SUPPLEMENTARY MATERIALS

^{177}Lu-Labeled Phosphoramidate-Based PSMA Inhibitors: The Effect of an Albumin Binder on Biodistribution and Therapeutic Efficacy in Prostate Tumor-Bearing Mice

Cindy J. Choy[#,1], Xiaoxi Ling[#,2], Jonathan J. Geruntho[2], Sophia K. Beyer[1], Joseph D. Latoche[2], Beatrice Langton-Webster[1], Carolyn J. Anderson[,2,3,4,5] and Clifford E. Berkman*[,1]*

[#]Co-first authors, *Co-corresponding authors

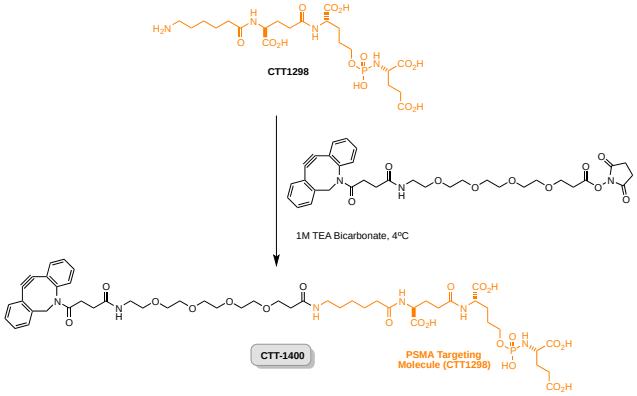
[1]CancerTargeted Technology, Woodinville, WA

Departments of [2]Medicine, [3]Radiology, [4]Bioengineering and [5]Pharmacology & Chemical Biology, University of Pittsburgh,

Table of Contents

Synthesis of 1400

CTT1298 was dissolved in ddH_2O to make a 0.43M solution. 125 µL of this solution was added to a 1 mL conical vial. 1M TEA-Bicarb buffer was added to the 1mL conical vial containing the CTT1298 solution. DBCO-PEG4-NHS (0.050 g) was dissolved in 0.300 µL of anhydrous DMSO (to make a 0.26M solution) and added to the vial dropwise. The resulting solution was stirred vigorously overnight at 4°C. The desired compound was obtained via RP-Prep HPLC with a 10-85% ACN (20.43 mg, 42.65%). Sodium bicarbonate (1.2 eq) was added to neutralize the ammonium acetate in the fractions. The ACN was removed by rotary evaporation with minimal heating, and the remaining water was lyophilized. The yield was determined with a spectrophotometer at 310 nm, ε_{310}=11,000 $M^{-1}Lcm^{-1}$. ^{1}H NMR (400 MHz, D_2O) δ 7.45 (d, *J* = 7.4, 1H), 7.36 - 7.20 (m, 6H), 7.16 (dd, *J* = 7.3, 1.6 Hz, 1H), 4.91 (d, *J* = 14.3 Hz, 1H), 3.95 (ddd, *J* = 13.9, 8.5, 4.9 Hz, 2H), 3.58 (ddd, *J* = 9.6, 5.6, 3.3 Hz, 5H), 3.51 - 3.38 (m, 12H), 3.33 (dt, *J* = 9.1, 6.2 Hz, 1H), 3.07 ? 2.88 (m, 4H), 2.32 (t, *J* = 6.1 Hz, 2H), 2.26 - 2.01 (m, 10H), 1.91 (d, *J* = 0.7 Hz, 2H), 1.74 - 1.60 (m, 4H), 1.62 - 1.27 (m, 8H), 1.15 (p, *J* = 7.6, 7.1 Hz, 2H). ^{31}P NMR (162 MHz, D_2O) δ 7.39. HRMS (MALDI): *m/z* calculated for $C_{51}H_{72}N_6O_{20}P$ [M+H] 1119.4539; found 1119.4542.



Scheme S1: Synthetic route for CTT 1400.

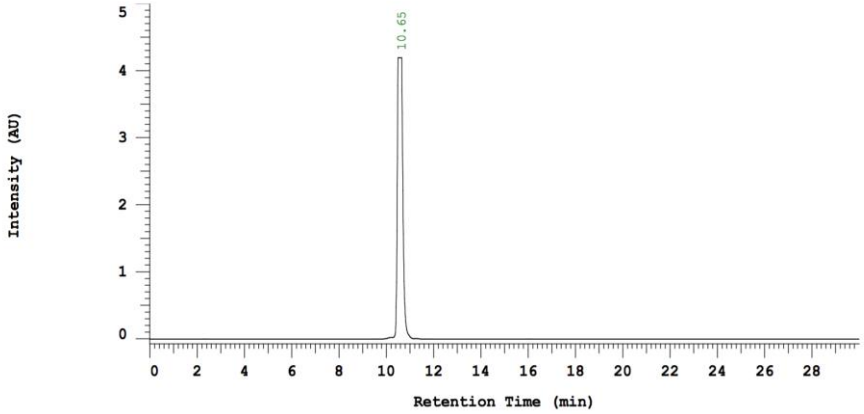
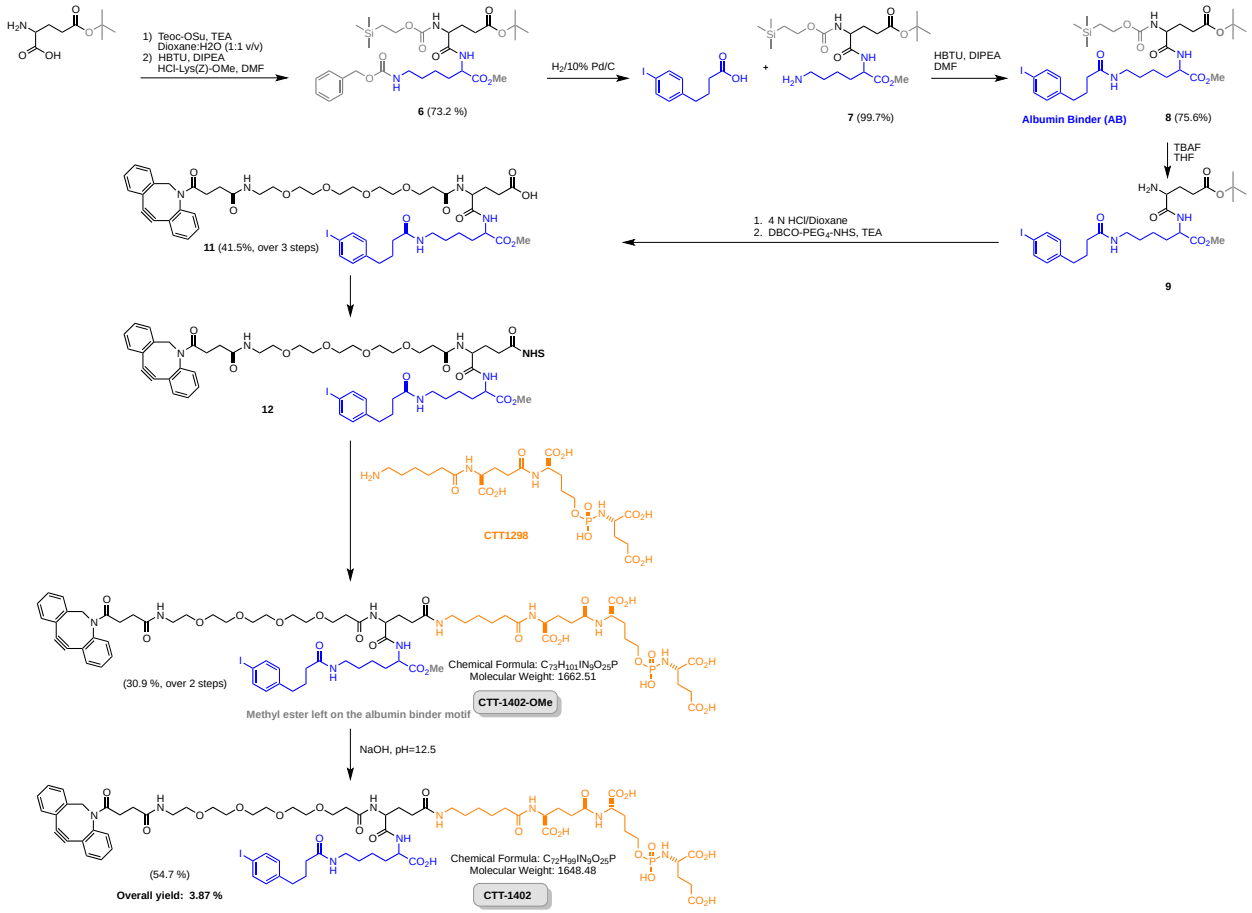


