Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for:

The inverse electron-demand Diels-Alder reaction as a new methodology for the synthesis of ^{225}Ac-labeled radioimmunoconjugates

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Methods and Materials:

All chemicals were acquired from Sigma-Aldrich (St. Louis, MO) and were used without further purification. All water used was ultrapure (>18.2 $M\Omega.cm^{-1}$ at 25 °C), all DMSO was of molecular biology grade (>99.9%), and all other solvents were of the highest grade commercially available. Acetonitrile (CH_3CN) and dimethylformamide (DMF) were purchased from Acros Organics (Waltham, MA) as extra dry over molecular sieves. Amine-reactive *trans*-cyclooctene [(E)-cyclooct-4-enyl 2,5-dioxo-1-pyrrolidinyl carbonate; TCO-NHS)] and amine-reactive tetrazine (2,6-dioxo-1-pyrrolidinyl 5-[4-(1,2,4,5-tetrazin-3-yl)benzylamino]- 5-oxopentanoate; Tz-NHS) were purchased from Sigma-Aldrich (St. Louis, MO). The p-SCN-Bn-DOTA chelator was purchased from Macrocyclics, Inc. (Dallas, TX). The DOTA-NHS-ester chelator was purchased from CheMatech (Dijon, France). The 5B1 mAb was produced at MabVax Therapeutics as previously described.[1] The ^{225}Ac used in this research was supplied by the United States Department of Energy Office of Science by the Isotope Program in the Office of Nuclear Physics. All experiments using laboratory animals were performed in accordance with a protocol approved by the Memorial Sloan Kettering Institutional Animal Care and Use Committee (protocol 08-07-013)

Instrumentation:

All instruments were calibrated and maintained in accordance with standard quality-control procedures. UV-Vis measurements were taken on a Thermo Scientific NanoDrop 2000 Spectrophotometer. NMR spectroscopy was performed on a Bruker 600 MHz NMR with TopSpin 2.1 software for spectrum analysis. Low-resolution mass spectrometry was performed using a Waters liquid chromatography–mass spectrometer (LC–MS) consisting of a Waters ZQ quadrupole spectrometer equipped with an ESCI electrospray/chemical ionization ion source and a Waters 2695 HPLC system (Waters, Milford, MA). A Capintec CRC-15R Dose Calibrator (Capintec, Ramsey, NJ) was used for all activity measurements. Activities were measured with the calibrator set at 775 and multiplying the display activity value by 5. The parent ^{225}Ac activity was measured at secular equilibrium when ^{225}Ac is in equilibrium with its daughters. Accurate measurements of activity concentrations were performed on a Perkin Elmer (Waltham, MA) Automatic Wizard2 Gamma Counter and Tri-Carb Liquid Scintillation Analyser, model 1600TR (Packard Instrument Co., Inc.). All experimental samples were counted for at least 1 min to 10 min. Instant thin-layer chromatography (iTLC) for radio-iTLC experiments was performed on strips of glass-fiber, silica-impregnated paper (Agilent), read on a Bioscan AR-2000 radioTLC plate reader, and analyzed using Winscan Radio-TLC software (Bioscan Inc.).

HPLC: HPLC purifications (Buffer A: {H_2O + 0.1% TFA}, Buffer B: {CH_3CN + 0.1% TFA} were performed on a Shimadzu HPLC system equipped with a DGU-20A degasser, a SPD-M20A UV

detector, a LC-6AD pump system and a CBM-20A communication BUS module, using a C_{18} reversed phase XTerra® Preparative MS OBDTM column (10 µm, 19.2 mm × 250 mm).

Synthesis and Characterization

Tz-PEG$_7$-NH2 and **DOTA-PEG$_7$-Tz** were synthesized according to the previously reported method.[2]

Synthesis of 2,2',2''-(10-(1-(4-(1,2,4,5-tetrazin-3-yl)phenyl)-3,7,33-trioxo-11,14,17,20,23,26,29-heptaoxa-2,8,32-triazatetratriacontan-34-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid

Tz-PEG$_7$-NH$_2$ (32.6 mg; 0.05 mmol) was dissolved in dimethylformamide (DMF, 300 µL) and added to DOTA-NHS-ester (13.8 mg; 0.06 mmol; 1.2 equiv.). Triethylamine (20 µL; 0.15 mmol) was then added to this solution, and the solution was placed on an agitating thermomixer at 300 rpm for 60 minutes at room temperature. After 60 minutes, the reaction was purified via preparative C18 HPLC using a gradient of 5:95 {CH_3CN + 0.1 % TFA:H_2O + 0.1 % TFA} to 95:5 {CH_3CN + 0.1 % TFA:H_2O + 0.1 % TFA} over 30 min (t_R = 21.0 min). Lyophilization of the HPLC eluent yielded the purified product as a bright pink powder (10.3 mg; 0.01 mmol; 20 % yield). ^{1}H NMR (600 MHz, DMSO), δ (ppm): 12.80 (br.s, 1H), 10.60 (s, 1H), 8.48 (t, 1H, ^3J(H,H) = 6.0 Hz), 8.47 (d, 2H, ^3J(H,H) = 8.0 Hz), 7.90 (t, 1H, ^3J(H,H) = 6.0 Hz), 7.55 (d, 2H, ^3J(H,H) = 8.3 Hz), 6.57 (br.s, 5H), 4.40 (d, 2H, ^3J(H,H) = 6.0 Hz), 3.61 (br.s, 2H), 3.27-3.44 (m, 44H), 3.20 (br.s, 2H), 3.18 (q, 4H, ^3J(H,H) = 5.8 Hz), 3.00 (br.s, 1H), 2.18 (t, 3H, ^3J(H,H) = 8.0 Hz), 2.10 (t, 3H, ^3J(H,H) = 8.0 Hz), 1.76 (q, 2H, ^3J(H,H) = 7.6 Hz). ESI-MS(+): m/z (%) = 1039.0 $[M+H]^+$; 520.0 $[M+2H]^{2+}$.

TCO conjugation

5B1 (7.51 mg, 14.0 mg/mL, ~ 50.0 nmol) in PBS buffer (pH 6.0) was adjusted to pH 8.5 using a sodium bicarbonate solution (200 mM, pH 10.0). TCO-NHS (2 µmol; 25.0 mg/mL, 94 mM) in DMF were added. The reaction was incubated at 37°C for 90 min before purification with three successive PD-10 desalting columns (GE Healthcare) with a 20 mM sodium acetate, 150 mM sodium chloride solution (pH 6.7).

