Supplemental material to

Comparison of the octadentate bifunctional chelator DFO*-*p*Phe-NCS and the clinically used hexadentate bifunctional chelator DFO-*p*Phe-NCS for ^{89}Zr-immuno-PET

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Table of contents

General analyses

^{1}H-NMR and ^{13}C-NMR spectra were measured with a Bruker ARX-400 or Bruker ARX-500 at room temperature. Chemical shifts (δ) are reported in ppm (parts per million) and coupling constant *J* in Hz. Residual solvent peaks were used as an internal reference. Abbreviations for the peak multiplicities are s (singlet), d (doublet), t (triplet), m (multiplet), br (broad). High resolution ESI-MS spectra were recorded using a Bruker ESQUIRE-LC quadrupole ion trap instrument. Ultra-performance liquid chromatography mass spectrometry (UPLC-MS) was performed with a Waters Acquity UPLC System with an Acquity UPLC PDA Detector and an Acquity UPLC BEH C_{18} 1.7 µm reverse phase column (2.1 x 50 mm); flow rate: 0.6 mL/min with a gradient of A (H_2O containing 0.1% formic acid) and B (acetonitrile): t = 0 min, 5% B; t = 0.5 min, 5% B; t = 4 min, 100% B; t = 5 min, 100% B. DFO*-*p*Phe-NCS was purified by preparative HPLC on a Phenomenex Jupiter 4u Proteo 90A (21.2 x 250 mm) column at a flow rate of 20 mL/min with a gradient of A (acetonitrile; Sigma-Aldrich HPLC-grade) and B (distilled water containing 0.1% TFA): t=0 min, A 5%; t=12 min, A 15%; t=24 min, A 35%; t=26 min, A 100%; t=28 min, A 100%; t=30 min, A 15%.

Synthesis of DFO*-*p*Phe-NCS

DFO* (50 mg, 0.066 mmol) was dissolved in a mixture of *i*-PropOH/H_2O (4:1; 8 mL) and added dropwise to a solution of *p*-phenylenediisothiocyanate (126 mg, 0.66 mmol, 10 eq) and Et_3N (11 µL, 0.079 mmol) in $CHCl_3$ (8 mL). The reaction mixture was stirred at room temperature for 24 h, concentrated under reduced pressure and the resulting white solid was washed with Et_2O (4 x 10 mL). The product was purified by preparative HPLC to provide, after lyophilization, a white powder (6 mg, 10%, purity according to UPLC-MS at 275 nm >95%).^{1}H-NMR (600 MHz, DMSO-d6): δ(ppm) 9.64 (br m, 5 H), 7.90 (br s, 1 H), 7.77 (br s, 3 H), 7.54 (d, *J* = 12 Hz, 2 H), 7.36 (d, *J* = 6 Hz, 2 H), 3.41-3.49 (m, 10 H), 2.97-3.03 (m, 6 H), 2.55-2.60 (m, 6 H), 2.23-2.3 (m, 6 H), 1.97 (s, 3 H), 1.48-1.51 (m, 10 H), 1.36-1.39 (m, 6 H), 1.19-1.21 (m, 8 H). ^{13}C-NMR (125 MHz, DMSO-d6): δ(ppm) 180.1, 172.0, 171.3, 170.2, 139.3, 132.6, 126.2, 124.6, 123.1, 47.1, 46.8, 43.7, 38.4, 29.9, 28.8, 28.0, 27.6, 27.1, 26.0, 23.6, 23.5, 20.4. Some carbon signals were not observed due to overlapping signals. HR-ESI-MS: calcd for $C_{42}H_{68}N_{10}O_{11}S_2$ $[M+H^+]^+$ 953.45887, found 953.45832.

The moderate yield of isolated DFO*-pPhe-NCS does not reflect low conversion of substrates or reagents but the challenging purification by HPLC due to low solubility of the compound in water and most organic solvents.