Figure S1. *HPLC chromatogram of CTT-1400 post purification. Retention time =10.65 mins.*

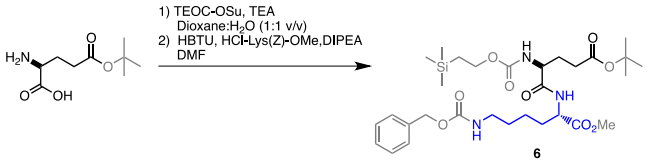
Synthesis of 1402



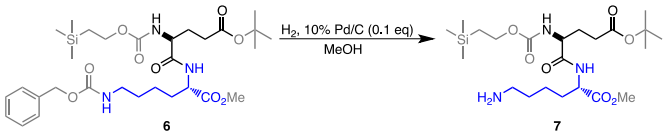
Scheme S2: Overall synthetic route for 1402.

(8S,11S)-methyl 11-(4-(((benzyloxy)carbonyl)amino)butyl)-8-(3-(tert-butoxy)-3-oxopropyl)-2,2-dimethyl-6,9-dioxo-5-oxa-7,10-diaza-2-siladodecan-12-oate (6). *Step 1:* To a stirred solution of Glu-(OtBu)-OH (2.089 g, 10.28 mmol) and triethylamine (0.2.15 mL, 15.43 mmol) in 1:1 Dioxane:water (v/v) (31 mL) Teoc-OSu (3.2 g, 12.34 mmol) was added in one portion. The mixture is stirred at room temperature overnight, then diluted with water (15 mL), acidified with 4 N HCl and 1 N HCl, and extracted with ethyl acetate (3 × 40 mL). The combined organic layers are washed with brine (60 mL), dried with magnesium sulfate, filtered and evaporated to give a crude oil (3.451 g, 96.6% yield) and dried overnight. *Step 2:* To the resultant crude solution (3.451, 9.929 mmol) in 20 mL of anh. DMF was added HBTU (3.765 g, 9.929 mmol) in one portion and stirred at room temperature for 30 min under inert atmosphere. After 30 min, to the reaction mixture, a solution of HCl-Lys(Z)-OMe (3.941 g, 11.914 mmol) and diisopropylethylamine (4.323 mL, 24.822 mmol) in 30 mL of anh. DMF was added drop-wise and stirred overnight at room temperature under inert atmosphere. Upon overnight stirring, the reaction mixture was taken up in ethyl acetate (100 mL) and the organic layer was washed with 1 N HCl (2X, 75 mL), followed by 10% $NaHCO_{3(aq)}$ (wt/v) (2X, 75 mL), then brine (1X, 75 mL). The organic layer was dried with magnesium sulfate, filtered and evaporated. The desired compound was obtained via silica chromatography (Silicycle 40 g cartridge) with 1:1

EtOAc:Hex (Rf=0.33) as the eluent (4.698 g, 75.9 %; 73.2 % over 2 steps). 1H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.27 (m, 5H), 6.89 (d, *J* = 7.7 Hz, 1H), 5.50 (d, *J* = 7.7 Hz, 1H), 5.10 (d, *J* = 2.1 Hz, 2H), 4.96 (s, 1H), 4.55 (dt, *J* = 7.9, 4.0 Hz, 1H), 4.22 (q, *J* = 7.3 Hz, 1H), 4.17 – 4.10 (m, 2H), 3.73 (s, 3H), 3.18 (p, *J* = 6.5 Hz, 2H), 2.38 (q, *J* = 7.1 Hz, 2H), 2.11 – 2.01 (m, 1H), 1.90 – 1.83 (m, 2H), 1.70 (dt, *J* = 14.1, 7.5 Hz, 1H), 1.58 – 1.49 (m, 2H), 1.45 (s, 9H), 1.36 (q, *J* = 7.7 Hz, 2H), 1.01 – 0.91 (m, 2H), 0.02 (s, 9H).

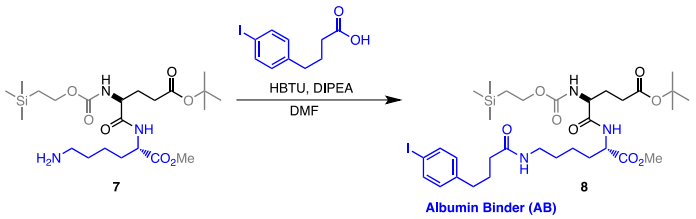


(8S,11S)-methyl 11-(4-aminobutyl)-8-(3-(tert-butoxy)-3-oxopropyl)-2,2-dimethyl-6,9-dioxo-5-oxa-7,10-diaza-2-siladodecan-12-oate (7). 10% Pd/C (0.797 g, 0.751 mmol) was added to a stirring solution of **6** (4.690 g, 7.518 mmol) in 70 mL of methanol at room temperature. The resultant solution was subjected to $H_{2(g)}$ atmosphere with a double-layered ballon and stirred overnight. Upon overnight stirring, the reaction was complete and filtered through a cellite plug and concentrated down to give **7** in quantitative yield (3.670 g, 99.7 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.56 (d, *J* = 8.0 Hz, 1H), 4.57 (td, *J* = 7.8, 4.9 Hz, 1H), 4.25 (q, *J* = 7.3 Hz, 1H), 4.19 – 4.12 (m, 2H), 3.75 (s, 3H), 2.74 (t, *J* = 6.6 Hz, 2H), 2.42 (q, *J* = 7.3 Hz, 2H), 2.08 (dq, *J* = 13.2, 6.4 Hz, 2H), 1.99 – 1.82 (m, 5H), 1.79 – 1.70 (m, 2H), 1.47 (s, 11H), 1.03 – 0.95 (m, 2H), 0.05 (s, 9H).

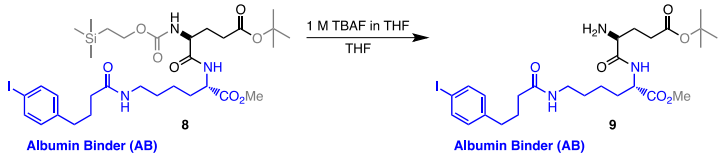


(8S,11S)-methyl 8-(3-(tert-butoxy)-3-oxopropyl)-11-(4-(4-(4-iodophenyl) butanamido)butyl)-2,2-dimethyl-6,9-dioxo-5-oxa-7,10-diaza-2-siladodecan-12-oate (8). To a solution of 4-(4-iodophenyl)butanoic acid (0.547 g, 1.89 mmol) in 7 mL of anh. DMF was added HBTU (0.716 g, 1.89 mmol) in one portion and stirred at room temperature for 30 min under inert atmosphere. After 30 min, to the reaction mixture, a solution of **7** (0.770 g, 1.57 mmol) and *N,N*-diisopropylethylamine (0.410 mL, 2.35 mmol) in 8 mL of anh. DMF was added drop-wise and stirred overnight at room temperature under inert atmosphere. Upon overnight stirring, the reaction mixture was taken up in ethyl acetate (100 mL) and the organic layer was washed with 1 N HCl (2X, 75 mL), followed by 10% $NaHCO_{3(aq)}$ (wt/v) (2X, 75 mL), then brine (1X, 75 mL). The organic layer was dried with magnesium sulfate, filtered and evaporated. The desired compound was obtained via silica chromatography (Silicycle 40g cartridge) with 65% EtOAc:Hex as the eluent (TLC developed with 75% EtOAc:Hex, Rf=0.33 with) (0.905 g, 75.6%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.57 – 7.52 (m, 2H), 7.07 (d, *J* = 7.8 Hz, 1H), 6.92 – 6.88 (m, 2H), 5.66 (d, *J* = 7.9 Hz, 1H), 4.47 (td, *J* = 8.1, 4.6 Hz, 1H), 4.22 (q, *J* = 7.4 Hz, 1H), 4.12 – 4.04 (m, 2H), 3.68 (s, 3H), 3.22 (dq, *J* = 13.2, 6.3 Hz, 1H), 3.11 (dq, *J* = 12.8, 6.1 Hz,

