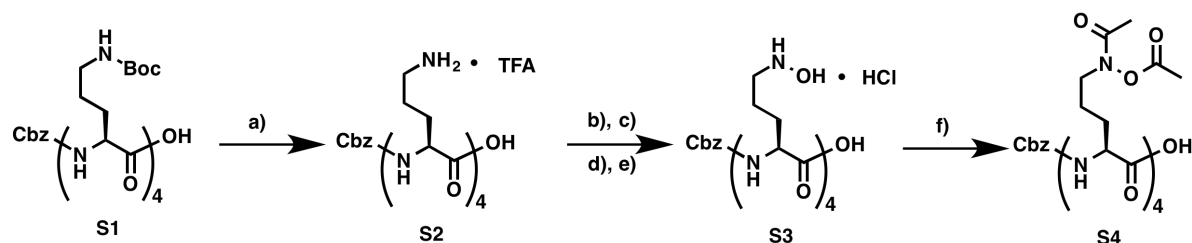


Multifunctional desferrichrome analogues as versatile $^{89}\text{Zr(IV)}$ chelators for immunoPET probe development: Supporting Information

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1. Experimental protocol for synthesis of precursors



Scheme S 1. Synthetic scheme for preparation of synthetic precursor S4. a) TFA/HOAc (7:3), 1 hr, b) benzaldehyde, KOH, MeOH, 3 Å molecular sieves, 24 hr, c) *m*CPBA, MeOH, 0-25°C, d) TFA/DCM (2:3), 1 hr, e) 1M HCl/DCM (1:1), 1 hr f) pH=4 KOAc/HOAc, Ac₂O, KHCO₃, 4 hr.

(9*S*,12*S*,15*S*,18*S*)-9-(((benzyloxy)carbonyl)amino)-12,15,18-tris(3-((tert-butoxycarbonyl)amino)propyl)-2,2-dimethyl-4,10,13,16-tetraoxo-3-oxa-5,11,14,17-tetraazanonadecan-19-oic acid, **S1**. The boc-protected poly-ornithine precursor (9*S*,12*S*,15*S*)-9-(((benzyloxy)carbonyl)amino)-12,15-bis(3-((tert-butoxycarbonyl)amino) propyl)-2,2-dimethyl-4,10,13-trioxo-3-oxa-5,11,14-triazahexadecan-16-oic acid (1.315 g, 1.67 mmol) was dissolved in 20 mL of THF, followed by NHS (1.315 g, 1.67 mmol). The solution was placed in an ice bath and cooled to 0 °C. To the solution at 0 °C was added DCC (0.349 g, 1.69 mmol) dissolved in 10 mL of THF. The reaction mixture was removed from the ice bath and stirred at room temperature overnight. The following day, the reaction mixture was filtered and the precipitated DCU was washed with THF.

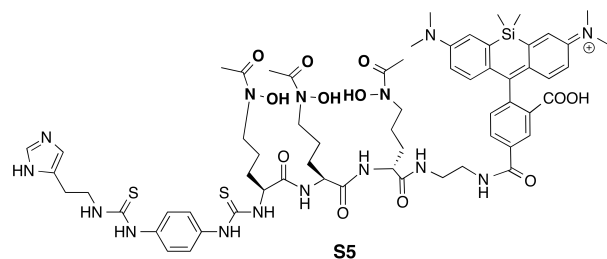
To a solution of N-delta-Boc-L-Ornithine (0.773 g, 3.32 mmol) and NaHCO₃ (0.605 g, 7.20 mmol) dissolved in 25 mL of H₂O/THF (1:4) was added the THF solution from above. The reaction mixture was stirred at room temperature for 6 hours. After 6 hours, the THF was removed and the aqueous portion was mixed with 50 mL of EtOAc. The pH of the mixture was brought down to 2 with a solution of 10% citric acid. The aqueous fraction was separated and extracted with 50 mL of EtOAc. The combined organic fractions were washed with brine and dried by filtering through Na₂SO₄. The EtOAc was evaporated, affording **S1** (1.43 g, 1.42 mmol, 85.1% yield) as a white foam. ¹H NMR (DMSO, 500 MHz, ppm): 8.04-8.03 (d, 1H, NH), 7.89-7.84 (m, 2H, NH), 7.38-7.35 (m, 5H, Ar-H), 7.32-7.30 (d, 1H, Cbz-NH), 6.77-6.72 (m, 4H, Boc-NH), 5.02-5.01 (s, 2H, Cbz-CH₂), 4.24 (s, 2H, αH), 4.13 (s, 1H, αH), 3.97 (s, 1H, αH), 2.88 (s, 8H, δH), 1.69-1.04 (m, 52H, βH/*t*-butyl-CH₃/γH). ¹³C NMR (DMSO, 125 MHz, ppm): 173.8 (COOH), 172.2-171.7 (3 signals, amide-C=O), 158.8 (amide-C=O), 156.4-156.1 (2 signals, amide-C=O), 137.5 (Ar-C), 128.8-128.1 (3 signals, Ar-C), 110.0 (Ar-C), 77.9 (*t*-butyl-C), 65.9 (Cbz-CH₂), 54.8-52.1 (3 signals, αC), 33.8 (δC), 28.7 (CH₃), 26.5-24.9 (3 signals, βC/γC). LC-ESI-MS (method E): calcd. for C₄₈H₈₀N₈O₁₅: 1008.6. Found: 909.4 [M+H-Boc]⁺, R_t=4.10 min.

(5*S*,8*S*,11*S*,14*S*)-5,8,11,14-tetrakis(3-aminopropyl)-3,6,9,12-tetraoxo-1-phenyl-2-oxa-4,7,10,13-tetraazapentadecan-15-oic acid, **S2**. **S1** (0.530 g, 0.526 mmol) was added to 3 mL of TFA/HOAc (7:3). The reaction mixture was stirred at room temperature for 4 hours. The reaction mixture was concentrated to a reduced volume (~1 mL) and partitioned between 5 mL of H₂O and 5 mL of EtOAc. The aqueous fraction was separated and the solvent was removed in vacuo to afford **S2** (0.546 g, 0.513 mmol, 97.7%) as a glassy, clear solid. ¹H NMR (DMSO, 400 MHz, ppm): 10.15 (s, 8H, NH₂), 8.25 (d, 1H, NH), 8.15 (d, 1H, NH), 8.05 (d, 1H, NH), 7.87 (s, 5H, Ar-H), 7.44 (d, 1H, Cbz-NH), 4.99 (m, 2H, Cbz-CH₂), 4.31-4.03 (m, 4H, αH), 2.84-2.68 (t, 8H, δH), 1.77-1.57 (m, 16H, βH/γH). ¹³C NMR (DMSO, 100 MHz, ppm): 176.1 (COOH), 175.1-174.3 (4 signals, amide-C=O), 159.1 (Cbz-C=O), 139.9 (Ar-C), 131.4-130.8 (3 signals, Ar-C), 68.7 (Cbz-CH₂), 57.0 (αC), 55.1-54.5 (3 signals, αC), 42.7 (δC), 32.0-30.9 (3 signals, βC), 26.7-26.4 (3 signals, γC). LC-ESI-MS (method E): calcd. for C₂₈H₄₈N₈O₇: 608.7. Found: 609.3 [M+H]⁺, R_t=0.75 min.