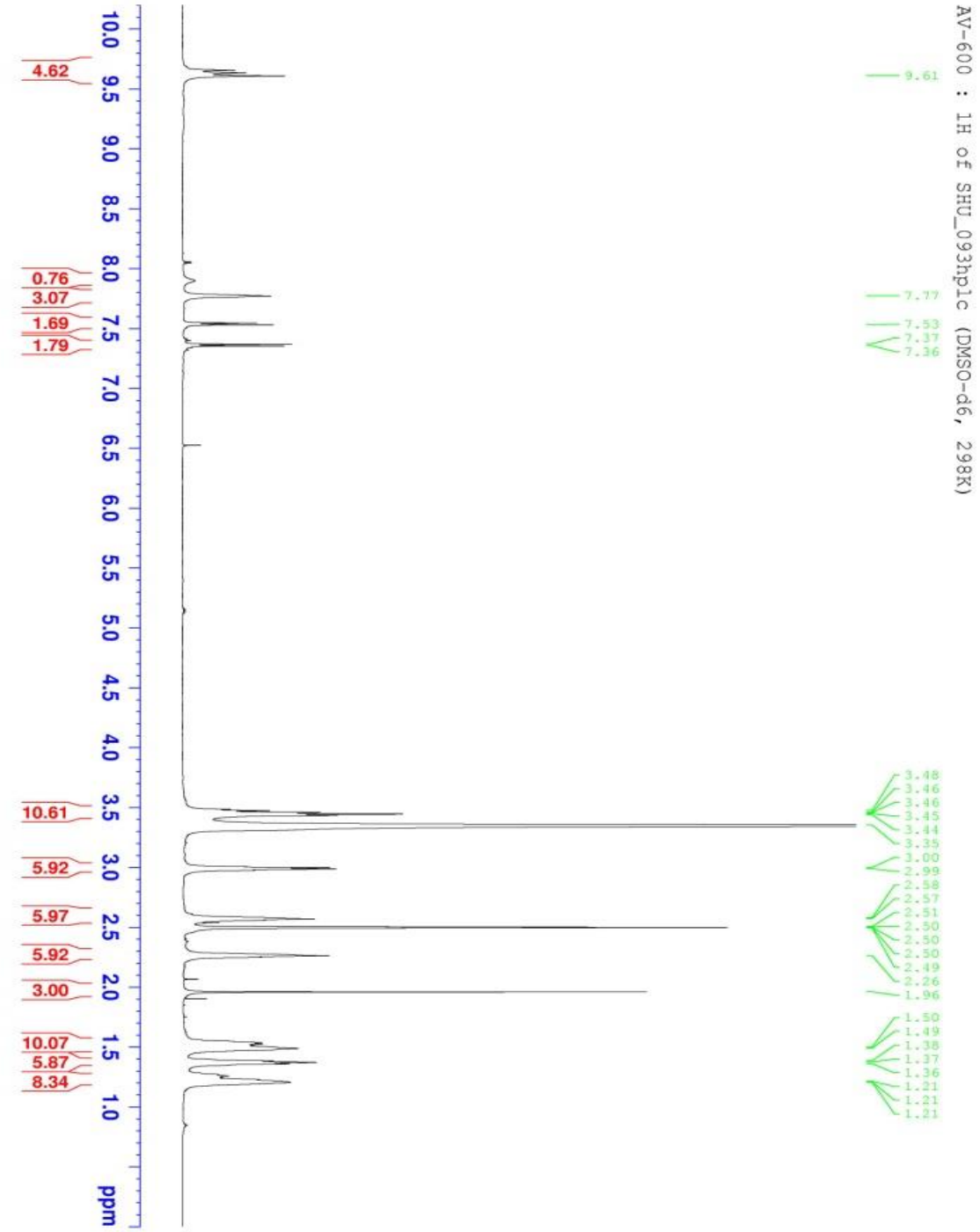


Fig. S1 ^{1}H-NMR spectrum of DFO*-*p*Phe-NCS

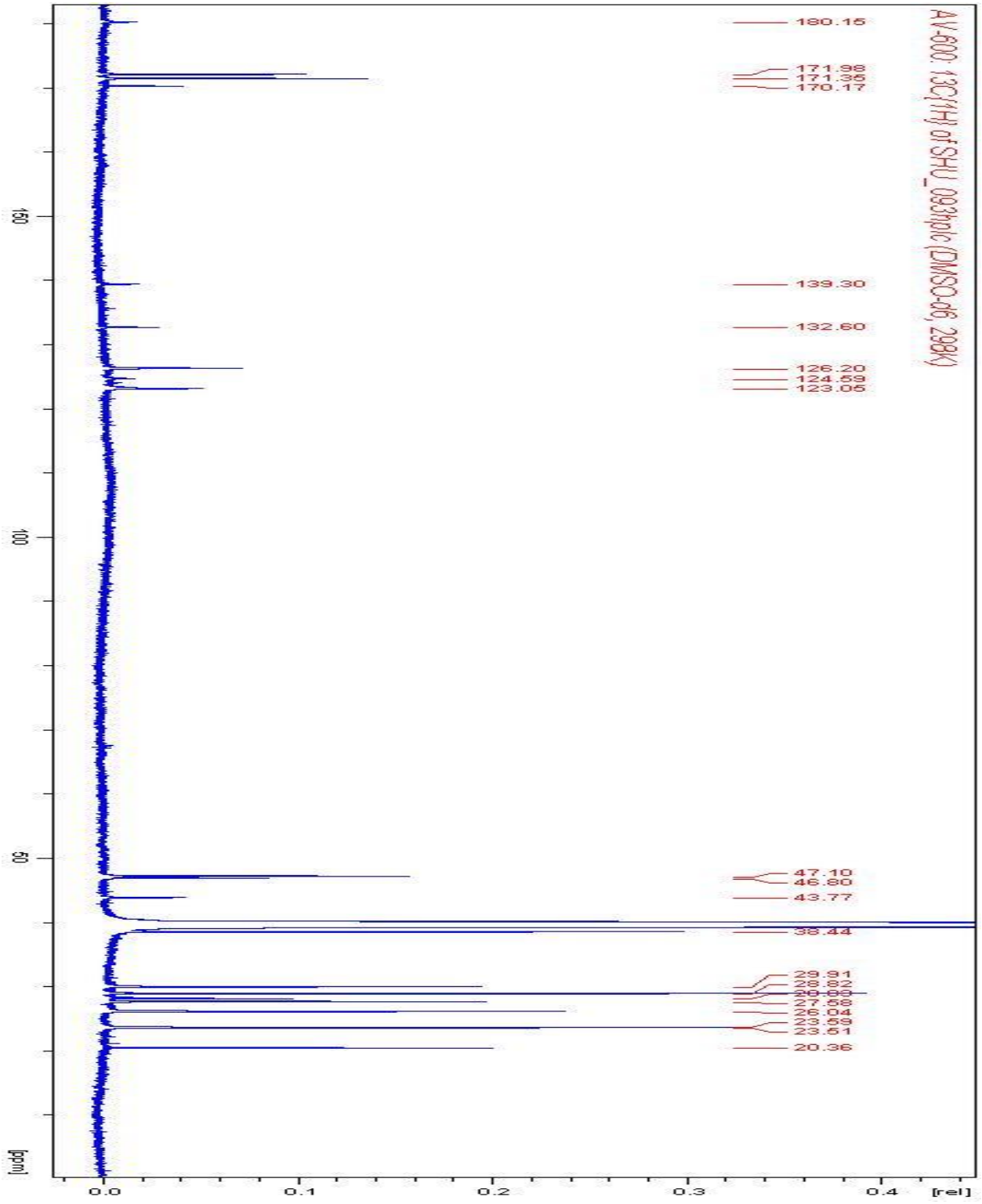


