Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Development of ^{18}F-Labeled hydrophilic *trans*-cyclooctene as a bioorthogonal tool for PET probe construction

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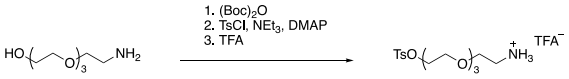
1. General

All chemicals that can be obtained commercially were used without further purification. A Luna C18 column (Phenomenex, 250 mm × 4.6 mm) was used for analytical RP HPLC, and the flow rate was 1 mL/min. A Gemini C18 column (Phenomenex, 250 mm × 10 mm) was used for semi-preparative RP HPLC, and the flow rate was 3.2 mL/min. Both HPLC shared the same mobile phase system with 0.1% TFA in water as solvent A and 0.1% TFA in acetonitrile as solvent B. The methods used in each run were listed in the supporting information. NMR spectra were obtained on Bruker AV400 (1H: 400 MHz, 13C: 101 MHz) and AV600 (1H: 600 MHz, 13C: 151 MHz) instruments. High-resolution mass spectra were acquired on a Q Exactive HF-X (Thermo Fisher, Bremen, Germany) system or a Waters GCT Premier and Thermo Q-Exactive Orbitrap. PD-10 column (Cytiva) was used for the purification of protein conjugation. The 3.5 – 4 mL fraction from the PD-10 column and used in further experiments, such as imaging studies and other conjugations. All animal experiments and procedures were performed in accordance with the United States' National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" and in compliance with a protocol approved by the University of North Carolina Institutional Animal Care and Use Committee.

2. Chemistry

Compound 1



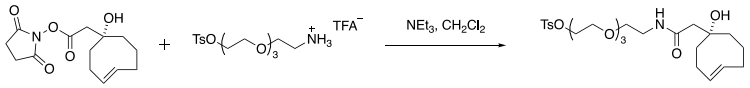
1

2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate TFA salt (1) An oven-dried 20 mL vial equipped with a magnetic stir bar was charged with amino-PEG_4-alcohol (300 mg, 1.55 mmol) and CH_2Cl_2 (9.7 mL) and stirred briefly before di-tert-butyl dicarbonate (338 mg, 1.55 mmol) was added. The reaction was stirred for 1 hour before concentrating to obtain the boc-protected-amino-PEG_4-alcohol intermediate as a clear, colorless oil that was immediately utilized in the next step.

The Boc-protected amino-PEG_4-alcohol was transferred to a round bottom flask equipped with a magnetic stir bar where CH_2Cl_2 (3.5 mL), 4-dimethyl-aminopyridine (1.7 mg, 0.014 mmol), and NEt_3 (0.45 mL, 3.2 mmol) were added and stirred. The reaction was chilled to 0 °C, then 4-toluenesulfonyl chloride (419 mg, 2.20 mmol) in 3.6 mL of CH_2Cl_2 was added dropwise, and the reaction was stirred overnight at room temperature. The reaction was quenched with 1.2 mL of 2M citric acid. The organic layer was washed with water, dried with $MgSO_4$, filtered, and concentrated. The crude was purified by silica gel chromatography (0-3% MeOH in CH_2Cl_2) to afford a pale-yellow oil as the Boc-protected-amino-PEG_4-OTs that was utilized directly in the next step.

To a 25 mL vial equipped with a magnetic stir bar containing the Boc-protected-amino-PEG_4-OTs intermediate, 4.1 mL of CH_2Cl_2 was added and stirred. Trifluoroacetic acid (2 mL) was next added dropwise, and the reaction was stirred for 25 minutes before concentrating. To the concentrated product, 4 mL toluene was added and concentrated again (repeated 3x) before drying under a high vacuum overnight. A clear, colorless oil (**1**, 790 mg) was obtained that contained traces of trifluoroacetic acid but was utilized in the next steps as it was otherwise pure by NMR analysis. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.1 Hz, 2H), 7.48 (s, 3H), 7.36 (d, *J* = 8.0 Hz, 2H), 4.18 – 4.14 (m, 2H), 3.87 – 3.82 (m, 2H), 3.78 – 3.72 (m, 2H), 3.72 – 3.63 (m, 8H), 3.30 (m, 2H), 2.45 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.7 (C), 131.9 (C), 130.1 (CH), 127.9 (CH), 70.4 (CH_2), 70.0 (CH_2), 69.9 (CH_2), 69.8 (CH_2), 69.6 (CH_2), 68.5 (CH_2), 66.8 (CH_2), 40.2 (CH_2), 21.7 (CH_3). FTMS (ESI+) calculated [M+H]+ for $C_{15}H_{26}NO_6S$, 348.1481; found 348.1468.

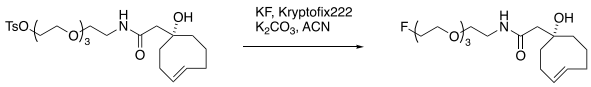
Compound 2



1 2

(E)-1-(1-hydroxycyclooct-4-en-1-yl)-2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl 4-methylbenzenesulfonate (2) A round bottom flask equipped with a magnetic stir bar was charged with **1** (354 mg, 0.767 mmol), CH_2Cl_2 (8.5 mL), and NEt_3 (0.20 mL, 1.4 mmol) and stirred for 20 minutes. (E)-N-hydroxysuccinyl 2-(1-ax-hydroxycyclooct-4-en-1-yl)acetate (120 mg, 0.427) was added in one portion, and the reaction was monitored by TLC. After 10 minutes, the reaction was loaded directly onto a silica gel chromatography column for purification (0-2% MeOH in CH_2Cl_2) to afford 212 mg (**2**, 0.413 mmol, 97% yield) of the title compound as a clear, colorless oil. ^{1}H NMR (600 MHz, C_6D_6) δ 7.75 (d, J = 8.3 Hz, 2H), 6.71 (d, J = 8.0 Hz, 2H), 6.09 (ddd, J = 15.4, 11.3, 3.7 Hz, 1H), 5.88 (br s, 1H), 5.55 (ddd, J = 15.4, 11.4, 3.3 Hz, 1H), 5.34 (s, OH), 3.88 (t, J = 4.7 Hz, 2H), 3.30 (dd, J = 5.8, 3.4 Hz, 2H), 3.29 – 3.18 (m, 10H), 3.14 (t, J = 5.1 Hz, 2H), 2.81 (qd, J = 11.8, 4.7 Hz, 1H), 2.35 – 2.29 (m, 1H), 2.16 (qd, J = 12.5, 4.5 Hz, 1H), 2.10 – 2.02 (m, 1H), 1.97 – 1.88 (m, 2H), 1.88 – 1.78 (m, 5H), 1.74 – 1.67 (m, 1H), 1.59 (dd, J = 15.0, 6.7 Hz, 1H), 1.40 (td, J = 13.1, 4.7 Hz, 1H), 1.32 (dd, J = 15.0, 11.7 Hz, 1H). ^{13}C NMR (101 MHz, C_6D_6) δ 173.2 (C), 144.4 (C), 135.7 (CH), 134.1 (C), 131.7 (CH), 129.9 (CH), 128.4 (CH), 71.5 (C), 70.9 (CH_2), 70.8 (CH_2), 70.6 (CH_2), 70.4 (CH_2), 70.0 (CH_2), 69.2 (CH_2), 68.8 (CH_2), 50.9 (CH_2), 48.4 (CH_2), 40.0 (CH_2), 39.2 (CH_2), 35.0 (CH_2), 30.8 (CH_2), 27.8 (CH_2), 21.2 (CH_3). FTMS (ESI+) calculated [M+H]+ for $C25H40O_8NS$, 514.2475; found 514.2471

