Supplemental Data

1 Preparation of non-radioactive compounds

Affibody compounds general

Recombinant HER2 Affibody $Z_{HER2:2891}$-Cys produced in *E. coli* was purchased from Affibody AB, Sweden. The Affibody molecule was provided lyophilised from 10 mM ammonium acetate, pH 6.0. Eei-aminooxyacetic acid succinic ester was purchased from IRIS Biotech (Germany). Di-*tert*-butyldifluorosilane was purchased from Fluorochem (UK). All other Reagents and solvents were obtained from IRIS Biotech (Germany), Merck (Germany), Romil (UK) and Fluka (Germany). 4-(Di-*tert*-butylfluorosilyl)benzaldehyde was prepared according to the literature (*1*).

Analytical LC-MS spectra were recorded on a Thermo Finnigan MSQ instrument by electrospray ionisation (ESI) operated in positive mode coupled to a Thermo Finnigan Surveyor PDA chromatography system using the following conditions: Solvent A = H_2O/0.1 % TFA and solvent B = MeCN/0.1 % TFA if not otherwise stated, flow rate: 0.6 mL/min, column: Phenomenex Luna 3 µm C18 (2) 20 x 2 mm, detection: UV 214/254 nm.

Semi-preparative reversed-phase HPLC runs were performed on a Beckman System Gold chromatography system using the following conditions: Solvent A = H_2O/0.1 % TFA and solvent B = MeCN/0.1 % TFA if not otherwise stated, flow rate: 10 mL/min, column: Phenomenex Luna 5 µm C18 (2) 250 x 21.2 mm, detection: UV 214 nm.

Preparative reversed-phase HPLC runs were performed on a Waters Prep 4000 system using the following conditions: Solvent A = H_2O/0.1% TFA and solvent B = MeCN/0.1 % TFA if not otherwise stated, flow rate: 50 mL/min, column: Phenomenex Luna 10 µm C18 (2) 250 x 50 mm, detection: UV 214/254 nm.

Affibody molecule synthesis ($Z_{HER2:2891}$-Cys (1))

The sequence AEAKYAKEMRNAYWEIALLPNLTNQQKRAFIRKLYDDPSQSSELLSEAKKLNDSQAPKVDC was assembled on a CEM Liberty microwave peptide synthesiser using Fmoc chemistry starting with H-Cys(Trt) HMPB NovaPEG resin (0.05 mmol). Amino acids (0.5 mmol) were added in each coupling step (5 min at 75 ^{o}C) using HBTU (0.45 mmol), HOAt (0.45 mmol), and DIPEA (1.0 mmol) for *in situ* activation. Fmoc was removed by piperazine (5 %) in DMF. Double coupling of both Arg was applied. Asp-Ser and Leu-Ser pseudoproline dipeptides (0.5 mmol) were incorporated into the sequence.

The simultaneous removal of the side-chain protecting groups and cleavage of the peptide from the resin was carried out in TFA (40 mL) containing TIS (2.5 %), EDT (2.5 %), EMS (2.5 %) and water (2.5 %) for 1 hr. The resin was removed by filtration, washed with TFA and the combined filtrates were evaporated *in vacuo*. Diethyl ether was added to the residue, the formed precipitate washed with diethyl ether and dried. The cleavage procedure was repeated once more. The dried precipitates were dissolved in acetonitrile/water (20 % v/v) and left overnight in order to remove remaining Trp protecting groups. The solution was lyophilised affording 148 mg (42 %) crude peptide **1**. The crude peptide **1** was purified by semi-preparative HPLC (4 runs, gradient: 25-30 % B over 40 min) affording 33 mg (9 %) pure **1**. LC-MS (gradient 10-40 % B over 5 min) t_R: 3.40 min, found m/z: 1758.3, expected MH_4^{4+}: 1758.4.