Fig. S2 ^{13}C-NMR spectrum of DFO*-*p*Phe-NCS

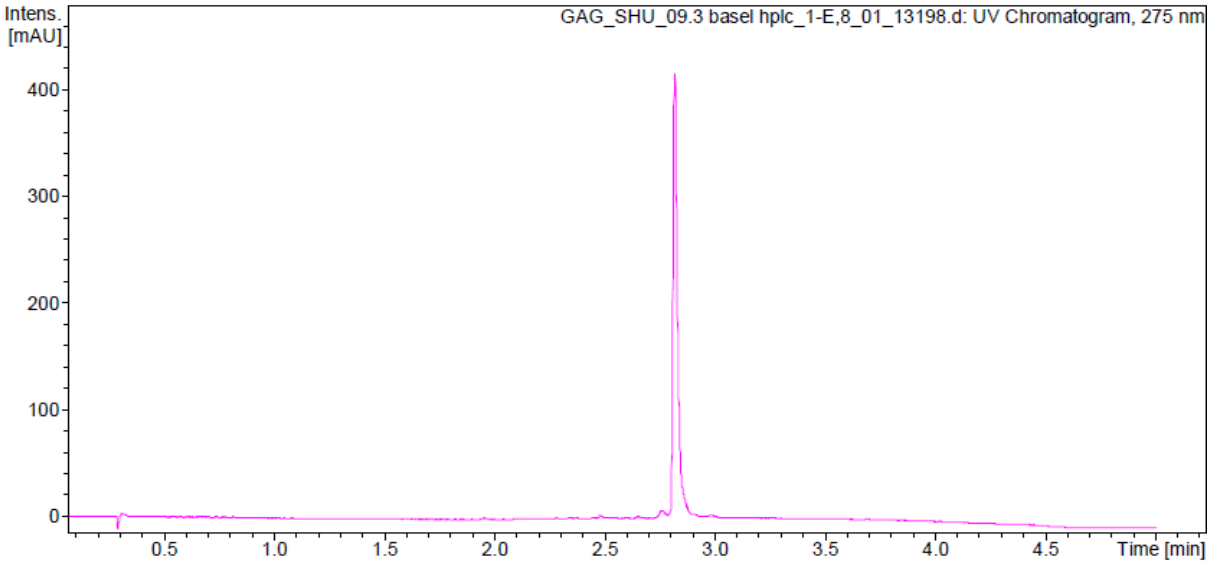
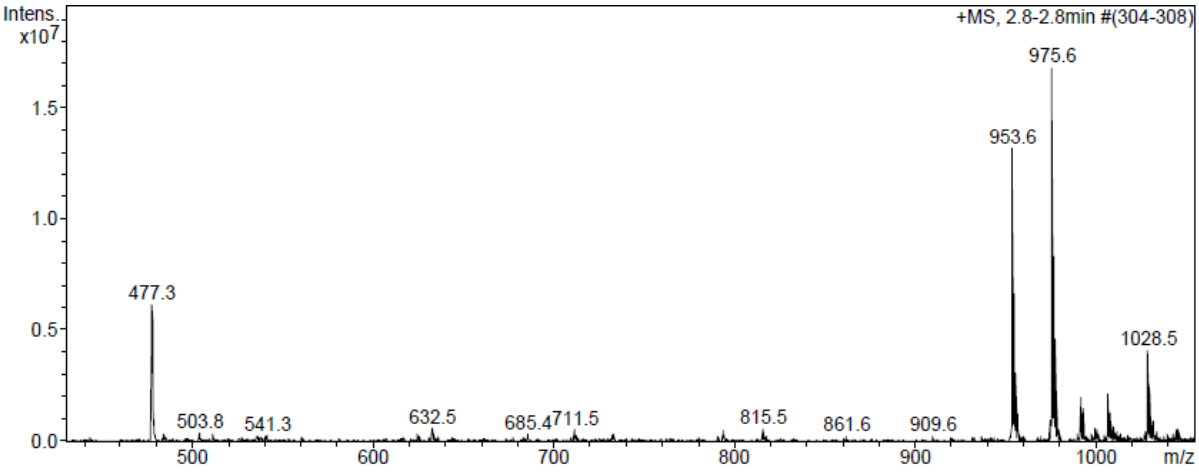


Fig. S3 HPLC analysis of DFO*-*p*Phe-NCS at 275 nm



HR-ESI calcd for C42H68N10O11S2/z $[M+H^+]^+$ 953.45887, found 953.45832, calcd for C42H68N10O11NaS2/z $[M+Na^+]^+$ 975.44027, found 975.44027