1H), 2.55 (t, *J* = 7.6 Hz, 2H), 2.35 (td, *J* = 7.2, 5.8 Hz, 2H), 2.14 (dd, *J* = 7.9, 6.5 Hz, 2H), 2.05 (ddd, *J* = 14.2, 7.1, 5.6 Hz, 1H), 1.94 – 1.78 (m, 5H), 1.71 - 1.63 (m, 1H), 1.49 – 1.44 (m, 2H), 1.41 (s, 9H), 1.35 – 1.26 (m, 2H), 0.97 – 0.89 (m, 2H), 0.01 (s, 9). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 172.99, 172.91, 172.48, 171.72, 156.67, 141.32, 137.47, 130.71, 91.04, 81.07, 63.58, 54.21, 52.48, 52.13, 38.76, 35.76, 34.79, 31.71, 31.48, 28.96, 28.24, 28.18, 27.01, 22.41, 17.78, -1.38.

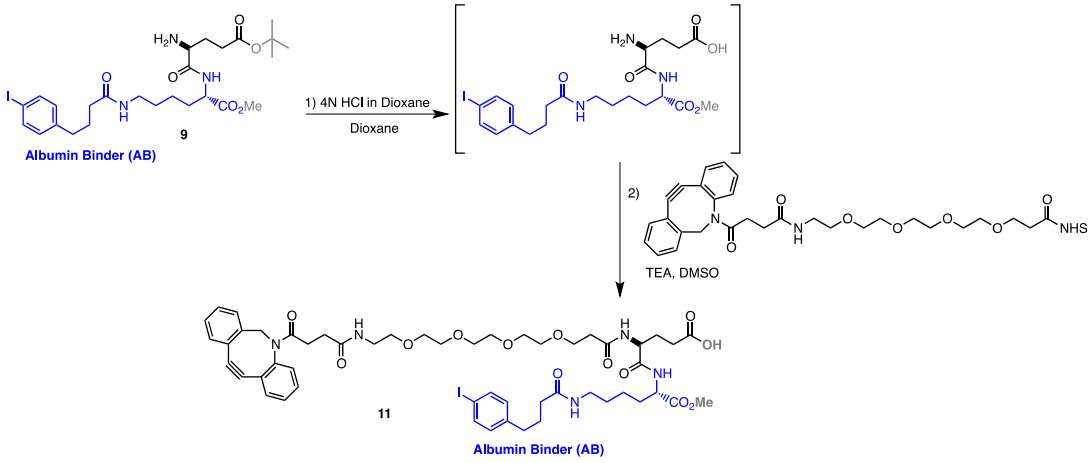


(S)-methyl 2-((S)-2-amino-5-(tert-butoxy)-5-oxopentanamido)-6-(4-(4-iodophenyl)butanamido)hexanoate (9). 1 M TBAF in THF (1.864 mL, 1.864 mmol) was added to at stirring solution of **8** (0.710 g, 0.932) in 9 mL of anh. THF at room temperature under inert atmosphere. The resultant solution was heated to 44 °C and stirred for 5 hrs, until completion. Upon completion, the reaction was cooled to room temperature and quenched with 5% $KHCO_{3(aq)}$ (wt/v) (15 mL) and extracted with ethyl acetate (2X, 50 mL). The combined organic layers were washed with brine (2X, 25 mL), dried with magnesium sulfate, filtered and evaporated and used in the next step without further purification (TLC developed in 20% MeOH:EtOAc, Rf=0.33) (0.5538 g, 96.1 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.73 (d, *J* = 8.4 Hz, 1H), 7.63 – 7.56 (m, 2H), 6.97 – 6.89 (m, 2H), 5.73 (d, *J* = 7.0 Hz, 1H), 4.56 (td, *J* = 8.4, 4.9 Hz, 1H), 3.73 (s, 3H), 3.42 (dd, *J* = 7.4, 5.2 Hz, 1H), 3.22 (td, *J* = 6.8, 5.7 Hz, 2H), 2.58 (t, *J* = 7.5 Hz, 2H), 2.35 (td, *J* = 7.4, 2.0 Hz, 2H), 2.15 (dd, *J* = 8.2, 6.8 Hz, 2H), 2.12 – 2.03 (m, 1H), 1.98 – 1.65 (m, 5H), 1.60 (s, 2H), 1.56 – 1.47 (m, 2H), 1.44 (s, 9H), 1.36 (dt, *J* = 8.3, 6.5 Hz, 2H).



(S)-1-(11,12didehydrodibenzo[b,f]azocin-5(6H)-yl)-22-(((S)-6-(4-(4-iodophenyl)butanamido)-1-methoxy-1-oxohexan-2-yl)carbamoyl)-1,4,20-trioxo-8,11,14,17-tetraoxa-5,21-diazapentacosan-25-oic acid (11). *Step 1:* 4 N HCl in Dioxane (5.0 mL, 20.08 mmol) was added dropwise to a solution of **9** (0.310 g, 0.502 mmol) in 5.0 mL of anhydrous Dioxane at 4°C for 30 mins then allowed to warm to room temperature. After 3 hrs, another aliquot of 4 N HCl in Dioxane (2.5 mL, 10.04 mmol) was added at room temp. Upon completion (approximately additional 30 min), the reaction was concentrated down and dried overnight under high vacuum and used in the next step without further purification. *Step 2:* DBCO-PEG_4-NHS (0.300 g, 0.462 mmol) in 2 mL of anhydrous Dioxane was added dropwise to the crude carboxylic acid (0.502 mmol) mixture from step 1 and TEA (0.104, 0.753 mmol) in 1.0 mL of anhydrous DMSO

and 4 mL of anhydrous Dioxane under inert atmosphere. The resulting solution was stirred overnight. Upon over night stirring, the reaction was taken up in 100 mL of EtOAc and washed with 1 N HCl (50 mL). The combined organic layer was collected and the aqueous layer was back extracted with EtOAc (100 mL). The combined organic layer was dried with $MgSO_4$, filtered and evaporated down. Compound **11** was isolated with a 0-4% H_2O in 3:7 ACN:MeOH gradient to yield a foamy pinkish orange solid (0.228 g, 41.5%, over 2 steps). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.93 (s, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.63 - 7.56 (m, 2H), 7.53 - 7.41 (m, 3H), 7.41 - 7.29 (m, 2H), 7.27 (d, *J* = 7.3 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 2H), 5.14 (d, *J* = 14.0 Hz, 1H), 4.38 (td, *J* = 8.6, 5.3 Hz, 2H), 3.71 (d, *J* = 7.9 Hz, 2H), 3.69 (s, 3H), 3.60 - 3.56 (m, 8H), 3.55 - 3.53 (m, 2H), 3.52 - 3.46 (m, 2H), 3.28 - 3.21 (m, 2H), 3.20 - 3.08 (m, 3H), 2.57 (t, *J* = 7.7 Hz, 2H), 2.55 - 2.43 (m, 3H), 2.40 (t, *J* = 7.7 Hz, 2H), 2.28 (t, *J* = 6.1 Hz, 2H), 2.18 (t, *J* = 7.5 Hz, 2H), 2.06 (tt, *J* = 15.3, 7.0 Hz, 3H), 1.89 (dq, *J* = 14.6, 7.8, 7.1 Hz, 4H), 1.78 – 1.59 (m, 3H), 1.54 - 1.45 (m, 2H), 1.45 - 1.36 (m, 3H), 1.08 - 1.00 (m, 2H). HRMS-MALDI: m/Z calculated for $C_{52}H_{66}IN_5NaO_{13}$ [M+Na] 1118.3599; found 1118.3577.