Compound 3

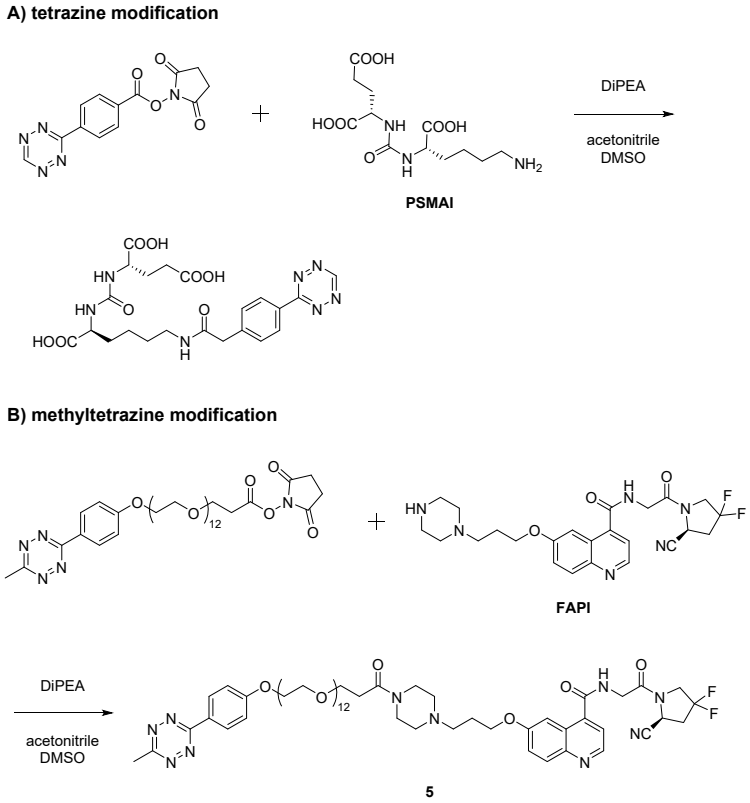


2 3

(E)-N-(2-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)ethyl)-2-(1-hydroxycyclooct-4-en-1-yl)acetamide (3) A round bottom flask equipped with a magnetic stir bar and a condenser was charged with KF (4.0 mg, 0.069 mmol), Kryptofix® 222 (25 mg, 0.066 mmol), K_2CO_3 (5.4 mg, 0.039 mmol), and acetonitrile (0.4 mL). TCO **2** (20 mg, 0.039 mmol) in acetonitrile (0.5 mL) was added and the reaction was heated to 45∞C for 1 hour and monitored by TLC before diluting with CH_2Cl_2, cooling, and concentrating. The crude was purified by silica gel chromatography (0-20% acetone in CH_2Cl_2) to afford 9.5 mg (**3**, 61% yield by NMR) as a clear, colorless oil that was deemed ~90% pure by NMR analysis. ^{1}H NMR (600 MHz, CD_3CN) δ 6.77 (br s, 1H), 5.64 (ddd, J = 15.9, 10.7, 3.4 Hz, 1H), 5.53 (ddd, J = 16.0, 11.1, 3.3 Hz, 1H), 4.85 (s, OH), 4.52 (dm, J_{HF} = 48.1 Hz, 2H), 3.67 (dm, J_{HF} = 31.4 Hz, 2H), 3.63 – 3.48 (m, 8H), 3.47 (t, J = 5.5 Hz, 2H), 3.30 (q, J = 5.5 Hz, 2H), 2.40 (app qd, J = 12.5, 11.9, 11.6, 4.7 Hz, 1H), 2.16 – 2.11 (m, 2H), 2.03 – 1.96 (m, 1H), 1.89 – 1.82 (m, 1H), 1.82 – 1.72 (m, 2H), 1.70 – 1.56 (m, 4H), 1.43 (app dd, J = 15.0, 10.7 Hz, 1H).^{13}C NMR (101 MHz, CD_3CN) δ 174.1 (C), 135.7 (CH), 132.4 (CH), 84.2 (d, J_{CF} = 165.5 Hz), 72.1 (C), 71.2 (CH_2), 71.02 (CH_2), 71.00 (CH_2), 70.81 (CH_2), 70.80 (CH_2), 70.01(CH_2), 51.2 (CH_2), 48.6 (CH_2), 39.8 (CH_2), 39.5 (CH_2), 34.8 (CH_2), 30.9 (CH_2), 28.0 (CH_2). FTMS (ESI+) calculated [M+H]+ for $C18H33O_5NF$, 362.2343; found 362.2331.

Compound 4 – 6

Scheme S1. Synthesis of tetrazine-modified small molecules



Tetrazine-NHS ester or methyltetrazine-PEG_{12}-NHS ester (Broadpharm) was dissolved in DMSO to make a tetrazine stock solution. Compounds **4** and **5** were separately dissolved in DMSO to make stock solutions. Each compound solution was mixed with tetrazine solution with a molar ratio of 1:1.1, followed by the addition of acetonitrile (ACN) and diisopropyl ethyl amine (DiPEA). The reaction vessels were placed on a thermomixer (Eppendorf) at 25 ℃ for 1 h. The products were purified using semi-preparative HPLC and lyophilized to store for further usage. Tetrazine-modified ligands were shown as follows. (Scheme S1)

PSMAI-NH_2 (3.0 mg, TargetMol), tetrazine-NHS ester (3.6 mg, Broadpharm), ACN (100 uL), DMSO (100 uL), and DiPEA (5 uL) were used to get compound **4** (1.5 mg, yield).**5** was synthesized in a tracer amount of **FAPI**, and the yield was estimated using an HPLC chromatogram (260 nm) as 21%.

3. Synthesis of tetrazine-modified proteins

Methyltetrazine-PEG_{12}-NHS ester (Broadpharm) was dissolved in DMSO to make a stock solution with a 5 ug/uL concentration. Proteins of interest were dissolved in water to 2 ug/uL. Tetrazine stock solutions were added to the protein solutions with a molar ratio of 1:10, and the pH of each reaction solution was adjusted to 8.5 using borate buffer (20 x). Protein was purified by PD-10 columns and stored under 4 degrees for future use. (tetrazine-modified mouse serum albumin **8**, tetrazine-modified anti-CD8 diabody **9**, tetrazine-modified anti-CD4 diabody **10**, and tetrazine-modified HER2-binding protein ligand **11**)

4. Radiochemistry

Synthesis of [^{18}F]3 The a-TCO precursor **2** was dissolved in DCM as a stock solution with a 35 μg/μL concentration. 5 μL of stock solution was mixed with 37 MBq [^{18}F]TBAF acetonitrile solution. The radiolabeling reaction was monitored by radio HPLC, and the product [^{18}F]**3** was collected and measured. The additional testing solvent, such as DMSO, DMF, and tert-butyl alcohol, was added to the reaction by 5 μL. The best condition was found when the reaction was heated for 10 minutes at 80 ℃ in dichloromethane and acetonitrile, and the decay-corrected radiochemistry yield (RCY) was 52%. The rest of conditions tested are listed in **Table S1**.

Table S1. Scope of the radiolabeling condition

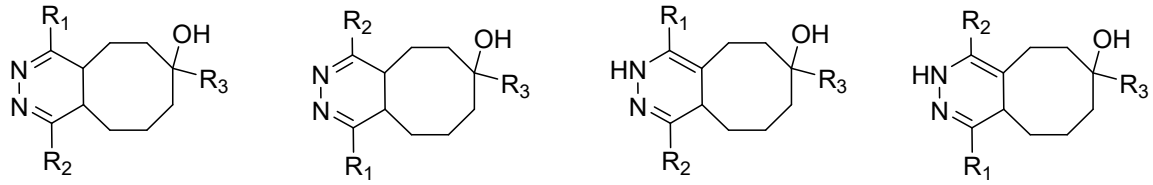
Solvent*	Temperature	Duration	RCY**
DCM + ACN	60 ℃	15 min	24%
DCM + ACN + DMF	60 ℃	15 min	1.3%
DCM + ACN + DMSO	60 ℃	15 min	12%
DCM + ACN + t-BuOH	60 ℃	15 min	8.7%
DCM + ACN	40 ℃	15 min	7.0%
DCM + ACN	80 ℃	15 min	46%
DCM + ACN	95 ℃	15 min	15%
DCM + ACN	80 ℃	5 min	34%
DCM + ACN	80 ℃	10 min	52%
DCM + ACN	80 ℃	30 min	14%

* DCM from the stock solution was not removed

** Radiochemical yield (RCY) was decay-corrected.

Tetrazine ligation of [^{18}F]3 For small molecule labeling, the [^{18}F]**3** solution obtained from HPLC was directly added into the tetrazine-modified small molecule stock solution. The mixture was mixed thoroughly using the micropipette, and the product was purified through HPLC to get the pure labeling product.

For protein labeling, the [^{18}F]**3** solution got from HPLC was diluted with 10 ml of water, and the resulting solution was pushed into a C18 cartridge by a 12 ml syringe slowly. Another 10 ml of water was used to wash the cartridge to make sure the acetonitrile was fully removed. 1 ml of ether was pushed through the cartridge to wash off all [^{18}F]**3**. And then, the ether was blown away by a nitrogen stream, and the residue was redissolved in water to make a stock solution. The stock solution was then measured and added to the protein stock solution. The mixture was well mixed, and the ^{18}F labeled protein was separated from the unreacted small molecules using the PD-10 column. Potential isomers of aTCO-tetrazine ligation (These isomers have the same mass but different retention time on HPLC):



Hydrophilicity We conducted the partition experiment to test the solubility of [^{18}F]**3**. 5.70 MBq (154 μCi) of [^{18}F]**3** was dissolved in 150 μL 1-octanol and 150 μL 1x PBS buffer, and the two layers were mixed thoroughly. Two layers were separated precisely with a pipette, and the radioactivity was measured for each part. 1.96 MBq (53 μCi, with decay-correction) was measured in the 1x PBS buffer part, and 3.74 MBq (101 μCi, with decay-correction) was measured in the 1-octanol part. After calculation, the LogP value was obtained as 0.28.

Protein conjugation analysis using SDS-PAGE 10 μg unlabeled protein samples and 0.185 MBq (5 μCi) radiolabeled protein samples were mixed with loading buffer and heated for 1 min. Then the loading solutions were loaded in the wells of a vertical SDS-PAGE system. 80 mV voltage and running for 90 minutes or until the smallest marker has reached the bottom. The gel was attached to an X-ray film and together placed in a cassette for exposure for around one hour. Sapphire biomolecular imager (Azure Biosystems) was used to obtain the autoradiography using phosphor imaging mode. The gel was then stained with Coomassie blue and imaged for analysis. All radiolabeled proteins have very similar molecular weights to the unlabeled tetrazine-modified proteins.

5. Animal xenograft model

About 4-week-old nude mice were used to establish mouse tumor models. The tumor cells U87 (4 x 10^6 counts), H1299 (10 x 10^6 counts), PC3-PSMA (2 x 10^6 counts), and B16F10 (2 x 10^6 counts) harvested from cell culture were inoculated into each mouse. Matrigel was mixed with U87 and H1299 tumor cells to make cell suspension before inoculation. Usually, the tumor starts growing 7 days after inoculation if it's not shrinking, and the xenograft model is used for imaging studies after it reaches 150 mm^3.

6. Small animal imaging

SuperArgus PET/CT 4R (Sedecal) was used for PET and CT imaging. About 3.7 MBq (100 μCi) of each tracer was injected into the tail veins of the mouse. Static PET and CT images were acquired at 0.5 h, 1.5 h, and 3.0 h or 4.0 h post-injection with mice under isoflurane anesthesia for 15 mins. The regions of interest (ROIs) were analyzed using Amide software, and organ uptakes were calculated as percentage injected dose per gram (%ID/g). The tumor-to-organ ratio is calculated by using the PET-derived tumor uptake divided by the organ uptake for each animal. Then, we calculated the mean and standard deviation based on individual ratios.

Table S2. Tumor/kidney ratio comparison of several PET tracers targeting PSMA

Tracer	Model	Tumor/Kidney Ratio	Reference
[^{68}Ga]Ga-PSMA-11	PC3-PIP	~ 0.2 at 1, 2, and 3 h p.i.	(1)
[^{68}Ga]Ga-PSMA-11	PC3-PIP	0.135, 0.134, and 0.169 at 0.5, 1.5, and 4 h p.i.	(2)
[^{18}F]F-PSMA-1007	LNCaP	0.096 at 1 h p.i.	(3)

[^{18}F]DCFPyL	60 ℃	0.63, 1.04, 2.51, and 4.93 at 0.5, 1, 2, and 4 h p.i.	(4)

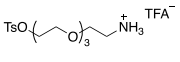
(1) S. Ray Banerjee, Z. Chen, M. Pullambhatla, A. Lisok, J. Chen, R. C. Mease and M. G. Pomper, *Bioconjugate Chem.*, 2016, **27**, 1447–1455.

