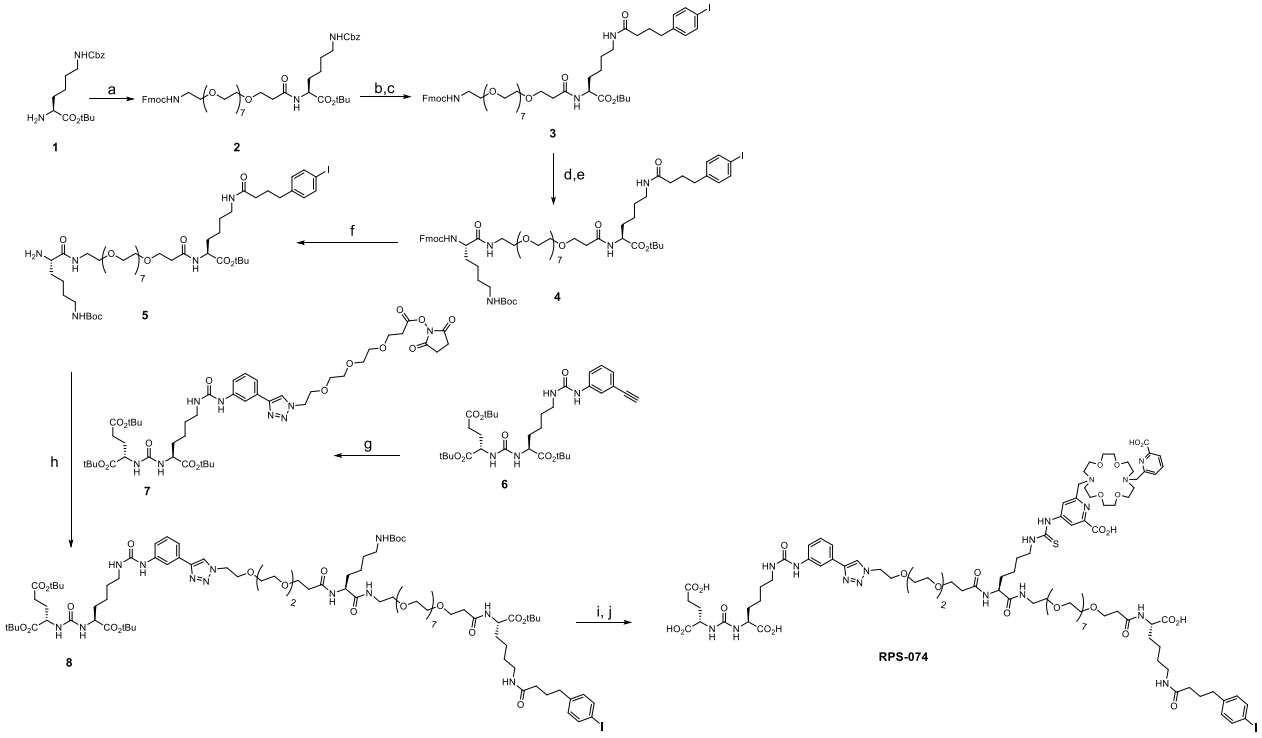
Synthesis of RPS-074

All solvents and reagents were purchased from commercial vendors and used without further purification. The intermediate di-*tert*-butyl (((*S*)-1-(*tert*-butoxy)-6-(3-(3-ethynylphenyl)ureido)-1-oxohexan-2-yl)carbamoyl)-L-glutamate (**6**) *(1)* and macropa-NCS *(2)* were synthesized according to literature procedures. Compounds were purified using silica chromatography on VWR® High Purity Silica Gel 60 Å preparative TLC on silica-coated glass plates (Analtech), or flash chromatography using a CombiFlash Rf+ (Teledyne Isco) system. Preparative HPLC was performed using an XBridge™ Prep C18 5µm OBD™ 19 x 100 mm column (Waters) on a dual pump Agilent ProStar HPLC fitted with an Agilent ProStar 325 Dual Wavelength UV-Vis Detector. UV absorption was monitored at 220 nm and 280 nm. A binary solvent system was used, with solvent A comprising H_2O + 0.01% TFA and solvent B consisting of 90% v/v MeCN/H_2O + 0.01% TFA. Purification was achieved using the following gradient HPLC method: Solvent A with 0%B 0-1 min., a linear increase of 0-100%B from 1-28 min., and a linear decrease of 100-0%B from 28-30 min.