CTT-1402-OMe. *Step 1:* EDCI-HCl (0.029 g, 0.153 mmol) followed by N-hydroxysuccinamide (0.014 g, 0.122 mmol) was added to a solution of **11** (0.067 g, 0.061 mmol) in 1.0 mL of anhydrous DMF under inert atmosphere. The reaction was stirred for 1 hr at 50°C and another aliquot of EDCI-HCl (0.029 g, 0.153 mmol) and N-hydroxysuccinamide (0.014 g, 0.122 mmol) was added and stirred until completion. The crude mixture was diluted with 20 mL of EtOAc and washed with 1 N HCl (aq) to removed unreacted EDCI-HCl. The organic layer was dried through a pad of anhydrous sodium sulfate and concentrated down to yield a glassy pink solid. The solid, **12**, was dried under high vacuum for an hr and used in the next step without further purification. *Step 2:* Compound **12** in 1 mL of anhydrous DMF was added dropwise to a stirring solution of CTT 1298 (0.419 mL, 0.108 mmol) in 0.839 mL of 1 M TEA-Bicarbonate at 4°C. The resulting solution was stirred overnight at 4°C. The desired compound **CTT-1402-OMe** was obtained via RP-Prep HPLC with a 10-85% ACN (29.6 mg, 30.9%). Sodium bicarbonate (1.2 eq) was added to neutralize the ammonium acetate in the fractions. The ACN was removed by rotary evaporation with minimal heating, and the remaining water was lyophilized. The yield was determined with a spectrophotometer at 310 nm, ε_{310}=11,000 $M^{-1}Lcm^{-1}$. ^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.47 (d, *J* = 7.6 Hz, 1H), 7.35 - 7.06 (m, 8H),

7.01 (d, *J* = 7.4 Hz, 1H), 6.66 (d, *J* = 8.0 Hz, 2H), 4.32 (t, *J* = 7.3 Hz, 1H), 4.27 (t, *J* = 7.2 Hz, 1H), 4.10 (dd, *J* = 8.6, 5.0 Hz, 1H), 4.06 (dd, *J* = 8.4, 5.0 Hz, 1H), 3.71 (p, *J* = 6.2 Hz, 2H), 3.65 (t, *J* = 6.1 Hz, 2H), 3.61 (s, 3H), 3.59 (dd, *J* = 4.1, 2.4 Hz, 2H), 3.55 - 3.35 (m, 13H), 3.11 (t, *J* = 7.1 Hz, 3H), 3.04 (s, 2H), 2.97 (t, *J* = 7.3 Hz, 2H), 2.46 (q, *J* = 6.8 Hz, 2H), 2.42 - 2.21 (m, 10H), 2.17 (dt, *J* = 10.3, 6.1 Hz, 3H), 2.11 - 2.00 (m, 4H), 1.83 - 1.70 (m, 6H), 1.70 - 1.61 (m, 4H), 1.61 - 1.52 (m, 4H), 1.46 (p, *J* = 7.2 Hz, 2H), 1.37 (s, 2H), 1.33 - 1.24 (m, 6H). ^{31}P NMR (243 MHz, D_2O) δ 7.42. HRMS-MALDI: m/Z calculated for $C_{73}H_{100}D_{12}IN_9O_{25}P$ [M-H] 1660.5613; found 1660.5560

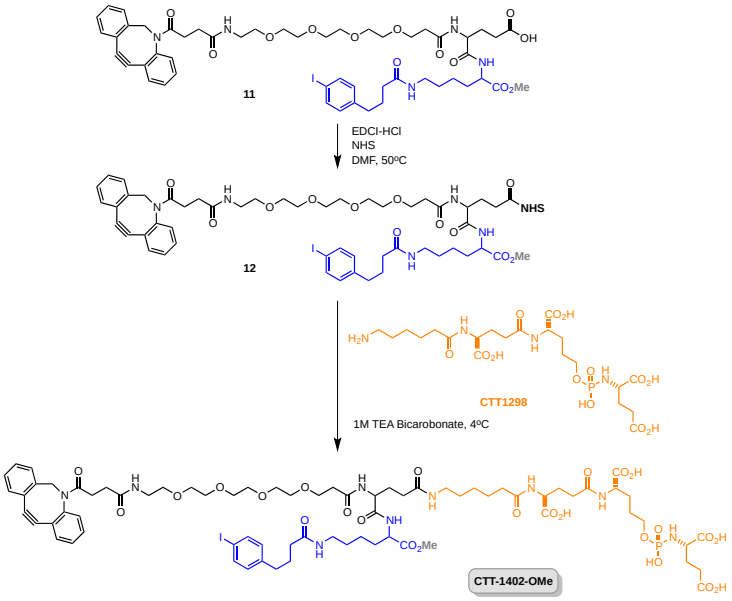


Table S1. Analytical HPLC method for CTT1402-OMe

- Column: Phenomenex Luna 5 um C18(2) 100 Å (cat. No. 00F-4252-E0)
- Dimensions: 150 × 4.6 mm
- Wavelength: 310 nm

Time	% 10 mM NH_4OAc	% Acetonitirle	Flow Rate (mL/min)
0.0	90	10	1
5.0	90	10	1
20.0	15	85	1
20.1	5	95	1
25.0	5	95	1
25.1	90	10	1
30	90	10	1

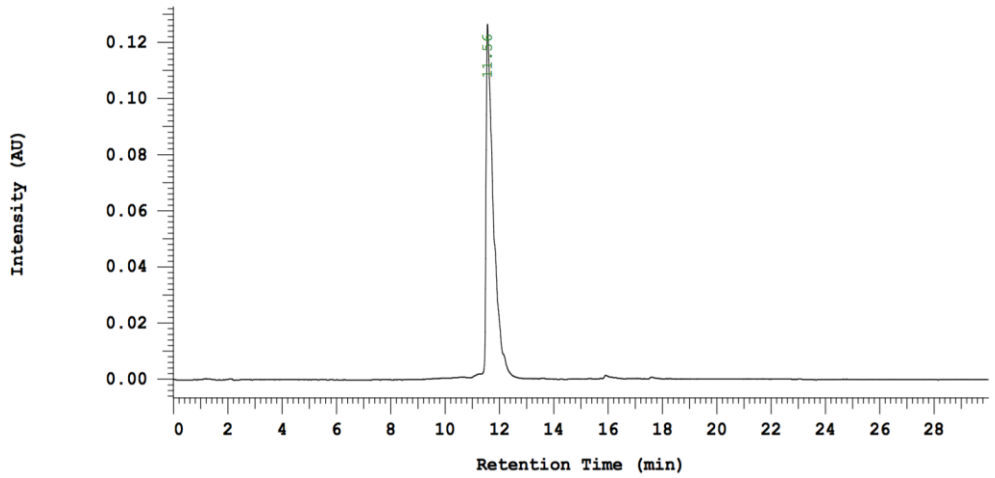


Figure S2: *HPLC chromatogram of CTT-1402-OMe post-purification. Retention time =11.56 min.*

Table S2. Preparative HPLC method for CTT1402-OMe

- Column: Phenomenex Luna 10 um C18(2) 100 Å (cat. No. 00B-4253-P0-AX)
- Dimensions: 50 × 21.2 mm
- Wavelength: 310 nm

Time	% 10 mM NH_4OAc	% Acetonitirle	Flow Rate (mL/min)
0.0	90	10	15
5.0	90	10	15
20.0	15	85	15
20.1	5	95	15
25.0	5	95	15
25.1	90	10	15
30	90	10	15