Eei-aminooxyacetylmaleimide (2)

N-(2-Aminoethyl)maleimide trifluoroacetate (51 mg, 0.20 mmol) and 2,5-dioxopyrrolidin-1-yl 2-(((1-ethoxyethylidene)amino)oxy)acetate (77 mg, 0.30 mmol) were dissolved in NMP (2 mL). Sym-collidine (80 µL, 0.6 mmol) was added and the reaction mixture stirred for 70 min. The reaction mixture was diluted with water (7 mL) and the product purified by semi-preparative HPLC (gradient: 15-30 % B over 40 min where A = water/0.1% acetic acid and B = MeCN) affording 43 mg (75 %) pure **2**. LC-MS (gradient 10-40 % B over 5) t_R: 1.93 min, found m/z: 284.1, expected MH^+: 284.1; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 6.72 (s, 2H), 6.65 (broad s, 1H), 4.32 (s, 2H), 3.97 (q, *J*=7.1 Hz, 2H), 3.75-3.71 (m, 2H), 3.55-3.50 (m, 2H), 2.02 (s, 3H), 1.27 (t, *J*=7.1 Hz, 3H).

$Z_{HER2:2891}$-Cys-aminooxy (3)

Recombinant Affibody molecule **1** (144 mg, 0.205 mmol) and maleimide **2** (17 mg, 0.60 mmol) were dissolved in water (3 mL). The solution was adjusted to pH 6 by addition of ammonium acetate and the reaction mixture shaken for 90 min. The reaction mixture was diluted with water (7 mL) and the product purified by semi-preparative HPLC affording 126 mg lyophilised Eei-protected product. The Eei-protected product was treated with TFA/water (2.5 % v/v, 16 mL) under a blanket of argon for 20 min. The solution was diluted with water (144 mL), frozen using isopropanol/dry-ice bath under a blanket of argon and lyophilised affording 149 mg (100 %) Affibody molecule **3**. Lyophilised **3** was analysed by LC-MS (gradient 10-40 % B over 5 min) t_R: 3.28 min, found m/z: 1811.8, expected MH_4^{4+}: 1811.4.

SiFA-aminooxyacetylmaleimide (4)

Eei-aminooxyacetylmaleimide **2** (20 mg, 71 µmol) was added to 4-(di-*tert*-butylfluorosilyl)-benzaldehyde in water/MeCN (0.1% TFA, 100 mL) (as obtained from preparative HPLC). Dilute HCl (1 M, 1 mL) was added and the reaction mixture stirred overnight. The product was purified by semi-

preparative HPLC (gradient: 40-80 % B over 40 min) affording 15 mg (45 %) pure SiFA-aminooxyacetylmaleimide. LC-MS (gradient 40-70 % B over 5 min) t_R: 3.00 min, found *m/z*: 462.1, expected MH^+: 462.2. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.24 (s, 1H), 7.67-7.58 (m, 4H), 6.64 (broad t, *J*=5.2 Hz, 1H), 6.52 (s, 2H), 4.62 (s, 2H), 3.76-3.71 (m, 2H) 3.55-3.50 (m, 2H), 1.07 (d, *JHF*=1.0 Hz, 18H); ^{19}F NMR (470 MHz, $CDCl_3$): δ_F -193.1 ppm.

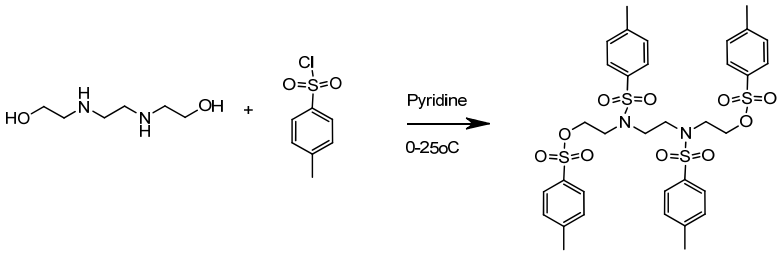
$Z_{HER2:2891}$-Cys-SiFA (5)

Recombinant Affibody molecule **1** (24 mg, 3.4 µmol) and maleimide **4** (4.7 mg, 10 µmol) were dissolved in MeCN/water (50 % v/v, 1 mL). The solution was adjusted to pH 6 by adding ammonium acetate and the mixture shaken for 1 h. The reaction mixture was diluted with MeCN/water/TFA (10 %/90 %/0.1% v/v/v, 8 mL) and the product was purified using semi-preparative HPLC (gradient: 20-40 % B over 40 min) affording 26 mg (>99 %) pure Affibody molecule **5**. LC-MS (gradient 10-40% B over 5 min) t_R: 3.87 min, found *m/z*: 1873.6, expected MH_4^{4+}: 1873.5.