(5*S*,8*S*,11*S*,14*S*)-14-(3-aminopropyl)-5,8,11-tris(3-(hydroxyamino)propyl)-3,6,9,12-tetraoxo-1-phenyl-2-oxa-4,7,10,13-tetraazapentadecan-15-oic acid, **S3**. **S2** (0.546 g, 0.513 mmol) was dissolved in 10 mL of MeOH. To this solution was added KOH (0.1664 g, 2.97 mmol), followed by benzaldehyde (0.237 g, 2.23 mmol, 0.228 mL) and 3 Å molecular sieves. The reaction mixture was allowed to stir overnight. The following day, the reaction mixture was filtered, and the white precipitate and sieves were washed with MeOH. The MeOH solution was placed in an ice bath and cooled to 0 °C. To this solution was added *m*CPBA (0.706 g, 4.09 mmol) in 5 mL of MeOH dropwise over 30 minutes. The reaction mixture was

stirred for an additional hour at 0 °C. After one hour, the solution was concentrated to a small volume (~3 mL) and partitioned between 15 mL of H₂O and 15 mL of EtOAc. The pH of the mixture was lowered to 2 by dropwise addition of 1 M HCl. The aqueous fraction was separated and washed with 15 mL of EtOAc. The combined organic fractions were washed with brine and dried by filtering through Na₂SO₄. The EtOAc was removed to yield a white solid. To this white solid were added 4 mL of TFA, followed by 6 mL of DCM. The reaction mixture was stirred at room temperature for 1 hour, after which the volatiles were removed, affording a white solid. To this solid were added 5 mL of 1 M HCl followed by 5 mL of DCM. The reaction mixture was stirred for 1 hour at room temperature. The aqueous fraction was extracted and washed with 5 mL of DCM, then 5 mL of hexane. The aqueous fraction was isolated and the solvent was removed in vacuo to afford **S3** (0.089 g, 0.109 mmol, 21.3%) as a white solid, which was used without further purification for the synthesis of **1b**. **Note:** Incomplete oxidation of all imines resulted in an appreciable amount of undesired side product. We note that fresh batches of *m*CPBA resulted in purer product and significantly higher yields (78%). LC-ESI-MS (method E): calcd. for C₂₈H₄₈N₈O₁₁: 672.74. Found: 673.3 [M+H]⁺, R_t=0.49 min.

(8*S*,11*S*,14*S*,17*S*)-11,14,17-tris(3-(*N*-acetoxyacetamido)propyl)-4-acetyl-8-(((benzyloxy)carbonyl)amino)-2,9,12,15-tetraoxo-3-oxa-4,10,13,16-tetraazaoctadecan-18-oic acid, **1b**. **S3** (0.089 g, 0.109 mmol) was dissolved in 5 mL of pH=4 KOAc/HOAc buffer solution and the pH was adjusted to 4 by addition of solid KHCO₃. Ac₂O (0.795 g, 7.79 mmol, 0.736 mL) was then added dropwise to the solution while maintaining the pH at 4 by addition of solid KHCO₃. The solution was then stirred at room temperature for 5 hours. After 5 hours, the solvent was removed in vacuo to afford a clear yellow oil. To this oil were added 5 mL of 0.5 M HCl and 4 mL of EtOAc. The layers were separated and the aqueous layer was washed with 4 mL of EtOAc. The combined organic fractions were washed with brine and dried by filtering through Na₂SO₄. The EtOAc was removed, affording **1b** (0.060 g, 0.0595 mmol, 54.6%) as a yellow oil. **Note:** After extraction with EtOAc, it was found that the aqueous layer still contained an appreciable amount of desired product. The solvent from the aqueous layer was removed in vacuo and the solid was washed with 5 mL of EtOAc. The EtOAc was removed, affording additional **1b** (0.011 g). ¹H NMR (DMSO, 400 MHz, ppm): 12.05 (s, 1H, COOH), 8.09 (s, 1H, NH), 7.90 (s, 1H, NH), 7.87 (s, 1H, NH), 7.39 (s, 1H, Cbz-NH), 7.27 (s, 5H, Ar-H), 4.99 (s, 2H, Cbz-CH₂), 4.24-3.99 (m, 4H, αH), 3.55 (s, 8H, δH), 2.16 (s, 12H, CH₃), 1.87 (s, 12H, CH₃), 1.50 (s, 16H, βH/γH). ¹³C NMR (DMSO, 100 MHz, ppm): 176.3 (COOH), 175.1 (amide-C=O), 174.7-173.4 (4 signals, acetyl-C=O), 159.0 (Cbz-C=O), 140.08 (Ar-C), 133.8 (Ar-C), 131.2-130.8 (4 signals, Ar-C), 68.5 (Cbz-CH₂), 57.2 (αC), 54.8 (αC), 49.7 (δC), 32.2 (βC), 26.2 (γC), 24.1 (acetyl-CH₃), 21.3 (acetyl-CH₃). LC-ESI-MS (method E): calcd. for C₄₄H₆₄N₈O₁₉: 1008.4. Found: 1009.4 [M+H]⁺, R_t=3.17 min.



N-(10-(4-(((5*S*,8*S*,11*S*)-11-(3-(4-(3-(2-(1*H*-imidazol-5-yl)ethyl)thioureido)phenyl)thioureido)-15-hydroxy-5,8-bis(3-(*N*-hydroxyacetamido)propyl)-4,7,10,16-tetraoxo-3,6,9,15-tetraazaheptadecyl)carbamoyl)-2-carboxyphenyl)-7-(dimethylamino)-5,5-dimethyldibenzo[*b,e*]silin-3(5*H*)-ylidene)-*N*-methylmethanaminium, **S5. 8** (0.002 g, 0.002 mmol) was dissolved in 1 mL of isopropanol/H₂O (6:1). To this solution was added histamine (0.001 g, 0.009 mmol) dissolved in 1 mL of chloroform, followed by 2 μL of triethylamine. The solution was stirred for 2 hours. The volatiles were removed and to the resulting residue was added 2 mL of H₂O/acetonitrile (1:1) + 0.1% TFA. The precipitate was filtered off and the filtrate was purified using preparative HPLC and method A, with the product eluting at 15.3 minutes. The fractions containing product were pooled and the solvent was removed in vacuo to afford **product S5** (0.001 g, 0.0007 mmol). LC-ESI-MS (E): calcd. for C₆₃H₈₄N₁₅O₁₂S₂Si⁺: 1334.6. Found: 668.9 [M+H]²⁺, 446.3 [M+H]³⁺, R_t=2.14 min