CTT-1402. **CTT-1402-OMe** was dissolved in 0.9 mL of MQ water. An aqueous solution of sodium hydroxide (1 N) was added until the pH of the solution was 12.5 and stirred overnight at room temperature. The final compound, CTT-1402, was obtained via RP-Prep HPLC with a 10-85% ACN (16.3 mg, 54.7%). Sodium bicarbonate (1.2 eq) was added to neutralize the ammonium acetate in the fractions. The ACN was removed by rotary evaporation with minimal heating, and the remaining water was lyophilized. The yield was determined with a spectrophotometer at 310 nm, ε_{310}=11,000 $M^{-1}Lcm^{-1}$. ^{1}H NMR ^{1}H NMR (600 MHz, D_2O) δ 7.51 (d, *J* = 7.7 Hz, 1H), 7.36 - 7.12 (m, 8H), 7.05 (d, *J* = 7.4 Hz, 1H), 6.70 (d, *J* = 7.8 Hz, 2H), 4.87 (d, *J* = 14.1 Hz, 1H), 4.35 (t, *J* = 7.2 Hz, 1H), 4.13 (dddd, *J* = 17.6, 13.5, 8.7, 4.9 Hz, 3H), 3.76 (q, *J* = 6.3 Hz, 2H), 3.66 (q, *J* = 5.9 Hz, 2H), 3.54 - 3.40 (m, 12H), 3.31 (d, *J* = 13.9 Hz, 2H), 3.20 - 3.05 (m, 5H), 3.03 - 2.93 (m, 2H), 2.48 (d, *J* = 5.9 Hz, 2H), 2.40 - 2.17 (m, 12H), 2.15 - 2.04 (m, 4H), 1.88 - 1.78 (m, 7H), 1.74 - 1.56 (m, 8H), 1.54 - 1.40 (m, 5H), 1.36 - 1.27 (m, 5H). ^{31}P NMR (243 MHz, D_2O) δ 7.47. HRMS (MALDI): *m/z* calculated for $C_{72}H_{98}IN_9O_{25}P$ [M-H] 1646.5456; found 1646.5381

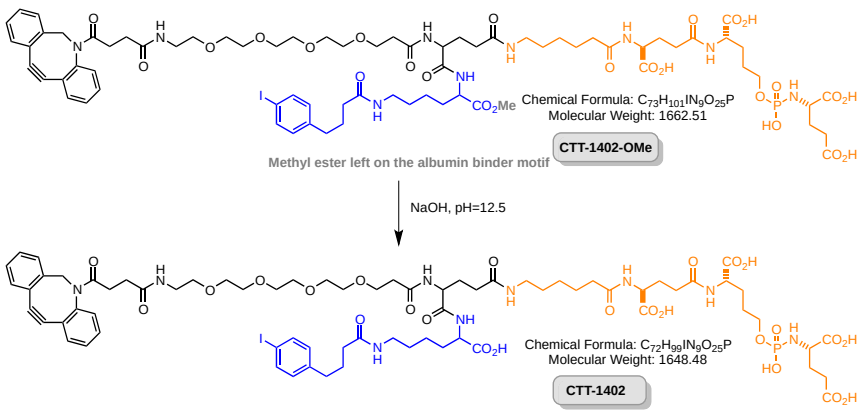


Table S3. Analytical HPLC method for CTT1402

- Column: Phenomenex Luna 5 um C18(2) 100 Å (cat. No. 00F-4252-E0)
- Dimensions: 150 × 4.6 mm
- Wavelength: 310 nm

Time	% 10 mM NH_4OAc	% Acetonitirle	Flow Rate (mL/min)
0.0	90	10	1
5.0	90	10	1
20.0	15	85	1
20.1	5	95	1
25.0	5	95	1
25.1	90	10	1
30	90	10	1

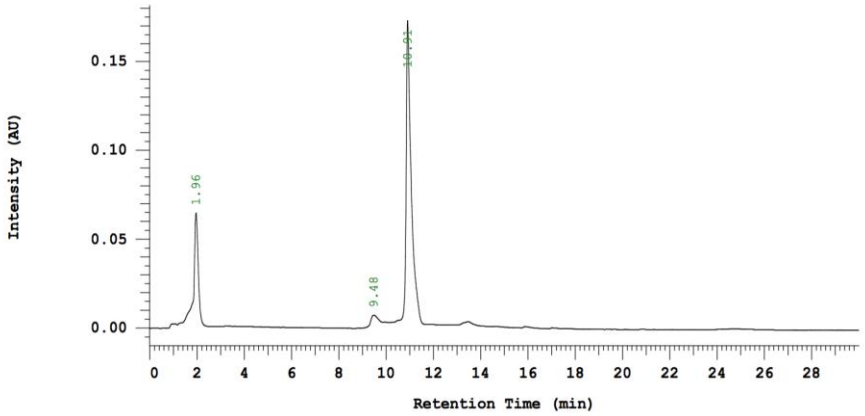


Figure S3: *HPLC chromatograph of CTT-1402 post purification. Peak at 1.96 is solvent front. Desired product has RT = 10.91 min. (Gradient tables for analytical and prep-HPLC are the same for See CTT-1402-OMe)*

Table S4. Preparative HPLC method for CTT1402

- Column: Phenomenex Luna 10 um C18(2) 100 Å (cat. No. 00B-4253-P0-AX)
- Dimensions: 50 × 21.2 mm
- Wavelength: 310 nm

Time	% 10 mM NH_4OAc	% Acetonitirle	Flow Rate (mL/min)
0.0	90	10	15
5.0	90	10	15
20.0	15	85	15
20.1	5	95	15
25.0	5	95	15
25.1	90	10	15
30	90	10	15

Table S5. Analytical data for non-radioactive standards

Substance	Molecular formula	Calcd. mass	Found m/z
CTT1401	$C_{70}H_{106}N_{14}O_{27}P^{+}$ $(M+H)^{+}$	1605.7084	1605.5396
Lu-CTT1401	$C_{70}H_{104}LuN_{14}O_{27}P^{+}$ $(M+H)^{+}$	1777.6257	1777.4727
CTT1403	$C_{91}H_{131}IN_{17}O_{32}P^{2-}$ $(M-2H)^{2-}$	1065.8970	1065.9008
Lu-CTT1403	$C_{91}H_{131}ILuN_{17}NaO_{32}P^{2+}$ $(M+H+Na)^{2+}$	1165.3628	1165.3541

Table S6. Analytical HPLC Method for ^{177}Lu-DOTA-Azide.

- Column: Phenomenex Luna 5 u C18(2) 100 Å (cat. No. 00F-4252-E0)
- Dimensions: 150 × 4.6 mm
- Wavelength: 214 and 280 nM

Time	%10 mM NH_4OAc	%Acetonitrile	Flow Rate (mL/min)
0.01	99.0	1.0	1.0
5.00	99.0	1.0	1.0
10.00	90.0	10.0	1.0
14.00	90.0	10.0	1.0
15.00	99.0	1.0	1.0

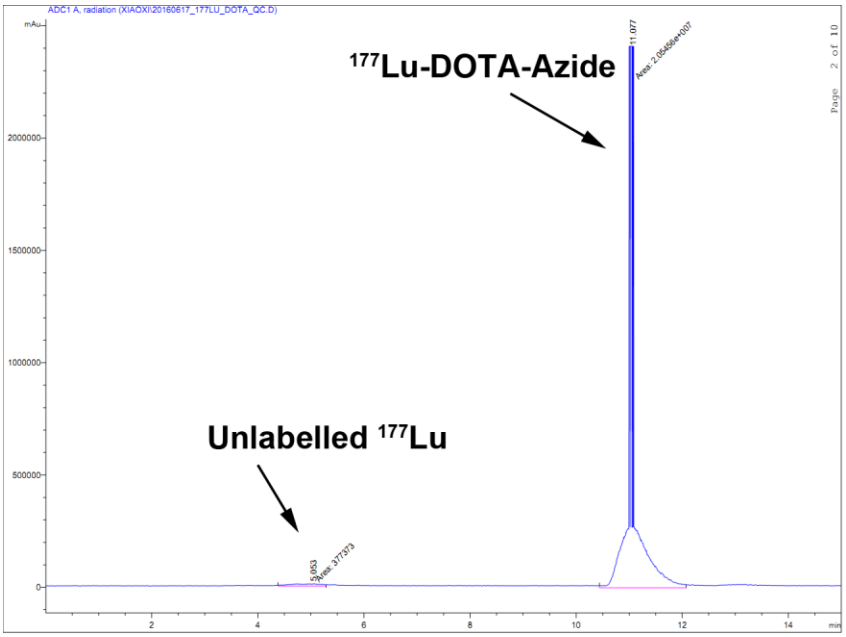


Figure S4: *Quality control HPLC chromatograph of ^{177}Lu-DOTA-Azide. Retention time =11.07 min.*

Table S7. Preparative and analytical HPLC method for CTT1401.