Final products were identified and characterized using thin layer chromatography, analytical HPLC and mass spectrometry. NMR spectroscopy was used to confirm the structure of compound **6** and macropa-NCS. NMR analyses were performed using a Bruker Avance III 500 MHz spectrometer. Spectra are reported in $CDCl_3$ or DMSO-d6. Analytical HPLC was performed using an XSelect™ CSH™ C18 5µm 4.6 x 50 mm column (Waters). Mass determinations were performed by LCMS analysis using a Waters ACQUITY UPLC® coupled to a Waters SQ Detector 2. The purity of all compounds evaluated in the biological assay was > 95% purity as judged by analytical HPLC.



Supplemental Figure 1. **The synthesis of RPS-074**. a. FmocHN-PEG8-NHS, NEt_3; b. H_2, Pd/C; c. 2,5-dioxopyrrolidin-1-yl 4-(4-iodophenyl)butanoate, NEt_3; d. $HNEt_2$; e. Fmoc-L-Lys(Boc)-OSu, NEt_3; f. $HNEt_2$; g. Azido-PEG3-NHS, $CuSO_4$, sodium ascorbate; h. NEt_3; i. TFA; j. macropa-NCS, NEt_3.

***tert*-Butyl N^2-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16,19,22,25,28-nonaoxa-4-azahentriacontan-31-oyl)-N^6-((benzyloxy)carbonyl)-*L*-lysinate (2)**

To a stirred mixture of Fmoc-*N*-amido-PEG-8-acid (663 mg, 1.0 mmol), N^{ε}-*Z*-Lys-OtBu hydrochloride (446 mg, 1.2 mmol) and HATU (456 mg, 1.2 mmol) in DMF (10 mL) was added DIPEA (260 mg, 2.0 mmol), and the reaction was stirred overnight at room temperature under N_2. The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography (0-10% MeOH in CH_2Cl_2) to give compound **2** as a colorless oil (845 mg, 86%). Mass (ESI+): 983.0 $[M+H]^+$. Calc. Mass: 981.5.

***tert*-Butyl N^2-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16,19,22,25,28-nonaoxa-4-azahentriacontan-31-oyl)-N^6-(4-(4-iodophenyl)butanoyl)-*L*-lysinate (3)**

Compound **2** (1.45 g, 1.48 mmol) was dissolved in MeOH (25 mL). 10% Palladium on charcoal (15 mg) was added, and the suspension was stirred in a three-neck flask at room temperature for 10 min. The flask was evacuated and then placed under an H_2 atmosphere. The suspension was then stirred at room temperature for 5 h before it was filtered through celite. The filter cake was washed with MeOH, and the combined filtrate was concentrated under reduced pressure to give the amine as a yellow oil (1.17 g, 93%) that was used without further purification. Mass (ESI+): 849.4 $[M+H]^+$. Calc. Mass: 848.0.

To a solution of the amine (865 mg, 1.01 mmol) and 2,5-dioxopyrrolidin-1-yl 4-(4-iodophenyl)butanoate (387 mg, 1.00 mmol) in CH_2Cl_2 (20 mL) was added NEt_3 (167 µL, 1.20 mmol). The resulting solution was stirred at room temperature under Ar for 4 h. The solution was then washed successively with 1% v/v $AcOH/H_2O$ and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a yellow oil. The crude product was purified by flash chromatography (0-30% MeOH in CH_2Cl_2) and compound **3** was isolated as a yellow oil (360 mg, 32%). Mass (ESI+): 1120.9 $[M+H]^+$. Calc. Mass: 1119.5.