HPLC-MS purity data for SiR-conjugates 6, 8 and S5

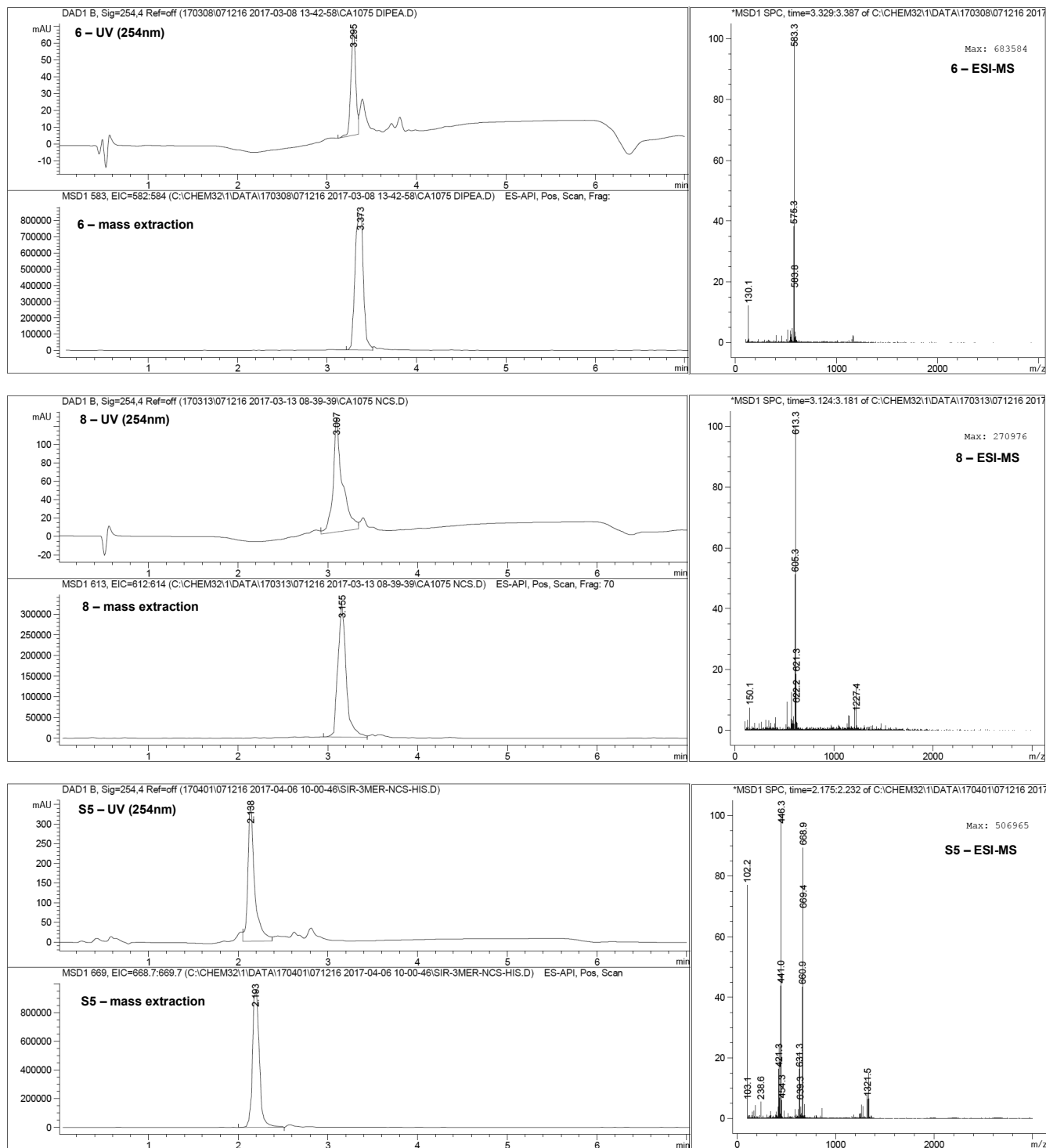


Figure S 1. HPLC-MS traces of compounds 6, 8 and S5. For each compound, the UV-Absorption trace at 254 nm, mass extraction for the corresponding product and the mass spectrum are shown.

2. ^1H spectrum of 2a and Zr-2a

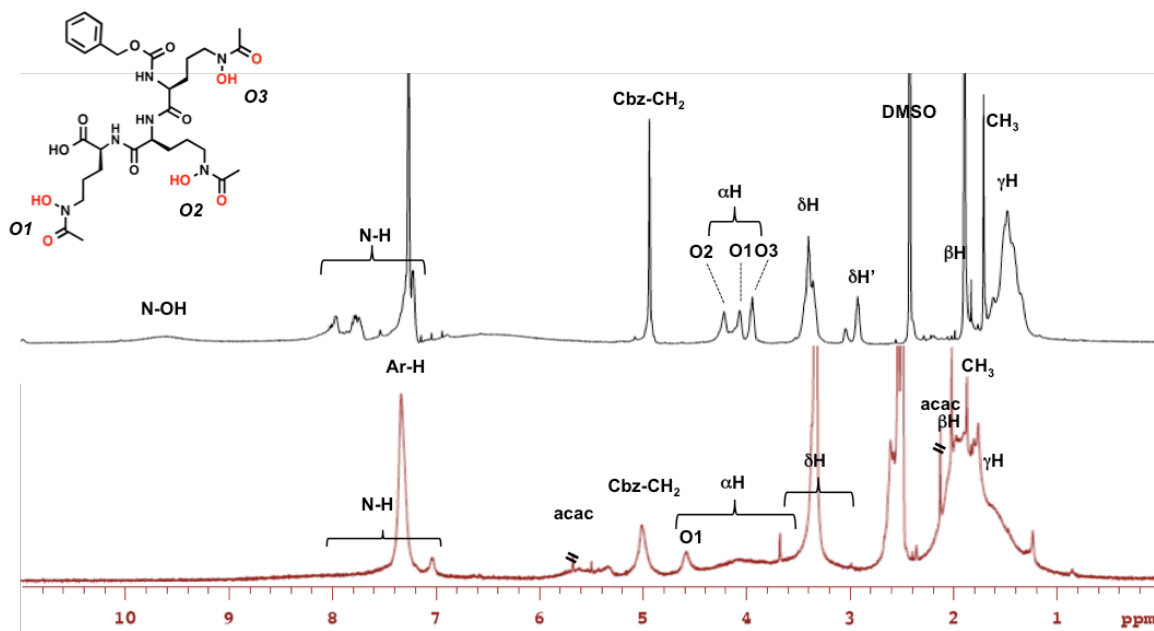


Figure S 2. ^1H -NMR spectra of **2a** (top) and **Zr-2a** (bottom) at 500 MHz in d_6 -DMSO at 60 °C. Chemical shift changes are indicated for amide and alpha carbon protons. Impurities are denoted with (*), as well as residual acetylacetonate (acac) and solvent.