Fig. S4 HR-MS spectrum of DFO*-*p*Phe-NCS

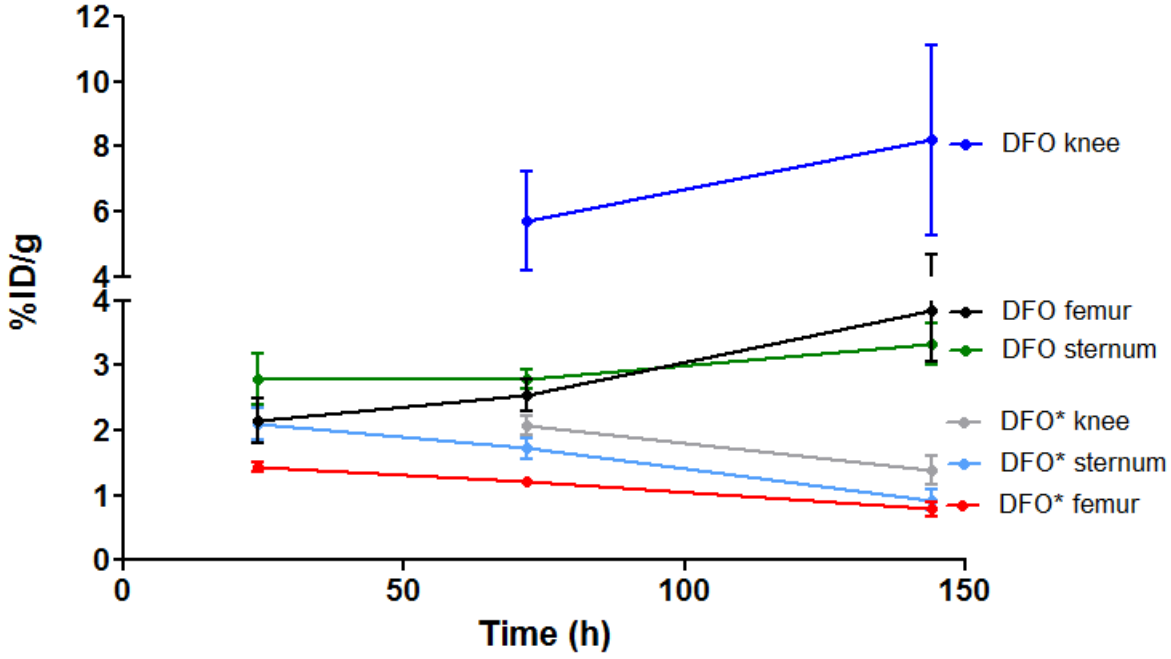


Fig. S5 %ID/g in sternum, femur and knee for ^{89}Zr-DFO*-trastuzumab and ^{89}Zr-DFO-trastuzumab over time.

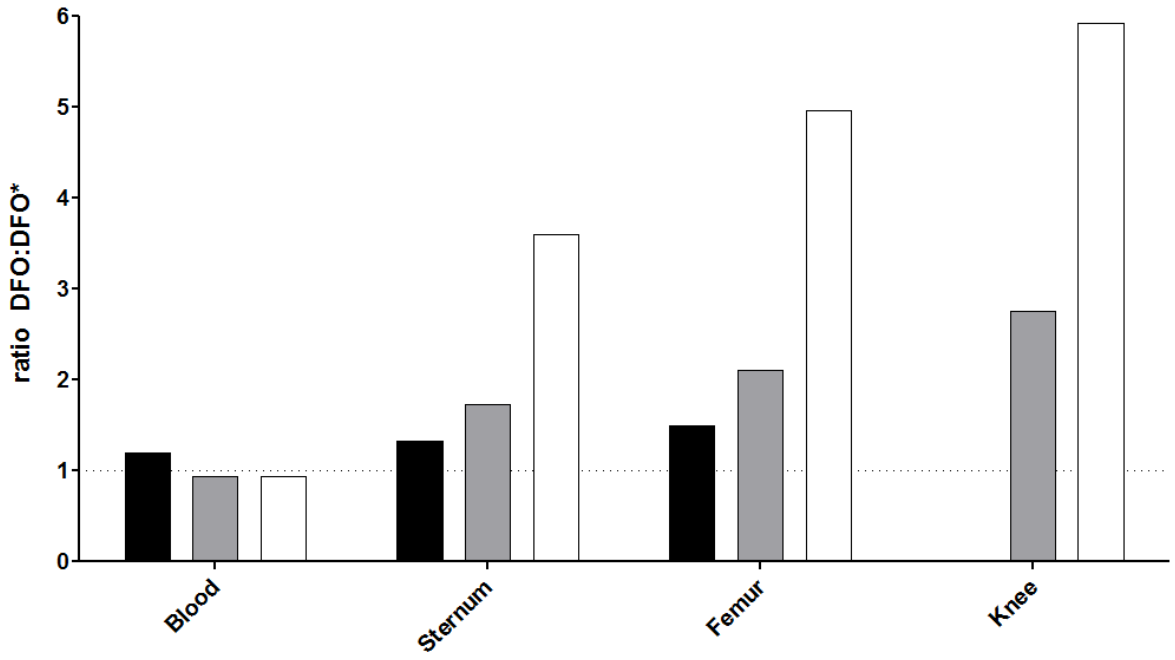


Fig. S6 Ratio of ^{89}Zr-DFO-trastuzumab/^{89}Zr-DFO*-trastuzumab in N87 tumor bearing nude mice at 24 h (black bar); 72 h (grey bar) and 144 h (white bar) after administration in blood, sternum, femur and knee.

