Electronic Supplementary Material

Preclinical Dosimetry, Imaging, and Targeted Radionuclide Therapy Studies of Lu-177 Labeled Albumin-Binding, PSMA-Targeted CTT1403

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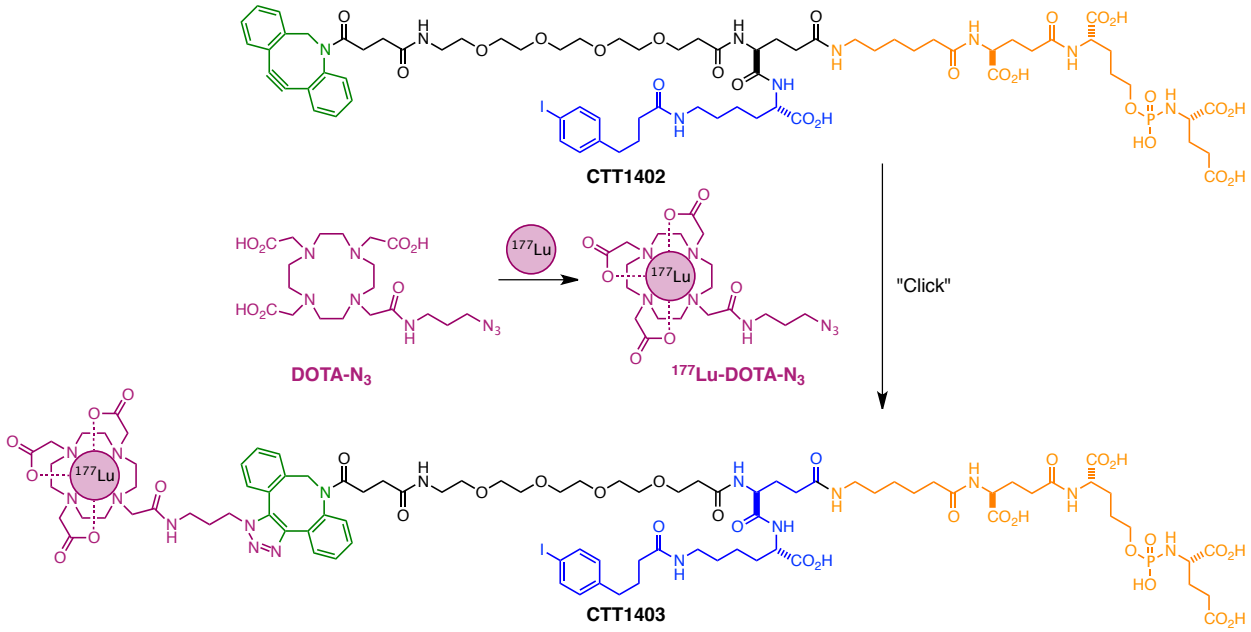
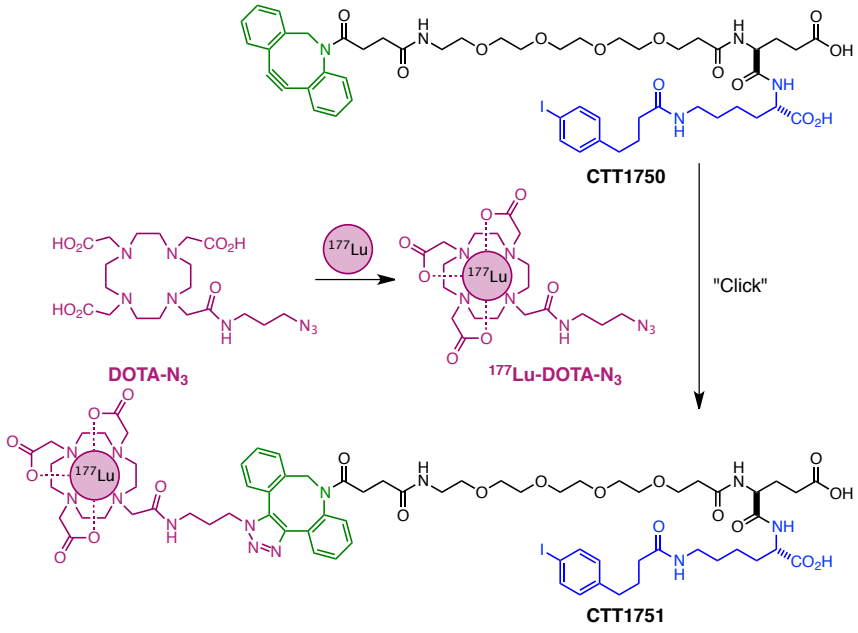
Radiolabeling of CTT1403.



