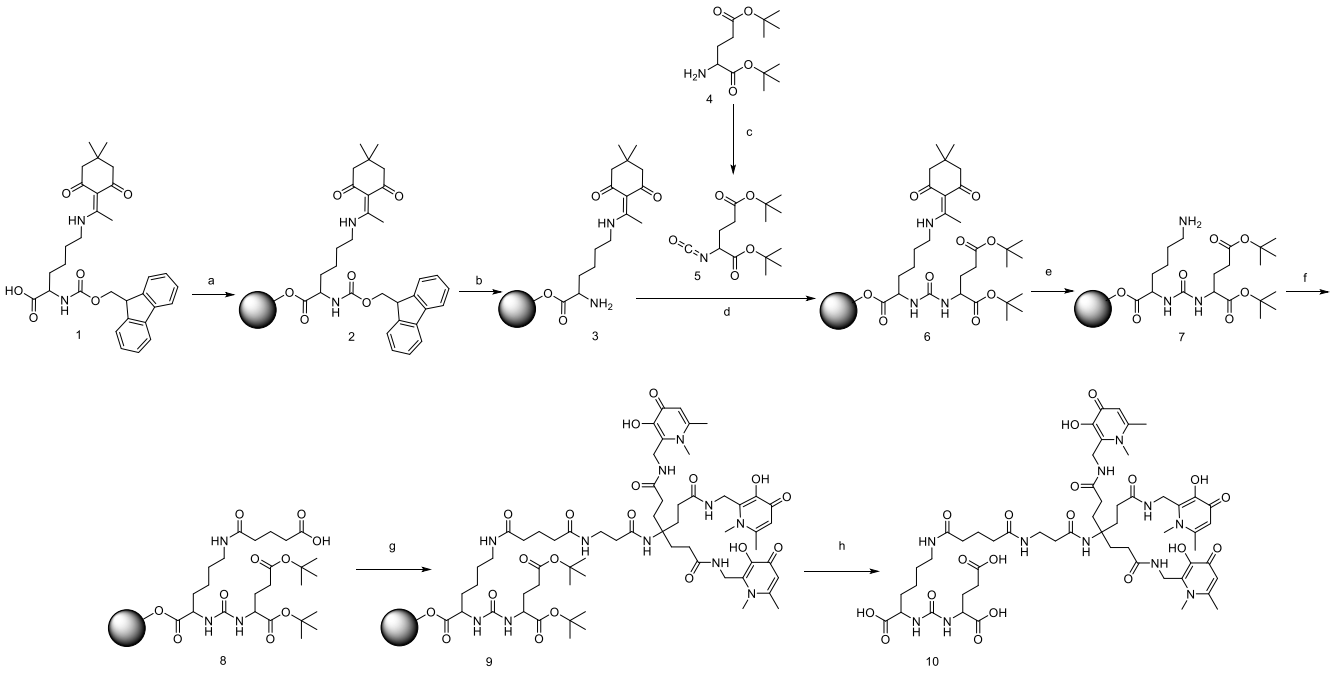
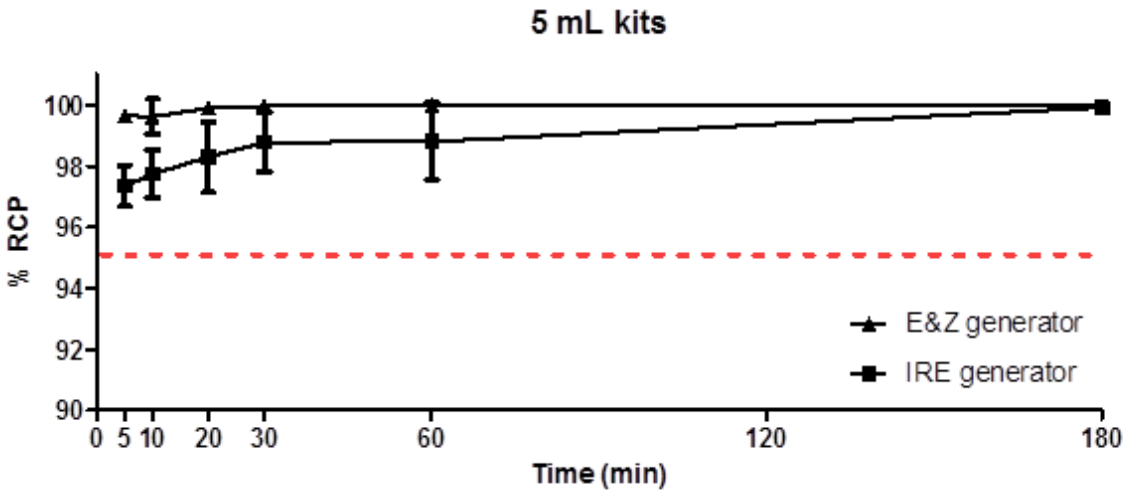