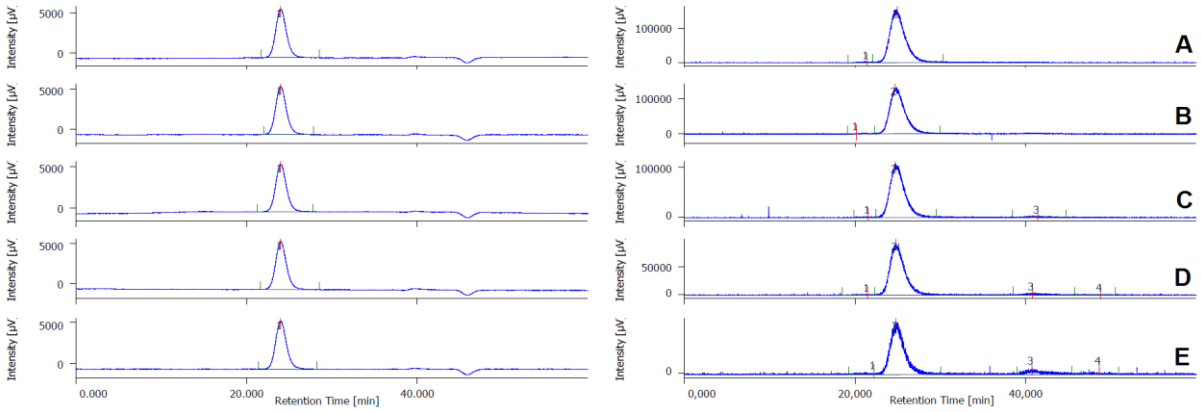


Fig. S7 SE-HPLC chromatogram of ^{89}Zr-DFO*-trastuzumab directly after formulation (A) and after storage at 2-8˚C for 24 h (B), 48 h (C), 72 h (D) and 168 h (E) in 0.9% NaCl. Left: UV at 280 nm and right: radioactive read-out.

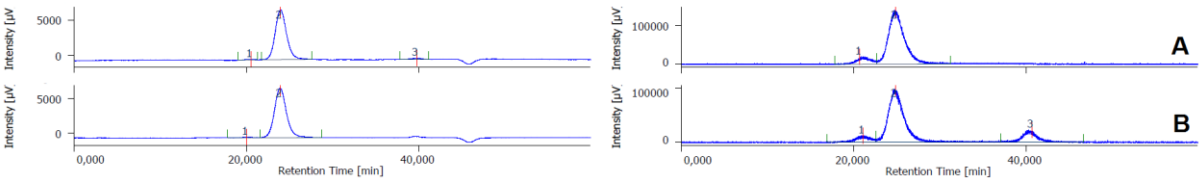


Fig. S8 SE-HPLC chromatogram of ^{89}Zr-DFO-trastuzumab directly after formulation (A) and after storage at 2-8˚C for 24 h (B) in 0.9% NaCl. Left: UV at 280 nm and right: radioactive read-out.

Table S1 *In vitro* stability of ^{89}Zr-DFO*-trastuzumab (A) and ^{89}Zr-DFO-trastuzumab (B) in 20 mM histidine+ 240 mM sucrose and 0.9% NaCl at room temperature

A	20 mM Histidine/240 mM sucrose		0.9% NaCl	
	21°C		21°C	
	Radiochemical purity (%)	Immunoreactive fraction (%)	Radiochemical purity (%)	Immunoreactive fraction (%)
0 h	99.0	98	98.0	97
24 h	98.3	96	97.3	95
48 h	96.8	95	93.5	91
72 h	94.7	93	90.7	87
168 h	84.1	81	76.5	72
B	20 mM Histidine/240 mM sucrose		0.9% NaCl	
	21°C		21°C	
	Radiochemical purity (%)	Immunoreactive fraction (%)	Radiochemical purity (%)	Immunoreactive fraction (%)
0 h	97.8	96	98.6	97
24 h	96.5	94	75.2	nd
48 h	95.6	92	57.3	nd
72 h	92.9	92	41.1	nd
168 h	87.5	85	42.7	nd

nd = not determined

Radiochemical purity of buffer samples determined by spin filter.