(2) T. Zhang, J. Cai, M. Xu, X. Ma, H. Wang, M. Wang, Z. Han, J. Wang, E. Smith, Z. Li and Z. Wu, *Mol. Pharmaceutics*, 2021, **19**, 720–727.

(3) J. Cardinale, M. Schäfer, M. Benešová, U. Bauder-Wüst, K. Leotta, M. Eder, O. C. Neels, U. Haberkorn, F. L. Giesel and K. Kopka, *J. Nucl. Med.*, 2016, **58**, 425–431.

(4) Y. Chen, M. Pullambhatla, C. A. Foss, Y. Byun, S. Nimmagadda, S. Senthamizhchelvan, G. Sgouros, R. C. Mease and M. G. Pomper, *Clin. Cancer Res.*, 2011, **17**, 7645–7653.

7. Spectra of non-radioactive materials



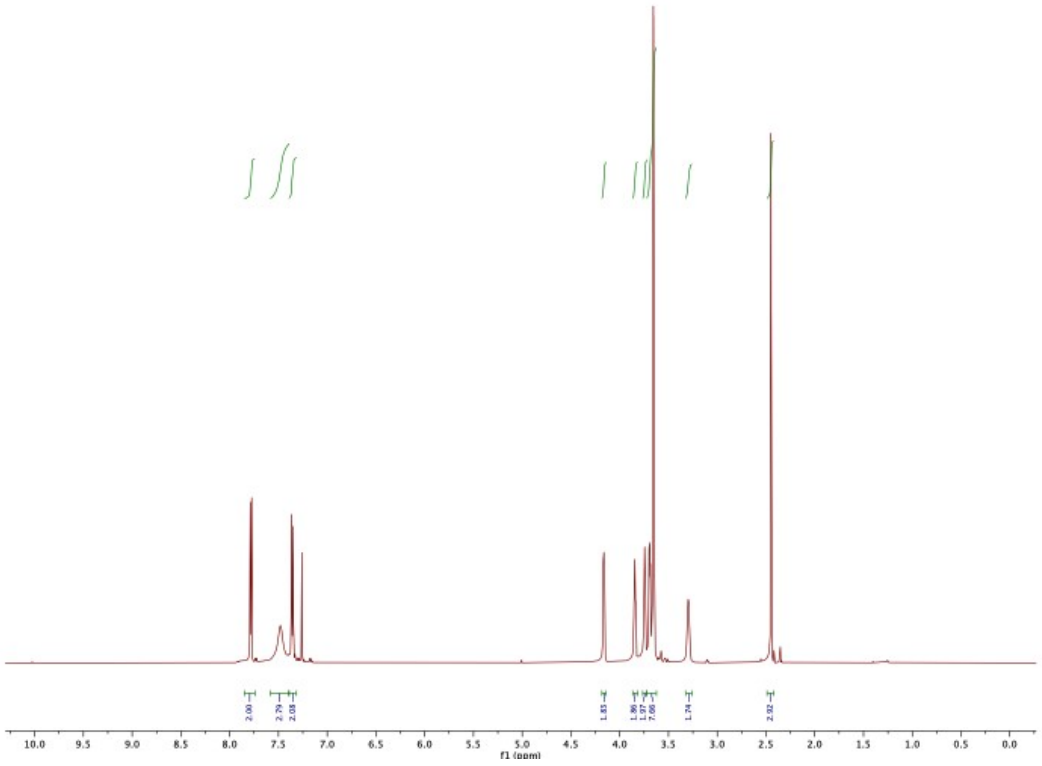
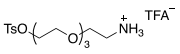