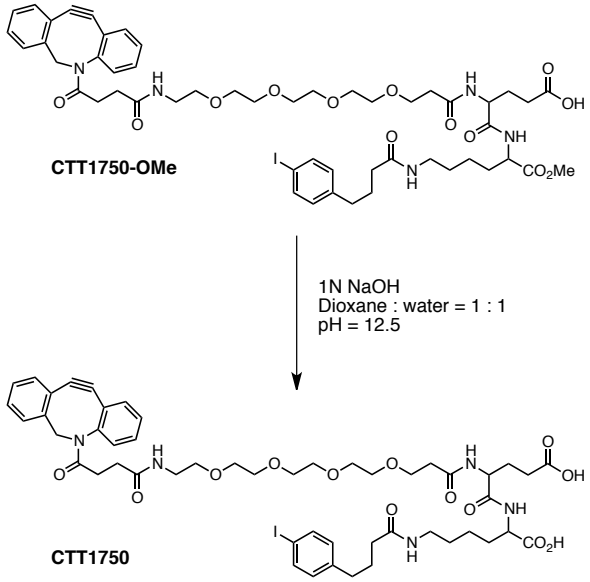
The radiolabeling procedure of CTT1403 was reported previously[1]. Briefly, ^{177}Lu-DOTA-N_3 was first prepared by mixing DOTA-N_3 (5.3 mM, 10 – 25 µL), $^{177}LuCl_3$ (10 – 20 mCi), and gentisic acid (56 mM, 10 µL) in a 0.5 M ammonium acetate buffer (pH 4.7 – 4.8, 100 – 150 µL) and agitated at 95 °C for 1 h. After confirmation of formation of ^{177}Lu-DOTA-N_3 by radio-HPLC, CTT1402 (20 mM, 5 – 20 µL) was added to the mixture and the resulting mixture was agitated at 37 °C for 1 h. CTT1403 was then isolated from the mixture using radio-HPLC. The solvent was removed using a nitrogen flow under mild heating (37 – 45 °C). The final product was reconstituted in saline before administration.

Radiolabeling of CTT1751.