Radiochemical purity of serum samples determined by SEC-HPLC.

Summary of radiolabeling results and QC data of ^{89}Zr-DFO*-cetuximab and rituximab

^{89}Zr-DFO*-cetuximab

Radiolabeling efficiency: 87%

Radiochemical purity (HPLC): 100%

(spin filter): 99.7%

Immunoreactive fraction: 99% (A431 fixated cells)

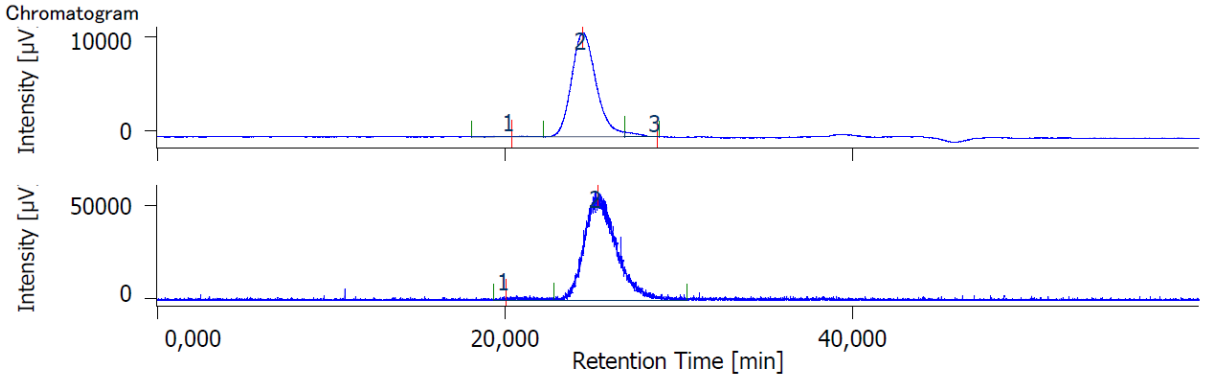


Fig. S9 SE-HPLC chromatogram of ^{89}Zr-DFO*-rituximab. Top: UV at 280 nm and bottom: radioactive read-out.

^{89}Zr-DFO*-rituximab

Radiolabeling efficiency: 89%

Radiochemical purity (HPLC): 100%

(spin filter): 99.4%

Immunoreactive fraction: 90% (SU-DHL4 fixated cells)

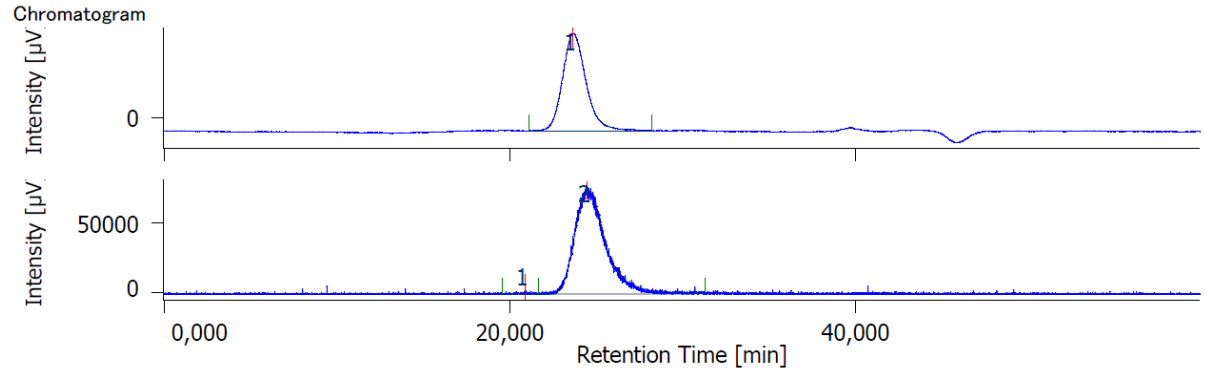


Fig. S10 SE-HPLC chromatogram of ^{89}Zr-DFO*-cetuximab. Top: UV at 280 nm and bottom: radioactive read-out.