Determining the TCO occupancy of 5B1-TCO

The average number of TCO moieties per antibody was estimated by incubating the antibody-TCO conjugate (100 µg, PBS) with >150-fold excess of a Tz-functionalized AlexaFluor680 for 180 minutes at room temperature.[2] After purification on PD-10 gel filtration column and concentration via centrifugal filtration, the degree of labelling (DOL) was determined by UV-Vis analysis.

DOL (5B1-TCO) = 1.8 ± 0.4 (n = 3)

Radiolabeling

^{225}Ac was received as a nitrate salt and reconstituted using 0.2 M Optima grade HCl solution (10 µL/37MBq; pH 1).

Preparation of ^{225}Ac-DOTA/DO3A-PEG$_7$-Tz: A solution of DOTA-PEG$_7$-Tz/DO$_3$A-PEG$_7$-Tz (15−450 µg; 10−370 nmol) in NH_4OAc buffer (0.25 M, pH 5.5, 200 µL) was first prepared. Then, the desired amount of activity (20−900 µCi, 0.7-33.0 MBq) was added to the reaction mixture, and the solution was placed on an agitating thermomixer at 300 rpm for 1h at 37 °C. After incubation, the progress of the ^{225}Ac-DOTA/DO3A-PEG$_7$-Tz radiolabeling was determined by iTLC-SG. The paper strips were developed using a 50 mM EDTA solution. The R_f of the radiolabeled chelates were 0 and free metal species were characterized by a R_f of 1. The same paper strip was read twice, once 5 min after its elution and a second time after secular equilibrium (20h after elution). According to the radiochemical purity observed, the ^{225}Ac-complexes were either purified using a C_{18}-light cartridge eluted with 300 µL of ethanol or without further purification. Molar activities are determined when secular equilibrium is reached (24 h post-radiolabeling) with decay correction and considering a recovery of 80% of DOTA/DO3A-PEG$_7$-Tz after the C_{18}-light cartridge.

Conjugation of ^{225}Ac-DOTA/DO3A-PEG$_7$-Tz to 5B1/huA33-TCO: The ^{225}Ac-DOTA-PEG$_7$-Tz/DO$_3$A-PEG$_7$-Tz chelation reaction was mixed with a solution of 5B1/huA33-TCO (30 µg – 3.0 mg) in 20 mM sodium acetate, 150 mM sodium chloride (pH 6.7). Different ratios of chelator to 5B1/huA33-TCO conjugate were used (3 to 55) to determine the influence on the specific activity and radiochemical yields. The reaction was left at room temperature for 5 min, the progress of the radioconjugation was determined by iTLC-SG. The paper strips were developed using an Ethanol/Water (50/50) mixture to ensure a good separation of ^{225}Ac-DOTA/DO3A-PEG$_7$-Tz and the ^{225}Ac-DOTA/DO3A-5B1/huA33 conjugates. The R_f of ^{225}Ac-DOTA/DO3A-5B1/huA33 conjugates were 0 and ^{225}Ac-DOTA/DO3A-PEG$_7$-Tz were characterized by a R_f of 1. The reaction mixture was purified by passage over a PD-10 desalting columns (GE Healthcare) using a 1% BSA, 0.9 % saline solution as a mobile phase. A small amount or the purified final product was used to determine the radiochemical purity (1.0 µL) using iTLC-SG and the same eluent mixture. Radiochemical purity was >98 %. Specific activities are determined when secular equilibrium is reached (24 h post-conjugation) with decay correction and considering a recovery of 80% of the mAb after the PD-10 desalting column.

Samples for injections to mice were injected at least 4 h after the purification of the radiotracers to allow actinium to reach a state close to secular equilibrium and have a good reading of the activity injected.

Saline and human serum stability of ^{225}Ac-complexes

The stability of ^{225}Ac-DOTA/DO3A-5B1 conjugates was investigated in vitro by incubation at 37 ºC in saline and human serum for up to 10 days (n=3 for all measurements). The radiochemical purity and the percentage of activity on proteins were determined via radio-TLC with an eluent of 50 mM EDTA pH 5.0.

Immunoreactivity measurements

The immunoreactivity of ^{225}Ac-DOTA-PEG$_7$-5B1 and ^{225}Ac-DO3A-PEG$_7$-5B1 were determined using CA19.9-positive (BxPC3) and CA19.9-negative (MIAPaCa-2) human pancreatic cancer cells via a protocol adapted from the Lindmo binding assay. 50 µL (200 ng/mL) of a stock solution of radiolabeled construct (0.9 % NaCl, 0.1 % BSA) was added to suspensions of cells (5 x 10^5 to 5 x10^6 cells/mL, 500 µL in PBS, pH 7.4) and incubated at room temperature for 1 h with a slight agitation (n =4). Cells were then centrifuged (600 x g for 2 min) and washed with ice cold PBS (x 3). Cell pellets were resuspended in 500 µL of PBS and transferred to 4.5 mL of liquid scintillation cocktails (Ultima Gold, Perkin Elmer). The activity of ^{225}Ac-DOTA-PEG$_7$-5B1 and ^{225}Ac-DO3A-PEG$_7$-5B1 bound to the cells was measured on a liquid scintillation analyser. The ratio of the total to bound activity was plotted against the inverse of the normalized cell concentration and a linear regression analysis was performed to determine the immunoreactivity.

^{225}Ac-DO3A-PEG$_7$-5B1 (MIAPaCa-2): 0.54 ± 0.11

^{225}Ac-DOTA-PEG$_7$-5B1 (MIAPaCa-2): 0.23 ± 0.01

^{225}Ac-DO3A-PEG$_7$-5B1 (BxPC3): 69.4 ± 0.96

^{225}Ac-DOTA-PEG$_7$-5B1 (BxPC3): 61.3 ± 4.52

Results are slightly lower than one could expect and could be due to a loss of activity during the transfer to liquid scintillation flasks.

Biodistribution

The percentage of injected dose (%ID) was determined by counting standards prepared from the formulated radiotracer together with the tissue samples. The same standards were used to convert the injected weight of the formulated solution into counts. The counts from each sample were decay corrected and background corrected and the count in each sample was converted to % ID/g.