NOTA-$(COOH)_2$ maleimide (6)

Synthesis of NOTA(mono acid, bis-tBu)

Synthesis of tetratosyl-*N,N'*-bis(2-hydroxyethyl)ethylene diamine

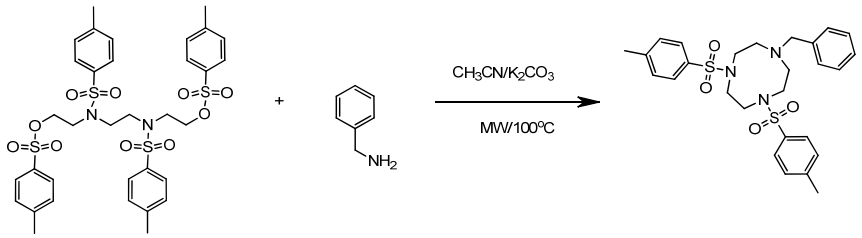


Supplemental Scheme 1. Synthesis of tetratosyl-*N,N'*-bis(2-hydroxyethyl)ethylene diamine.

N,N'-bis(2-hydroxyethyl)-ethylenediamine (Aldrich, 14.8 g, 100 mmol) and pyridine (Fluka, 200 mL) were stirred at 0 °C under nitrogen while a solution of toluene-4-sulfonyl chloride (Fluka, 77 g, 400 mmol) dissolved in pyridine (Fluka, 100 mL) was dropped into the solution over a period of 75 minutes. The temperature was slowly raised to room temperature and continued being stirred for 4 hours. The reaction mixture was poured into a mixture of ice (250 mL) and hydrochloric acid (concentrated, 250 mL) while stirring to afford a dark sticky oil. Solvents were removed by decantation, and the product crude was washed with water, decanted and re-dissolved in methanol

(250 mL). The resulting slurry was isolated by filtration and the crude product was re-dissolved in hot methanol (60 °C, 600 mL) and cooled down. A solid product was filtered off and dried in vacuo. Yield 36.36 g (47.5%).

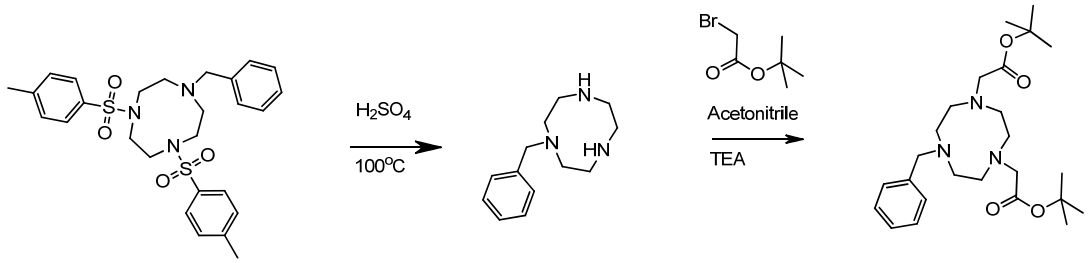
Synthesis of 1-benzyl-4,7-ditosyl-1,4,7-triazonane



Supplemental Scheme 2. Synthesis of 1-benzyl-4,7-ditosyl-1,4,7-triazonane.

A mixture of tetratosyl-*N,N'*-bis(2-hydroxyethyl)ethylene diamine (2.0 g, 2.6 mmol), benzyl amine (500 µL, 4.6 mmol), potassium carbonate (Fluka, 792 mg, 5.7 mmol) and acetonitrile (Merck, 25 mL) was heated to 100 °C and stirred overnight. The solvents were removed from the solid product by filtration. The solid was washed with acetonitrile (2 x 10 mL) and solvents were evaporated off. The solids were dissolved in hot ethanol (15 mL) and left for three days in room temperature. Crystals were collected by filtration and dried in vacuum overnight. The product was confirmed by LC-MS (column: Phenomenex Luna C18(2) 2.0×50 mm, 3 µm); solvents: A = water/0.1 % trifluoroacetic acid and B = acetonitrile/0.1 % trifluoroacetic acid; gradient 10-80 % B over 5 min; flow rate 0.6 mL/min, UV detection at 214 and 254 nm, ESI-MS) t_R = 3.66 min. Yield 1.0 g (72 %).