3. DFT coordinates

Table S1. DFT-optimized Coordinates (Cartesian, Å) of $[\text{Zr}(\text{AHA})_3(\text{OH}_2)]^+$

Zr	1.51384463281581	7.77531069738314	-0.41464051351479
C	5.62357464072277	7.10765786343352	0.91358786778076
C	4.22410980644053	6.54766553529121	-1.09309211849490
C	5.32084419721429	5.88735539072948	-1.88136501005486
N	4.41054266998389	7.05172771796200	0.11093170074797
O	3.30946870701122	7.58787154831882	0.71028340118582
O	3.03399018007483	6.65798757278315	-1.57607150376289
C	-0.29167616770511	7.51632475878353	3.52508607750672
C	-0.16459996781804	6.09366584830338	1.43845436115025
C	-0.93792502832448	4.90643095032294	1.92768978363908
N	0.07651439902995	7.17724456469016	2.15566010107741
O	0.78019763551241	8.16202963201805	1.53050602423858
O	0.31331063861800	6.08007765327102	0.23878102717322
C	1.47688181426713	12.01370806262171	-1.36195738707598
C	-0.21489597220849	10.14573520645984	-1.27590428841850
C	-1.39587075520307	11.00932781167938	-1.63043175093971
N	1.01235394432039	10.64663761926575	-1.18529794661320
O	1.99581017082912	9.75270678870138	-0.85929981814625
O	-0.35415995698260	8.89827991834943	-1.04652537154601
H	-1.26588589147750	11.49356633633121	-2.60460842513409
H	-1.55688713235734	11.79080833806473	-0.87904994133849
H	-2.28242812336432	10.37405260950736	-1.66760573053303
H	-1.76145624009714	4.71676054659478	1.23054800497243
H	-0.28477918366880	4.02648848864472	1.90882849961189
H	-1.34560136508520	5.02258185676809	2.93187060148959
H	5.76367084882730	5.04748779757780	-1.33492979120223
H	4.89552962163761	5.51456777530136	-2.81473377972886
H	6.11993066834094	6.59772543405708	-2.12304265753465
H	5.45661813132025	6.56366522183030	1.84777491271432
H	6.45228693533695	6.65963310623960	0.36387671372325
H	-0.91542973254724	8.41449283430752	3.50748164403700
H	-0.8351805945045	6.69440114778707	3.98878022921677
H	2.21807884439814	12.03927959942192	-2.16642840728939
H	0.63433750133769	12.65780096618146	-1.61628104955798
H	5.84796260195469	8.15407749027692	1.13887243152032
H	0.62372843984681	7.72165038797699	4.08653170119240
H	1.93807770811915	12.35713021538750	-0.43111453114759
O	0.70562763051349	6.97324983565093	-2.45509191364145
H	1.14600188358015	6.17990834439418	-2.80650681886812
H	-0.25605014070965	6.88819752733062	-2.56810732843476

Table S2. DFT-optimized Coordinates (Cartesian, Å) of [Zr(DFC)(OH₂)]⁺

Zr	1.176155	7.490533	-0.194612
C	5.927025	11.706187	2.001278
C	5.530739	10.277848	2.444083
C	5.687951	9.289363	1.271657
C	5.599431	7.825544	1.721399
C	5.419285	6.832500	0.568330
C	3.924019	7.148175	-1.447753
C	5.040018	7.156487	-2.456285
N	4.185002	10.224920	3.005388
N	4.149808	7.031036	-0.145772
O	6.798991	11.890212	1.151707
O	3.028002	6.998704	0.638771
O	2.701008	7.250088	-1.818016
C	3.884514	9.658358	4.211939
C	2.377163	9.474953	4.518964
C	2.083162	7.959274	4.472122
C	0.678161	7.503856	4.909881
C	-0.473447	7.695089	3.904534
C	-0.351553	6.019490	2.010020
C	-1.021625	4.879632	2.714885
N	1.489610	10.252109	3.654899
N	-0.159944	7.208282	2.549712
O	4.729601	9.262174	5.007012
O	0.458778	8.125509	1.738485
O	0.068082	5.862318	0.800019
C	1.215253	11.565796	3.944302
C	0.156042	12.271778	3.071847
C	0.123868	11.877575	1.577544
C	1.389944	12.250230	0.797328
C	1.329787	11.839776	-0.679271
C	-0.040192	9.900818	-1.505547
C	-1.011049	10.721323	-2.307326
N	0.306532	13.709378	3.240684
N	0.976328	10.424081	-0.845767
O	1.747932	12.142832	4.885420
O	1.775658	9.538944	-0.175428
O	-0.175694	8.618959	-1.432640
C	-0.718030	14.543011	2.936578
C	-0.480356	16.056276	3.055313
O	-1.822747	14.143320	2.562861
N	0.874433	16.494009	3.347199
C	1.848233	16.528054	2.394409
C	3.206277	17.083051	2.862922
O	1.666056	16.173534	1.233171
N	4.308812	16.240112	2.429278
C	4.347267	14.938828	2.808827
C	5.530057	14.108150	2.313287
O	3.485723	14.452948	3.545335
N	5.267933	12.718005	2.615334
H	-0.500725	11.372163	-3.024590
H	-1.631678	11.348767	-1.656850
H	-1.662281	10.035308	-2.851926
H	-1.269018	5.093067	3.755144
H	-1.939949	4.617912	2.176160

H	-0.359806	4.007512	2.680392
H	5.616133	6.224781	-2.428359
H	4.601037	7.271343	-3.448837
H	5.730181	7.988864	-2.279624
H	5.421472	5.803282	0.947653
H	6.219691	6.927655	-0.170046
H	4.773001	7.684300	2.423840
H	6.514340	7.539331	2.255725
H	6.646686	9.491505	0.782851
H	4.902586	9.508544	0.533602
H	6.449768	14.459723	2.804835
H	5.682378	14.228670	1.234155
H	3.237344	17.192100	3.953025
H	3.340391	18.073144	2.417457
H	-1.148954	16.437361	3.833035
H	-0.800635	16.489682	2.103641
H	2.809288	7.499460	5.150308
H	2.307542	7.568678	3.471495
H	0.384325	8.014641	5.836892
H	0.737966	6.435064	5.155291
H	-0.738684	8.745971	3.778800
H	-1.370047	7.175423	4.251538
H	2.298302	12.000609	-1.166305
H	0.575854	12.423031	-1.212987
H	2.276176	11.797089	1.253008
H	1.544429	13.335366	0.824518
H	-0.744989	12.387925	1.145635
H	-0.077327	10.802558	1.493531
H	1.123273	16.696721	4.306579
H	4.937155	16.577689	1.712329
H	4.557665	12.533012	3.317826
H	3.408198	10.415278	2.383253
H	1.083914	9.791737	2.845224
H	1.191370	14.061402	3.597978
H	2.244293	9.842555	5.541314
H	-0.822149	11.993280	3.491851
H	6.207712	9.995641	3.260309
O	0.423319	5.879972	-1.714770
H	0.973490	5.674981	-2.489482
H	0.077178	5.055867	-1.330477