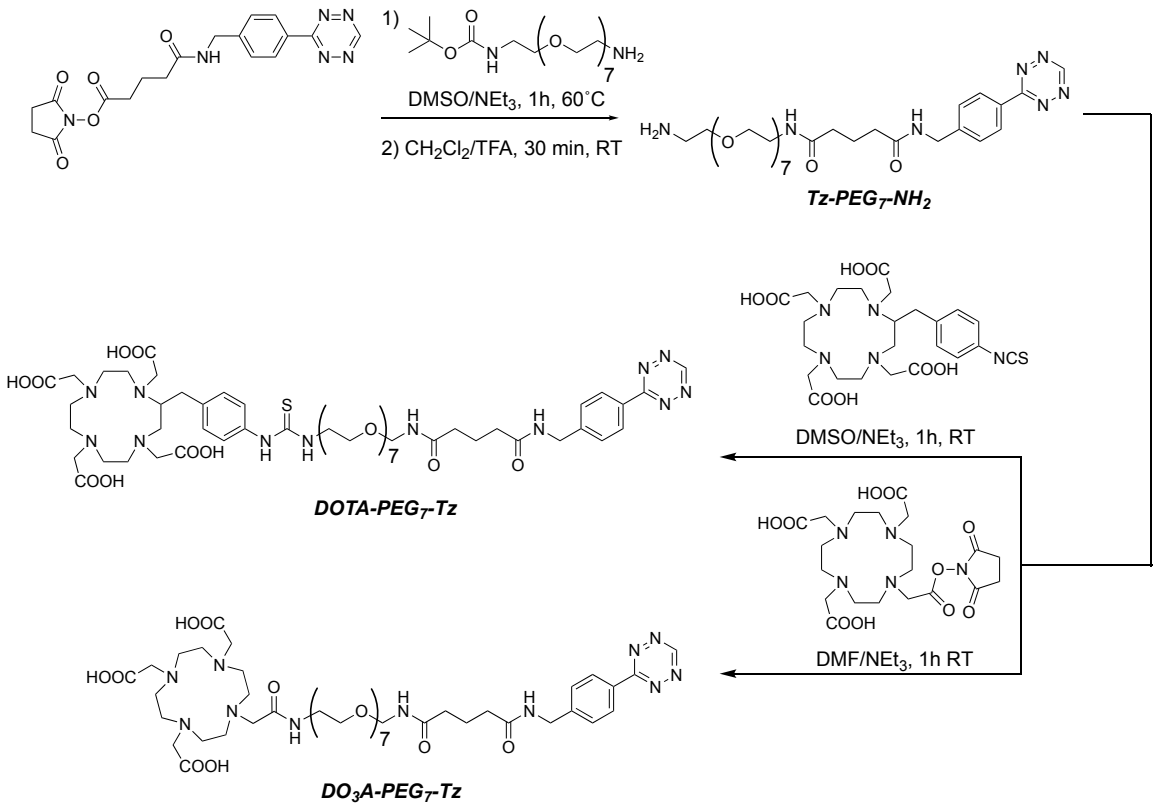

Figure S1. Synthesis scheme of DOTA-PEG_7-Tz and DO_3A-PEG_7-Tz (**1**)

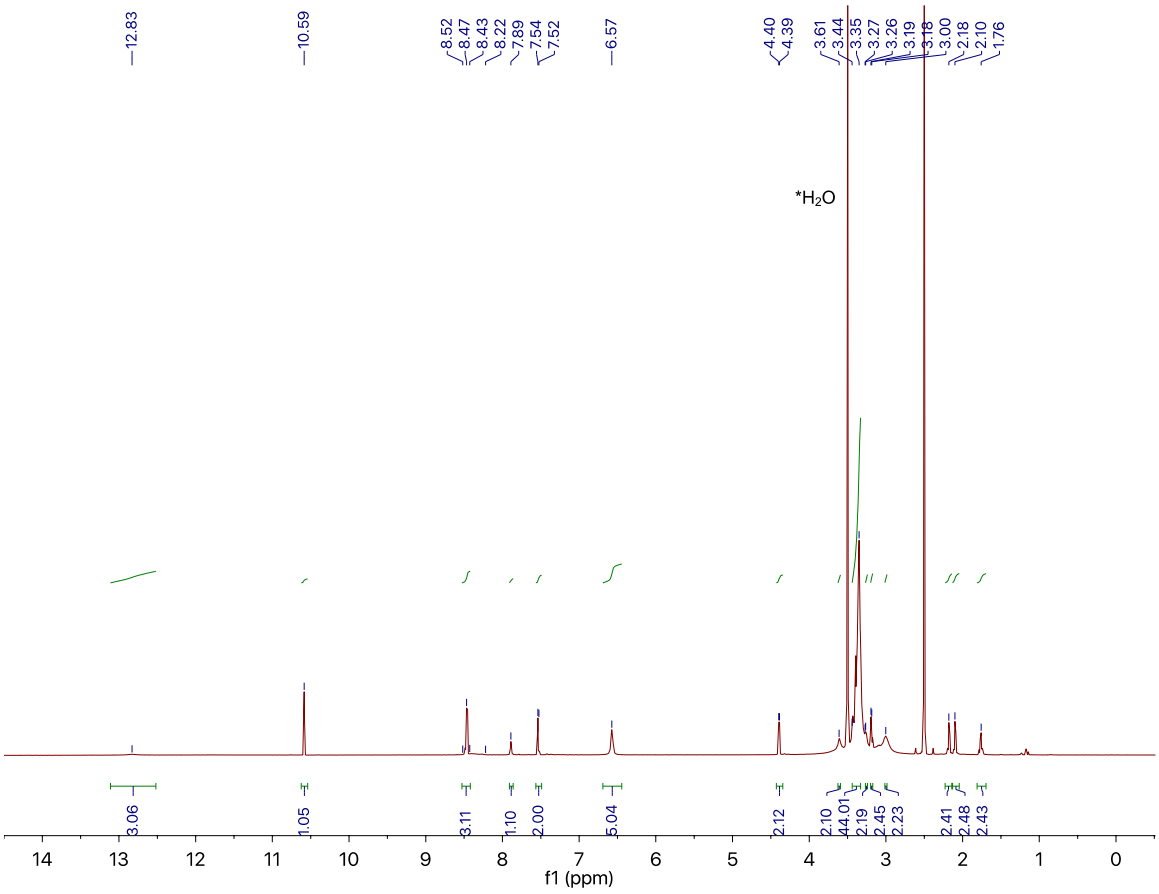

Figure S2. ^{1}H-NMR spectra of DO3A-PEG$_7$-Tz (**1**).