Figure S1. ^{1}H NMR of compound 1



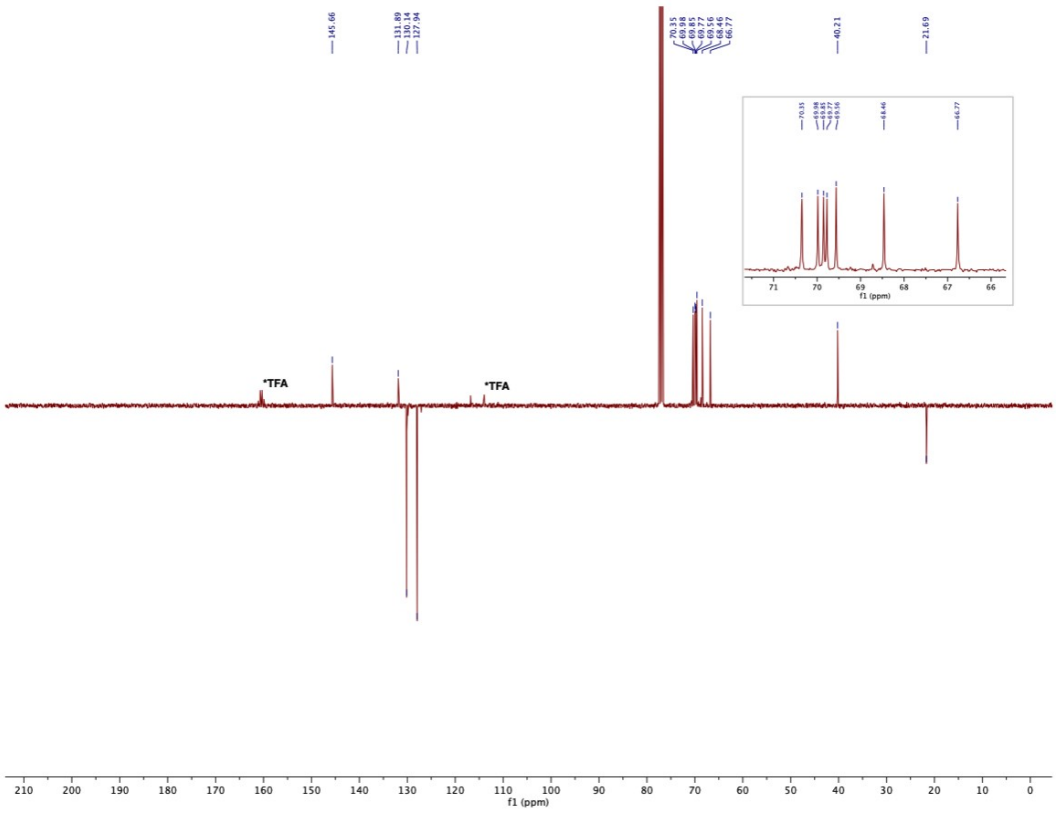


Figure S2. ^{13}C NMR of compound 1

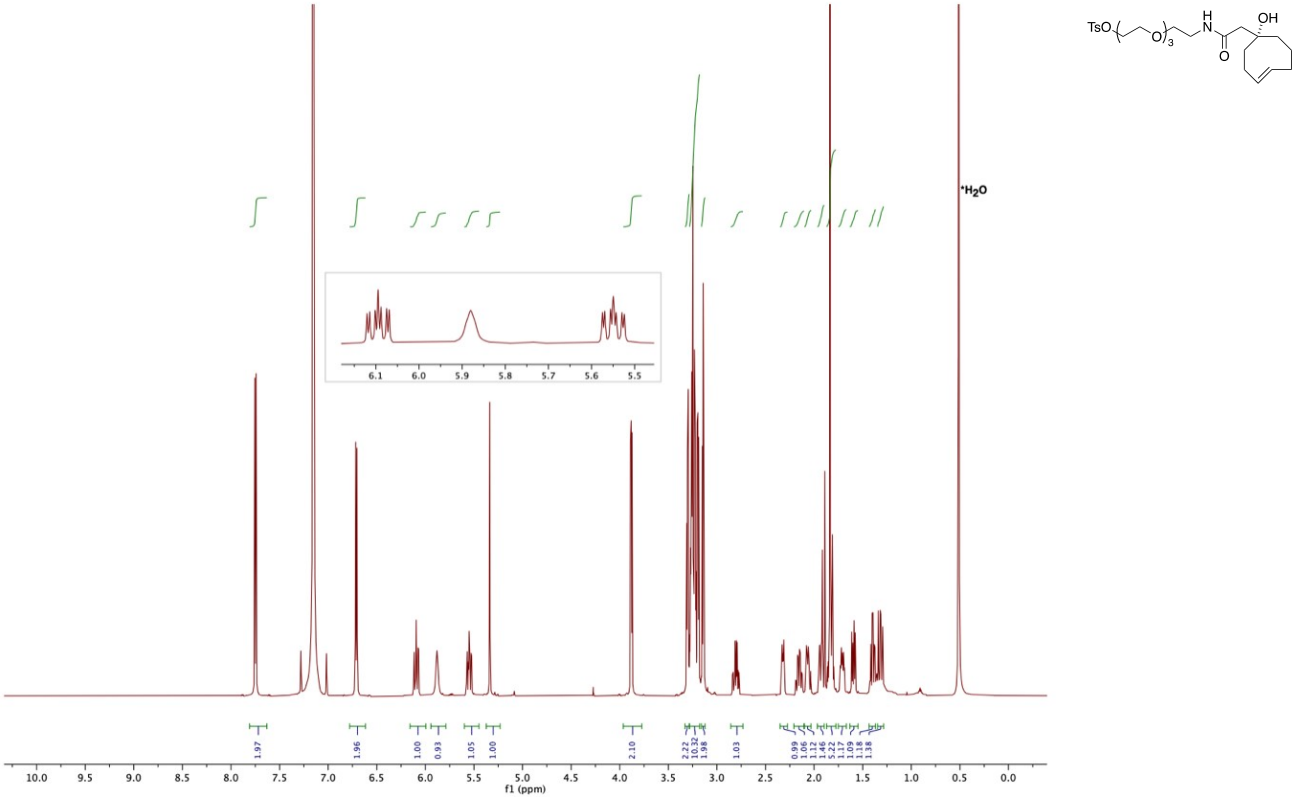


Figure S3. ^{1}H NMR of compound 2

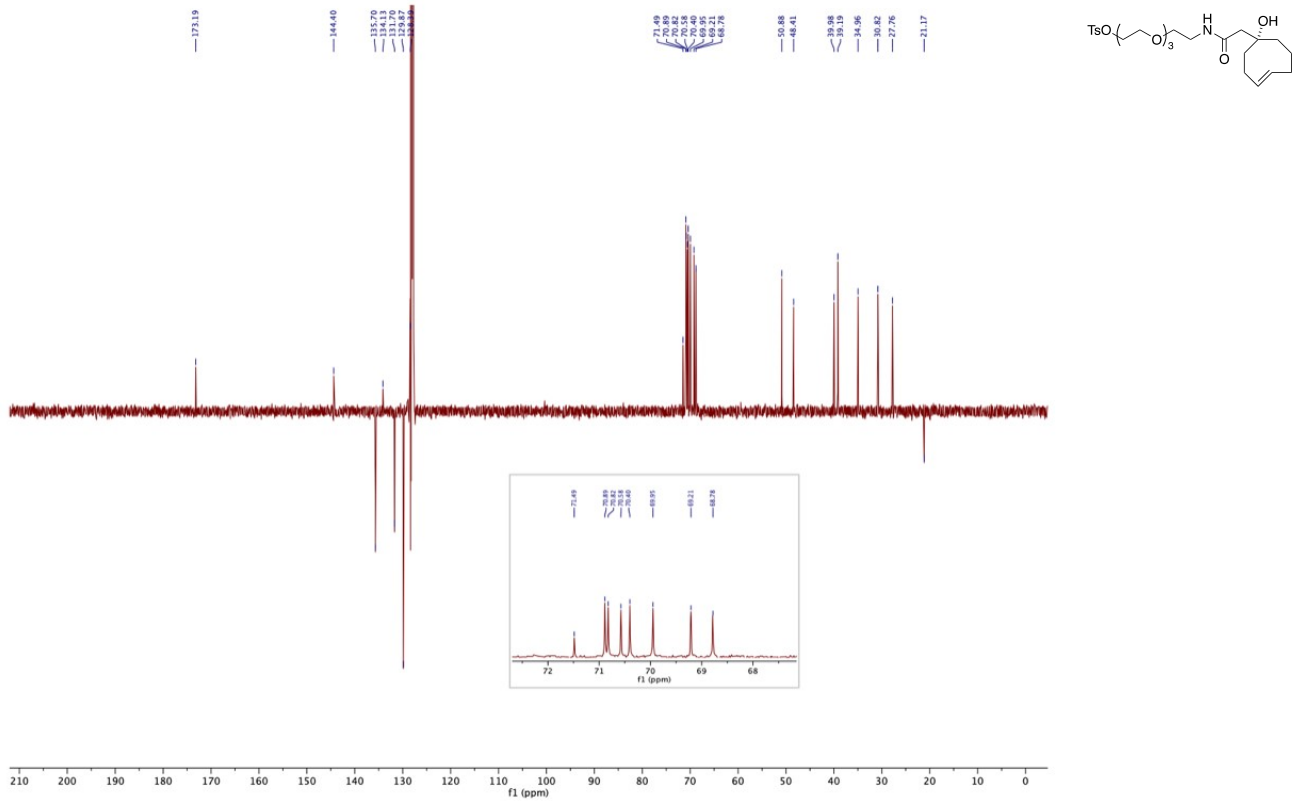


Figure S4. ^{13}C NMR of compound 2

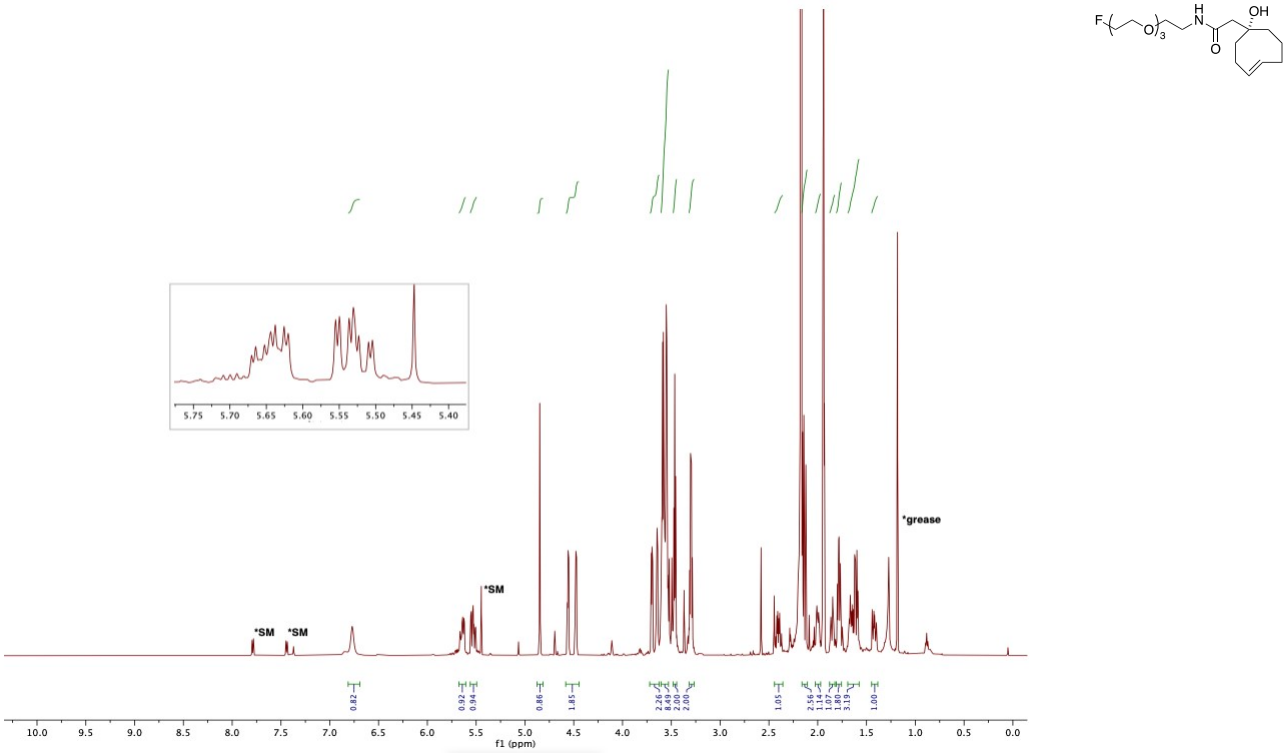


Figure S5. ^{1}H NMR of compound 3

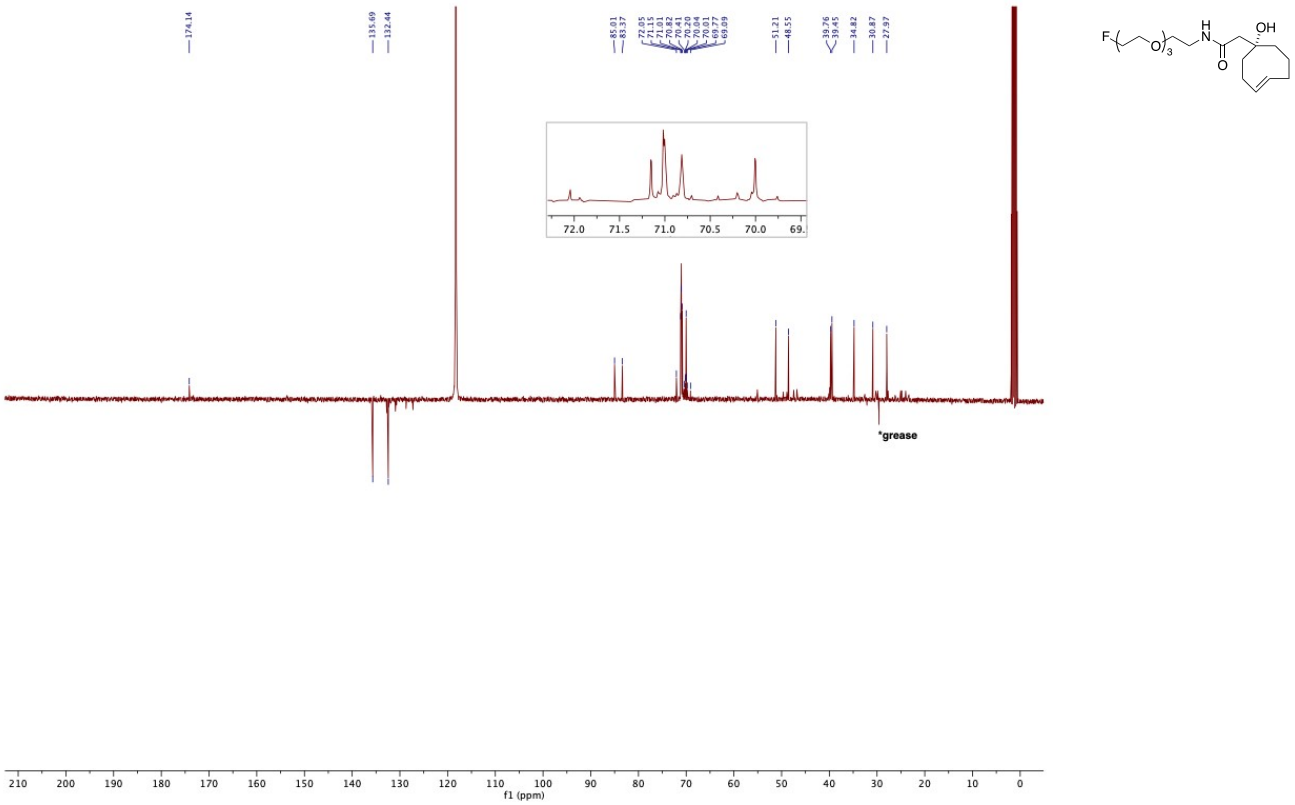
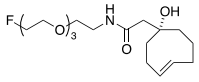
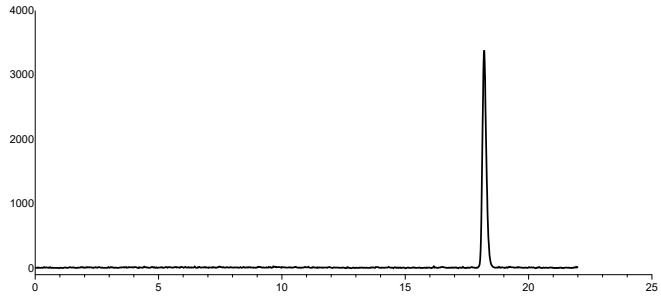


Figure S6. ^{13}C NMR of compound 3

(A)





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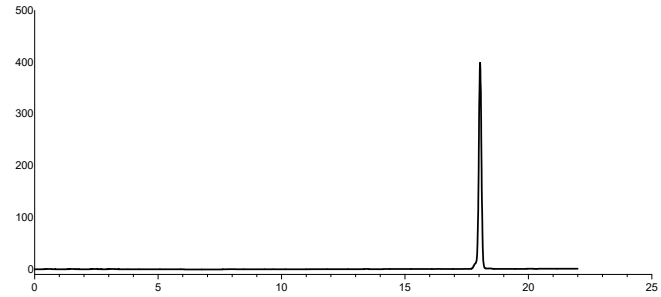
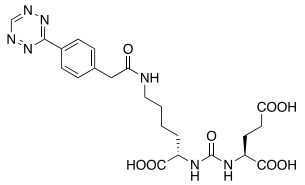


Figure S7. Coinjection HPLC profile of compound **3** (20 - 60%, 20 min, 1 mL/min, without TFA). (A) Radio chromatogram, the retention time is 18.21 min. (B) UV chromatogram (212 nm), the retention time is 18.05 min.