Table S3. DFT-optimized Coordinates (Cartesian, Å) of [Zr(Orn3-hx)(OH₂)]⁺

Zr	0.63705074196322	7.57320342530294	2.18792312977759
C	2.89328147630994	11.53815884000819	-2.84478493788812
C	3.28794330973284	10.14451389218658	-2.35822326074706
C	2.07269197729552	9.18956186392379	-2.49941916522344
C	2.47185532744629	7.72016778829597	-2.30559622236677
C	1.29080675024852	6.77765219946649	-2.05284189401699
C	-0.68457150435149	7.19469024584445	-0.52735089879505
C	-1.71666176436372	7.18085556672085	-1.62217382497710
N	3.81485161936102	10.13620756233363	-1.00644557475669
N	0.61178197276301	7.05511868954990	-0.77838279144738
O	2.56626672012250	11.77538329572642	-3.98616465776688
O	1.41843527075452	7.03820214919160	0.32838714017577
O	-1.02795300873745	7.33556738590111	0.69805038584553
C	4.97490878661224	9.51237393224220	-0.66689468371601
C	5.31917130979600	9.47935683907059	0.84163367699718
C	5.30760644601848	7.99206690207301	1.26259920581514
C	5.75382813772226	7.65631070403999	2.69716366119482
C	4.73261512466816	7.89258207233928	3.82643621425320
C	2.86775122972593	6.17887907632837	3.75026547481165
C	3.59117567467644	5.07201938345109	4.45471676329047
N	4.46212226517637	10.31714632334863	1.68036435453636
N	3.38782100135924	7.37016576769746	3.52501138276014
O	5.69334182466451	8.92763229671233	-1.47377898781436
O	2.56431079455055	8.25272751260542	2.87415624385632
O	1.66140327495034	5.98943732179467	3.33237692051997
C	4.79062236819412	11.62295256942627	1.94253436508166
C	3.97761754438152	12.37554851483808	3.02324296028418
C	2.48753155199337	11.99137299432920	3.15085519695426
C	1.65078489154078	12.30743455335952	1.90531259824419
C	0.17376445140545	11.91879572626111	2.03969724921411
C	-0.64384466159212	9.98574184409295	3.42848600549435
C	-1.42019684298266	10.81331443499248	4.41465919661256
N	4.11099473189854	13.80910946504565	2.77157825823082
N	0.00246539849926	10.50493430504523	2.40098048611358
O	5.74869705415623	12.16775455867248	1.40162299605524
O	0.63240372485253	9.61460227328433	1.57739860702322
O	-0.58362461768817	8.70345689026703	3.56082836328666
H	-2.13695592389000	11.47512606546939	3.91795147118365
H	-0.75305310772558	11.42959498063887	5.02865384510657
H	-1.96394401135875	10.13215309609381	5.07169580878405
H	3.04777043349226	4.81759342262843	5.37223797823298
H	3.58688846304312	4.18429372970243	3.81294244686744
H	4.62283396567960	5.31720955919248	4.70898245286526
H	-1.74195956559294	6.21364733297299	-2.13726466330959
H	-2.69335524357968	7.36572104553227	-1.17170640579860
H	-1.52128412533530	7.95794178720003	-2.36889277436440
H	1.64020465760719	5.73860847686271	-2.01435116133114
H	0.53694822635243	6.85502515028360	-2.84073620501856
H	3.17180004561175	7.61978073443996	-1.47247637046986
H	2.99643242433640	7.35470839468458	-3.19708860240414
H	1.62504744308485	9.33898155297215	-3.48912993155089
H	1.32380939283437	9.48241800696283	-1.74958732926861
H	5.99111731823118	7.49011751322194	0.57121995783714
H	4.31495324594324	7.56424239950689	1.07146342398181

H	6.66055708265612	8.21847128587605	2.95870070360704
H	6.03705001685128	6.59544147802346	2.71542283715114
H	4.58545822988618	8.95426517055652	4.03006991263553
H	5.07665547253607	7.42897023511203	4.75471182583092
H	-0.35500775824583	12.08464467044453	1.09379063194265
H	-0.31931083926331	12.50928035208320	2.81602437537228
H	2.06366397396511	11.80602525870725	1.02517048491248
H	1.70018023799555	13.38161313629577	1.69845396308836
H	2.08596707608961	12.55264078853528	4.00769309748459
H	2.39834484344378	10.93068682690261	3.41882803926888
H	3.31331064032290	10.63291738633874	-0.28278671258740
H	3.64795017975817	9.88429959695642	2.10664084388341
H	6.33605174935465	9.87696673777532	0.92185706827696
H	4.46463435491241	12.07149590384083	3.97084138437030
H	4.09519348985828	9.80183787698090	-3.01495073655797
H	5.00031176617318	13.98663894037474	2.30483685442600
O	2.90183187606344	12.47315882886098	-1.86562322747195
H	2.65814652127593	13.32744111013605	-2.27244199115996
H	4.10115797735646	14.33669862571807	3.64124453429428
O	-0.84749542832309	5.94459797019298	2.95825933420879
H	-0.46556317834479	5.13781556314749	3.34480177360107
H	-1.64331227618033	5.72353084300609	2.44644912516633

Table S4. DFT-optimized Coordinates (Cartesian, Å) of [Zr(AHA)₄]⁺

Zr	-0.22471255763656	7.65054493550714	0.77110030501446
C	3.00971276345052	4.61332901989798	1.01597637196926
C	0.93625797887160	5.00267259312602	-0.33055038707558
C	1.08859916245736	3.75565027334027	-1.17131095529206
N	1.80599104150824	5.31430129881091	0.62131026986591
O	1.57174400470160	6.46583196743485	1.30866144676283
O	-0.04006087253042	5.78940410785810	-0.52270449258585
C	0.00829979417692	7.43605811384489	5.14069794730311
C	-1.12724206820788	6.17369156069530	3.30330287080718
C	-1.67567443208003	5.09151887997289	4.20442270397952
N	-0.40628911688420	7.17804279819303	3.77616025761202
O	0.00699811339721	8.11998929043089	2.89014325620540
O	-1.34863502879923	6.13871027414319	2.04853362112787
C	-3.60452722155071	10.50752663601459	0.40635719969275
C	-3.03223610737074	8.19454475576683	-0.41344958233938
C	-4.31752014868460	7.93360282945982	-1.15768203844521
N	-2.77699602389364	9.33154029850367	0.22257164407218
O	-1.57758935143006	9.40679188821649	0.86190896786329
O	-2.14801691362112	7.28458215483627	-0.37333475537666
H	-4.06845836302236	7.39387577151363	-2.07617231595176
H	-4.87612202493323	8.83571983429758	-1.41676852303898
H	-4.96283723299795	7.28038492348231	-0.55686529229634
H	-2.24214193669956	5.49835533888502	5.04971582791139
H	-2.34032551518133	4.46692584933900	3.60384322056346
H	-0.87384843956703	4.45857814934572	4.60503740832416
H	1.11067962831997	2.84703430840724	-0.55814384903221
H	0.22913355258633	3.70404669854514	-1.84257472713431
H	2.00346983799391	3.78187293324531	-1.77585788294595
H	2.94214884569739	4.34549573655488	2.07678699721037
H	3.13673505669803	3.70927266069516	0.41672082382763
H	-0.39591735858618	8.40261377486146	5.46209421273499
H	-0.34753102261277	6.64526664311883	5.80430203923714
H	-3.07743936154611	11.38285624498029	0.01025533271069
H	-4.56312939459867	10.39296576890623	-0.10156802166249
C	2.19003463900013	11.24562357329535	0.10811096025954
N	1.44853762287233	10.02644285926933	-0.14508686767150
H	3.14553643085259	10.99243942636819	0.58113201090200
H	2.36854940577122	11.78511831876620	-0.82433498334861
O	1.18761079154028	9.30109299996139	0.97248797085650
C	1.01929269687011	9.54022377522681	-1.29886369647341
C	1.28871854796804	10.24145464661044	-2.61013379420075
O	0.35949031650690	8.44983763986721	-1.27631366229470
H	0.94601928922912	9.58472309693111	-3.41247882987803
H	2.35343193810095	10.45421343956301	-2.75909119776264
H	0.73924371026407	11.18868450379852	-2.67902739189944
H	3.87350578596607	5.27347593577012	0.87645576015111
H	1.10197092487341	7.48282742152119	5.17336749678948
H	1.61213748466998	11.87343032329854	0.79463871212683
H	-3.77324487190991	10.65734172752161	1.47859661082478