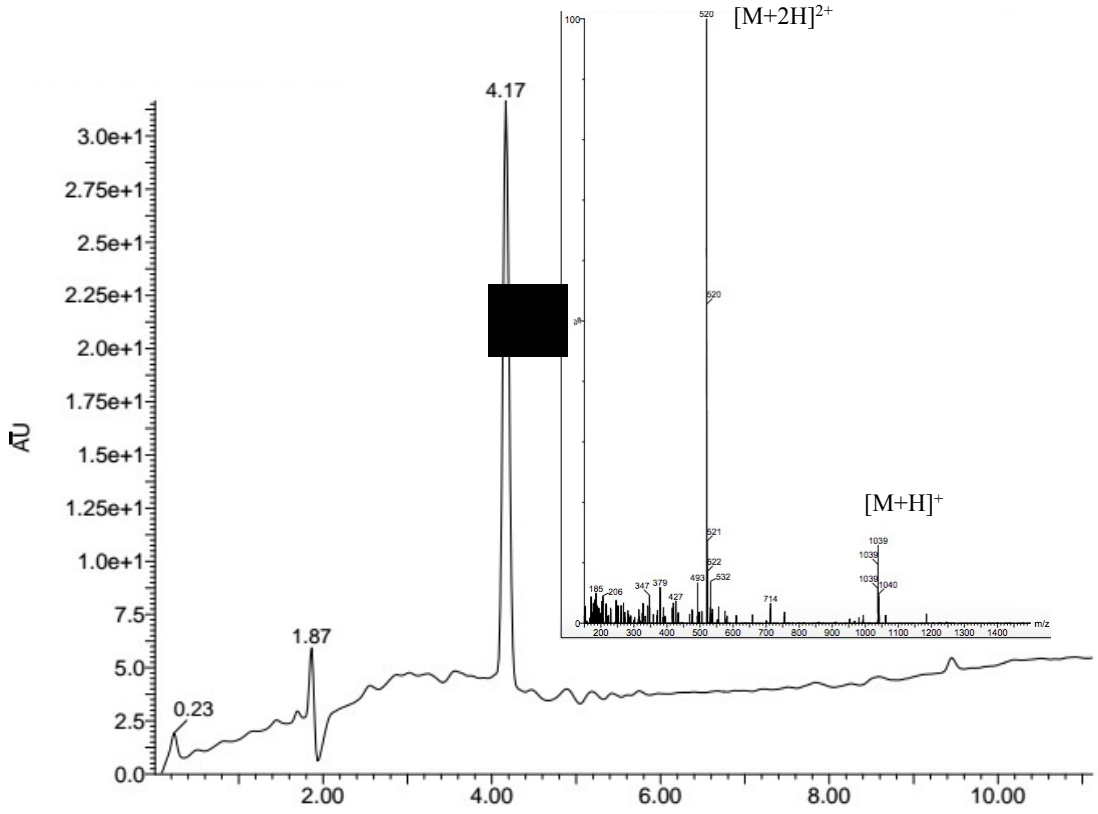

Figure S3. LC-MS analysis of DO3A-PEG$_7$-Tz (**1**).

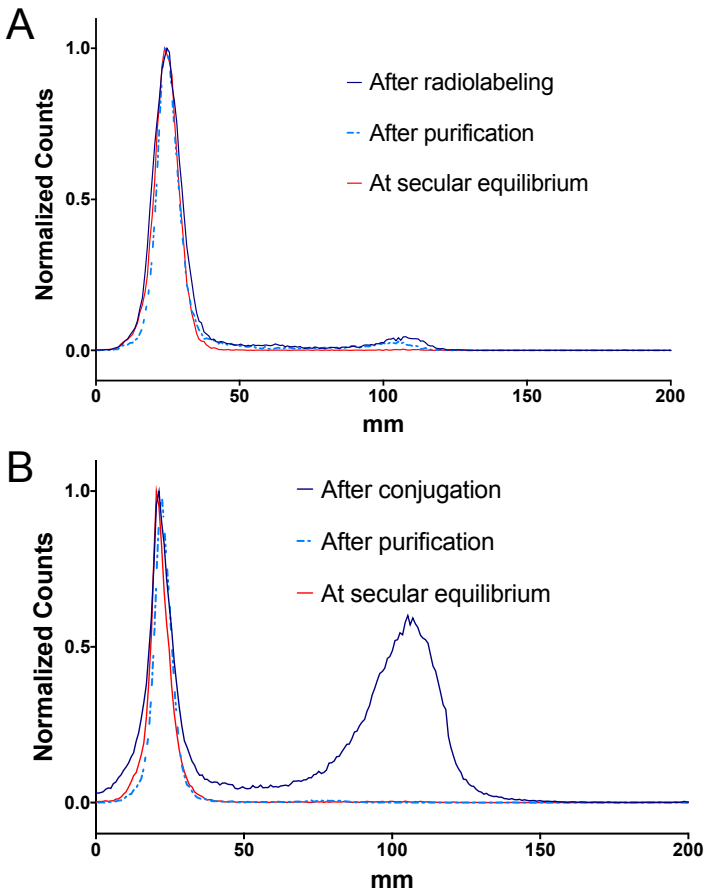

Figure S4. Two-step ^{225}Ac-radiolabelling method based on the IEDDA reaction **(A)** Representative iTLCs of ^{225}Ac-DOTA-PEG7-Tz after elution in 50 mM EDTA solution after the radiolabelling, after purification on C18 light cartridge and once secular equilibrium was reached. The R_f of the radiolabelled DOTA-PEG$_7$-Tz was 0, and free metal species were characterized by a R_f of 1. The difference between the iTLCs after purification and at secular equilibrium highlights the presence of free non-equilibrium daughters; **(B)** Representative iTLCs of ^{225}Ac-DOTA-PEG$_7$-5B1 after elution in a water/ethanol mixture after conjugation, after purification on a PD-10 desalting column, and once secular equilibrium was reached. The R_f of ^{225}Ac-DOTA-PEG$_7$-5B1 was 0, and ^{225}Ac-DOTA-PEG$_7$-Tz was characterized by R_f of 1.

