	100
24	95	98	100	100	100	100	100	100	90	98	100
48	88	94	100	100	98	100	100	100	80	91	100
72	80	90	100	100	99	100	100	100	78	85	100

Table S4: *p*-values of the compounds of the Competitive Cell Binding Assays.

	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PSMA-617
PS1	-	0.11	0.03	0.0016	0.64	0.0064	0.041	<0.0001	0.35	0.86	0.0018	0.0008
PS2	-	-	0.49	0.057	0.88	0.12	0.066	0.0012	0.24	0.58	0.038	0.022
PS3	-	-	-	0.22	0.63	0.33	0.15	0.004	0.12	0.12	0.11	0.0079
PS4	-	-	-	-	0.36	0.91	0.50	0.0035	0.063	0.062	0.36	0.22
PS5	-	-	-	-	-	0.46	0.35	0.10	0.31	0.78	0.33	0.31
PS6	-	-	-	-	-	-	0.52	0.093	0.12	0.12	0.39	0.26
PS7	-	-	-	-	-	-	-	0.048	0.092	0.083	0.92	0.80
PS8	-	-	-	-	-	-	-	-	0.022	0.0080	0.019	0.0043
PS9	-	-	-	-	-	-	-	-	-	0.42	0.087	0.082
PS10	-	-	-	-	-	-	-	-	-	-	0.068	0.575
PS11	-	-	-	-	-	-	-	-	-	-	-	0.84
PSMA-617	-	-	-	-	-	-	-	-	-	-	-	-

Table S5: Surface Binding and Internalization of the ^{68}Ga-labeled compounds. Specific Surface Binding and Internalization were calculated by subtracting the unblocked values from the blocked values for each substance. Values are expressed as mean ± standard deviation.

	Surface Binding	Surface Binding Block	Internalization	Internalization Block	Specific Surface Binding	Specific Internalization
	[%Binding / %Internalization]					
PS1	1.15 ± 0.17	0.069 ± 0.012	0.66 ± 0.14	0.314 ± 0.024	1.09 ± 0.34	0.35 ± 0.3
PS2	1.01 ± 0.14	0.183 ± 0.024	0.55 ± 0.04	0.21 ± 0.036	0.83 ± 0.27	0.34 ± 0.24
PS3	1.32 ± 0.11	0.069 ± 0.129	0.67 ± 0.14	0.112 ± 0.022	1.26 ± 1.97	0.56 ± 0.42
PS4	1.43 ± 0.12	0.080 ± 0.009	0.81 ± 0.29	0.149 ± 0.047	1.35 ± 0.21	0.66 ± 0.68
PS5	1.41 ± 0.10	0.082 ± 0.029	0.67 ± 0.06	0.155 ± 0.022	1.33 ± 0.42	0.51 ± 0.25
PS6	0.76 ± 0.15	0.049 ± 0.02	0.60 ± 0.13	0.092 ± 0.032	0.71 ± 0.59	0.51 ± 0.58
PS7	0.64 ± 0.08	0.060 ± 0.021	0.40 ± 0.08	0.094 ± 0.025	0.58 ± 0.47	0.31 ± 0.49
PS8	0.99 ± 0.11	0.055 ± 0.011	0.71 ± 0.26	0.092 ± 0.032	0.94 ± 0.33	0.62 ± 0.72
PS9	0.94 ± 0.02	0.037 ± 0.009	0.59 ± 0.06	0.082 ± 0.005	0.90 ± 0.28	0.51 ± 0.17
PS10	0.57 ± 0.04	0.027 ± 0.005	0.51 ± 0.07	0.205 ± 0.020	0.55 ± 0.29	0.31 ± 0.24
PS11	0.81 ± 0.04	0.123 ± 0.015	0.66 ± 0.09	0.077 ± 0.009	0.69 ± 0.17	0.58 ± 0.26
PSMA-617	0.79 ± 0.13	0.062 ± 0.013	0.50 ± 0.05	0.210 ± 0.036	0.72 ± 0.38	0.28 ± 0.27

Table S6: *p*-values of the ^{68}Ga-labeled compounds of the Surface Binding Assay.