Table S5. DFT-optimized Coordinates (Cartesian, Å) of [Zr(Orn4-hx)₄]⁺

Zr	-0.26949414305451	7.46067635099584	0.61602413984343
C	4.52206360283568	10.39797193865465	2.14863483375628
C	4.25785349022623	9.08176590214796	2.92756477126626
C	4.32070867815862	7.88970250440826	1.94246707637627
C	4.08289436996509	6.53458918005282	2.62932561972135
C	3.51864015842434	5.44556414306599	1.70852216226579
C	1.82166758280696	5.40414911603041	-0.12162655427075
C	2.65266648869098	4.47679085416375	-0.97514391001764
N	2.96988876168525	9.10395444168519	3.62259102485640
N	2.26277311720262	5.83945657086627	1.05278444704881
O	5.45907730850239	10.48811558200779	1.35237275977779
O	1.39089622310830	6.59626042056357	1.79499724840165
O	0.67802832260507	5.78700386952222	-0.53240173583284
C	2.82957133608016	8.93174736882227	4.97458814235418
C	1.37564300474618	8.71284372645576	5.47045930568917
C	1.06390288831820	7.20567038636332	5.31538586555078
C	-0.30936026677207	6.71225254015490	5.80652632376418
C	-1.51115294451474	6.95843160775466	4.86721686773049
C	-1.47850220987565	5.59586776744732	2.75173029203912
C	-2.02768124432751	4.36537848137568	3.43498960298795
N	0.38734674980203	9.54869792066078	4.79078789114052
N	-1.24352529620327	6.70710121359744	3.44983588525954
O	3.77302897255573	8.88857348187890	5.75722000491303
O	-0.82246596884272	7.80974715703120	2.73326063605391
O	-1.25551093056263	5.58579302903158	1.50823812643279
C	0.07146776711873	10.79595672148424	5.23564610456713
C	-1.03166635964287	11.58450465382813	4.47775953357821
C	-1.23548346018725	11.14881077127223	3.02619805383264
C	-2.49437081709987	11.67747553045705	2.32481078722265
C	-2.56267866758591	11.22795266501847	0.84870852157560
C	-3.02832042560441	8.77725069123771	0.37512917753842
C	-4.52422949784703	8.90406963575674	0.24961979242713
N	-0.64938598078871	13.01405488307479	4.56892308546989
N	-2.23953674928628	9.81401116503561	0.64212432905960
O	0.58559154379542	11.30354961737093	6.23416489546695
O	-0.89477837187138	9.56100612683488	0.67124961434577
O	-2.49103546095414	7.63387708333159	0.21561887718772
O	1.95028327770214	14.51232746479755	1.81280368036209
C	2.30996704210512	13.44204861647715	2.26596695107498
C	3.49452116021030	12.64614760364872	1.68234932503858
O	1.72679441678001	12.80756923424103	3.28527377624611
N	3.62774105075506	11.38317383696493	2.39914700320576
H	-4.80961889573866	8.72483470035601	-0.79413877310488
H	-4.91940556267940	9.87171754109068	0.56436480177662
H	-4.98956877778524	8.11802730356843	0.85300776929621
H	-3.11576407927310	4.44860421690138	3.55568139774894
H	-1.82344074192542	3.50631358593011	2.79247713402676
H	-1.58476380211905	4.18955176714272	4.41962150844403
H	2.97315229792623	3.58501267598906	-0.42495063692795
H	2.04003206699262	4.16984690720731	-1.82504169150693
H	3.54944806117996	4.98107278957964	-1.35609222406273
H	3.34042413790196	4.53320432067595	2.29722496600520
H	4.23431336130054	5.20126868873502	0.91786961193763
H	3.39231403924510	6.65367456828348	3.46667766303257

H	5.01873754837474	6.14490853988958	3.05248499990686
H	5.29365457937510	7.91671227998106	1.44091034964642
H	3.55540587792835	8.05196912653331	1.17514028073495
H	4.39540450284149	13.25803829852123	1.82553136481133
C	3.28988866517493	12.44239816527945	0.15791183632032
H	1.83788392339221	6.68070648712255	5.88864781876015
H	1.19233571629648	6.90473185463885	4.26894757160071
H	-0.55425163038704	7.14983878943072	6.78485566269841
H	-0.22369950229410	5.62906146562044	5.96831151706763
H	-1.85096798381342	7.99543789563478	4.92093120278563
H	-2.35216282759535	6.32964029465871	5.17788685709583
H	-1.82290736329621	11.77927617331037	0.25834557812619
H	-3.54866199457969	11.43773086243291	0.42673025275002
H	-3.38980362111651	11.32515447308528	2.85835367476438
H	-2.53424866611600	12.77654247633982	2.32339471065163
H	-1.30794216076950	10.06218166976427	2.98395503466633
H	-0.34739264184416	11.41979116779143	2.44820253492280
H	2.93339986012500	11.19625625601466	3.11148524660007
H	2.15954518700768	8.91192330314034	3.04109027858953
H	-0.06089635205732	9.15351764193434	3.96417883169676
H	1.38262780513054	8.99708911488150	6.52564747323944
H	-1.95861090295456	11.39222565097455	5.04938576811671
H	5.02893672858720	8.98846233787198	3.70046630724440
H	4.21360131099335	11.99689147060327	-0.22536425222450
H	3.19028357468402	13.43353323274546	-0.29593088949133
C	2.07144416396593	11.54826435062048	-0.17118979872597
C	2.40334222486552	10.39889975572120	-1.14103511863902
H	1.24846581337965	12.14719349431000	-0.58005428833292
H	1.69174552074309	11.08009814418195	0.74192230723750
N	1.41839340639949	9.32431290730560	-1.05954838214227
H	3.37126109332688	9.95535346835625	-0.88151303225228
H	2.45994969368494	10.74445569366852	-2.17744778211215
O	1.47994457847227	8.60484398644765	0.10980962261786
C	0.46655353459429	8.97928331728030	-1.92163789777690
C	0.35703631347637	9.66237835183086	-3.26479791468179
O	-0.34723037927204	8.05963726006164	-1.60208004169286
H	-0.44549531931644	9.17498590166936	-3.82185994354032
H	1.28572041596327	9.58441970678176	-3.84141951741096
H	0.11378059955493	10.72597675317272	-3.15506654194861
H	0.86444642705748	13.20417334546414	3.64112861003458
H	-0.35389948156697	13.19906503624088	5.53003593301989
H	-1.44373686260108	13.61717157765577	4.36407891329240

4. Complex inertness and concentration dependent radiolabeling yields

Table S6. Complex inertness chart for 1000x EDTA challenge experiment (n=3).