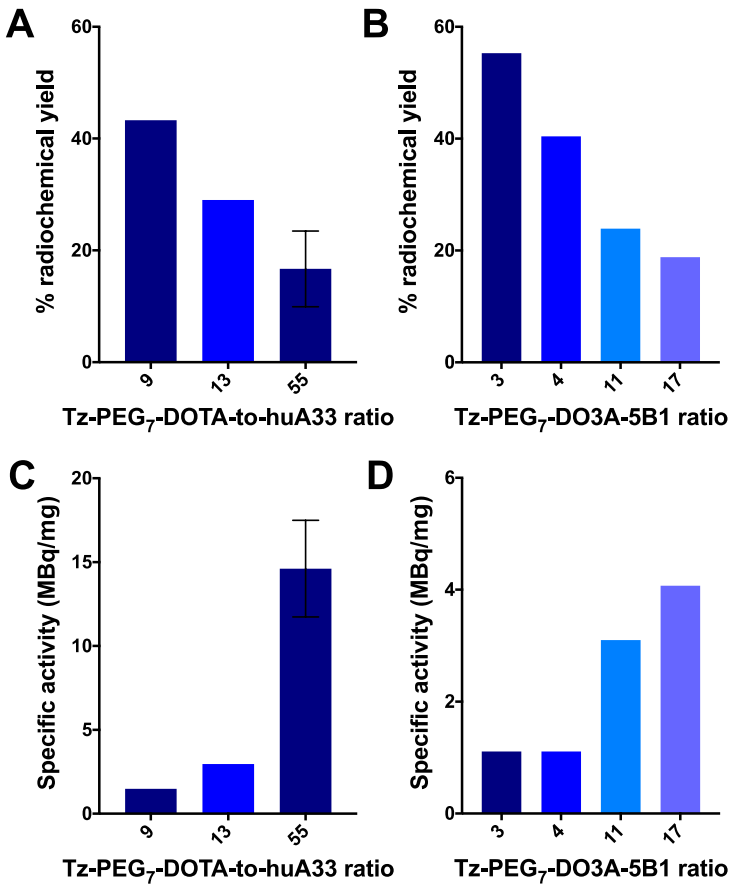

Figure S5. Second step of the ^{225}Ac-IEDDA-radiolabeling method. **(A)** Dependence of the radiochemical yield on the Tz-PEG$_7$-DOTA-to-huA33 ratio; **(B)** Dependence of the radiochemical yield on the Tz-PEG$_7$-DO3A-to-5B1 ratio; **(C)** Dependence of the specific activity on the Tz-PEG$_7$-DOTA-to-huA33 ratio; **(D)** Dependence of the specific activity on the Tz-PEG$_7$-DO3A-to-5B1 ratio.

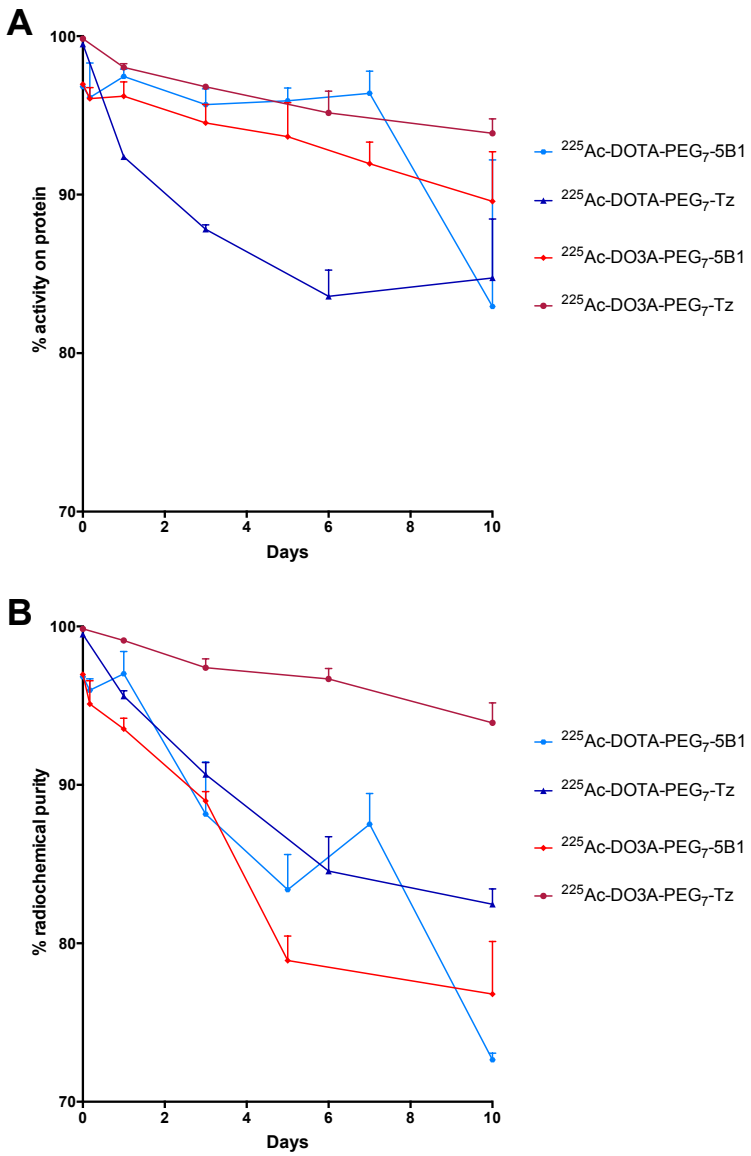

Figure S6. *In vitro* stability study at 37 ºC **(A)** in human serum to determine the percentage of protein-bound ^{225}Ac and **(B)** in saline to determine the radiochemical purity of the ^{225}Ac-conjugates up to 10 days. All data are ± SD, n = 3 per point.

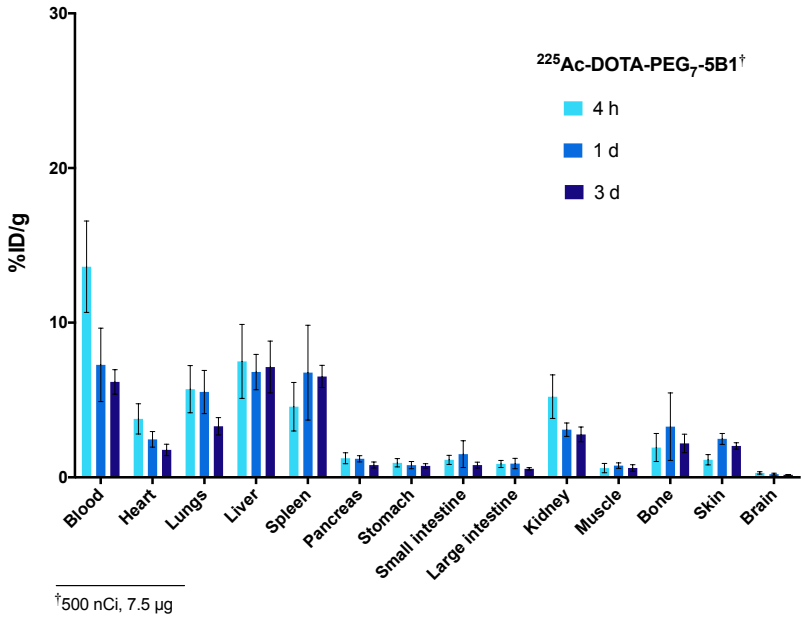

Figure S7. *In vivo* biodistribution in healthy athymic nude mice of ^{225}Ac-DOTA-PEG$_7$-5B1 at 4 h, 1 d and 3 d post-injection. Error bars represent the SD (n = 5).

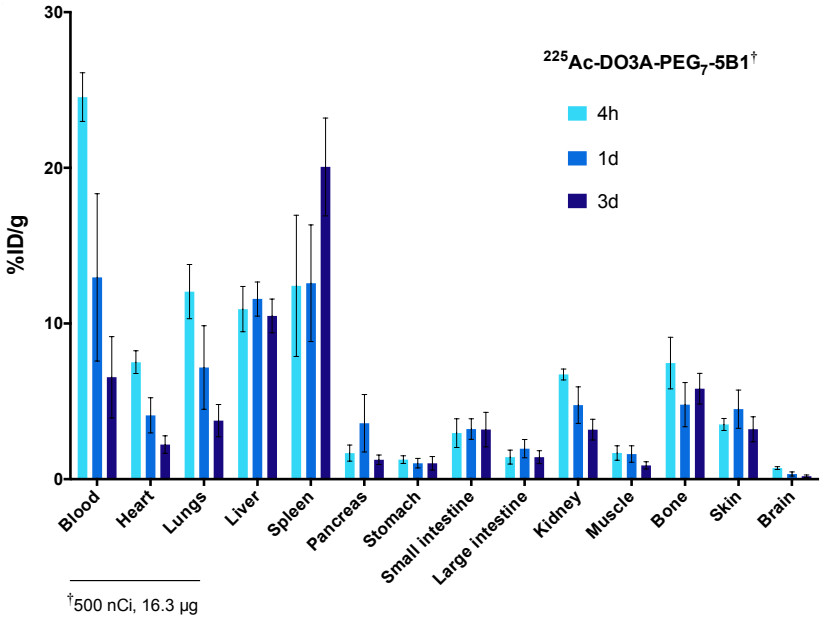

Figure S8. *In vivo* biodistribution in healthy athymic nude mice of ^{225}Ac-DO3A-PEG$_7$-5B1 at 4 h, 1 d and 3 d post-injection. Error bars represent the SD (n = 5).

	^{225}Ac-DOTA-PEG$_7$-5B1			^{225}Ac-DO3A-PEG$_7$-5B1		
	4 hours	1 day	3 days	4 hours	1 day	3 days
Blood	13.6 ± 2.9	7.3 ± 2.4	6.2 ± 0.8	24.5 ± 1.6	13.0 ± 5.4	6.5 ± 2.6
Heart	3.8 ± 1.0	2.5 ± 0.5	1.8 ± 0.4	7.5 ± 0.7	4.1 ± 1.1	2.2 ± 0.6
Lungs	5.7 ± 1.5	5.5 ± 1.4	3.3 ± 0.6	12.0 ± 1.7	7.2 ± 2.7	3.8 ± 1.0
Liver	7.5 ± 2.4	6.8 ± 1.1	7.1 ± 1.7	10.9 ± 1.5	11.6 ± 1.1	10.5 ± 1.0
Spleen	4.6 ± 1.6	6.8 ± 3.1	6.5 ± 0.7	12.4 ± 4.5	12.6 ± 3.7	20.1 ± 3.1
Pancreas	1.2 ± 0.4	1.2 ± 0.2	0.8 ± 0.2	1.7 ± 0.5	3.6 ± 1.8	1.2 ± 0.3
Stomach	0.9 ± 0.3	0.8 ± 0.2	0.7 ± 0.1	1.3 ± 0.2	1.0 ± 0.3	1.0 ± 0.4
Small intestine	1.1 ± 0.3	1.5 ± 0.9	0.8 ± 0.2	3.0 ± 0.9	3.2 ± 0.6	3.2 ± 1.1
Large intestine	0.9 ± 0.2	0.9 ± 0.3	0.6 ± 0.2	1.4 ± 0.4	2.0 ± 0.6	1.4 ± 0.4
Kidney	5.2 ± 1.4	3.1 ± 0.4	2.8 ± 0.5	6.7 ± 0.3	4.8 ± 1.2	3.1 ± 0.6
Muscle	0.6 ± 0.3	0.8 ± 0.2	0.6 ± 0.2	1.7 ± 0.5	1.6 ± 0.5	0.9 ± 0.2
Bone	1.9 ± 0.9	3.3 ± 2.2	2.2 ± 0.6	7.5 ± 1.7	4.8 ± 1.4	5.8 ± 1.0
Skin	1.1 ± 0.3	2.5 ± 0.4	2.0 ± 0.2	3.5 ± 0.4	4.5 ± 1.2	3.2 ± 0.8
Brain	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.2 ± 0.1

Table S1. Biodistribution data (% ID/g ± SD) of ^{225}Ac-DOTA-PEG$_7$-5B1 (18.5 kBq, 7.6 µg) and ^{225}Ac-DO3A-PEG$_7$-5B1 (18.5 kBq nCi, 16.3 µg) up to 3 days in healthy athymic nude mice (n = 5).