- Column: Phenomenex Luna 5 u C18(2) 100 Å (cat. No. 00F-4252-E0)
- Dimensions: 150 × 4.6 mm
- Wavelength: 214 and 280 nM

Time	%10 mM NH_4OAc	%Acetonitrile	Flow Rate (mL/min)
0.01	99.0	1.0	1.0
5.00	99.0	1.0	1.0
10.00	90.0	10.0	1.0
15.00	90.0	10.0	1.0
25.00	80.0	20.0	1.0
35.00	80.0	20.0	1.0
50.00	70.0	30.0	1.0
55.00	70.0	30.0	1.0
60.00	1.0	99.0	1.0
65.00	99.0	1.0	1.0
70.00	99.0	1.0	1.0

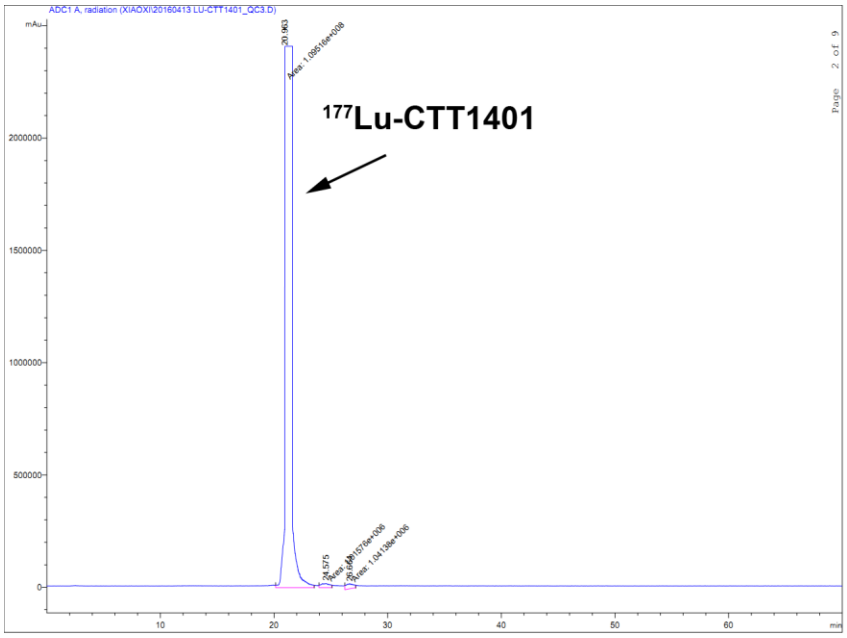


Figure S5: *Quality control HPLC chromatograph of CTT1401. Retention time =20.96 min.*

Table S8. Preparative and analytical HPLC method for CTT1403

- Column: Phenomenex Luna 5 u C18(2) 100 Å (cat. No. 00F-4252-E0)
- Dimensions: 150 × 4.6 mm
- Wavelength: 214, 254 and 280 nM

Time	%10 mM NH_4OAc	%Acetonitrile	Flow Rate (mL/min)
0.01	95.0	5.0	1.0
3.00	95.0	5.0	1.0
28.00	5.0	95.0	1.0
32.00	5.0	95.0	1.0
33.00	95.0	5.0	1.0
38.00	95.0	5.0	1.0

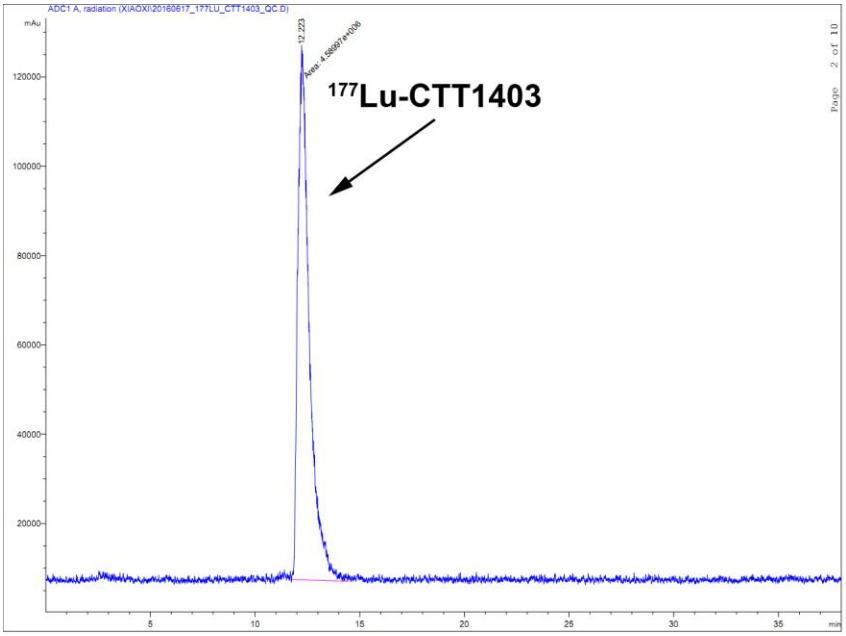


Figure S6: *Quality control HPLC chromatograph of CTT1403. Retention time =12.22 min.*

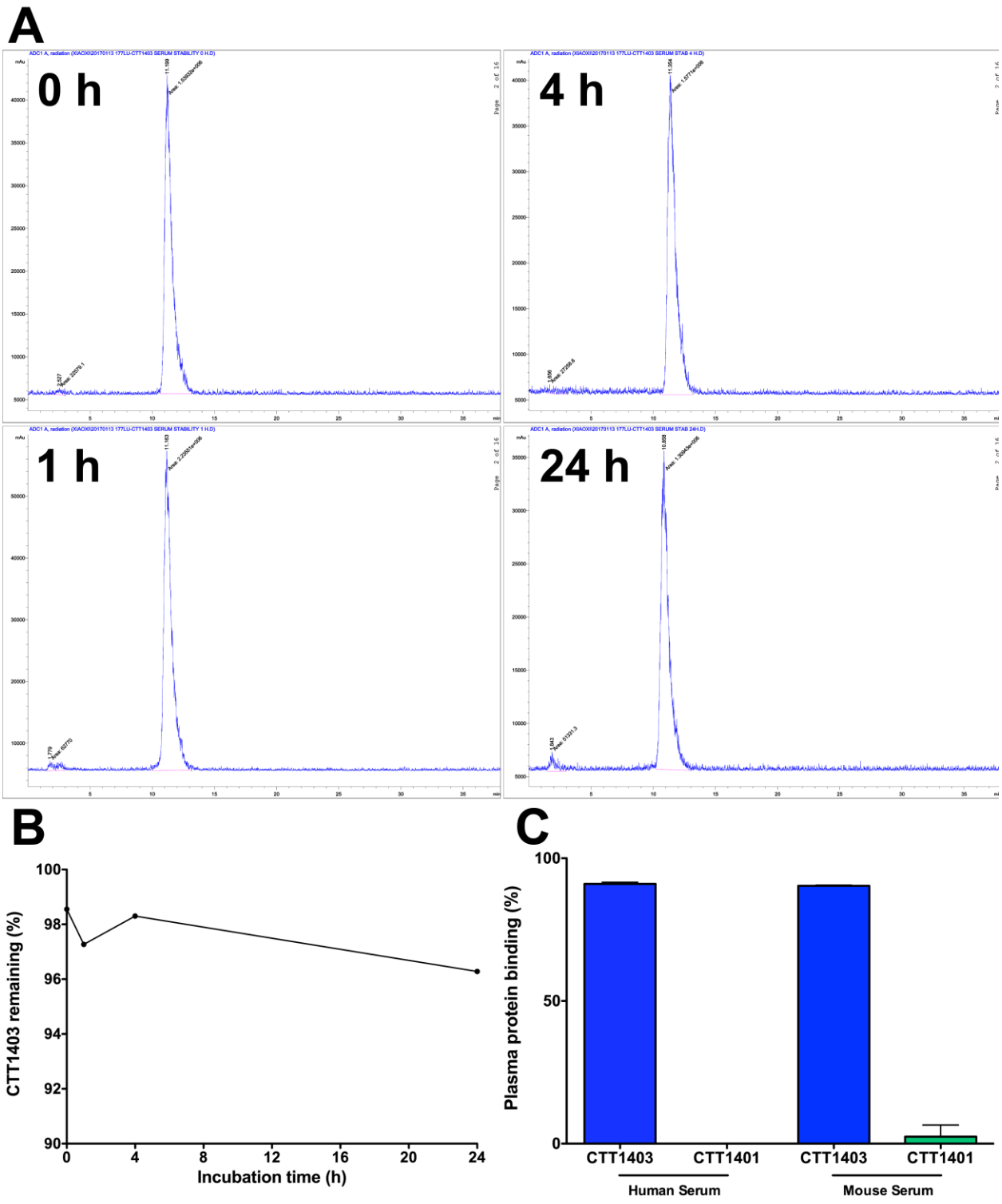


Figure S7: *(A) Stability of CTT1403 in mouse serum over time analyzed by radio HPLC, (B) remaining CTT1403 radioactivity after incubation in mouse serum over time, (C) results of ultrafiltration assay of CTT1401 and CTT1403 in human or mouse serum.*

Table S9. Comparison of CTT1401 and CTT1403, including ratio of the bound reagent internalized.