	⁸⁹ Zr-DFO	⁸⁹ Zr-2a	⁸⁹ Zr-2b	⁸⁹ Zr-DFC
time (h)				
0	1.000	1.000	1.000	1.000
12	0.830 ± 0.014	0.653 ± 0.042	0.783 ± 0.035	0.570 ± 0.071
24	0.735 ± 0.021	0.573 ± 0.058	0.737 ± 0.047	0.550 ± 0.042
48	0.680 ± 0.028	0.450 ± 0.070	0.710 ± 0.046	0.525 ± 0.021
72	0.665 ± 0.021	0.417 ± 0.058	0.667 ± 0.029	0.530 ± 0.014
96	0.655 ± 0.035	0.390 ± 0.056	0.643 ± 0.021	0.520 ± 0.014
120	0.665 ± 0.021	0.387 ± 0.051	0.643 ± 0.012	0.525 ± 0.007

Table S7. Radiochemical yield in dependence of complex concentration (n=2) as determined by radioTLC.

conc. [μM]	⁸⁹ Zr-2a	⁸⁹ Zr-2b	⁸⁹ Zr-DFC
1500	1.000 ± 0	1.000 ± 0	1.000 ± 0
150	0.990 ± 0	0.990 ± 0	0.990 ± 0
15	0.990 ± 0	0.990 ± 0	0.990 ± 0
1.5	0.945 ± 0.021	0.990 ± 0	0.965 ± 0.007
0.15	0.745 ± 0.021	0.925 ± 0.007	0.855 ± 0.021
0.015	0.565 ± 0.035	0.775 ± 0.021	0.720 ± 0.014

5. Fluorescence spectroscopy

Fluorescence measurements were acquired with a Horiba Dual FL fluorimeter (Horiba Scientific, New Jersey, USA). To obtain the absorption and emission fluorescence spectra of unfunctionalized silicon-rhodamine, a 1 mM stock solution of fluorophore was diluted to 0.1 μ M using water and subsequently scanned. Excitation spectra were recorded between 400 and 675 nm. Emission spectra were recorded between 640 – 850 nm. The obtained excitation and emission spectra demonstrate that functionalization of the SiR fluorophore with a closely associated metal complex does not lead to significant changes in fluorescence properties.

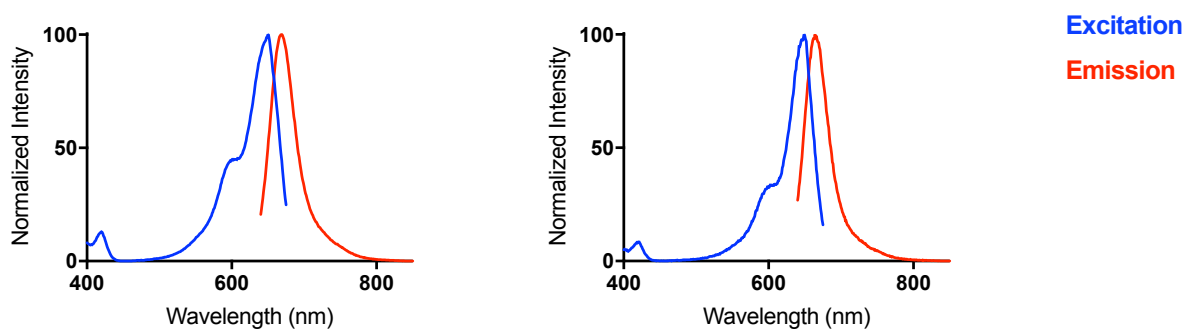


Figure S 3. Excitation and emission spectra obtained for SiR (left, $\text{Excitation}_{\text{max}} = 650 \text{ nm}$, $\text{Emission}_{\text{max}} = 669 \text{ nm}$) and model complex [Zr(S5)] (right, $\text{Excitation}_{\text{max}} = 649 \text{ nm}$, $\text{Emission}_{\text{max}} = 664 \text{ nm}$).

6. Cell imaging

Multichannel images were collected on a Nikon eclipse Ti inverted microscope equipped with a Nikon C-HGFI intensilight fiber illuminator and a Cy5.5 excitation and emission filter (Ex = 678 nm and Em = 694). Cell culture: BT474 cells (HER2+, a kind gift from the lab of Prof. Umar Mahmood) were cultivated in EMEM media supplemented with 10 % fetal bovine serum and 1 % Penicillin/Streptomycin solution under standard culture conditions. For imaging experiments, 5×10^4 cells were plated in 12-well plates (Thermo Fisher Scientific) grown for 48 hours, reaching 60% confluence. Subsequently, wells were incubated with 60 μ L of 8-trastuzumab in 2 mL culture media per well for 24 hours, followed by 3 subsequent washes with PBS. Images were acquired with a 10 second delay time to compensate for the low quantum yield and excitation efficiency in the Cy5.5 channel. Cellular uptake of probe is indicated by the observation of puncta inside the cells.