***tert*-Butyl N^2-((*S*)-10-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2,2-dimethyl-4,11-dioxo-3,15,18,21,24,27,30,33,36-nonaoxa-5,12-diazanonatriacontan-39-oyl)-N^6-(4-(4-iodophenyl)butanoyl)-*L*-lysinate (4)**

A solution of **3** (360 mg, 0.32 mmol) and diethylamine (0.67 mL, 6.48 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 7 h. The solution was concentrated under reduced pressure and the crude residue was purified by flash chromatography (0-30% MeOH in CH_2Cl_2). The fractions containing the product were combined and concentrated to give the amine as a yellow oil (96 mg, 33%). Mass (ESI+): 899.2 $[M+H]^+$. Calc. Mass: 897.4.

To a solution of the amine (96 mg, 107 µmol) and Fmoc-*L*-Lys(Boc)-OSu (62 mg, 110 µmol) in CH_2Cl_2 (5 mL) was added NEt_3 (28 µL, 200 µmol). The mixture was stirred overnight at room temperature under Ar. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (0-30% MeOH in CH_2Cl_2). The desired product co-eluted with a minor impurity, therefore the mixture was purified a second time by prep TLC (10% v/v $MeOH/CH_2Cl_2$). Compound **4** was isolated as a colorless oil (78 mg, 51%). Mass (ESI+): 1349.0 $[M+H]^+$. Calc. Mass: 1347.6.

***tert*-Butyl N^2-((*S*)-10-amino-2,2-dimethyl-4,11-dioxo-3,15,18,21,24,27,30,33,36-nonaoxa-5,12-diazanonatriacontan-39-oyl)-N^6-(4-(4-iodophenyl)butanoyl)-*L*-lysinate (5)**

A solution of **4** (73 mg, 54 μmol) and diethylamine (0.5 mL, 4.83 mmol) in CH_2Cl_2 (2 mL) was stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude product was dissolved in MeOH and purified by prep TLC (10% v/v MeOH in CH_2Cl_2). Amine **5** was isolated as a pale oil (25 mg, 41%). Mass (ESI+): 1127.7 $[M+H]^+$. Calc. Mass: 1126.2.

Di-*tert*-butyl (((*S*)-1-(tert-butoxy)-6-(3-(3-(1-(2-(2-(2-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)phenyl)ureido)-1-oxohexan-2-yl)carbamoyl)-*L*-glutamate (7)

A solution of 0.5M $CuSO_4$ (100 μL) and 1.5M sodium ascorbate (100 μL) was mixed until the brown color was converted to orange. This mixture was then added to a solution of **6** (315 mg, 0.5 mmol) and azido-PEG3-NHS (177 mg, 0.5 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 2 h. It was then diluted with CH_2Cl_2 and washed with H_2O. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a pale oil. The crude product was purified by flash chromatography (0-30% MeOH in CH_2Cl_2) to give compound **7** as a clear oil (460 mg, 95%). Mass (ESI+): 975.9 $[M+H]^+$. Calc. Mass: 974.5.

Di-*tert*-butyl (((*S*)-1-(*tert*-butoxy)-6-(3-(3-(1-((14*S*,45*S*)-45-(*tert*-butoxycarbonyl)-14-(4-((*tert*-butoxycarbonyl)amino)butyl)-54-(4-iodophenyl)-12,15,43,51-tetraoxo-3,6,9,19,22,25,28,31,34,37,40-undecaoxa-13,16,44,50-tetraazatetrapentacontyl)-1*H*-1,2,3-triazol-4-yl)phenyl)ureido)-1-oxohexan-2-yl)carbamoyl)-*L*-glutamate (8)

To a solution of amine **5** (25 mg, 22 μmol) in CH_2Cl_2 (4 mL) was added a solution of ester **7** (24 mg, 25 μmol) and NEt_3 (7 μL, 50 μmol) in CH_2Cl_2 (1 mL). The reaction was stirred for 5 h at room temperature under Ar. Then the reaction was concentrated under reduced pressure and the crude residue was dissolved in EtOAc (1 mL) and purified by prep TLC (90% EtOAc in hexanes) to give compound **8** as a pale oil (33 mg, 76%). Mass (ESI+): 994.3 $[(M+2H)/2]^+$. Calc. Mass: 1986.2.