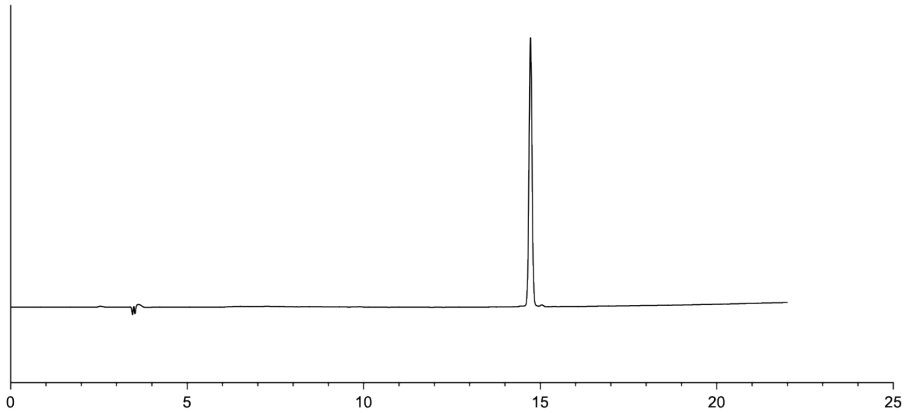


Figure S8. HPLC profile of compound **4** (10 - 50%, 20 min. 1 mL/min.). The retention time is 14.7 min.

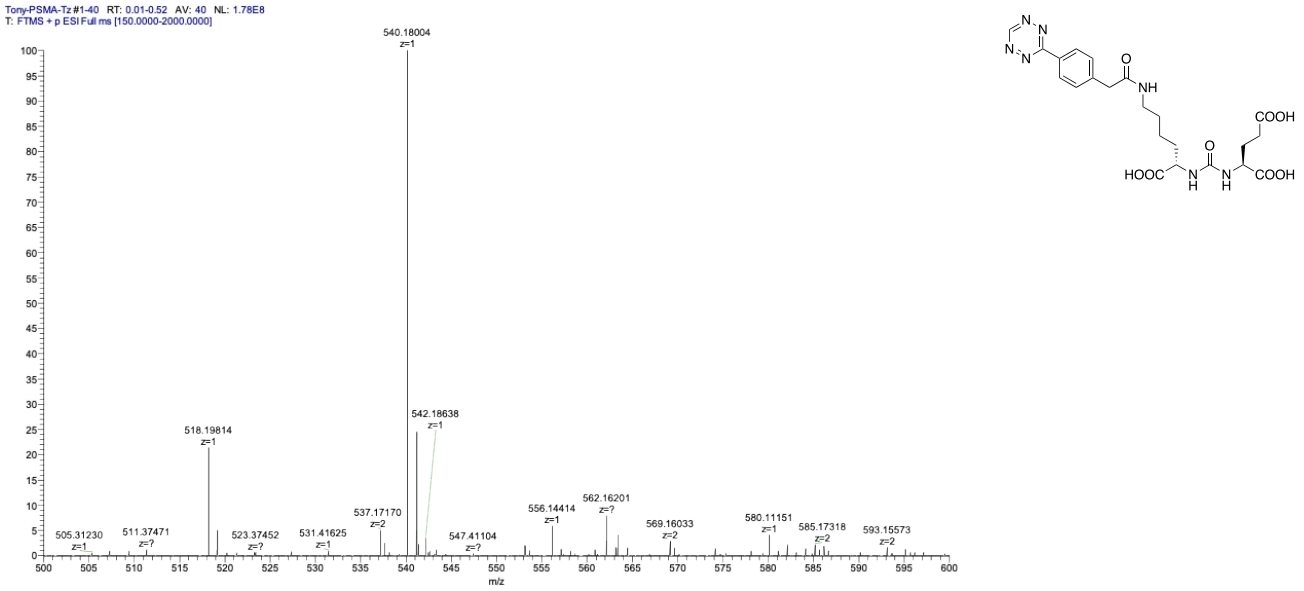


Figure S9. High-resolution mass spectrum (ESI) of compound **4**. m/z = 540.18004, assigned chemical formula: $C_{22}H_{27}N_7O_8Na$ $[M+Na]^+$, mass error = -2.4 ppm.

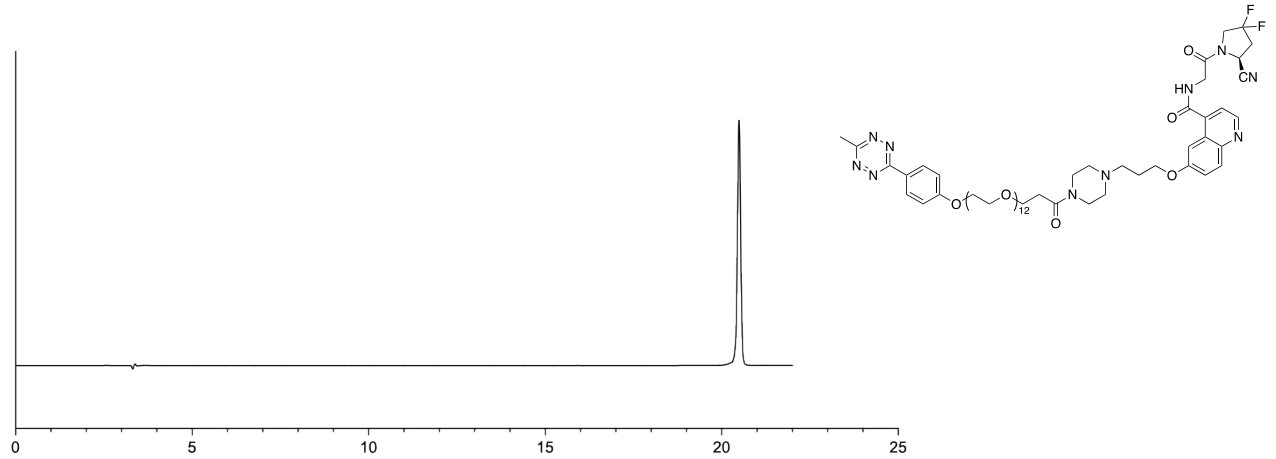


Figure S10. HPLC profile of compound **5** (10 - 50%, 20 min. 1 mL/min.). The retention time is 20.5 min.

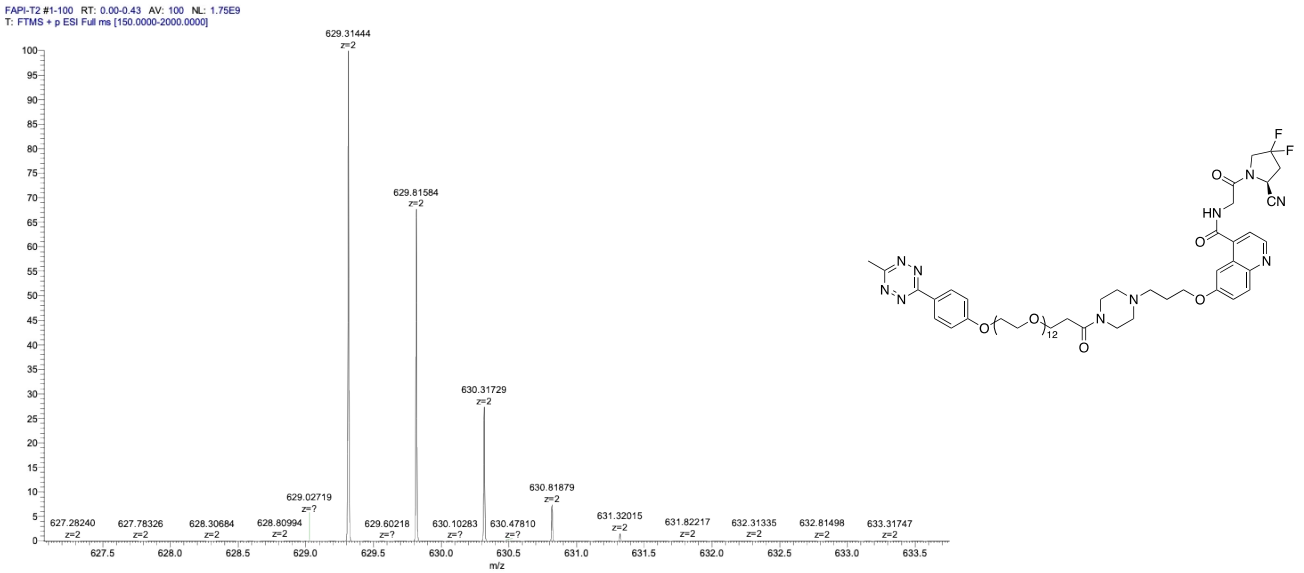
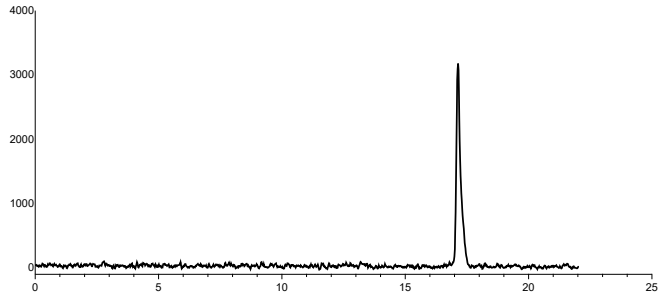
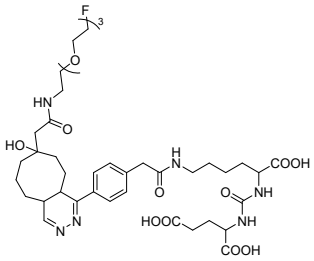


Figure S11. High-resolution mass spectrum (ESI) of compound **5**. m/z = 629.31444, assigned chemical formula: $C_{60}H_{88}N_{10}O_{17}F_2$ $[M+2H]^{2+}$, mass error = -0.6 ppm.