SUPPLEMENTAL FIGURE 1: Reaction scheme for synthesis of THP-PSMA. a. 2-chlorotrityl polymer bound resin beads, DIPEA, DCM; b. Piperidine, DMF; c. Triphosgene, DIPEA, DCM, 0 °C; d. Compound **3**, Compound **5**, DCM; e. Hydrazine hydrate, DMF; f. Glutaric anhydride, DIPEA, DMF; g. THP-NH_2, HATU, DIPEA, DMF, DMSO; h. TFA, phenol, TIPS, H_2O

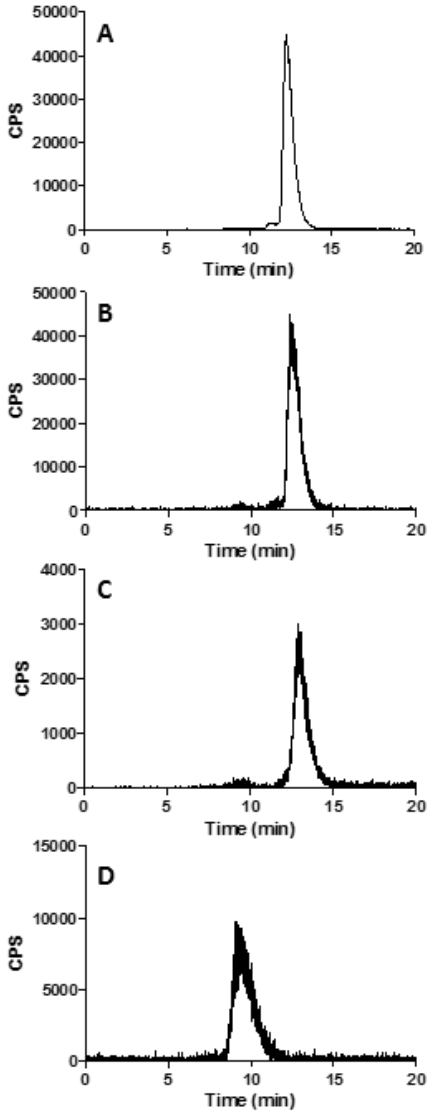
^{1}H NMR: (D_2O, 700 MHz)	1.37 (m, 2H), 1.50 (m, 2H), 1.7 (m, 1H), 1.78 (m, 2H), 1.85 (m, 1H), 1.92 (t, *J* = 8.09, 6H), 1.96 (m, 1H), 2.14 (m, 1H), 2.18 (q, *J*= 6.59, 4H), 2.22 (t, *J* = 8.09, 6H), 2.35 (t, *J* =6.64, 2H), 2.49 (t, *J* = 7.49, 2H), 2.57 (s, 9H), 3.13 (t, *J* = 6.98, 2H), 3.36 (t, *J* = 6.64, 2H), 3.91 (s, 9H), 4.15 (dd, *J* = 8.99, 5.59 1H), 4.23 (dd, *J* = 8.70, 4.97 1H), 4.67 (s, 9H), 7.00 (s, 3H)
^{13}C NMR: (D_2O, pH 3, 175 MHz)	20.5, 21.9, 22.4, 26.5, 27.8, 29.3, 29.4, 30.1, 30.7, 34.8, 35.5, 35.9, 36.1, 38.5, 39.0, 52.8, 113.6, 138.1, 143.2, 149.9, 159.4, 161.0, 173.2, 175.5, 175.7, 176.2, 176.6, 177.4
ESI-MS: m/z $[C_{54}H_{77}N_{11}O_{19} + H]^{+}$	observed monoisotopic peak = 1184.5494 calculated = 1184.5475
ESI-MS: m/z $[C_{54}H_{77}N_{11}O_{19} + 2H]^{+2}$	observed monoisotopic peak = 592.7768, calculated = 592.7788
ESI-MS: m/z $[C_{54}H_{77}N_{11}O_{19} + 3H]^{+3}$	observed monoisotopic peak = 395.5202, calculated = 395.5221
m/z $[C_{54}H_{74}N_{11}O_{19}Ga + H]^{+}$	observed monoisotopic peak = 1250.45092 calculated = 1250.449102
m/z $[C_{54}H_{74}N_{11}O_{19}Ga + 2H]^{+2}$	observed monoisotopic peak = 625.72846, calculated = 625.72819