(((*S*)-1-Carboxy-5-(3-(3-(1-((14*S*,45*S*)-45-carboxy-14-(4-(3-(2-carboxy-6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)pyridin-4-yl)thioureido)butyl)-54-(4-iodophenyl)-12,15,43,51-tetraoxo-3,6,9,19,22,25,28,31,34,37,40-undecaoxa-13,16,44,50-tetraazatetrapentacontyl)-1*H*-1,2,3-triazol-4-yl)phenyl)ureido)pentyl)carbamoyl)-*L*-glutamic acid (RPS-074)

Compound **8** (33 mg, 16 μmol) was dissolved in CH_2Cl_2 (2 mL). Then TFA (0.5 mL) was added and the reaction was stirred overnight at room temperature. The solvent was removed under N_2 flow and the crude product was lyophilized to give a white residue (22 mg, 83%). Mass (ESI+): 832.0 $[(M+2H)/2]^+$. Calc. Mass: 1661.8.

To a solution of the free amine (13 mg, 7.8 μmol) and TEA (0.22 mL, 1.56 mmol) in DMF (1 mL) was added a solution of macropa-NHS (6 mg, 10 μmol) in DMF (1 mL). The resulting mixture was stirred for 90 min at room temperature. The reaction was concentrated under reduced pressure and the crude product was purified by prep HPLC. The peak corresponding to the desired product was collected and lyophilized to give **RPS-074** as a white powder (4.5 mg, 26%). Mass (ESI+): 2252.6 $[M+H]^+$; 1126.6 $[(M+2H)/2]^+$. Calc. Mass: 2251.3.

References

1. Kelly J, Amor-Coarasa A, Nikolopoulou A, et al. Synthesis and pre-clinical evaluation of a new class of high-affinity ^{18}F-labeled PSMA ligands for detection of prostate cancer by PET imaging. Eur J Nucl Med Mol Imaging 2017;44:647-61.

2. Thiele NA, Brown V, Kelly JM, et al. An Eighteen-Membered Macrocyclic Ligand for Actinium-225 Targeted Alpha Therapy. Angew Chem Int Ed. 2017;56:14712-14717.

Synthesis of DOTA-Lys-IPBA

2,5-dioxopyrrolidin-1-yl 4-(4-iodophenyl)butanoate (9)

A solution of 4-(4-iodophenyl)butanoic acid (1.16 g, 4.0 mmol), N-hydroxysuccinimide (483 mg, 4.2 mmol), EDC.HCl (768 mg, 4.0 mmol) and 4-DMAP (5.8 mg, 47 µmol) in CH_2Cl_2 (30 mL) was stirred for 20 h. Then the reaction mixture was washed successively with 1M HCl, saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give NHS ester **9** as a white powder (1.29 g, 83%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.61 (d, 2H, J = 7.2 Hz). 6.95 (d, 2H, J = 7.6 Hz), 2.83 (s, 4H), 2.67 (t, 2H, J = 7.6 Hz), 2.59 (t, 2H, J = 7.3 Hz), 2.03 (quint, 2H, J = 7.3 Hz).

***N*^{2}-(tert-butoxycarbonyl)-*N*^{6}-(4-(4-iodophenyl)butanoyl)-*L*-lysine (10)**

Boc-*L*-Lys-OH (871 mg, 3.53 mmol) was suspended in DMF (10 mL) and stirred at room temperature. To the stirred suspension was slowly added a solution of NHS ester **9** (1.29 g, 3.33 mmol) and NEt_3 (557 µL, 4.00 mmol) in DMF (5 mL). The resulting suspension was stirred overnight at room temperature. The reaction was quenched with 1M HCl (2 mL), and the solvent was removed under reduced pressure. The crude residue was dissolved in CH_2Cl_2 and washed successively with 1M HCl, saturated $NaHCO_3$ solution and brine. The organic fraction was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give Boc-Lys-IPBA (**10**) as a clear foam (1.25 g, 72%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.57 (d, 2H, J = 7.7 Hz), 6.91 (d, 2H, J = 7.8 Hz), 5.94 (br s, 1H), 5.32 (br s, 1H), 4.21 (m, 1H), 3.21 (m, 2H), 2.56 (t, 2H, J = 7.6 Hz), 2.15 (t, 2H, J = 7.1 Hz), 1.90 (quint, 2H, J = 7.5 Hz), 1.88 (m, 1H), 1.69 (m, 1H), 1.51 (m, 2H), 1.42 (s, 9H), 1.41 (m, 2H). Mass (ESI+): 519.3 $(M+H)^+$. Calc. Mass: 518.4.