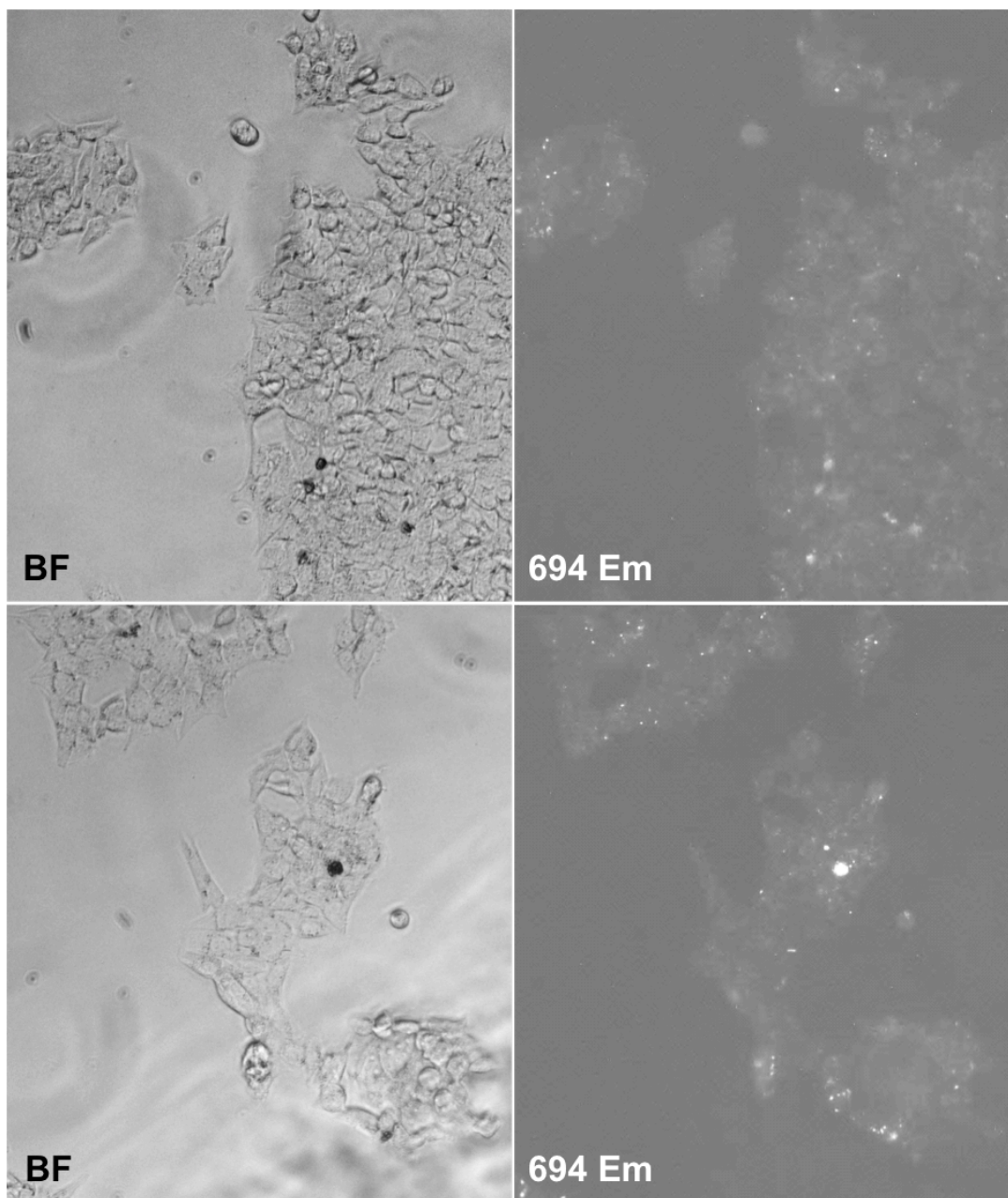


Figure S 4. Bright field (left) and fluorescence emission image of BT474 cells after 24 hours of incubation with 8a-trastuzumab.

7. Multimodal gel electrophoresis

Purified samples of radiolabeled immunoconjugates were loaded onto an electrophoresis gel under reducing conditions. Subsequently, the plastic-encased gel was placed on a photoplate for 30 minutes. The photo plate was subsequently imaged using a packard cyclone phosphorimager. After autoradiography, the gel was removed from the casing and subjected to fluorescence imaging using an Alpha Innotech Fluorchem Q Multimode III Western Blot Imager in order to detect fluorescent bands with a SiR label. The Cy5 channel was used (excitation at 649 nm, emission at 666 nm) to visualize SiR. After fluorescence image acquisition, the gel was stained using conventional coumassie staining procedure to visualize protein and ladder within the gel.

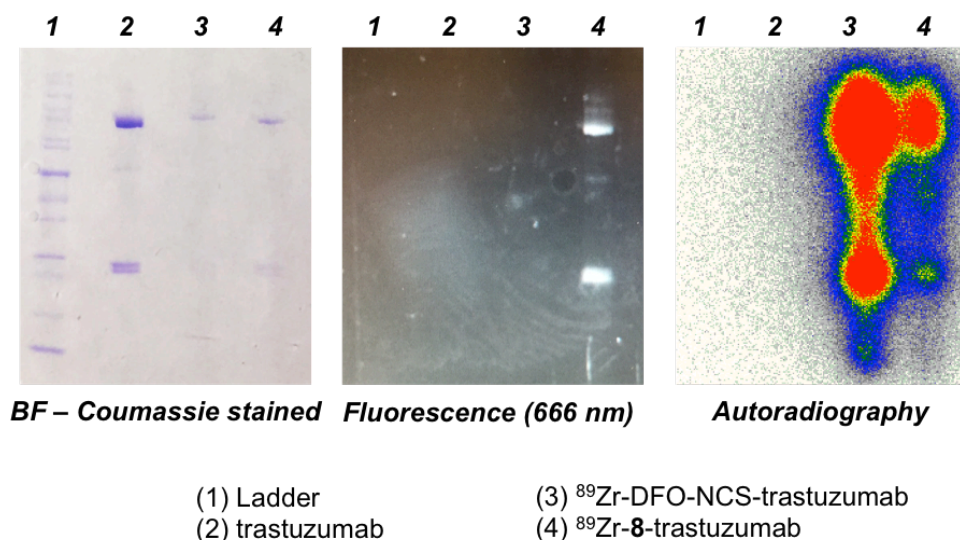


Figure S 5. Multimodal gel electrophoresis of radiolabeled immunoconjugates.

8. Ex vivo imaging

For ex vivo fluorescence imaging, an IVIS Spectrum imaging system was used to record the images (exposure time, 5 s; bin=8, $f=1$, FOV=D). Select organs and tissue samples (bone, liver, spleen, kidney and probe phantom) were placed in culture dishes (60 mm diameter), and samples were excited at 660 nm, with fluorescence emission monitored at 700 nm. Subsequently, the same select organs were imaged in a microPET/X-ray Sofie Biosciences G4 PET scanner (Culver City, CA, USA), by acquisition of a 10 min static microPET image.

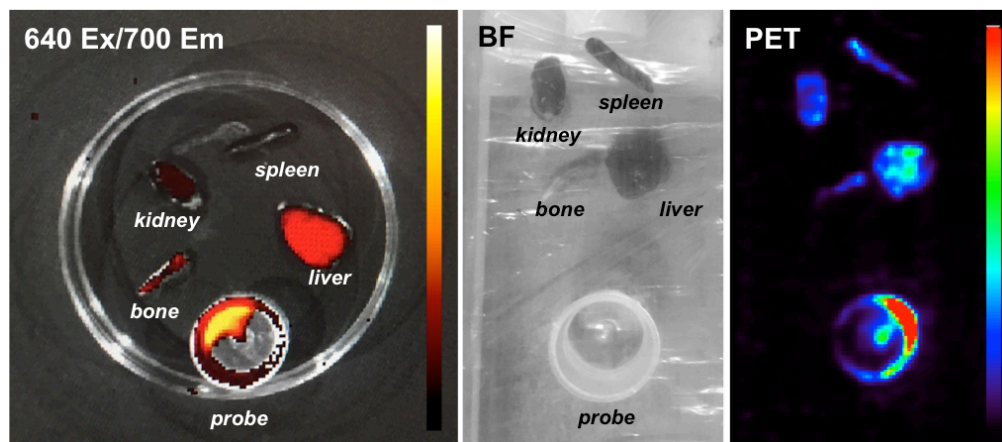


Figure S 6. Fluorescence, bright field and PET image of select excised organs after administration of Zr-8-trastuzumab.