SUPPLEMENTAL TABLE 1: ^{1}H NMR, ^{13}C NMR and ESI-MS data for THP-PSMA confirming its identity.



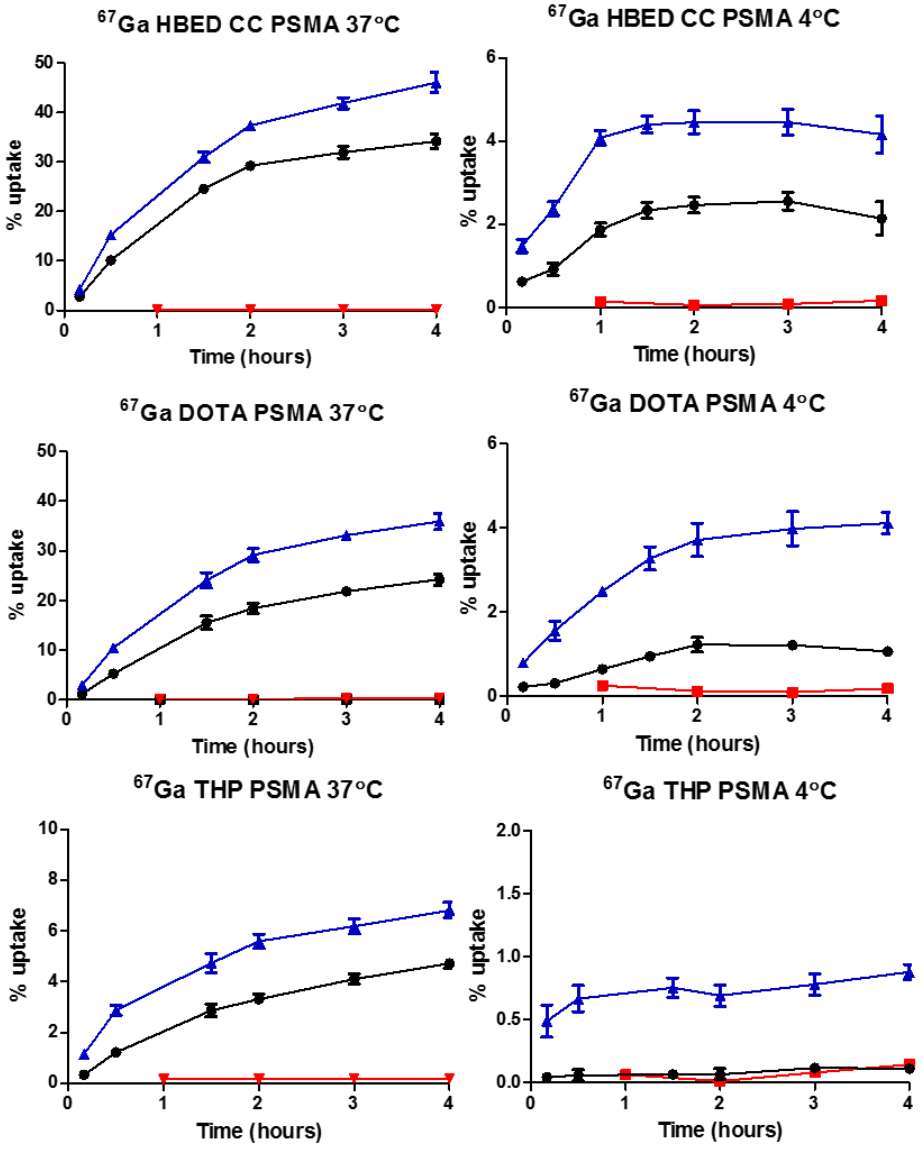
SUPPLEMENTAL FIGURE 2: Radiochemical purity as measured by iTLC of ^{68}Ga-THP-PSMA labeled with 5mL one step kits as a function of incubation time at room temperature, with both an IRE generator and an E&Z generator. Broken red line represents quality control threshold of 95% (n=3, mean ± SD)