(A)





(B)

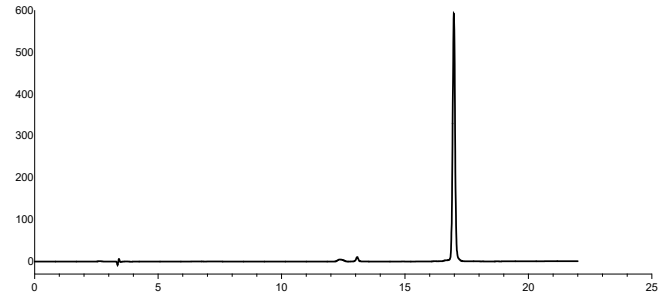
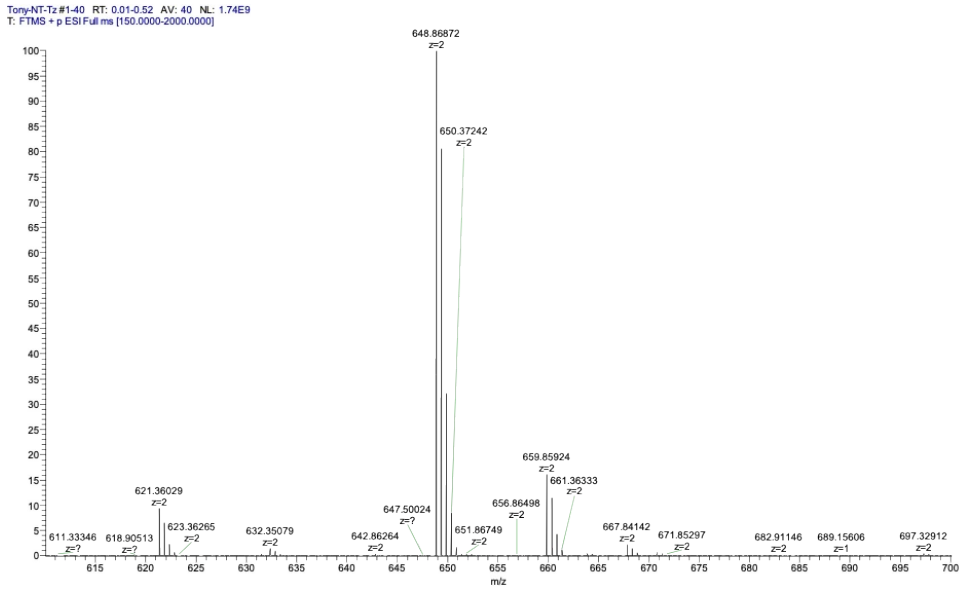


Figure S12. (A) Radio- and (B) UV- HPLC profile of compound **6** (10 - 50%, 20 min. 1 mL/min.). The retention time is 20.5 min.



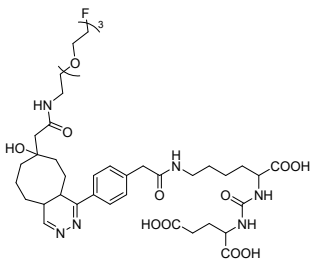
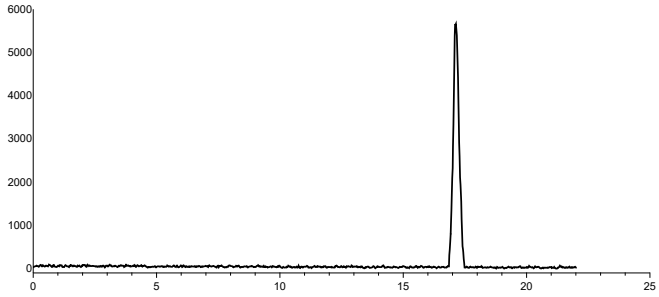
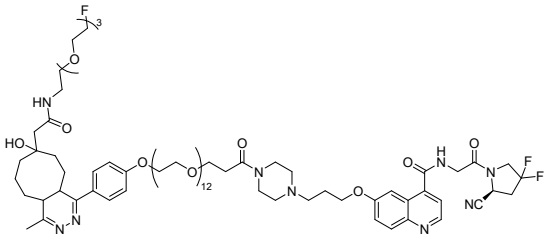


Figure S13. High-resolution mass spectrum (ESI) of compound **6**. m/z = 648.86872, assigned chemical formula: $C_{62}H_{95}N_{19}O_{12}$ $[M+2H]^{2+}$, mass error = -1.7 ppm.

(A)





(B)

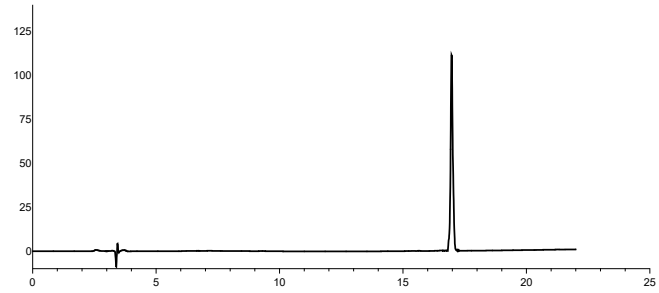
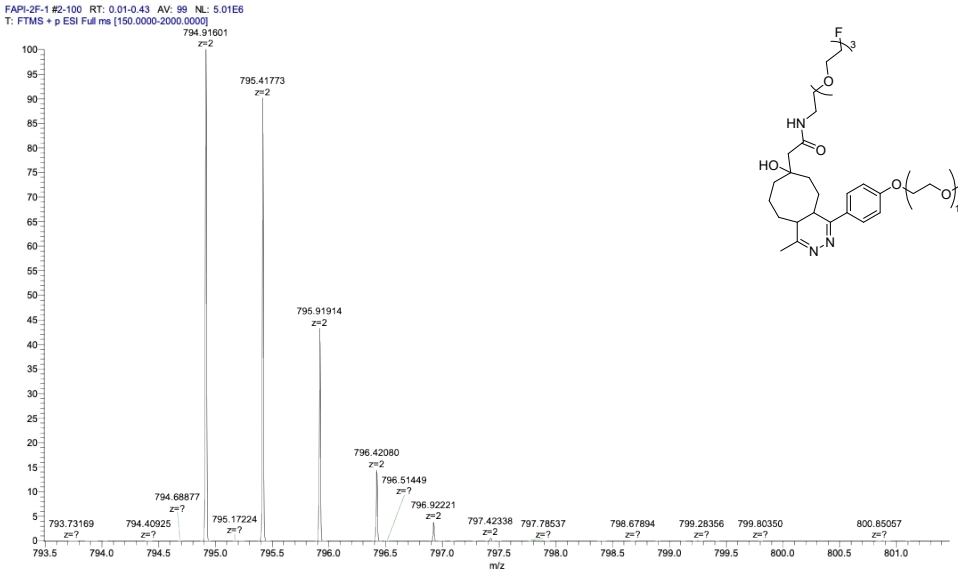


Figure S14. (A) Radio- and (B) UV- HPLC profile of compound **7** (10 - 50%, 20 min. 1 mL/min.). The retention time is 17.1 min.



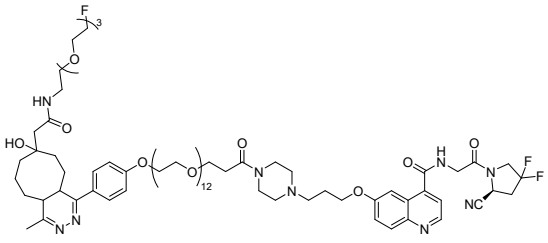


Figure S15. High-resolution mass spectrum (ESI) of compound **7**. m/z = 794.91601, assigned chemical formula: $C_{78}H_{118}N_9O_{22}F_3$ $[M+2H]^{2+}$, mass error = -0.6 ppm.

8. Radio-HPLC diagram

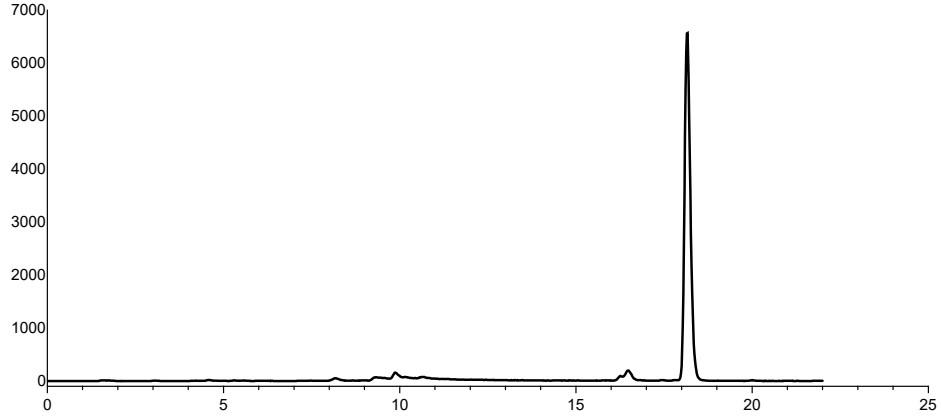


Figure S16. Crude product chromatogram of compound [^{18}F]**3** (20 - 60%, 20 min, 1 mL/min, without TFA). The retention time is 18.17 min.