	CTT-1401				CTT-1403			
Time (min)	Surface	Internalized	Total	Ratio Internalized	Surface	Internalized	Total	Ratio Internalized
15	2.17 ±0.37%	11.57 ±0.22%	15.71 ±0.85%	82.11 ±1.79%	5.29 ±0.96%	18.67 ±0.52%	25.13 ±1.23%	77.03 ±3.11%
30	2.96 ±0.20%	19.17 ±1.19%	24.08 ±1.53%	84.80 ±0.91%	8.72 ±0.52%	31.13 ±0.63%	41.65 ±0.92%	77.09 ±1.13%
60	3.70 ±0.17%	30.05 ±0.48%	35.76 ±0.42%	87.57 ±0.84%	4.73 ±0.92%	43.55 ±4.55%	50.05 ±3.66%	88.98 ±2.68%
240	1.28 ±0.09%	44.21 ±2.52%	46.26 ±2.56%	96.77 ±0.34%	0.39 ±0.03%	83.43 ±3.53%	84.65 ±3.55%	99.17 ±0.07%

Table S10. Biodistribution Data for CTT1401.

	CTT1401							
	PC3-PIP (PSMA+)						PC3 WT (PSMA−)	
	1 h p.i.	4 h p.i.	24 h p.i.	48 h p.i.	72 h p.i.	4 h blocked	4 h p.i.	24 h p.i.
Blood	0.25 ± 0.25	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Kidney	11.91 ± 3.09	9.28 ± 4.16	14.90 ± 5.44	9.68 ± 4.10	7.25 ± 4.33	4.46 ± 1.52	9.54 ± 2.70	7.95 ± 2.56
Liver	0.11 ± 0.07	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.01
Lung	0.39 ± 0.24	0.06 ± 0.03	0.05 ± 0.03	0.03 ± 0.02	0.01 ± 0.01	0.06 ± 0.01	0.10 ± 0.07	0.14 ± 0.06
Spleen	0.21 ± 0.14	0.05 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.03	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Muscle	0.45 ± 0.27	0.03 ± 0.01	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02	0.03 ± 0.01	0.00 ± 0.00
Heart	0.19 ± 0.14	0.02 ± 0.00	0.01 ± 0.01	0.00 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Bone	0.22 ± 0.16	0.04 ± 0.04	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
Tumor	2.78 ± 0.48	3.00 ± 0.84	2.11 ± 0.52	1.63 ± 0.86	0.98 ± 0.08	1.12 ± 0.17	0.05 ± 0.01	0.01 ± 0.00
Prostate	3.37 ± 4.98	0.34 ± 0.34	0.06 ± 0.03	0.06 ± 0.10	-0.01 ± 0.04	0.17 ± 0.16	0.53 ± 0.71	0.15 ± 0.21
Small Intestine	0.09 ± 0.05	0.20 ± 0.17	0.17 ± 0.07	0.35 ± 0.23	0.23 ± 0.15	0.18 ± 0.12	0.24 ± 0.17	0.12 ± 0.07
Large Intestine	0.37 ± 0.26	0.06 ± 0.06	0.04 ± 0.02	0.09 ± 0.08	0.08 ± 0.05	0.09 ± 0.06	0.25 ± 0.23	0.17 ± 0.11
Stomach	0.07 ± 0.04	0.02 ± 0.02	0.05 ± 0.04	0.14 ± 0.09	0.08 ± 0.03	0.11 ± 0.08	0.10 ± 0.05	0.08 ± 0.06
Lacrimal Gland	0.53 ± 0.41	0.10 ± 0.06	0.08 ± 0.03	0.14 ± 0.05	0.12 ± 0.06	0.11 ± 0.05	0.17 ± 0.07	0.09 ± 0.04

Table S11. Biodistribution Data for CTT1403 in PC3-PIP cells.

	CTT1403							
	PC3-PIP (PSMA+)							
	1 h p.i.	4 h p.i.	24 h p.i.	48 h p.i.	72 h p.i.	120 h p.i.	168 h p.i.	4 h blocked
Blood	25.81 ± 4.22	19.11 ± 3.94	8.63 ± 0.63	5.82 ± 1.57	2.88 ± 0.93	1.25 ± 0.25	0.54 ± 0.14	21.02 ± 2.58
Kidney	12.35 ± 3.24	24.07 ± 9.17	34.13 ± 8.0	52.76 ± 11.54	47.86 ± 12.72	49.13 ± 16.91	34.59 ± 8.60	12.49 ± 3.82
Liver	5.27 ± 1.04	3.74 ± 0.59	1.77 ± 0.24	1.25 ± 0.46	0.61 ± 0.20	0.29 ± 0.09	0.16 ± 0.04	4.09 ± 0.44
Lung	11.13 ± 2.38	8.79 ± 1.33	5.05 ± 0.86	3.56 ± 1.05	1.63 ± 0.62	0.69 ± 0.16	0.35 ± 0.08	10.84 ± 2.34
Spleen	4.69 ± 0.55	4.04 ± 0.51	2.02 ± 0.23	1.49 ± 0.52	0.86 ± 0.31	0.44 ± 0.10	0.29 ± 0.07	4.38 ± 0.43
Muscle	1.86 ± 0.34	1.97 ± 0.36	1.05 ± 0.11	0.69 ± 0.15	0.32 ± 0.09	0.15 ± 0.04	0.06 ± 0.01	2.05 ± 0.28
Heart	7.84 ± 1.31	6.80 ± 1.46	3.11 ± 0.58	2.13 ± 0.59	1.10 ± 0.52	0.49 ± 0.13	0.20 ± 0.09	8.09 ± 1.67
Bone	2.65 ± 0.47	2.60 ± 0.76	1.20 ± 0.11	0.86 ± 0.35	0.43 ± 0.11	0.22 ± 0.04	0.12 ± 0.02	2.35 ± 0.16
Tumor	5.02 ± 0.67	17.38 ± 6.75	37.67 ± 8.66	45.36 ± 6.24	46.48 ± 14.48	35.04 ± 13.23	24.23 ± 4.20	9.28 ± 3.11
Prostate	16.77 ± 5.20	9.35 ± 4.69	6.54 ± 1.69	6.36 ± 3.85	1.88 ± 1.90	0.22 ± 0.05	0.12 ± 0.06	18.61 ± 7.74
Small Intestine	1.05 ± 0.13	1.24 ± 0.43	0.93 ± 0.14	0.69 ± 0.17	0.50 ± 0.15	0.26 ± 0.10	0.33 ± 0.10	1.35 ± 0.39
Large Intestine	2.20 ± 0.31	2.14 ± 0.42	1.20 ± 0.14	0.75 ± 0.21	0.43 ± 0.13	0.33 ± 0.11	0.89 ± 0.22	2.58 ± 0.63
Stomach	0.89 ± 0.11	1.69 ± 0.62	0.90 ± 0.13	0.58 ± 0.20	0.34 ± 0.11	0.24 ± 0.09	0.46 ± 0.17	1.85 ± 0.43
Lacrimal Gland	18.95 ± 5.40	19.69 ± 3.99	10.65 ± 4.63	6.70 ± 2.41	2.98 ± 2.30	0.75 ± 0.12	1.17 ± 0.48	22.36 ± 5.76

Table S12. Biodistribution Data for CTT1403 in PC3 WT tumor-bearing mice.