	^{225}Ac-DOTA-PEG$_7$-5B1					
	4 hours BxPC3	1 day BxPC3	3 days BxPC3	3 days MIAPaCa-2	7 days BxPC3	10 days BxPC3
Blood	16.7 ± 2.3	8.4 ± 2.9	3.7 ± 2.9	5.3 ± 2.8	0.7 ± 0.6	0.4 ± 0.3
Heart	5.1 ± 0.6	2.5 ± 0.9	1.0 ± 0.6	1.7 ± 0.9	0.3 ± 0.2	0.2 ± 0.1
Lungs	7.5 ± 0.9	4.8 ± 1.3	1.5 ± 0.8	2.5 ± 1.1	0.5 ± 0.4	0.3 ± 0.2
Liver	10.5 ± 1.7	9.4 ± 2.8	7.8 ± 1.0	6.2 ± 0.9	7.3 ± 1.5	5.8 ± 2.6
Spleen	6.7 ± 1.4	6.5 ± 2.2	3.9 ± 1.5	5.4 ± 2.3	5.6 ± 1.6	4.0 ± 1.1
Pancreas	1.6 ± 0.5	1.4 ± 0.5	0.3 ± 0.2	0.6 ± 0.3	0.2 ± 0.1	0.1 ± 0.1
Stomach	1.0 ± 0.2	0.8 ± 0.2	0.4 ± 0.2	0.6 ± 0.3	0.2 ± 0.1	0.1 ± 0.1
Small intestine	1.5 ± 0.5	1.2 ± 0.4	0.4 ± 0.1	0.7 ± 0.3	0.2 ± 0.1	0.2 ± 0.1
Large intestine	1.3 ± 0.5	1.0 ± 0.2	0.5 ± 0.3	0.5 ± 0.2	0.2 ± 0..1	0.2 ± 0.1
Kidney	5.3 ± 0.6	3.3 ± 0.9	1.4 ± 0.5	2.3 ± 0.9	0.6 ± 0.3	0.5 ± 0.1
Muscle	0.7 ± 0.2	0.8 ± 0.3	0.2 ± 0.1	0.5 ± 0.3	0.1 ± 0.1	0.1 ± 0.1
Bone	2.1 ± 0.4	2.3 ± 0.7	2.2 ± 1.0	4.4 ± 1.7	1.0 ± 0.4	1.6 ± 0.8
Skin	2.8 ± 0.4	3.6 ± 0.9	2.2 ± 1.0	4.0 ± 0.5	0.8 ± 0.4	0.5 ± 0.3
Tumor	15.4 ± 3.5	35.9 ± 13.9	31.1 ± 21.4	5.3 ± 2.7	21.9 ± 8.9	32.4 ± 9.8
Carcass	1.8 ± 0.1	1.8 ± 0.2	0.9 ± 0.2	1.4 ± 0.5	0.4 ± 0.1	0.3 ± 0.1

Table S2. Biodistribution data (%ID/g ± SD) of ^{225}Ac-DOTA-PEG$_7$-5B1 (18.5 kBq, 8.6 µg, 0.06 nmol) up to 10 days post-injection in BxPC3 (CA19.9-positive) and MIAPaCa-2 (CA19.9-negative) tumor-bearing athymic nude mice (n = 5).

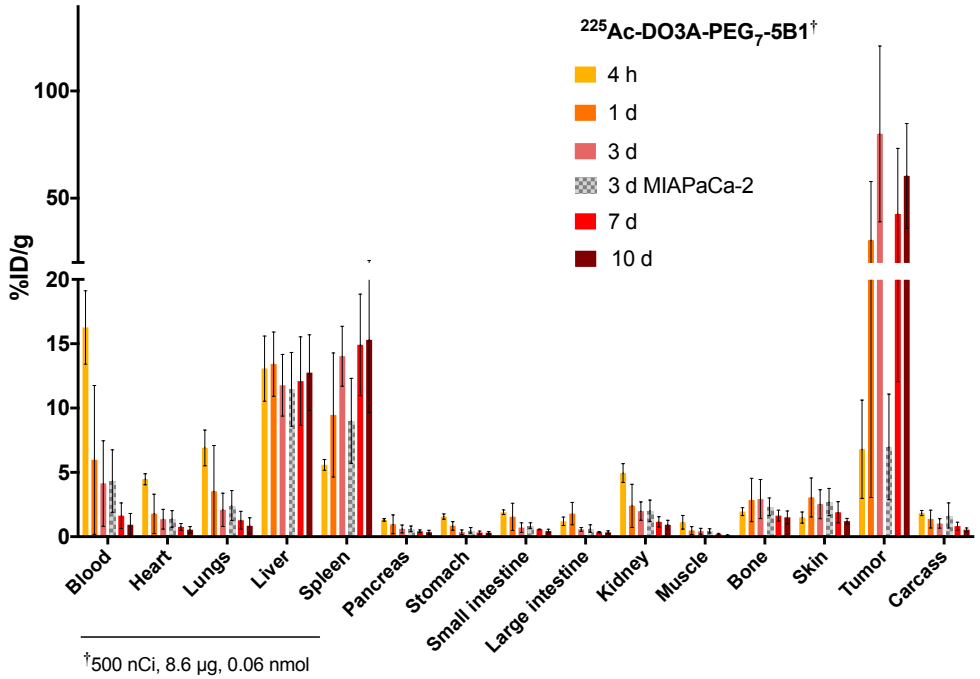

Figure S9. *In vivo* biodistribution in BxPC3 (CA19.9-positive) and MIAPaCa-2 (CA19.9-negative) tumor-bearing athymic nude mice of ^{225}Ac-DO3A-PEG$_7$-5B1 up to 10 days post-injection. Error bars represent the SD (n = 5).

	^{225}Ac-DO3A-PEG$_7$-5B1					
	4 hours BxPC3	1 day BxPC3	3 days BxPC3	3 days MIAPaCa-2	7 days BxPC3	10 days BxPC3
Blood	16.3 ± 2.9	6.0 ± 5.8	4.14 ± 3.3	4.3 ± 2.4	1.6 ± 1.0	0.9 ± 0.9
Heart	4.5 ± 0.4	1.8 ± 1.5	1.4 ± 0.7	1.4 ± 0.6	0.8 ± 0.3	0.5 ± 0.2
Lungs	6.9 ± 1.4	3.5 ± 3.5	2.1 ± 1.3	2.4 ± 1.2	1.3 ± 0.7	0.9 ± 0.6
Liver	13.1 ± 2.5	13.4 ± 2.5	11.8 ± 2.4	11.5 ± 2.9	12.1 ± 3.4	12.8 ± 3.0
Spleen	5.6 ± 0.4	9.5 ± 4.8	14.0 ± 2.3	9.0 ± 3.3	14.9 ± 4.0	15.3 ± 5.7
Pancreas	1.3 ± 0.1	1.0 ± 0.7	0.6 ± 0.3	0.6 ± 0.2	0.4 ± 0.1	0.4 ± 0.1
Stomach	1.6 ± 0.2	0.8 ± 0.3	0.3 ± 0.2	0.5 ± 0.3	0.3 ± 0.1	0.3 ± 0.1
Small intestine	1.9 ± 0.2	1.6 ± 1.0	0.7 ± 0.4	0.9 ± 0.2	0.6 ± 0.1	0.4 ± 0.1
Large intestine	1.2 ± 0.3	1.8 ± 0.8	0.6 ± 0.2	0.6 ± 0.3	0.4 ± 0.1	0.3 ± 0.1
Kidney	5.0 ± 0.7	2.4 ± 1.7	2.0 ± 0.7	2.0 ± 0.8	1.2 ± 0.4	0.9 ± 0.4
Muscle	1.1 ± 0.5	0.5 ± 0.3	0.4 ± 0.2	0.5 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
Bone	2.0 ± 0.3	2.9 ± 1.7	2.9 ± 1.5	2.3 ± 0.7	1.6 ± 0.4	1.5 ± 0.5
Skin	1.5 ± 0.4	3.1 ± 1.5	2.5 ± 1.1	2.7 ± 1.1	1.9 ± 0.8	1.2 ± 0.2
Tumor	6.8 ± 3.8	30.4 ± 27.3	80.0 ± 41.0	7.0 ± 4.1	42.6 ± 30.6	60.4 ± 24.5
Carcass	1.8 ± 0.2	1.4 ± 0.7	1.0 ± 0.4	1.7 ± 1.0	0.8 ± 0.3	0.5 ± 0.1