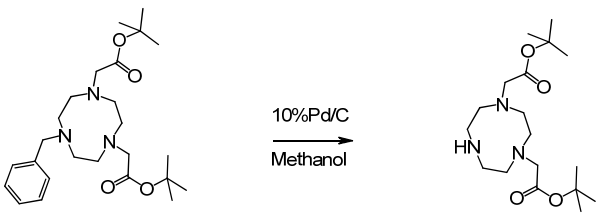
Synthesis of (4-Benzyl-7-*tert*-butoxycarbonylmethyl-[1,4,7]triazonane-1-yl)-acetic acid *tert*-butyl ester



Supplemental Scheme 3. Synthesis of (4-benzyl-7-*tert*-butoxycarbonylmethyl-[1,4,7]triazonane-1-yl)-acetic acid *tert*-butyl ester.

Sulphuric acid (Sigma, concentrated, 25 mL) was added to 1-benzyl-4-(4-methanesulfonyl-phenyl)-7-(toluene-4-sulfonyl)-[1,4,7]triazonane (2.5 g, 4.7 mmol) while stirring and heating to 100 °C. After 20 hours the reaction mixture was cooled to room temperature and added slowly into diethyl ether (VWR, 500 mL). The product was filtered off and washed with acetonitrile, chloroform and dichloromethane. Solvents were removed in vacuo. The crude product (986.3 mg, 4.5 mmol) was mixed with triethylamine (Fluka, 1.4 mL, 10 mmol) in acetonitrile (50 mL). *Tert*-butyl bromoacetate (Fluka, 1.47 mL, 10 mmol) was dissolved in acetonitrile (25 mL) and added dropwise. The reaction mixture was stirred at room temperature overnight. The pH was controlled and triethylamine added if necessary. Solvents were removed in vacuo and crude material dissolved in dichloromethane (150 mL) and washed with water (2 x 25 mL), 0.1 M hydrochloric acid (1 x 25 mL) and water (1 x 25 mL). The organic phase was separated and the solvent was evaporated off. The crude material was dissolved in acetonitrile/water (1:1 v/v) and purified by preparative HPLC (column Phenomenex Luna C18 (2) 5µm 250x 21.2 mm); solvents: A = water/0.1 % trifluoroacetic acid and B = acetonitrile/0.1 % trifluoroacetic acid; gradient 10-80 % B over 60 min) and lyophilized. The product was confirmed by LC-MS (column: Phenomenex Luna C18(2) 2.0×50 mm, 3 µm); solvents: A = water/0.1 % trifluoroacetic acid and B = acetonitrile/0.1 % trifluoroacetic acid; gradient 10-80 % B over 5 min; flow rate 0.6 mL/min, UV detection at 214 and 254 nm, ESI-MS) t_R = 3.99 min, (MH+) 447.4.

Synthesis of (4-*tert*-Butoxycarbonylmethyl-[1,4,7]triazonane-1-yl)-acetic acid *tert*-butyl ester

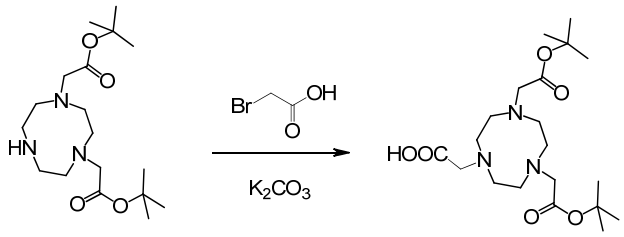


Supplemental Scheme 4. Synthesis of (4-*tert*-butoxycarbonylmethyl-[1,4,7]triazonane-1-yl)-acetic acid *tert*-butyl ester.