	CTT1403	
	PC3 WT (PSMA−)	
	4 h p.i.	24 h p.i.
Blood	19.55 ± 6.92	7.55 ± 1.56
Kidney	26.78 ± 7.97	29.49 ± 9.99
Liver	3.93 ± 1.14	1.51 ± 0.36
Lung	8.74 ± 2.89	4.19 ± 0.98
Spleen	3.62 ± 1.01	1.54 ± 0.48
Muscle	2.10 ± 0.85	0.92 ± 0.25
Heart	8.55 ± 6.85	2.78 ± 0.75
Bone	2.39 ± 0.96	0.98 ± 0.34
Tumor	7.81 ± 3.52	4.49 ± 0.69
Prostate	10.61 ± 6.66	4.90 ± 2.06
Small Intestine	1.07 ± 0.33	0.70 ± 0.19
Large Intestine	1.95 ± 0.78	0.84 ± 0.20
Stomach	1.47 ± 0.88	0.60 ± 0.20
Lacrimal Gland	21.14 ± 6.52	10.93 ± 2.69

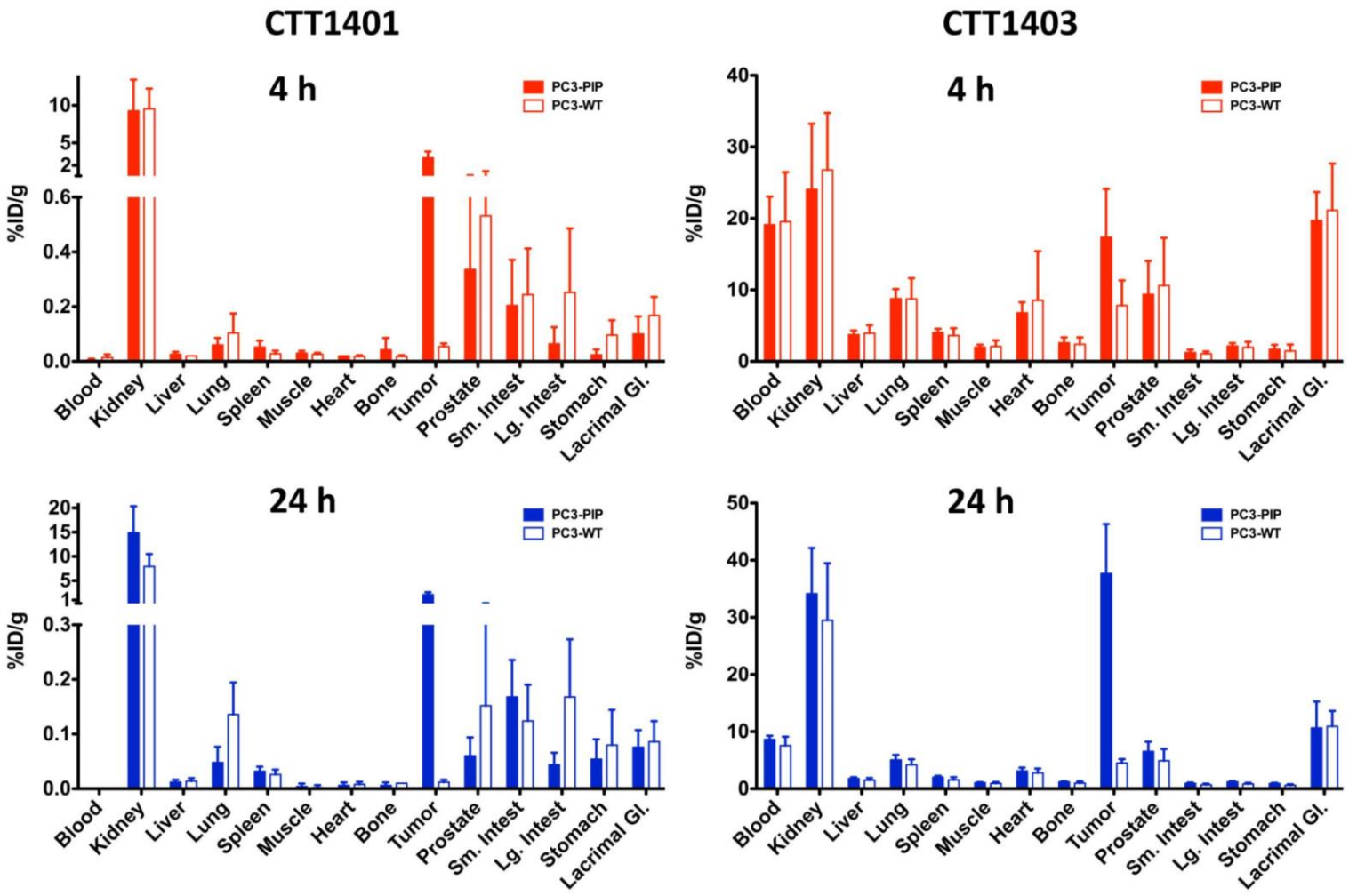


Figure S8: *Biodistribution of CTT1401 and CTT1403 in PC3-PIP and PC3-WT tumor-bearing mice.*

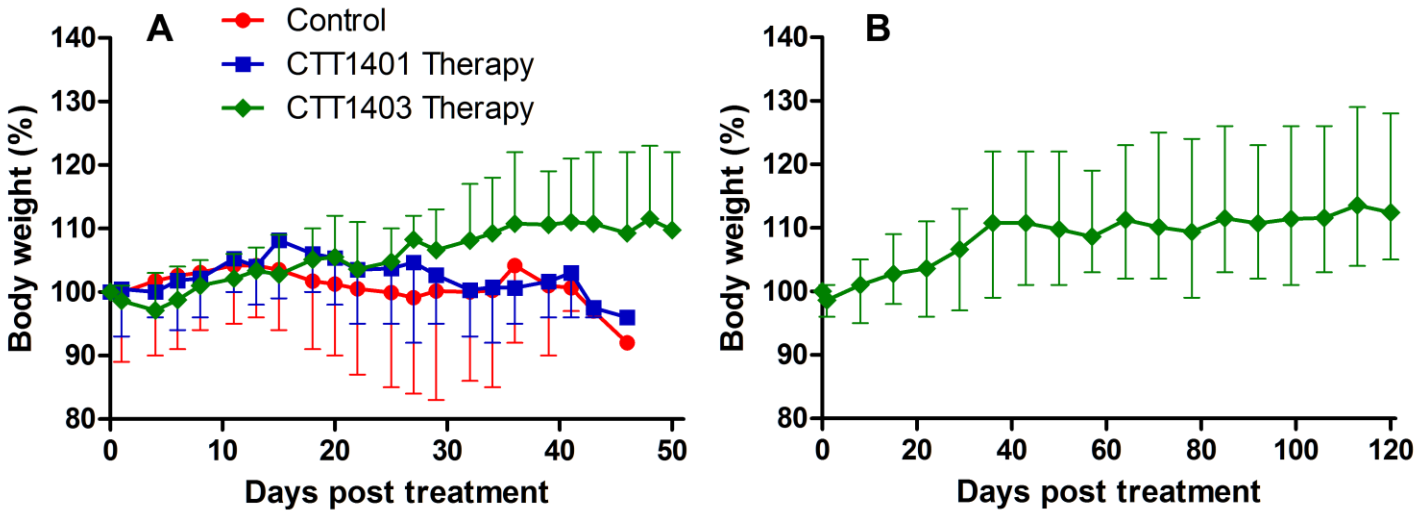


Figure S9: (A) *Body weight changes of mice injected with either saline (control), 29 MBq of CTT1401(CTT1401 Therapy), and 29 MBq of CTT1403(CTT1403 Therapy) over 50 days post-treatment. (B) Body weight changes of mice injected with 29 MBq of CTT1403(CTT1403 Therapy) out to 120 days post-treatment.*