Table S3. Biodistribution data (%ID/g ± SD) of ^{225}Ac-DO3A-PEG$_7$-5B1 (18.5 kBq, 8.6 µg, 0.06 nmol) up to 10 days post-injection in BxPC3 (CA19.9-positive) and MIAPaCa-2 (CA19.9-negative) tumor-bearing athymic nude mice (n = 5).

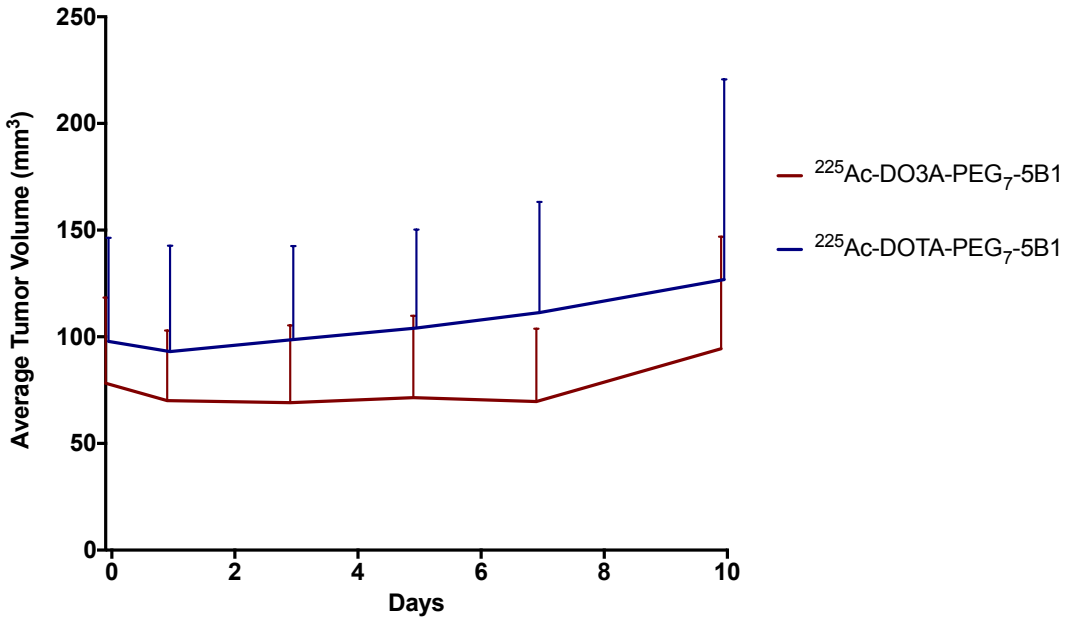

Figure S10. Plot of the average tumor volume of the 7 days and 10 days biodistribution BxPC3 (CA19.9-positive) tumor-bearing athymic nude mice groups.

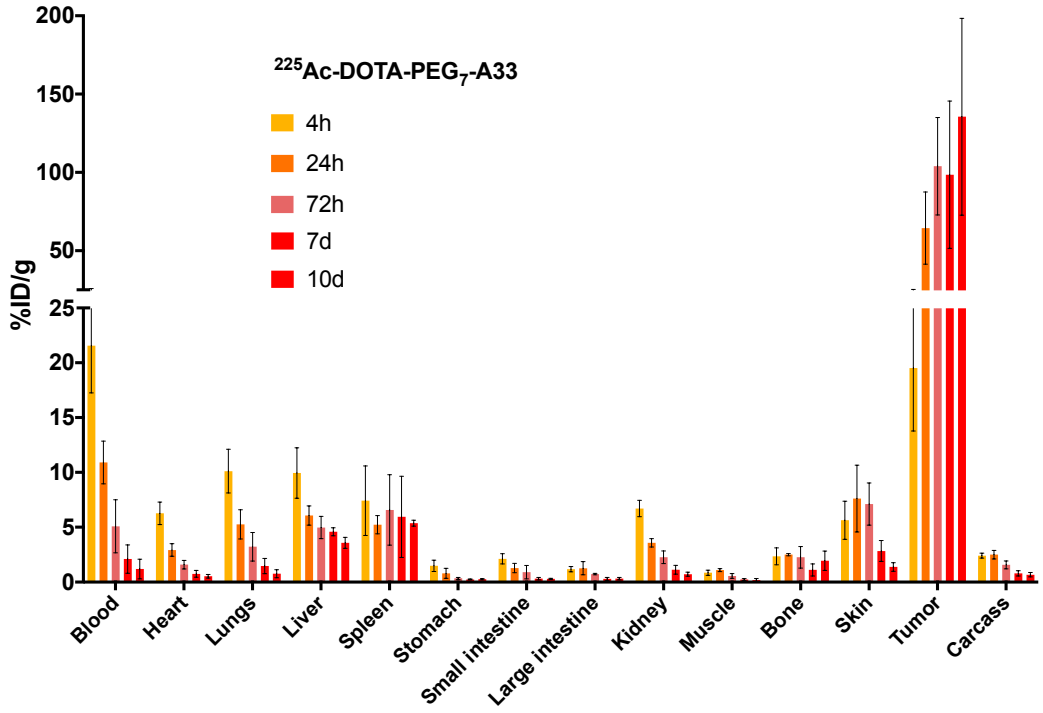

Figure S11. *In vivo* biodistribution in SW1222 (A33 positive) tumor-bearing athymic nude mice of ^{225}Ac-DOTA-PEG$_7$-huA33 up to 10 days post-injection. Error bars represent the SD (n = 5)

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