(4-Benzyl-7-*tert*-butoxycarbonylmethyl-[1,4,7]triazanonane-1-yl)-acetic acid *tert*-butyl ester, Pd/C (10%, 235 mg) and methanol (25 mL) were mixed and stirred under argon. Argon was then removed by vacuo and hydrogen gas was started to be supplied. Reaction mixture was left for three hours with stirring and a continuous supply of hydrogen gas. The catalyst was removed by centrifugation and solvents evaporated off. The crude product was purified by preparative HPLC (Phenomenex Luna C18 (2) 5µm 250x 21.2 mm, solvents: A = water/0.1% trifluoroacetic acid and B = acetonitrile/0.1% trifluoroacetic acid; gradient 2-80% B over 60 min). LC-MS (Phenomenex Luna

C18(2) 2.0×50 mm, 3 µm, solvents: A = water/0.1% trifluoroacetic acid and B = acetonitrile/0.1% trifluoroacetic acid; gradient 10-80% B over 5 min; flow rate 0.6 mL/min, UV detection at 214 and 254 nm, ESI-MS) t_R = 2.55 min, (MH+) 357.9.Yield 150 mg. Product confirmed by NMR.

Synthesis of (4,7-Bis-*tert*-butoxycarbonylmethyl-[1,4,7]triazonane-1-yl)-acetic acid



Supplemental Scheme 5. Synthesis of (4,7-Bis-*tert*-butoxycarbonylmethyl-[1,4,7]triazonane-1-yl)-acetic acid**.**

Di-*tert*-butyl 2,2'-(1,4,7-triazonane-1,4-diyl)diacetate (280 µmol, 100 mg) and bromoacetic acid (Fluka, 1 mmol, 138.21 mg) were dissolved in methanol (1 mL). Potassium carbonate dissolved in water (1 mL) was added with stirring. The reaction mixture was stirred at room temperature overnight and concentrated in vacuo. The residue was dissolved in water (2.5 mL), and the pH was adjusted to 4 with hydrochloric acid (1 M). The crude product was purified by preparative HPLC (column: Phenomenex Luna C18 (2) 5µm 250 x 21.2 mm); solvents: A = water/0.1 % trifluoroacetic acid and B = acetonitrile/0.1 % trifluoroacetic acid; gradient 10-80 % B over 60 min). LC-MS (column: Phenomenex Luna C18(2) 2.0×50 mm, 3 µm); solvents: A = water/0.1 % trifluoroacetic acid and B = acetonitrile/0.1 % trifluoroacetic acid; gradient 10-80 % B over 5 min; flow rate 0.6 mL/min, UV detection at 214 and 254 nm) t_R = 2.40 min. Yield 116 mg (99 %).

NOTA-$(COOH)_2$ maleimide (6)

N-(2-Aminoethyl)maleimide trifluoroacetate (23 mg, 0.090 mmol), NOTA(mono acid, bis-tBu ester) (30 mg, 0.072 mmol)(*2*) and PyAOP (51 mg, 0.10 mmol) were dissolved in DMF (2 mL). *Sym*collidine (29 µL, 0.40 mmol) was added and the reaction mixture shaken for 1 h. The mixture was diluted with water/ TFA (0.1% v/v, 6 mL) and the product purified by semi-preparative HPLC (gradient: 20-50% B over 60 min) affording 33 mg (87%) pure NOTA(di-*tert*-Bu)-maleimide. LC-MS (gradient 10-40% B over 5 min) t_R: 4.09 min, found m/z: 538.2, expected MH^+: 538.3.

NOTA(di-*tert*-Bu)-maleimide (33 mg, 61 µmol) was treated with a solution of TIS/water/TFA (2.5 %/2.5 %/95 % v/v/v, 10 mL) for 4.5 h. TFA was evaporated *in vacuo*, the residue dissolved in water/0.1 % TFA (8 mL) and the product purified by semi-preparative HPLC (gradient: 0-20 % B over

40 min) afforded 15 mg (58 %) pure NOTA(bis-acid)maleimide **6**. LC-MS (gradient 0-30 % B over 5 min) t_R: 1.34 min, found m/z: 426.0, expected MH^+: 426.2.