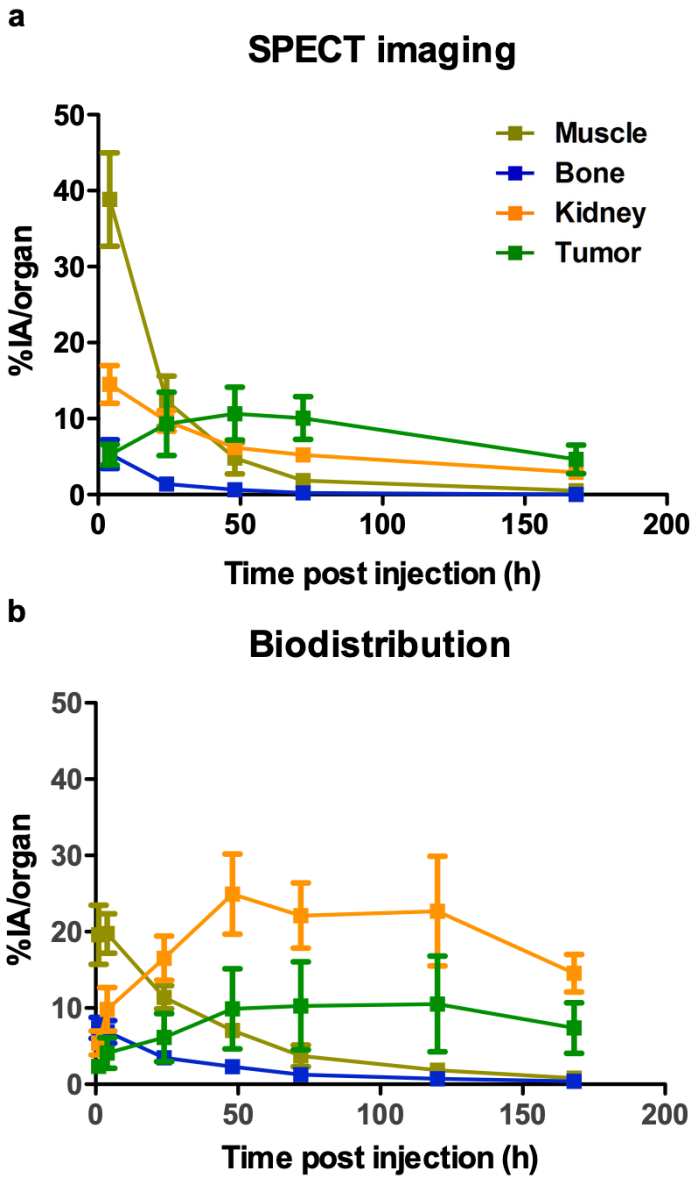


The radiolabeling of CTT1751 is similar to that of CTT1403 by replacing CTT1402 with CTT1750 (20 mM, 5 – 8 μL) in the click chemistry step. CTT1751 was isolated using the same radio-HPLC method. The solvent was removed using a nitrogen flow under mild heating (37 – 45 °C). The final product was reconstituted in saline before administration.

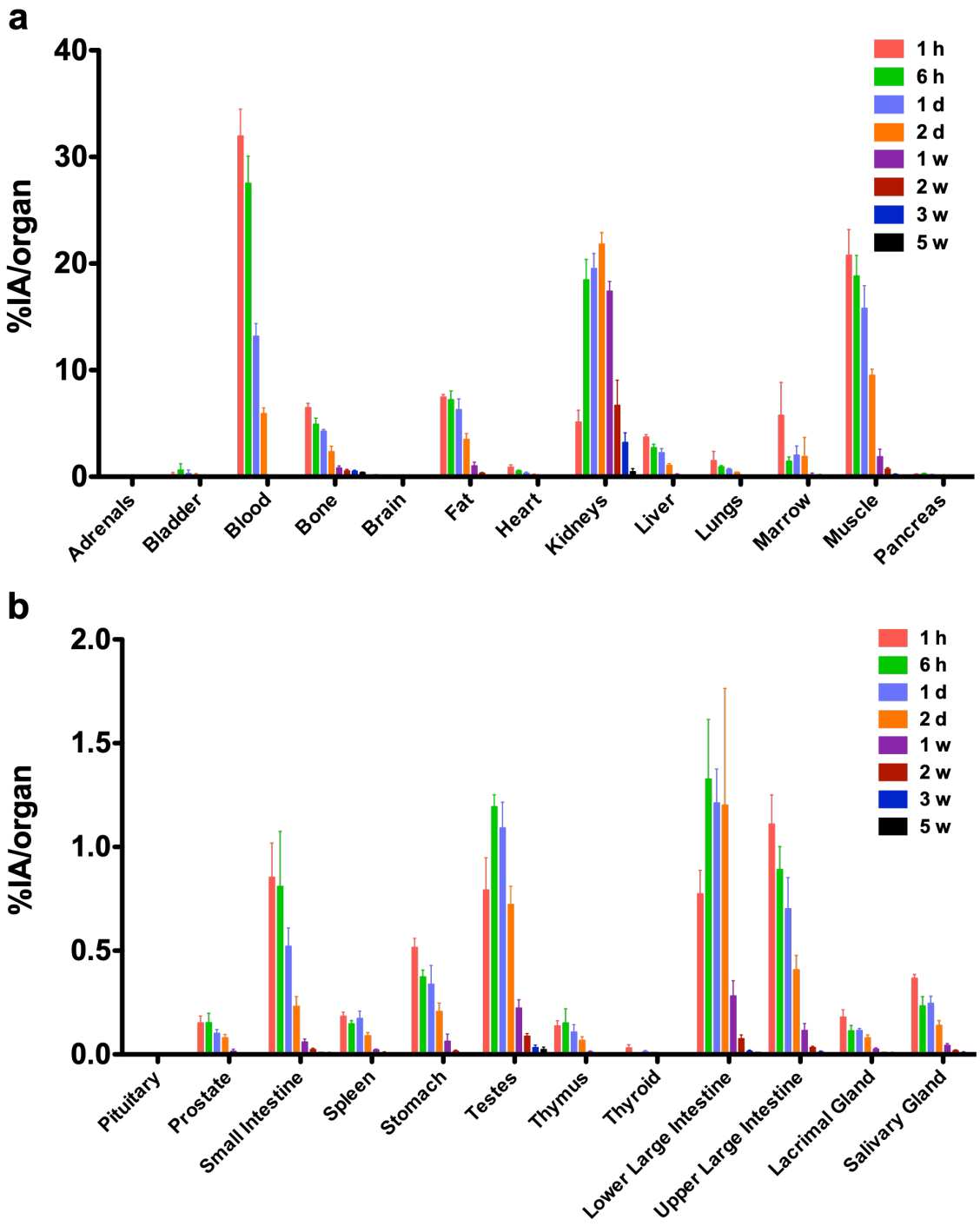
Synthesis of CTT1750.