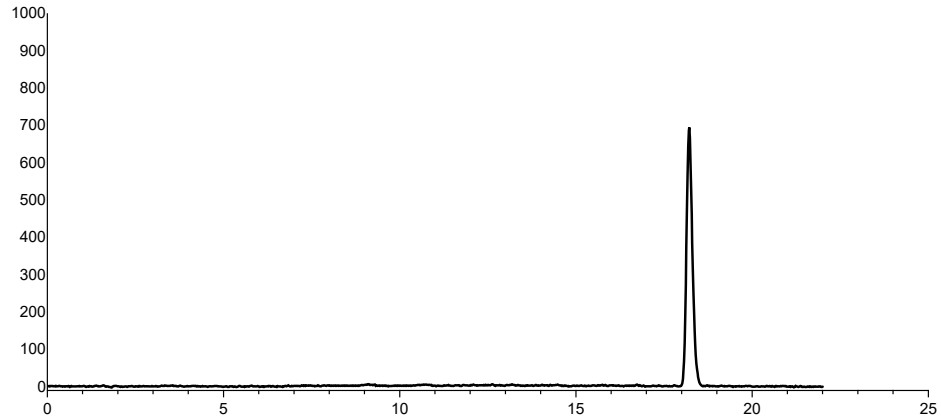


Figure S17. Quality control chromatogram of compound [^{18}F]**3** (20 - 60%, 20 min, 1 mL/min, without TFA). The retention time is 18.22 min.

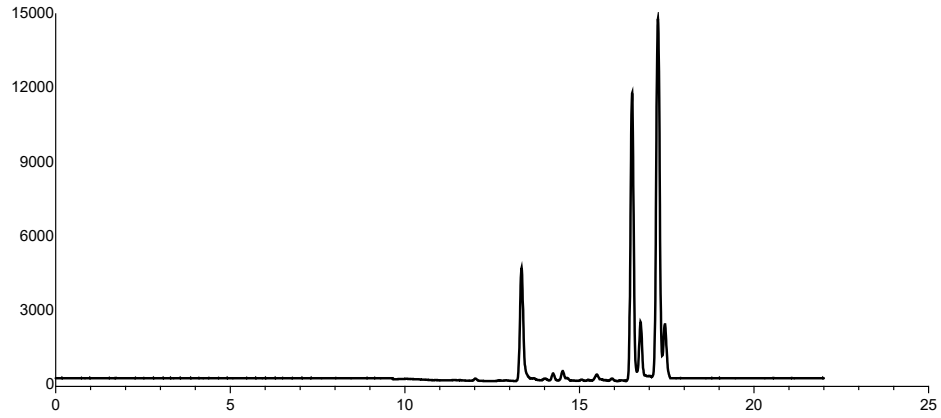


Figure S18. Crude product chromatogram of compound [^{18}F]**6** (10 - 50%, 20 min, 1 mL/min). The retention time is 17.2 min.

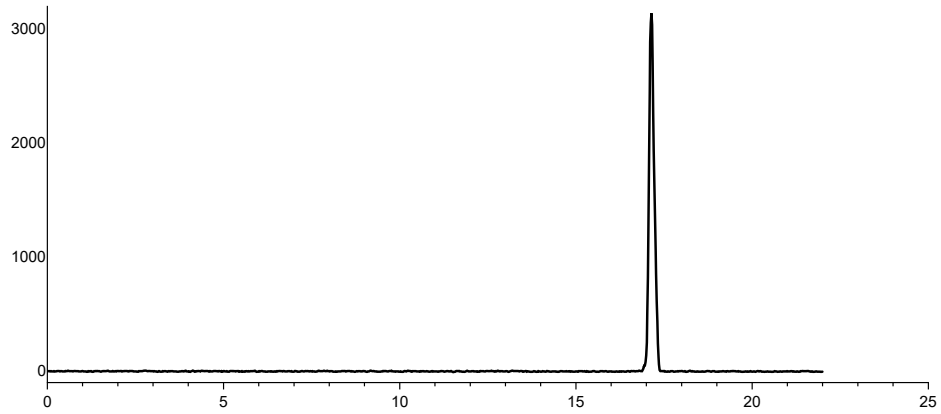


Figure S19. Quality control chromatogram of compound [^{18}F]**6** (10 - 50%, 20 min, 1 mL/min). The retention time is 17.2 min.

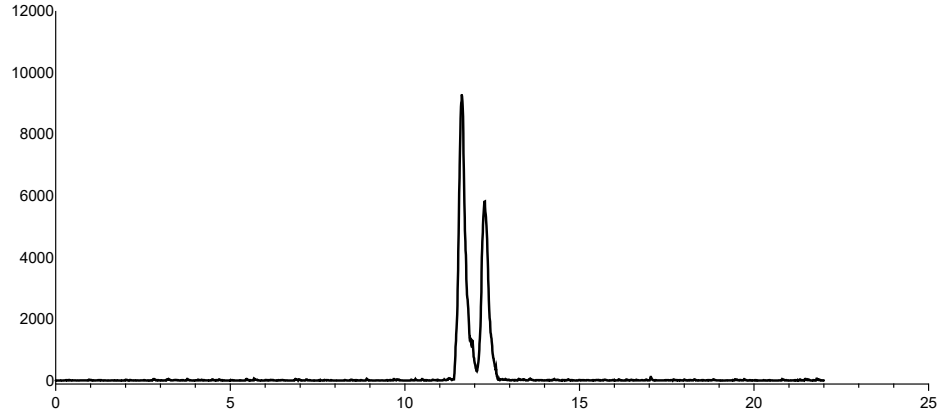


Figure S20. Crude product chromatogram of compound [^{18}F]**7** (25-35%, 20 min, 1 mL/min). The retention time is 11.7 min.

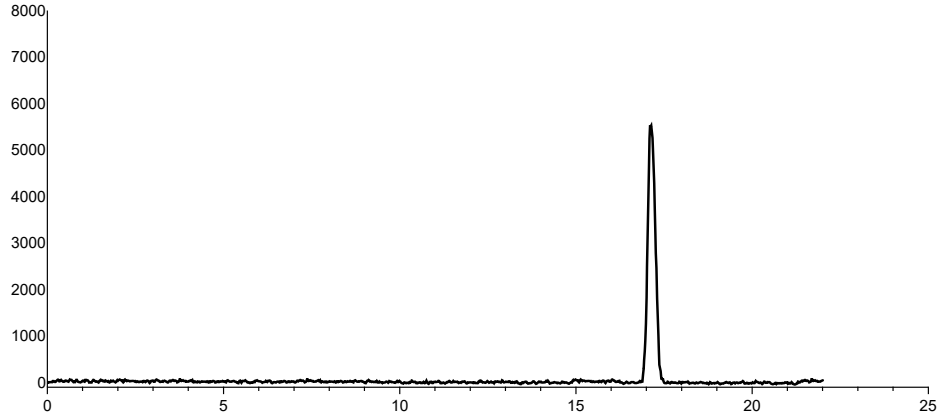
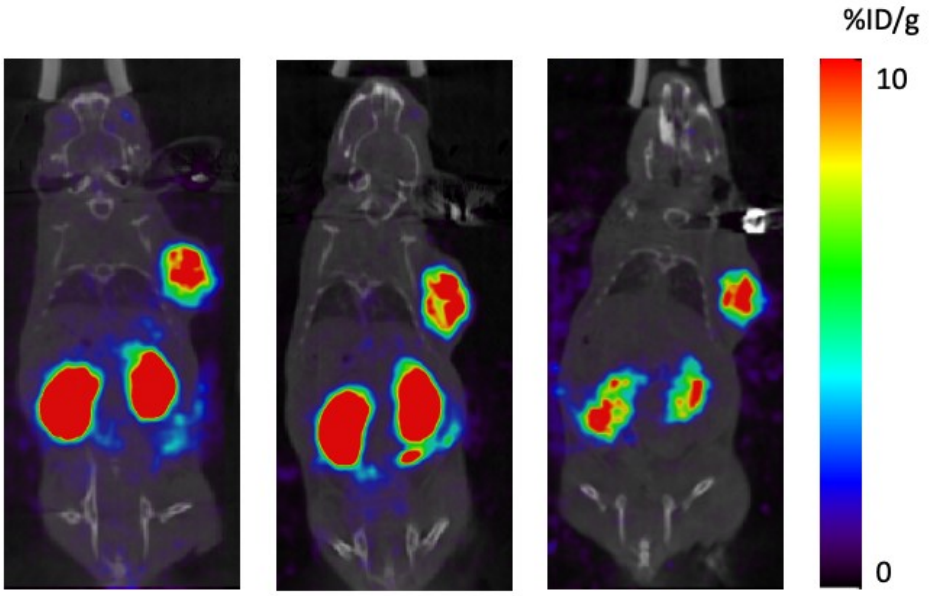
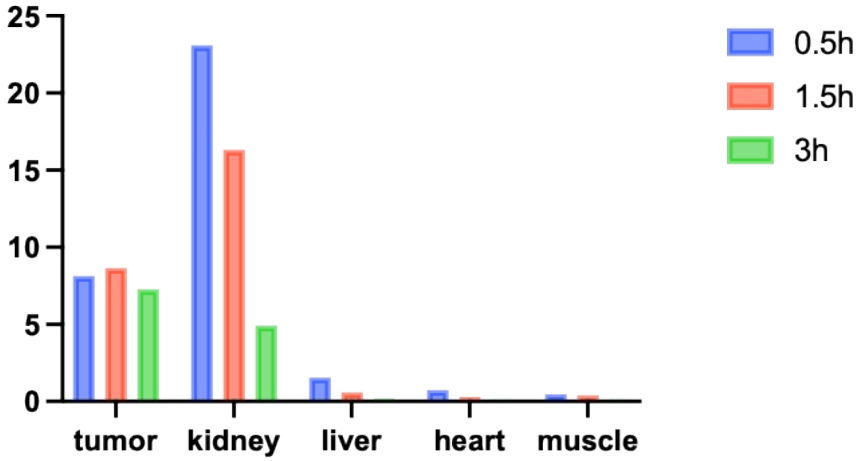


Figure S21. Quality control chromatogram of compound [^{18}F]**7**. (10 - 50%, 20 min. 1 mL/min.). The retention time is 17.1 min.