$Z_{HER2:2891}$-Cys-NOTA-$(COOH)_2$ (7)

Recombinant Affibody molecule **1** (40 mg, 5.7 µmol) and **6** (6.1 mg, 14 µmol) were dissolved in water (1.5 mL). The solution was adjusted to pH 6 by adding ammonium acetate and the mixture shaken for 1 h. The reaction mixture was diluted with water/0.1% TFA (6 mL) and the product purified using semi-preparative HPLC (gradient: 20-30 % B over 40 min) affording 38 mg (90 %) pure Affibody molecule **7**. LC-MS (gradient 10-40 % B over 5 min) t_R: 3.31 min, found m/z: 1864.5, expected MH_4^{4+}: 1864.5.

NOTA-$(COOH)_2$ maleimide (8)

PyAOP (96 mg, 0.18 mmol) dissolved in NMP (1 mL) was added to a solution of NOTA(tri-*tert*-Bu) (100 mg, 0.18 mmol)(*3*) and ethylenediamine (1.2 mL, 18 mmol) in NMP (1 mL). The reaction mixture was shaken for 1 h and a second aliquot of PyAOP (38 mg, 0.073 mmol) was added. Shaking was continued for 30 min. A solution of 20% MeCN/water (20%/80 % v/v, 5 mL) was added and the product purified by semi-preparative HPLC (gradient: 20-50% B over 40 min) affording 123 mg (98%) pure NOTA(tris-*tert*-Bu)-NH-CH_2CH_2-NH_2. LC-MS (gradient 20-50% B over 5 min) t_R: 1.95 min, found *m/z*: 586.4, expected MH^+: 586.4.

NOTA(tris-*tert*-Bu)-NH-CH_2CH_2-NH_2 (123 mg, 0.176 mmol), 3-maleimidopropionic acid NHS ester (70 mg, 0.26 mmol) and *sym*-collidine (346 µL, 2.60 mmol) were dissolved in NMP (2 mL). The reaction mixture was stirred for 6 h. Water (6 mL) was added and the product purified by semi-preparative HPLC (gradient: 20-50 % B over 40 min) affording 115 mg (87%) pure NOTA(tris-*tert*-Bu)-NH-CH_2CH_2-NH-maleimide. LC-MS (gradient 10-60 % B over 5 min) t_R: 3.36 min, found *m/z*: 737.4, expected MH^+: 737.4.

NOTA(tri-*tert-Bu*)-NH-CH_2CH_2-NH-maleimide (115 mg, 0.150 mmol) was treated with a solution of TIS/water/TFA (2.5 %/2.5%/95 % v/v/v, 10 mL) for 4 h. The solvents were evaporated *in vacuo*, the residue redissolved in water (8 mL) and the product purified by semi-preparative HPLC (gradient: 0-20% B over 40 min) affording 80 mg (90%) pure NOTA(tris-acid)-NH-CH_2CH_2-NH-maleimide. LC-MS (gradient 0-30% B over 5) t_R: 2.74 min, found *m/z*: 569.5, expected MH^+: 569.2.

$Z_{HER2:2891}$-Cys-NOTA-$(COOH)_3$ (9)

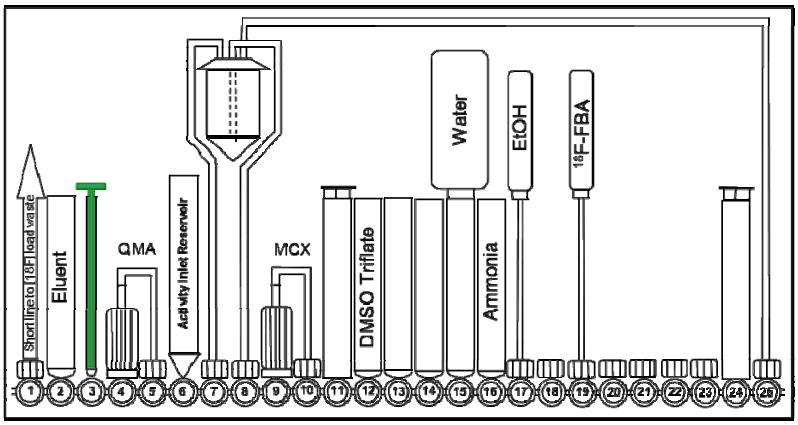
Synthetic Affibody molecule **1** (13.7 mg, 1.95 µmol) and NOTA(tris-acid)maleimide **8** (11 mg, 19.3 µmol) were dissolved in water (1 mL). The solution was adjusted to pH 6 by adding ammonium acetate and the mixture shaken for 3 h. The reaction mixture was diluted with water/0.1% TFA (6.5 mL) and the product purified using semi-preparative HPLC (gradient: 15-35 % B over 40 min) affording 8.4 mg (57 %) pure Affibody molecule **9**. LC-MS (gradient 10-40 % B over 5 min) t_R: 3.31 min, found *m/z*: 1900.7, expected MH_4^{4+}: 1900.2