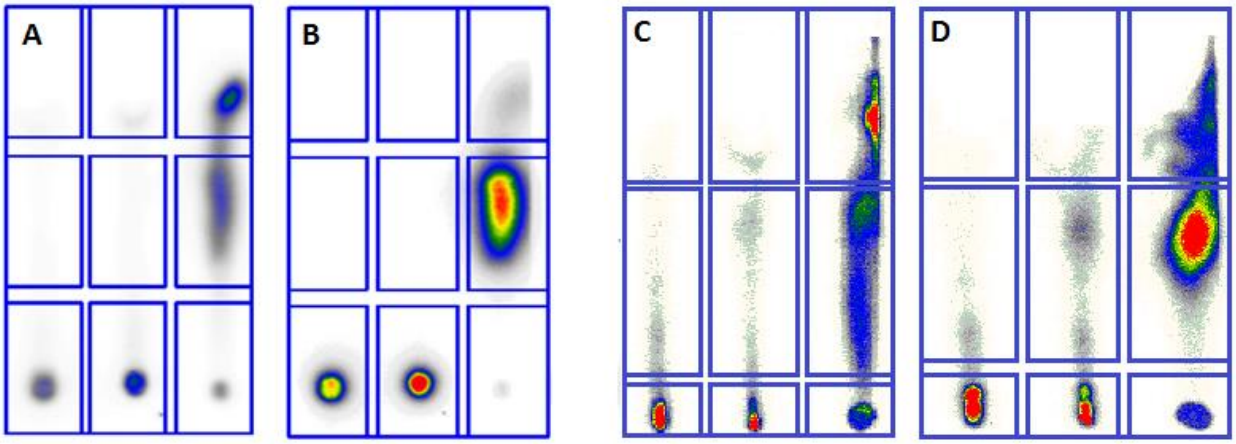
***N*^{6}-(4-(4-iodophenyl)butanoyl)-*L*-lysine (11)**

Boc-Lys-IPBA (518 mg, 1.0 mmol) was dissolved in 10 mL of a 20% v/v TFA/CH_2Cl_2 solution and stirred overnight at room temperature. The solvents were removed under a stream of N_2 and Lys-IPBA (**11**) was isolated as a colorless oil (402 mg; 96%).^{1}H NMR (DMSO, 500 MHz): δ 7.75 (br s, 1H), 7.61 (d, 2H, J = 7.8 Hz), 6.99 (d, 2H, J = 7.8 Hz), 3.79 (m, 1H), 2.99 (m, 2H), 2.02 (t, 2H, J = 7.3 Hz), 1.74 (quint, 2H, J = 7.4 Hz), 1.37 (m, 4H), 1.24 (m, 2H). Mass (ESI+): 419.2 $(M+H)^+$. Calc. Mass: 418.3.

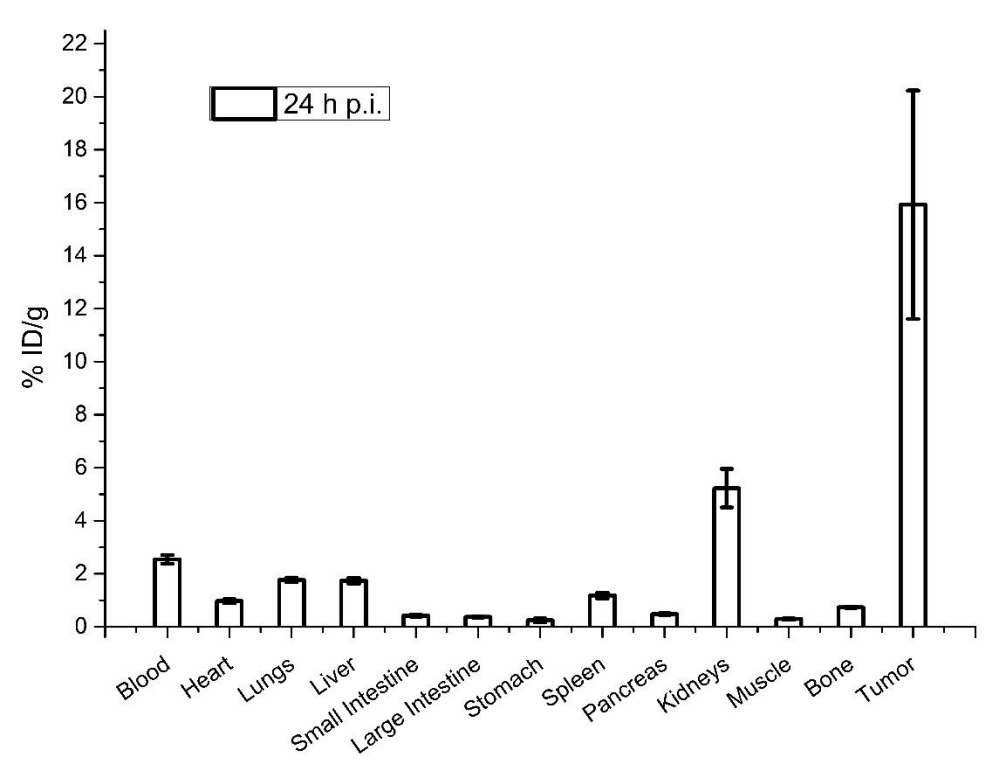
2,2',2'',2'''-(2-(4-(3-((*S*)-1-carboxy-5-(4-(4-iodophenyl)butanamido)pentyl)thioureido)benzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid (DOTA-Lys-IPBA)

To a solution of Lys-IPBA (11 mg, 26 µmol) and DIPEA (17 µL, 100 µmol) in DMF (1 mL) was added a solution of *p*-SCN-Bn-DOTA·2.5Cl·2.5H_2O (8 mg, 11.6 µmol) in H_2O (1 mL). The reaction was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and the crude residue was purified by prep HPLC. The peak corresponding to the product was collected and lyophilized to give

DOTA-Lys-IPBA as a white powder (5 mg, 43%). ^{1}H NMR (DMSO, 500 MHz): δ 9.72 (br s, 1H), 7.89 (d, 2H, J = 7.6 Hz), 7.77 (m, 1H), 7.61 (d, 2H, J = 7.1 Hz), 7.51 (d, 2H, J = 7.8 Hz), 7.24 (m, 2H), 6.99 (d, 2H, J = 7.8 Hz), 4.84 (m, 1H), 3.70-3.04 (m, 14H), 3.01 (m, 4H), 2.02 (t, 2H, J = 7.1 Hz), 1.76 (m, 4H), 1.39 (m, 2H), 1.31 (m, 2H). Mass (ESI+): 971.0 $(M+H)^{+}$. Calc. Mass: 969.9.



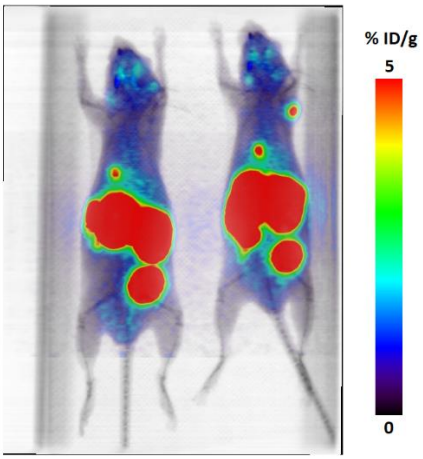
Supplemental Figure 2. Quality Control Analysis of ^{225}Ac-RPS-074 and ^{225}Ac-RPS-072 by radioTLC. A. Radiolabeling of ^{225}Ac-RPS-074 analyzed by phosphorimager 1 h after running the TLC plate in the mobile phase. B. Visualization 8 h after running the plate. C. Radiolabeling of ^{225}Ac-RPS-072 analyzed by phosphorimager 1 h after running the TLC plate in the mobile phase. D. Visualization 8 h after running the plate. Left lane: Purified reaction product. Center lane: Reaction prior to purification by SPE. Right lane: $^{225}Ac(NO_3)_3$ control.



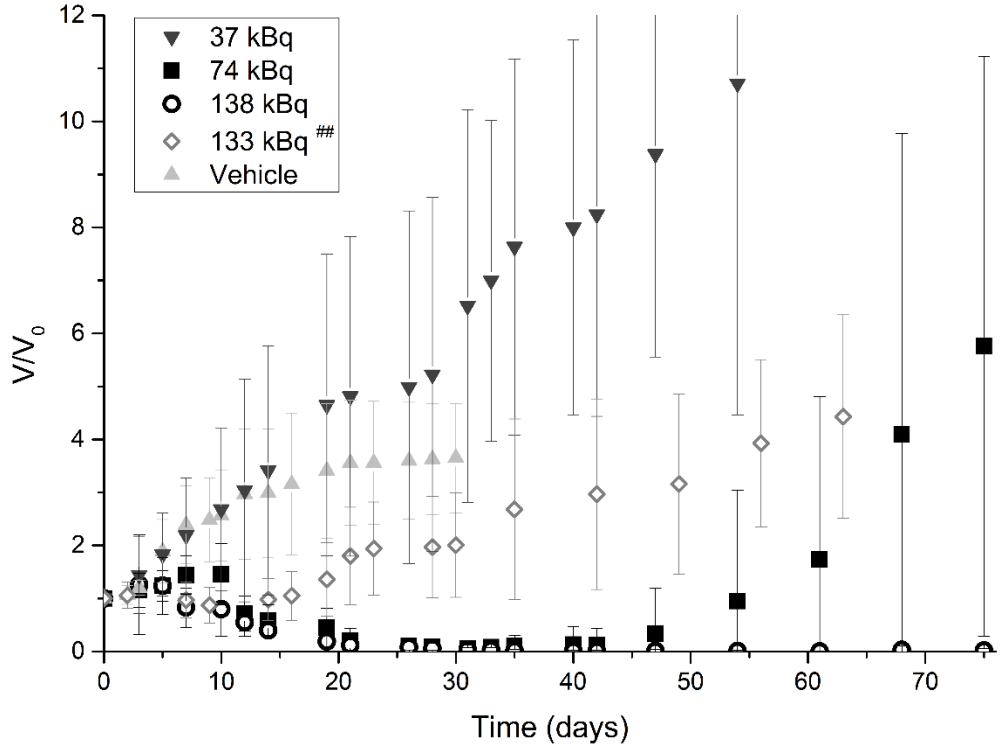
Supplemental Figure 3. Biodistribution of ^{225}Ac-RPS-072 at 24 h. Plot showing the tissue distribution of 105 kBq ^{225}Ac-RPS-072 in male BALB/c nu/nu mice bearing LNCaP xenograft tumors (n=4).

Target Organ	Alpha	Beta	Photon	Total (%ID/g) (mSv/MBq)	Total (%ID/organ) (mSv/MBq)
LLI Wall	1.56E+01	1.01E-02	4.30E-03	1.56E+01	2.11E+01
Small Intestine	6.12E+00	3.97E-03	2.02E-03	6.12E+00	1.05E+01
Stomach Wall	4.84E+00	3.14E-03	8.89E-04	4.84E+00	6.19E+00
ULI Wall	1.91E+01	1.24E-02	2.52E-03	1.91E+01	1.91E+01
Heart Wall	1.88E+01	1.22E-02	2.09E-03	1.89E+01	4.02E+01
Kidneys	5.71E+01	3.71E-02	8.44E-03	5.72E+01	8.37E+01
Liver	1.55E+01	1.00E-02	3.37E-03	1.55E+01	1.94E+01
Lungs	3.66E+00	2.38E-03	8.20E-04	3.66E+00	5.49E+00
Muscle	1.44E-02	9.36E-06	9.74E-04	1.54E-02	3.00E-02
Pancreas	7.95E+00	5.16E-03	1.83E-03	7.96E+00	1.37E+01
Red Marrow	0.00E+00	0.00E+00	6.92E-04	6.92E-04	6.92E-04
Osteogenic Cells	1.48E-02	1.37E-05	9.00E-04	1.57E-02	1.57E-02
Spleen	1.26E+01	8.17E-03	2.37E-03	1.26E+01	1.36E+01
Tumor	6.00E+02	3.90E-01	5.96E-02	6.01E+02	4.40E+02
Total Body	1.36E+00	8.84E-04	1.04E-03	1.36E+00	1.66E+00

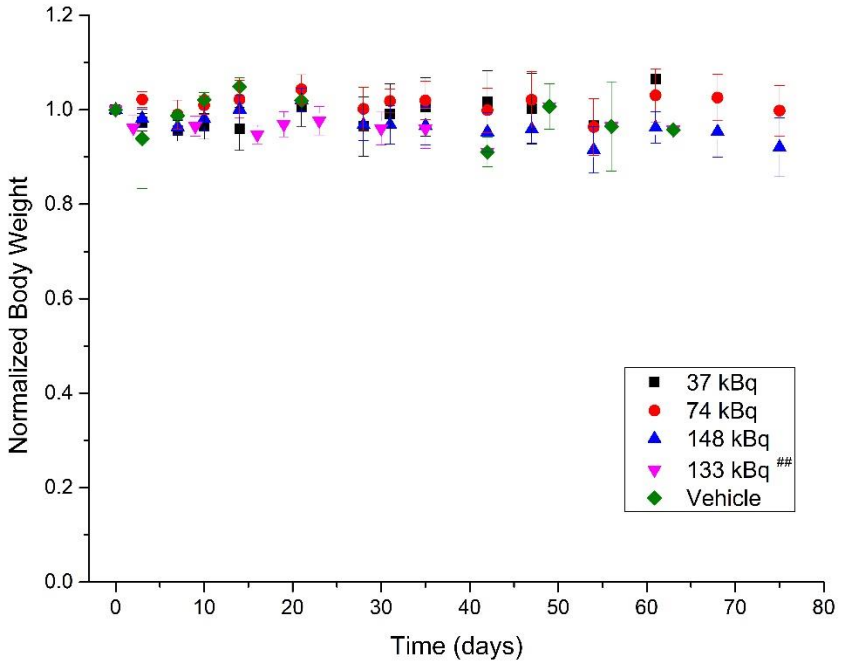
Supplemental Table 1. Normal Organ Absorbed Doses Calculated Using an OLINDA Male Adult Phantom.



Supplemental Figure 4. Visual Confirmation of Tumor Absence by µPET/CT Imaging. Mice treated with 148 kBq ^{225}Ac-RPS-074 (left) or 74 kBq ^{225}Ac-RPS-074 (right) were injected intravenously with ^{68}Ga-PSMA-11 75 days after injection of ^{225}Ac-RPS-074 and imaged by µPET/CT. Images were acquired 60 min post injection and were corrected for decay and for activity injected.



Supplemental Figure 5. Change in Normalized Tumor Volume in Treatment Groups. Mice were injected with either 37, 74 or 148 kBq ^{225}Ac-RPS-074, 133 kBq ^{225}Ac-DOTA-Lys-IPBA or an equal volume of vehicle on day 0 and tumor dimensions were measured by digital calipers at the indicated time points. ## = Activity of ^{225}Ac-DOTA-Lys-IPBA administered.



Supplemental Figure 6. Normalized Body Weights of All Mice Over the Duration of the Study. Weights are normalized to the starting weight of each mouse. Mice were injected with a dose of 37 kBq, 74 kBq or 148 kBq ^{225}Ac-RPS-074, 133 kBq ^{225}Ac-DOTA-Lys-IPBA (##) or vehicle.