	PS1	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PSMA-617**
PS1	-	0.32	0.22	0.073	0.081	0.029	0.0021	0.19	0.057	0.0031	0.0254	0.0088
PS2	-	-	0.044	0.019	0.0080	0.10	0.0070	0.85	0.33	0.0066	0.0759	0.056
PS3	-	-	-	0.34	0.35	0.0072	0.0003	0.016	0.0014	0.0005	0.0023	0.0011
PS4	-	-	-	-	0.83	0.0043	0.0002	0.0056	0.0006	0.0004	0.0015	0.0005
PS5	-	-	-	-	-	0.0011	<0.0001	0.0021	0.0002	<0.0001	0.0003	0.0001
PS6	-	-	-	-	-	-	0.23	0.072	0.066	0.10	0.63	0.85
PS7	-	-	-	-	-	-	-	0.0031	0.0006	0.25	0.028	0.11
PS8	-	-	-	-	-	-	-	-	0.41	0.0024	0.058	0.042
PS9	-	-	-	-	-	-	-	-	-	<0.0001	0.0068	0.0545
PS10	-	-	-	-	-	-	-	-	-	-	0.0034	0.039
PS11	-	-	-	-	-	-	-	-	-	-	-	0.75
PSMA-617	-	-	-	-	-	-	-	-	-	-	-	-

Table S7: *p*-values of the ^{68}Ga-labeled compounds of the Internalization Assay.

	PS1	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PSMA-617**
PS1	-	0.27	0.93	0.42	0.94	0.61	0.025	0.76	0.43	0.19	0.99	0.051
PS2	-	-	0.24	0.21	0.049	0.56	0.049	0.36	0.37	0.54	0.12	0.18
PS3	-	-	-	0.51	0.96	0.59	0.030	0.83	0.38	0.18	0.95	0.046
PS4	-	-	-	-	0.38	0.33	0.045	0.66	0.20	0.17	0.46	0.051
PS5	-	-	-	-	-	0.44	0.0034	0.76	0.17	0.040	0.96	0.0036
PS6	-	-	-	-	-	-	0.068	0.55	0.90	0.40	0.53	0.16
PS7	-	-	-	-	-	-	-	0.072	0.014	0.14	0.013	0.087
PS8	-	-	-	-	-	-	-	-	0.42	0.28	0.79	0.12
PS9	-	-	-	-	-	-	-	-	-	0.21	0.27	0.043
PS10	-	-	-	-	-	-	-	-	-	-	0.041	0.68
PS11	-	-	-	-	-	-	-	-	-	-	-	0.014
PSMA-617	-	-	-	-	-	-	-	-	-	-	-	-

Table S8: SUV values of the ^{68}Ga-labeled compounds 1 h and 2 h p.i. of the relevant organs in PET imaging in LNCaP tumor-bearing BALB/c nu/nu mice. Values are expressed as mean ± standard deviation due to the VOIs drawn in PMOD.

Abbr.	tumor [g/ml]		kidneys [g/ml]		muscle [g/ml]	
	1 h	**2h**	**1 h**	**2h**	**1 h**	**2h**
PSMA-617	0.63 ± 0.02	0.52 ± 0.16	1.25 ± 0.14	0.28 ± 0.06	0.073 ± 0.004	0.02 ± 0.01
PS1	0.43 ± 0.06	0.30 ± 0.11	1.71 ± 0.20	1.32 ± 0.15	0.09 ± 0.01	0.08 ± 0.02
PS2	0.78 ± 0.05	0.74 ± 0.06	1.64 ± 0.10	1.01 ± 0.09	0.11 ± 0.02	0.11 ± 0.01
PS3	0.93 ± 0.08	0.92 ± 0.06	3.05 ± 0.01	1.56 ± 0.13	0.13 ± 0.03	0.1 ± 0.01
PS4	1.15 ± 0.04	1.07 ± 0.09	3.81 ± 0.15	2.77 ± 0.20	0.11 ± 0.02	0.1 ± 0.03
PS5	0.86 ± 0.06	0.94 ± 0.07	3.13 ± 0.16	2.37 ± 0.27	0.12 ± 0.02	0.09 ± 0.02
PS6	0.69 ± 0.07	0.85 ± 0.08	1.53 ± 0.08	0.40 ± 0.05	0.11 ± 0.02	0.11 ± 0.02
PS7	0.71 ± 0.05	0.56 ± 0.08	1.60 ± 0.12	0.59 ± 0.06	0.09 ± 0.01	0.12 ± 0.02
PS8	1.32 ± 0.08	0.86 ± 0.39	3.89 ± 0.65	2.70 ± 0.89	0.08 ± 0.02	0.11 ± 0.02
PS9	0.21 ± 0.05	0.12 ± 0.03	0.98 ± 0.09	0.74 ± 0.09	0.082 ± 0.002	0.07 ± 0.03
PS10	0.65 ± 0.04	0.37 ± 0.05	8.13 ± 1.31	1.09 ± 0.06	0.21 ± 0.03	0.06 ± 0.02
PS11	0.82 ± 0.07	0.88 ± 0.09	1.24 ± 0.08	0.77 ± 0.13	0.11 ± 0.02	0.10 ± 0.03