CTT1750 was prepared by hydrolysis of intermediate compound CTT1750-OMe in alkaline conditions. CTT1750-OMe was an intermediate product when synthesize CTT1402. Its synthesis was described in previous publication.[1] Briefly, 11 mg of CTT1750-OMe (10 μmol) was dissolved in a 1 : 1 mixture of dioxane and water (100 μL). A 1 N sodium hydroxide solution (32 μL) was then added to this mixture and the pH was adjusted to 12.5. The resulting mixture was stirred 18 h at room temperature before the solvent were removed by lyophilization. The yield was determined with a spectrophotometer at 310 nm, ε_{310} =11,000 L· M^{-1}· cm^{-1}. ^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.58 (d, *J* = 7.6 Hz, 1H), 7.50 – 7.44 (m, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.41 – 7.31 (m, 4H), 7.28 (d, *J* = 7.4 Hz, 2H), 7.17 (d, *J* = 7.6 Hz, 1H), 7.05 (dq, *J* = 17.9, 9.7, 9.2 Hz, 1H), 6.82 (d, *J* = 7.9 Hz, 2H), 4.94 (d, *J* = 14.2 Hz, 1H), 4.33 (dd, *J* = 9.2, 5.3 Hz, 1H), 4.17 (dt, *J* = 9.2, 4.6 Hz, 1H), 3.79 – 3.71 (m, 1H), 3.70 (dd, *J* = 6.3, 2.4 Hz, 1H), 3.69 – 3.64 (m, 2H), 3.64 – 3.56 (m, 3H), 3.56 (s, 1H), 3.54 (s, 3H), 3.54 – 3.48 (m, 7H), 3.45 (d, *J* = 14.1 Hz, 1H), 3.19 – 3.15 (m, 1H), 3.10 (q, *J* = 6.0, 5.5 Hz, 2H), 3.04 (h, *J* = 7.0, 6.2 Hz, 1H), 2.56 – 2.49 (m, 2H), 2.49 – 2.37 (m, 3H), 2.37 – 2.32 (m, 1H), 2.31 – 2.19 (m, 4H), 2.13 (t, *J* = 7.5 Hz, 2H), 2.11 – 2.04 (m, 1H), 2.04 – 1.97 (m, 1H), 1.96 – 1.86 (m, 2H), 1.81 (dd, *J* = 12.8, 7.5 Hz, 1H), 1.75 (q, *J* = 7.6 Hz, 2H), 1.68 (td, *J* = 16.4, 14.3, 8.4 Hz, 1H), 1.51 – 1.42 (m, 2H), 1.36 – 1.29 (m, 2H). MS-MALDI: *m/z* calculated for $C_{51}H_{64}IN_5NaO_{13}$ $[M + Na]^+$ 1104.34; found 1104.33.



Supplemental Figure 1 Comparison of (a)SPECT/CT tumor/organ uptake value to (b) previously reported biodistribution study.[1]



Supplemental Figure 2 Rat dosimetry study of CTT1403 1 h, 6 h, 1 d, 2 d, 1 w, 2 w, 3 w and 5 w post injection in %IA/organ. (a) biodistribution in adrenals through pancreas; (b) biodistribution in organs pituitary through salivary glands.

Supplemental Table 1 Number of disintegrations in source organs (residence time):

Adrenals	1.96E-02	MBq-h/MBq or µCi-h/µCi

Brain	1.86E-01	MBq-h/MBq or µCi-h/µCi
Breasts	0.00E+00	MBq-h/MBq or µCi-h/µCi
Gallbladder Contents	0.00E+00	MBq-h/MBq or µCi-h/µCi
LLI	8.71E-02	MBq-h/MBq or µCi-h/µCi
Small Intestine	2.48E-01	MBq-h/MBq or µCi-h/µCi
Stomach	3.80E-02	MBq-h/MBq or µCi-h/µCi
ULI	7.18E-02	MBq-h/MBq or µCi-h/µCi
Heart Contents	0.00E+00	MBq-h/MBq or µCi-h/µCi
Heart Wall	2.80E-01	MBq-h/MBq or µCi-h/µCi
Kidneys	1.79E+01	MBq-h/MBq or µCi-h/µCi
Liver	9.14E-01	MBq-h/MBq or µCi-h/µCi
Lungs	1.16E+00	MBq-h/MBq or µCi-h/µCi
Muscle	9.99E+00	MBq-h/MBq or µCi-h/µCi
Ovaries	0.00E+00	MBq-h/MBq or µCi-h/µCi
Pancreas	7.99E-02	MBq-h/MBq or µCi-h/µCi
Red Marrow	7.29E-01	MBq-h/MBq or µCi-h/µCi
Cortical Bone	1.70E+00	MBq-h/MBq or µCi-h/µCi
Trabecular Bone	4.25E-01	MBq-h/MBq or µCi-h/µCi
Spleen	1.24E-01	MBq-h/MBq or µCi-h/µCi
Testes	4.09E-02	MBq-h/MBq or µCi-h/µCi
Thymus	1.46E-02	MBq-h/MBq or µCi-h/µCi
Thyroid	2.28E-02	MBq-h/MBq or µCi-h/µCi
Urinary Bladder Contents	1.44E-01	MBq-h/MBq or µCi-h/µCi
Uterus/Uterine Wall	0.00E+00	MBq-h/MBq or µCi-h/µCi
Remainder	1.28E+01	MBq-h/MBq or µCi-h/µCi

Reference

1. Choy, C. J.; Ling, X.; Geruntho, J. J.; Beyer, S. K.; Latoche, J. D.; Langton-Webster, B.; Anderson, C. J.; Berkman, C. E., (177)Lu-Labeled Phosphoramidate-Based PSMA Inhibitors: The Effect of an Albumin Binder on Biodistribution and Therapeutic Efficacy in Prostate Tumor-Bearing Mice. *Theranostics* **2017,** *7* (7), 1928-1939.