Serum Stability:

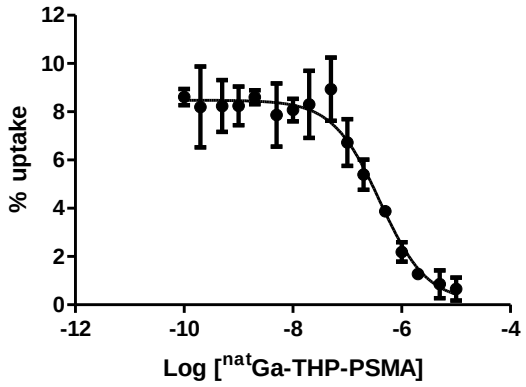


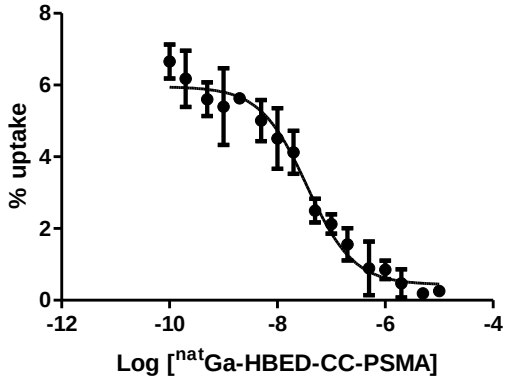
SUPPLEMENTAL FIGURE 3. Size exclusion HPLC chromatograms of **(A)** ^{68}Ga-THP-PSMA; **(B)** ^{68}Ga-THP-PSMA after 6 hours incubation in human serum at 37°C; <2% transchelation to serum **(C)** ^{67}Ga-THP-PSMA after 8 days incubation in human

serum at 37°C <5% transchelation to serum **(D)** $^{68}Ga^{3+}$ incubated in human serum for 6 hours. All chromatograms have been decay corrected.