9. Small animal PET imaging and organ distribution

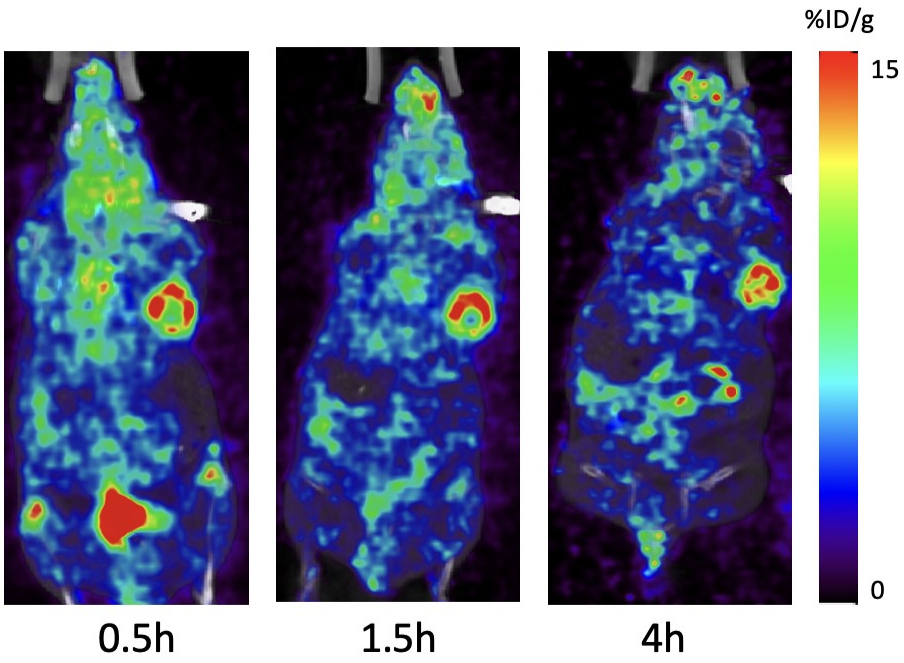


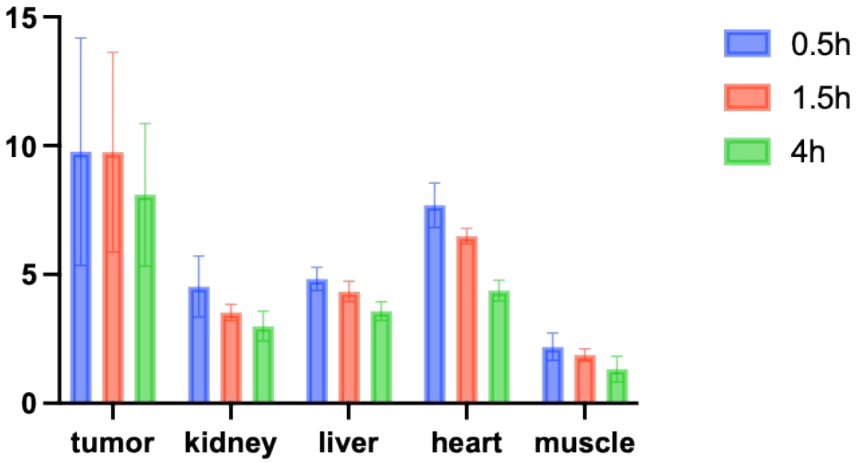


Organs	0.5 h post-injection (%ID/g)	1.5 h post-injection (%ID/g)	3 h post-injection (%ID/g)
Tumor	8.14	8.66	7.27
Kidney	23.1	16.3	4.93
Liver	1.56	0.599	0.191
Heart	0.749	0.312	0.137
Muscle	0.464	0.410	0.130

Ratio	0.5 h post-injection	1.5 h post-injection	3 h post-injection
Tumor-to-kidney	0.35	0.53	1.47

Table S3. Quantitative uptake and tumor-to-kidney ratio of compound [^{18}F]**7** in major organs derived from PET images of PC3-PIP tumor-bearing mice (n = 2).

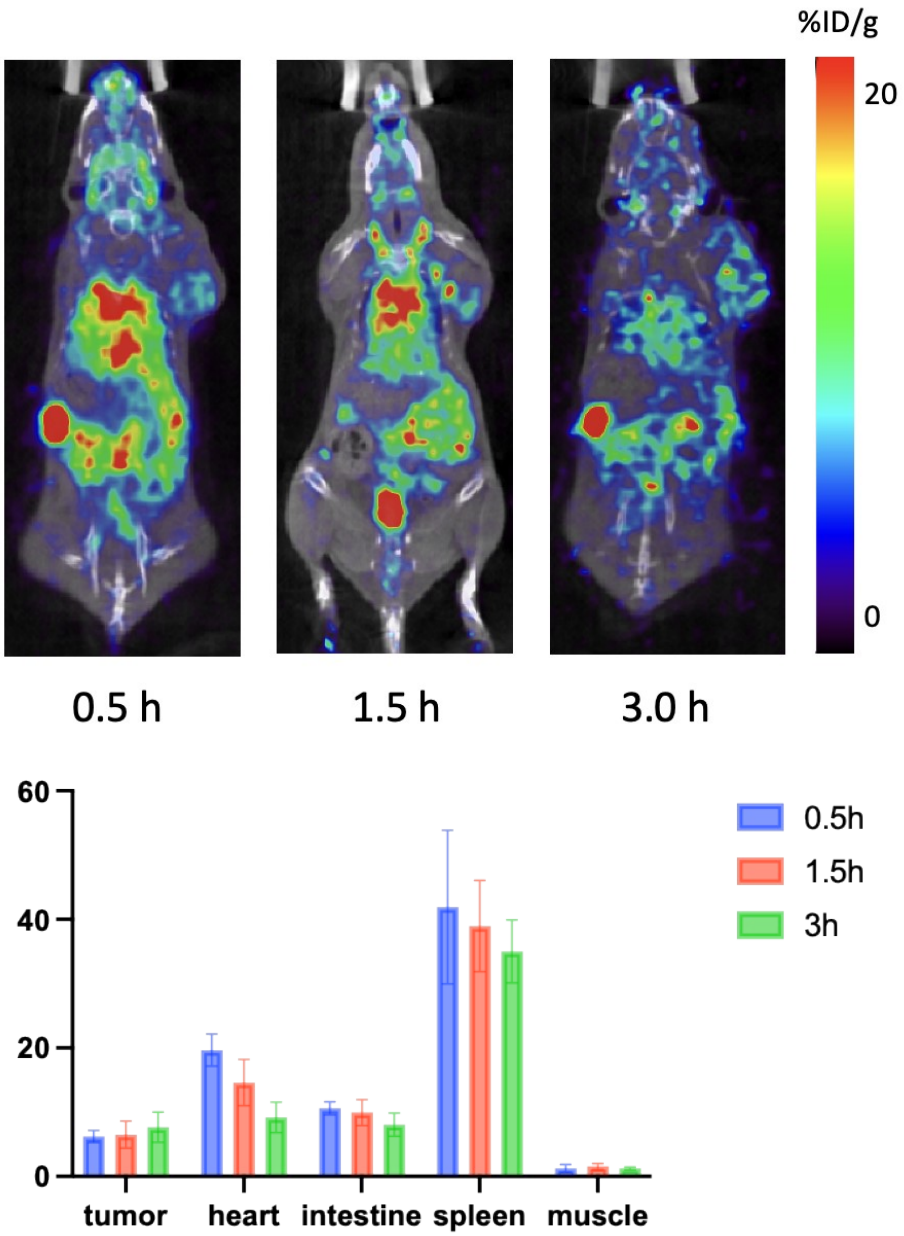




Organs	0.5 h post-injection (%ID/g)	1.5 h post-injection (%ID/g)	4 h post-injection (%ID/g)
Tumor	9.77 ± 4.41	9.75 ± 3.88	8.10 ± 2.77
Kidney	4.54 ± 1.18	3.53 ± 0.309	3.00 ± 0.579
Liver	4.83 ± 0.451	4.35 ± 0.399	3.59 ± 0.361
Heart	7.69 ± 0.865	6.50 ± 0.300	4.38 ± 0.397
Muscle	2.20 ± 0.531	1.88 ± 0.236	1.33 ± 0.498

Ratio	0.5 h post-injection	1.5 h post-injection	4 h post-injection
Tumor-to-kidney	2.14 ± 0.67	2.79 ± 1.10	2.70 ± 0.65

Table S4. Quantitative uptake and tumor-to-kidney ratio of compound [^{18}F]**8** in major organs derived from PET images of U87 tumor-bearing mice (n = 3).

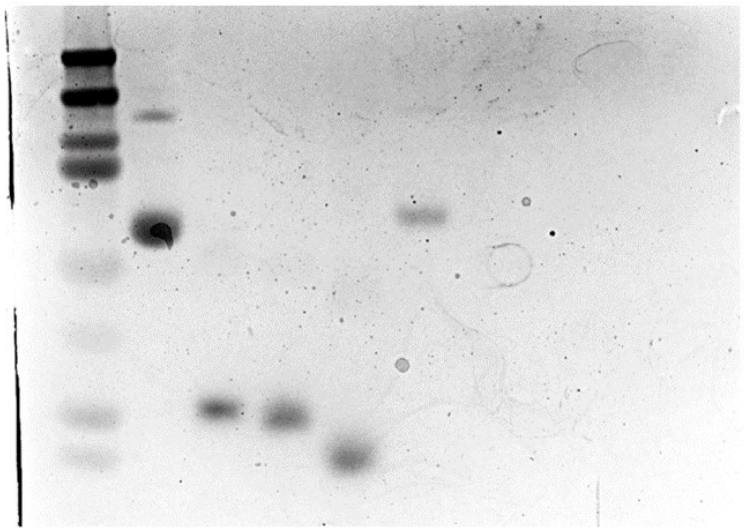


Organs	0.5h post injection (%ID/g)	1.5h post injection (%ID/g)	3h post injection (%ID/g)
Tumor	6.22 ± 0.958	6.52 ± 2.11	7.67 ± 2.35
Heart	19.7 ± 2.50	14.6 ± 3.60	9.20 ± 2.35
Intestine	10.6 ± 1.03	9.95 ± 2.03	8.09 ± 1.81
Spleen	41.9 ± 11.9	39.0 ± 7.09	35.0 ± 4.90
Muscle	1.28 ± 0.587	1.55 ± 0.473	1.26 ± 0.211

Table S5. Quantitative uptake of [^{18}F]**14** in major organs derived from PET images of B16F10 tumor-bearing mice (n = 3).

Raw data of Protein Gel and autoradiography

Protein Gel:



Autoradiography: