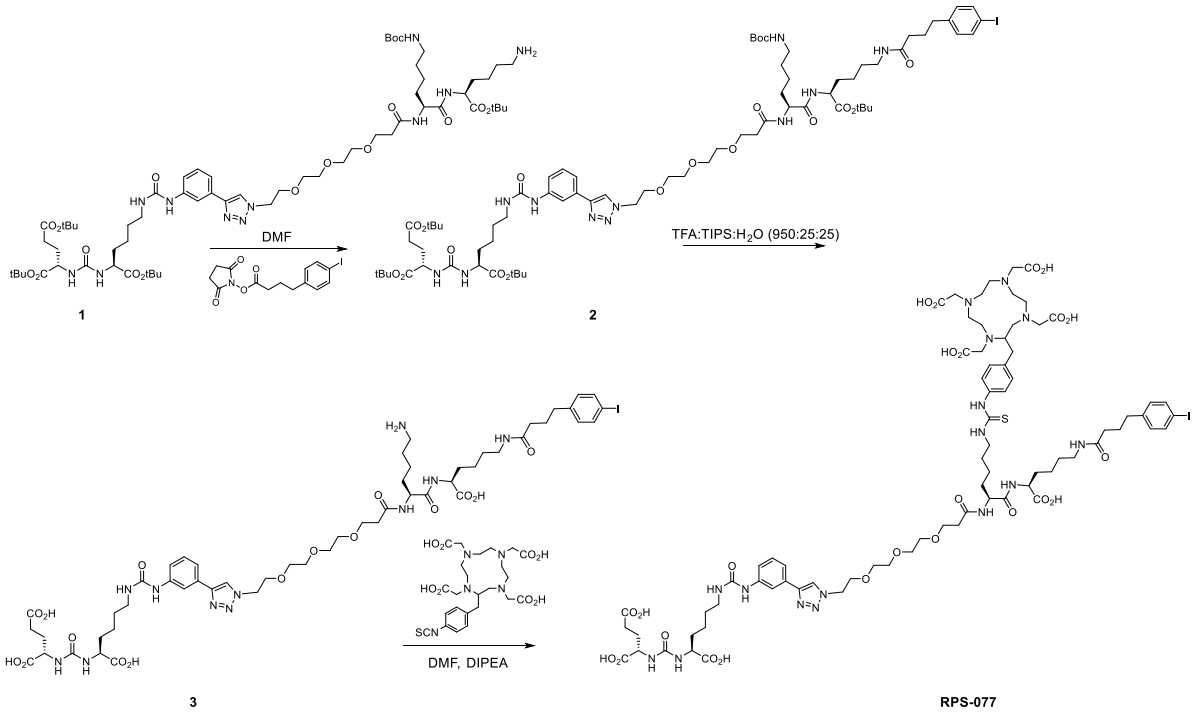
Synthesis of precursors

General Methods: All solvents were purchased from Sigma Aldrich and were of reagent grade quality unless otherwise indicated. Solvents were dried either by distillation over an activated stainless steel column (Pure Process Technology, LLC) column or by drying over activated molecular sieves. Reagents were purchased from Sigma Aldrich, except azido-PEG3-NHS ester and azido-PEG8-NHS ester, which were purchased from BroadPharm, and p-SCN-Bn-DOTA, which was purchased from Macrocyclics, Inc. The reagents were all of reagent grade and were used without any further purification.

All reactions described below were carried out in dried glassware. Purifications were performed by flash chromatography using a CombiFlash® Rf+ (Teledyne Isco) system or by preparative HPLC on a Agilent 1200 Series HPLC equipped on a Phenomenex Luna C18(2) 100Å, 250 cm x 21.20 mm I.D. 10 µm reverse phase column. A gradient of 10% MeCN/H_2O + 0.05% TFA to 90% MeCN/H_2O + 0.05% TFA over 40 min at a flow rate of 12 mL/min was used unless otherwise specified.

Reactions were monitored using thin layer chromatography (TLC) on glass-backed Silica gel 60 plates (EMD Millipore) or LCMS using a Waters ACQUITY UPLC® coupled to a Waters SQ Detector 2. Final products were characterized using analytical HPLC and LCMS. Analytical HPLC was performed using an XSelect™ CSH™ C18 5 µm 4.6 x 50 mm column (Waters). The purity of all compounds evaluated in the biological assay was > 95% purity as judged by analytical HPLC.

Synthesis of RPS-077, RPS-071 and RPS-072:



Supplemental Figure 1. Synthesis of **RPS-077** from amine **1**, a common intermediate with **RPS-063**.

General procedure for conversion of amines (1) and (11) to RPS-077, RPS-071 and RPS-072

To a solution of amine (1.0 eq) in DMF (3 mL) was added 2,5-dioxopyrrolidin-1-yl 2-(4-iodophenyl)acetate or 2,5-dioxopyrrolidin-1-yl 4-(4-iodophenyl)butanoate (2.0 eq) and the reaction was stirred at room temperature for 3 h. The reaction was concentrated under reduced pressure and the crude product was purified by flash chromatography (1-10% MeOH in CH_2Cl_2 over 25 min). The fractions corresponding to the product were collected and concentrated under reduced pressure.

The purified product (1.0 eq) was dissolved in TFA:H_2O:TIPS (950 µL:25 µL:25 µL) and stirred for 1 h at room temperature. The solvents were removed under reduced pressure and the resulting amine was recovered as the TFA salt after overnight drying under high vacuum and used without further purification.

The amine (as a TFA salt) (1.0 eq) was dissolved in DMF (2 mL). An excess of DIPEA (100 µL) was added, and the solution was stirred for 5 min. Then a solution of *p*-SCN-Bn-DOTA (1.1 eq) in H_2O (100 µL) was added, and the resulting mixture was stirred for 2 h at 40°C. The solvents were removed under reduced pressure, and the crude residue was purified by prep HPLC using a gradient of 10% MeCN/H_2O + 0.05% TFA to 70% MeCN/H_2O + 0.05% TFA. The peak corresponding to the product was collected and lyophilized to give the final product as a white powder.

Synthesis of RPS-077

Di-tert-butyl (((S)-1-(tert-butoxy)-6-(3-(3-(1-((14S,17S)-17-(tert-butoxycarbonyl)-14-(4-((tert-butoxycarbonyl)amino)butyl)-26-(4-iodophenyl)-12,15,23-trioxo-3,6,9-trioxa-13,16,22-triazahexacosyl)-1H-1,2,3-triazol-4-yl)phenyl)ureido)-1-oxohexan-2-yl)carbamoyl)-L-glutamate **(2)**

Compound **2** was synthesized from amine **1** and 2,5-dioxopyrrolidin-1-yl 4-(4-iodophenyl)butanoate according to the general procedure. Mass (ESI+): 1563.3 $[M+H]^+$. Calc. Mass: 1561.8.

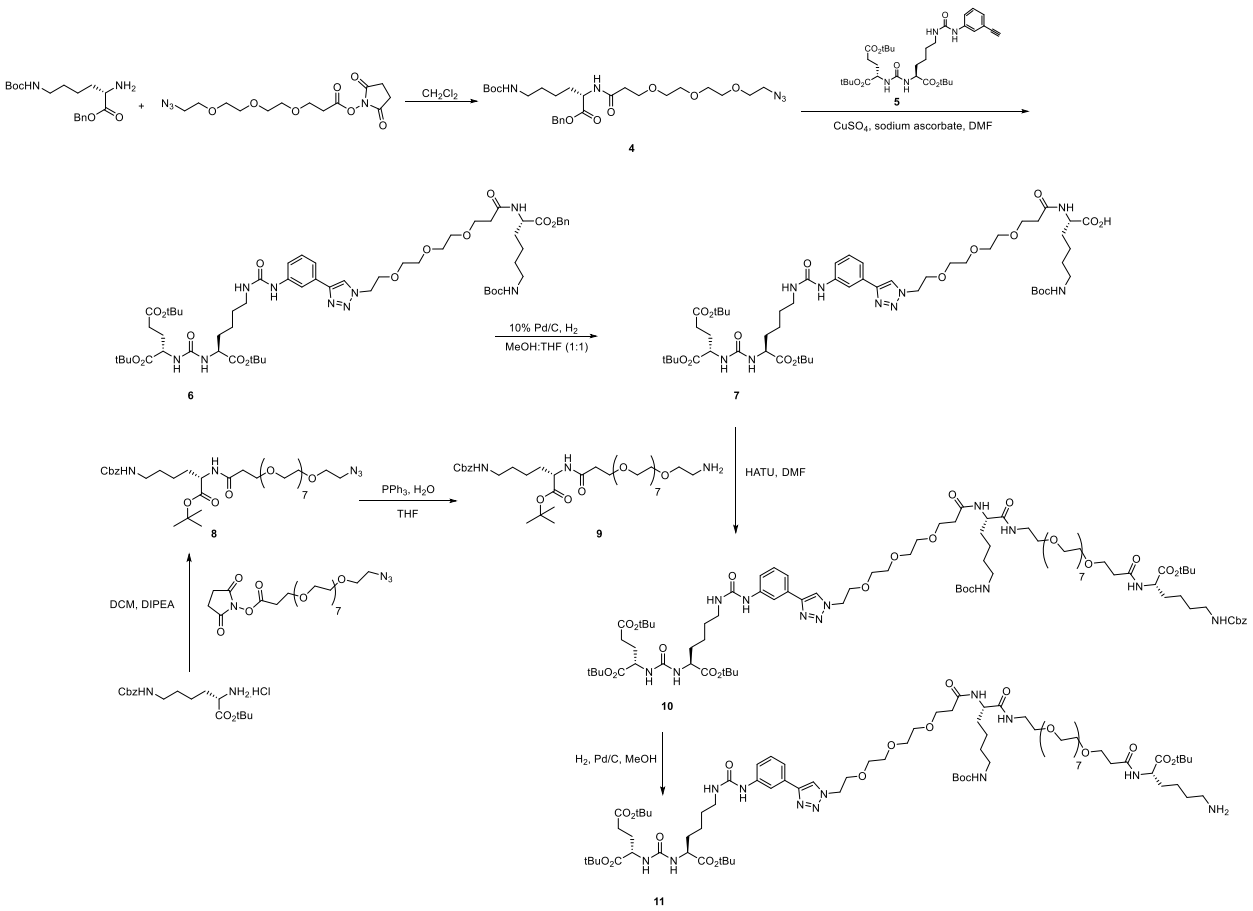
(((S)-5-(3-(3-(1-((14S,17S)-14-(4-aminobutyl)-17-carboxy-26-(4-iodophenyl)-12,15,23-trioxo-3,6,9-trioxa-13,16,22-triazahexacosyl)-1H-1,2,3-triazol-4-yl)phenyl)ureido)-1-carboxypentyl)carbamoyl)-L-glutamic acid **(3)**

Amine **3** was synthesized from **2** according to the general procedure. Mass (ESI+): 1238.4 $[M+H]^+$. Calc. Mass: 1237.5.

(((1S)-1-carboxy-5-(3-(3-(1-((14S,17S)-17-carboxy-26-(4-iodophenyl)-12,15,23-trioxo-14-(4-(3-(4-((1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10-tetraazacyclododecan-2-yl)methyl)phenyl)thioureido)butyl)-3,6,9-trioxa-13,16,22-triazahexacosyl)-1H-1,2,3-triazol-4-yl)phenyl)ureido)pentyl)carbamoyl)-L-glutamic acid (**RPS-077**)

RPS-077 was synthesized from amine **3** and *p*-SCN-Bn-DOTA according to the general procedure. Mass (ESI+): 1789.7 $[M+H]^+$. Calc. Mass: 1788.7.

Synthesis of RPS-071 and RPS-072:



Supplemental Figure 2. Synthesis of common precursor **11**.

Benzyl N^2-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)propanoyl)-N^6-(tert-butoxycarbonyl)-L-lysinate **(4)**

To a solution of benzyl N^6-(*tert*-butoxycarbonyl)-L-lysinate (336 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) was added dropwise a solution of Azido-PEG3-NHS ester (344 mg, 1.0 mmol) in CH_2Cl_2 (5 mL). The resulting mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure, and the crude product was purified by flash chromatography (1-10% MeOH in CH_2Cl_2 over 20 min). The fractions corresponding to the product were collected and concentrated to give **4** in 52% yield. Mass (ESI+): 566.2 $[M+H]^+$. Calc. Mass: 565.3.

Di-tert-butyl (((S)-6-(3-(3-(1-(((S)-10-((benzyloxy)carbonyl)-2,2-dimethyl-4,12-dioxo-3,15,18,21-tetraoxa-5,11-diazatricosan-23-yl)-1H-1,2,3-triazol-4-yl)phenyl)ureido)-1-(tert-butoxy)-1-oxohexan-2-yl)carbamoyl)-L-glutamate **(6)**

Compound **5** (315 mg, 0.5 mmol) was synthesized according to previously published procedures (*2*) and dissolved in DMF (5 mL). Compound **4** (252 mg, 0.5 mmol) was added, followed by a pre-mixed aqueous solution of 0.5 M $CuSO_4$ (0.25 mL) and 0.5 M sodium ascorbate (0.25 mL). The resulting mixture was stirred for 3 h at room temperature. The solvent was evaporated under reduced pressure and the crude compound was purified by flash chromatography (1-10% MeOH in DCM over 20 min). The fractions corresponding to the product were collected and concentrated to give triazole **6** in 48% yield. Mass (ESI+): 1196.9 $[M+H]^+$. Calc. Mass: 1195.7.

N^2-(3-(2-(2-(2-(4-(3-(3-((S)-6-(tert-Butoxy)-5-(3-((S)-1,5-di-tert-butoxy-1,5-dioxopentan-2-yl)ureido)-6-oxohexyl)ureido)phenyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)propanoyl)-N^6-(tert-butoxycarbonyl)-L-lysine **(7)**

Compound **6** (240 mg, 0.2 mmol) was dissolved in a mixture of MeOH:THF (1:1, 10 mL), and 10% Pd-C was added. The resulting suspension was stirred under an H_2 atmosphere for 3 h. The mixture was filtered through Celite and the filtrate was concentrated to afford acid **7** as a pale oil in 31% yield. The product was used without any further purification.

tert-Butyl N^2-(1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oyl)-N^6-((benzyloxy)carbonyl)-L-lysinate **(8)**

To a solution of *tert*-butyl N^6-((benzyloxy)carbonyl)lysinate hydrochloride (372 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) was added DIPEA (260 mg, 2.0 mmol) at room temperature followed by dropwise addition of Azido-PEG8-NHS ester. The mixture was stirred at room temperature for 3 h. The reaction was concentrated under reduced pressure, and the crude compound was purified by flash chromatography (1-10% MeOH in CH_2Cl_2 over 30 min). The fractions corresponding to the product were collected and concentrated under reduced pressure to give compound **7** in 44% yield Mass (ESI+): 786.2 $[M+H]^+$. Calc. Mass: 785.4.

tert-Butyl N^2-(1-amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oyl)-N^6-((benzyloxy)carbonyl)-L-lysinate **(9)**

To a suspension of compound **8** (392 mg, 0.5 mmol) in THF (10 mL) was added PPh_3 (314 mg, 1.2 mmol), and the resulting mixture was stirred for 3 h at room temperature. H_2O (0.5 mL) was added, and the reaction was stirred for a further 12 h at room temperature. The solvents were evaporated under reduced pressure and the crude compound was purified by flash chromatography (1-10% MeOH in CH_2Cl_2 over 20 min). The fractions corresponding to the product were collected and concentrated under reduced pressure to give amine **9** in 28% yield. Mass (ESI+): 760.6 $[M+H]^+$. Calc. Mass: 759.5.

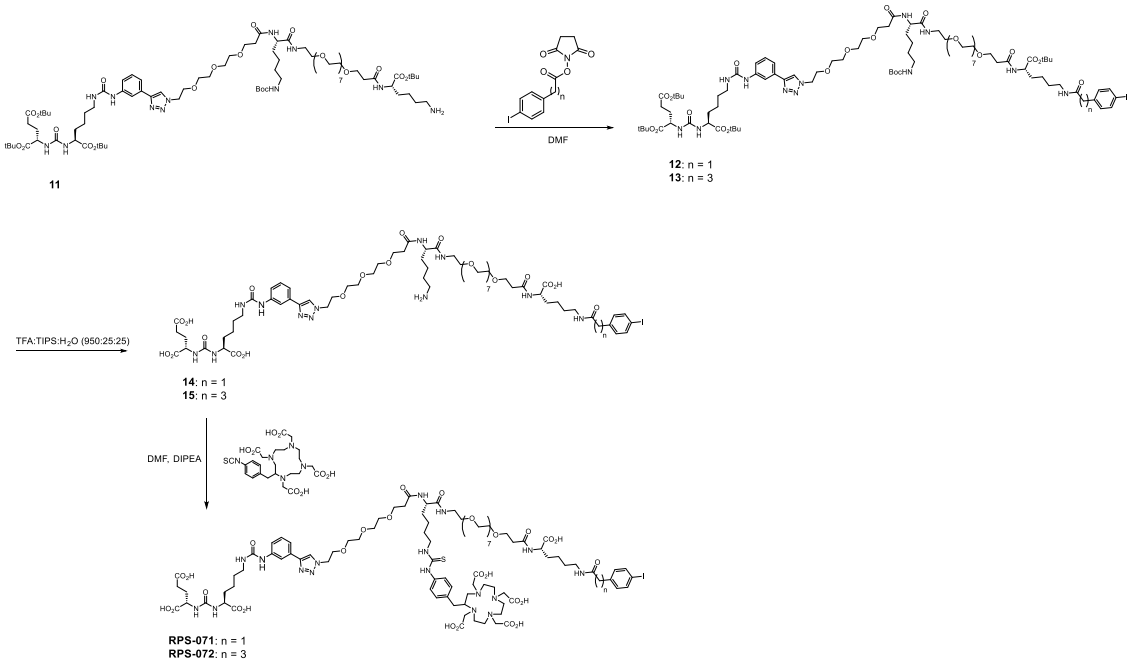
Di-tert-butyl (((S)-1-(tert-butoxy)-6-(3-(3-(1-((9S,40S)-9-(tert-butoxycarbonyl)-40-(4-((tert-butoxycarbonyl)amino)butyl)-3,11,39,42-tetraoxo-1-phenyl-2,14,17,20,23,26,29,32,35,45,48,51-dodecaoxa-4,10,38,41-tetraazatripentacontan-53-yl)-1H-1,2,3-triazol-4-yl)phenyl)ureido)-1-oxohexan-2-yl)carbamoyl)-L-glutamate **(10)**

To a stirred mixture of **9** (190 mg, 0.25 mmol), compound **7** (215 mg, 0.25 mmol) and HATU (95 mg, 0.25 mmol) in DMF (5 mL) was added DIPEA (65 mg, 0.5 mmol) and the reaction was stirred overnight at room temperature under N_2. The reaction was concentrated under reduced pressure and the resulting residue was purified by flash chromatography (1-10% MeOH in CH_2Cl_2 over 25 min). The fractions corresponding to the product were collected and concentrated under reduced pressure to give compound **10** in 42% yield. Mass (ESI+): 1849.4 $[M+H]^+$. Calc. Mass: 1848.2.

Di-tert-butyl (((S)-6-(3-(3-(1-((S)-10-(((S)-33-amino-29-(tert-butoxycarbonyl)-27-oxo-3,6,9,12,15,18,21,24-octaoxa-28-azatritriacontyl)carbamoyl)-2,2-dimethyl-4,12-dioxo-3,15,18,21-tetraoxa-5,11-diazatricosan-23-yl)-1H-1,2,3-triazol-5-yl)phenyl)ureido)-1-(tert-butoxy)-1-oxohexan-2-yl)carbamoyl)-L-glutamate **(11)**

Compound **10** (184 mg, 0.1 mmol) was dissolved in a mixture of MeOH:THF (1:1, 10 mL) and 10% Pd/C was added. The resulting suspension was stirred under H_2 atmosphere for 3 h. The mixture was filtered

through Celite and the filtrate was concentrated under reduced pressure to afford amine **11** as a colorless oil in 26% yield. The product was used without further purification.



Supplemental Figure 3. Synthesis of **RPS-071** and **RPS-072** from common precursor **11**.

Di-tert-butyl (((S)-1-(tert-butoxy)-6-(3-(3-(1-((14S,45S)-45-(tert-butoxycarbonyl)-14-(4-((tert-butoxycarbonyl)amino)butyl)-52-(4-iodophenyl)-12,15,43,51-tetraoxo-3,6,9,19,22,25,28,31,34,37,40-undecaoxa-13,16,44,50-tetraazadopentacontyl)-1H-1,2,3-triazol-5-yl)phenyl)ureido)-1-oxohexan-2-yl)carbamoyl)-L-glutamate **(12)**

Compound **12** was synthesized according to the general procedure described above from amine **11** and 2,5-dioxopyrrolidin-1-yl 2-(4-iodophenyl)acetate. Mass (ESI+): 1958.1 $[M+H]^+$. Calc. Mass: 1957.0.

Di-tert-butyl (((S)-1-(tert-butoxy)-6-(3-(3-(1-((14S,45S)-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)-1H-1,2,3-triazol-5-yl)phenyl)ureido)-1-oxohexan-2-yl)carbamoyl)-L-glutamate **(13)**

Compound **13** was synthesized according to the general procedure from amine **11** and 2,5-dioxopyrrolidin-1-yl 4-(4-iodophenyl)butanoate. Mass (ESI+): 1985.9 $[M+H]^+$. Calc. Mass: 1985.0

(((S)-5-(3-(3-(1-((14S,45S)-14-(4-aminobutyl)-45-carboxy-52-(4-iodophenyl)-12,15,43,51-tetraoxo-3,6,9,19,22,25,28,31,34,37,40-undecaoxa-13,16,44,50-tetraazadopentacontyl)-1H-1,2,3-triazol-5-yl)phenyl)ureido)-1-carboxypentyl)carbamoyl)-L-glutamic acid **(14)**

Compound **12** was deprotected as described in the general procedure, and the resulting compound **14** was recovered as the TFA salt and used without further purification.

(((S)-5-(3-(3-(1-((14S,45S)-14-(4-aminobutyl)-45-carboxy-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)-1H-1,2,3-triazol-5-yl)phenyl)ureido)-1-carboxypentyl)carbamoyl)-L-glutamic acid **(15)**

Compound **13** was deprotected as described in the general procedure, and the resulting compound **15** was recovered as the TFA salt and used without further purification.

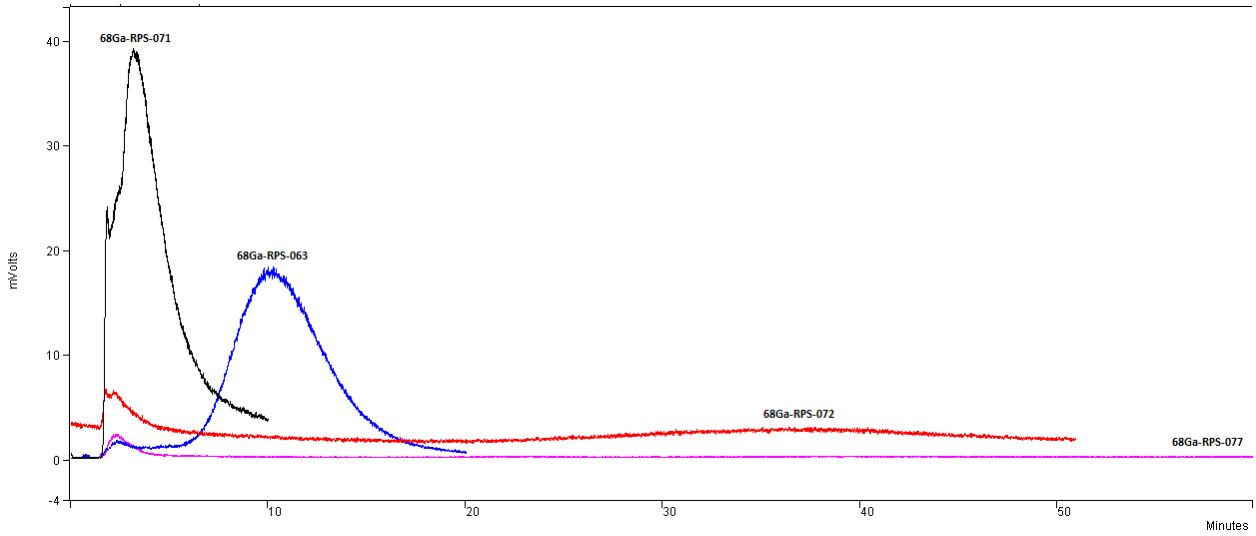
(((1S)-1-Carboxy-5-(3-(3-(1-((14S,45S)-45-carboxy-14-(4-(3-(4-(2-carboxy-2-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)ethyl)phenyl)thioureido)butyl)-52-(4-iodophenyl)-12,15,43,51-tetraoxo-3,6,9,19,22,25,28,31,34,37,40-undecaoxa-13,16,44,50-tetraazadopentacontyl)-1H-1,2,3-triazol-5-yl)phenyl)ureido)pentyl)carbamoyl)-L-glutamic acid **(RPS-071)**

RPS-071 was synthesized from the TFA salt of compound **14** and *p*-SCN-Bn-DOTA according to the general procedure. Mass (ESI+): 2185.3 $[M+H]^+$. Calc. Mass: 2183.9.

(((1S)-1-Carboxy-5-(3-(3-(1-((14S,45S)-45-carboxy-14-(4-(3-(4-(2-carboxy-2-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)ethyl)phenyl)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)-1H-1,2,3-triazol-5-yl)phenyl)ureido)pentyl)carbamoyl)-L-glutamic acid **(RPS-072)**

RPS-072 was synthesized from the TFA salt of compound **15** and *p*-SCN-Bn-DOTA according to the general procedure. Mass (ESI+): 2212.7 $[M+H]^+$. Calc. Mass: 2211.9.

Determination of HSA affinity by high performance affinity chromatography



Supplemental Figure 4. Analysis of ^{68}Ga-labeled compounds by high performance affinity chromatography. Analytes were injected as a 10% EtOH solution, with a maximum injected mass of 0.8 µg and a maximum injected volume of 40 µL. Analysis was performed on a Chiralpak® HSA™ Analytical HPLC column, 100 x 2 mm, 5 µm column using a 5% v/v isopropanol in 0.067M phosphate buffer (pH = 7.4) mobile phase at a constant flow rate of 0.3 mL/min.

Comparison of AUC in tumor, blood and kidneys of various ^{177}Lu-labeled radioligands in LNCaP or PC3-PIP xenograft models

	^{177}Lu-RPS-063#ǂ	^{177}Lu-RPS-071#	^{177}Lu-RPS-072#	^{177}Lu-RPS-077#	^{177}Lu-PSMA-617#ǂ	^{177}Lu-PSMA-617†	^{177}Lu-PSMA-Alb-02†	^{177}Lu-PSMA-Alb-56†
Tumor	2986 ± 76	689 ± 36	4932 ± 240	4571 ± 685	820 ± 15	3691 ± 156	6688 ± 485	8491 ± 537
Blood	26 ± 14	4.1 ± 15	400 ± 14	1240 ± 42	4.7 ± 0.4	52 ± 2	145 ± 6	341 ± 17
Kidney	5036 ± 16	496 ± 13	633 ± 16	3043 ± 85	233 ± 13	99 ± 11	1130 ± 62	809 ± 43
Tumor-to-Blood	115	168	12.1	3.7	174	71	46	25
Tumor-to-Kidney	0.6	1.4	7.8	1.5	3.5	37	5.9	11

Supplemental Table 1. AUCs in the tumor, kidney and blood at 192 h for ^{177}Lu-labeled ligands. Values are expressed as %ID/g · h. # = Extrapolated from data obtained over 96 h. ǂ = Extrapolated from data published by Kelly, et al. (*10*). † = Data obtained in a PC3-PIP xenograft model.

References

1. Kelly JM, Amor-Coarasa A, Ponnala S, Nikolopoulou A, Williams C., Jr, Schlyer D, et al. Trifunctional PSMA-targeting constructs for prostate cancer with unprecedented localization to LNCaP tumors. Eur J Nucl Med Mol Imaging. doi: 10.1007/s00259-018-4004-5.

2. Kelly J, Amor-Coarasa A, Nikolopoulou A, Kim D, Williams C., Jr, Ponnala S, Babich JW. 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.