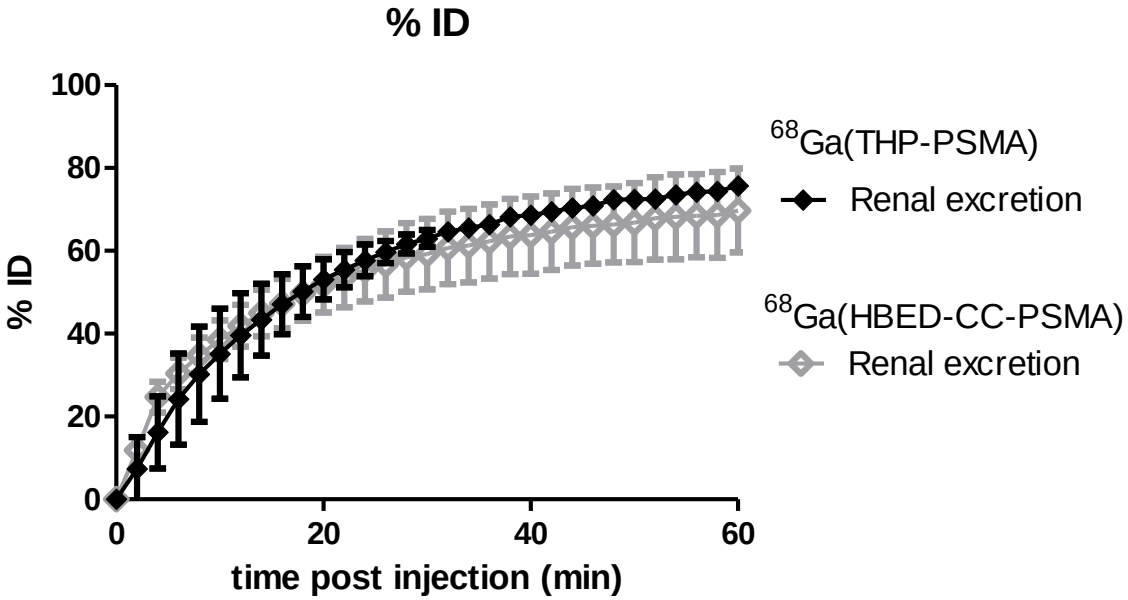


SUPPLEMENTAL FIGURE 4: Uptake of 1nM ^{67}Ga-HBED-CC-PSMA ^{67}Ga-DOTA-PSMA and ^{67}Ga-THP-PSMA over time at 4°C and 37°C, 1 × 10^6 cells/mL. Combined internalized and cell surface bound activity DU145-PSMA cells (blue); internalized activity DU145-PSMA cells (black); internalized and cell surface bound activity DU145 cells (red). (n=4, mean ± SD) per time point per tracer. DU145 and DU145-PSMA cells were provided by Dr Florian Kampmeier (*1*).





SUPPLEMENTAL FIGURE 5: Representative IC_{50} experiments for ^{nat}Ga-THP-PSMA and ^{nat}Ga-HBED-CC-PSMA with 1nM ^{68}Ga-DOTA-PSMA as the probe. (n=4) for each concentration. IC_{50} values in main text are the mean of at least 3 experiments.



SUPPLEMENTAL FIGURE 6. Time activity curves of SCID beige mice bearing DU145-PSMA tumors, derived from PET imaging data from mice imaged with ^{68}Ga-THP-PSMA or ^{68}Ga-HBED-CC-PSMA during one hour post injection. Plotted data is the mean and standard deviation of three mice calculated from the total % ID in the kidneys and bladder combined.

	^{68}Ga-THP-PSMA			^{68}Ga-HBED-CC-PSMA
	Group 1	Group 4	Group 3	Group 2
Tumor type	DU145-PSMA	DU145	DU145-PSMA Blocked PMPA	DU145-PSMA
	(n=3)	(n=3)	(n=3)	(n=3)
Tumor	16.4 ± 7.4	0.69 ± 0.7	1.40 ± 0.2	19.5 ± 7.6
Blood	0.73 ± 0.4	1.23 ± 1.7	1.11 ± 0.7	2.18 ± 1.2
Intestines	0.35 ± 0.1	0.64 ± 0.6	0.71 ± 0.3	1.20 ± 0.5
Kidney	40.4 ± 34	52.3 ± 77	5.20 ± 5.0	63.4 ± 23
Liver	0.45 ± 0.2	0.59 ± 0.7	0.60 ± 0.2	0.69 ± 0.1
Lungs	0.50 ± 0.2	0.94 ± 1.3	0.72 ± 0.3	1.29 ± 0.4
Muscle	0.51 ± 0.4	0.31 ± 0.4	0.21 ± 0.1	0.26 ± 0.1
Salivary glands	0.28 ± 0.1	0.48 ± 0.7	0.33 ± 0.2	0.76 ± 0.1
Spleen	3.70 ± 1.3	3.61 ± 3.1	0.75 ± 0.1	17.6 ± 6.1
Tumor to organ ratios				
Tumor to blood	30.8 ± 28	0.93 ± 0.4	1.64 ± 0.9	11.0 ± 6.0
Tumor to muscle	44.6 ± 28	3.16 ± 1.1	9.79 ± 6.8	77.0 ± 34
Tumor to spleen	4.38 ± 0.8	0.17 ± 0.1	1.87 ± 0.3	1.16 ± 0.42

SUPPLEMENTAL TABLE 2. Ex vivo biodistribution at 1 hour post injection for PET-imaged mice. Mice bearing DU145-PSMA tumors were injected with ^{68}Ga-THP-PSMA, ^{68}Ga-THP-PSMA plus blocking agent PMPA, or ^{68}Ga-HBED-CC-PSMA. Mice bearing control DU145 tumors were imaged with ^{68}Ga-THP-PSMA only.

REFERENCES

1. Kampmeier F, Williams JD, Maher J, Mullen GE, Blower PJ. Design and preclinical evaluation of a 99mTc-labelled diabody of mAb J591 for SPECT imaging of prostate-specific membrane antigen (PSMA). *EJNMMI Res.* 2014;4:13.