$Z_{HER2:2891}$-Cys-FBA (11)

4-Fluorobenzaldehyde (5 µL, 50 µmol) was added to Affibody molecule **1** (14 mg, 1.9 µmol) dissolved in water (2 mL), and the reaction mixture shaken for 20 min. The mixture was diluted with water (6 mL) and the product purified using semi-prep HPLC (gradient 25-40 % B over 40 min) affording 13.5 mg (95 %) pure Affibody molecule **11**. LC-MS (gradient 10-40 % B over 5 min) t_R: 3.67 min, found *m/z*: 1836.9, expected MH_4^{4+}: 1837.2

2 FASTlab preparation of ^{18}F-FBA

A FASTlab module was equipped with a cassette as shown in Supplemental Figure 1. Supplemental Table 1 lists the components of the cassette. The [^{18}F]-fluoride was trapped on a QMA cartridge and eluted using Kryptofix/potassium carbonate (2 mL) into the reaction vial. After drying for 9 min at 120 ^{o}C under a steady stream of nitrogen, 4-trimethylammonium benzaldehyde triflate(*4*) in DMSO was added (Supplemental Tab. 1) and the mixture heated at 105 ^{o}C for 7 minutes. The crude labelling mixture was diluted with ammonium hydroxide solution and loaded onto a pre-conditioned MCX SepPak cartridge. After washing with water and drying with nitrogen, the product [^{18}F]FBA was eluted with ethanol (1 mL) into the product collection vial.

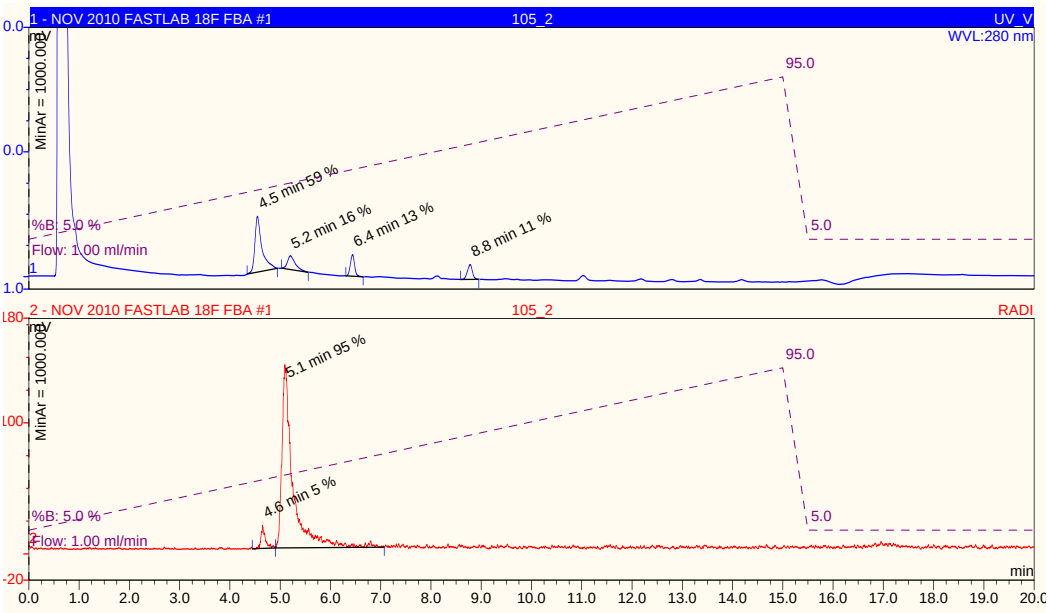


Supplemental Figure 1. Layout of a FASTlab cassette to produce [^{18}F]FBA.

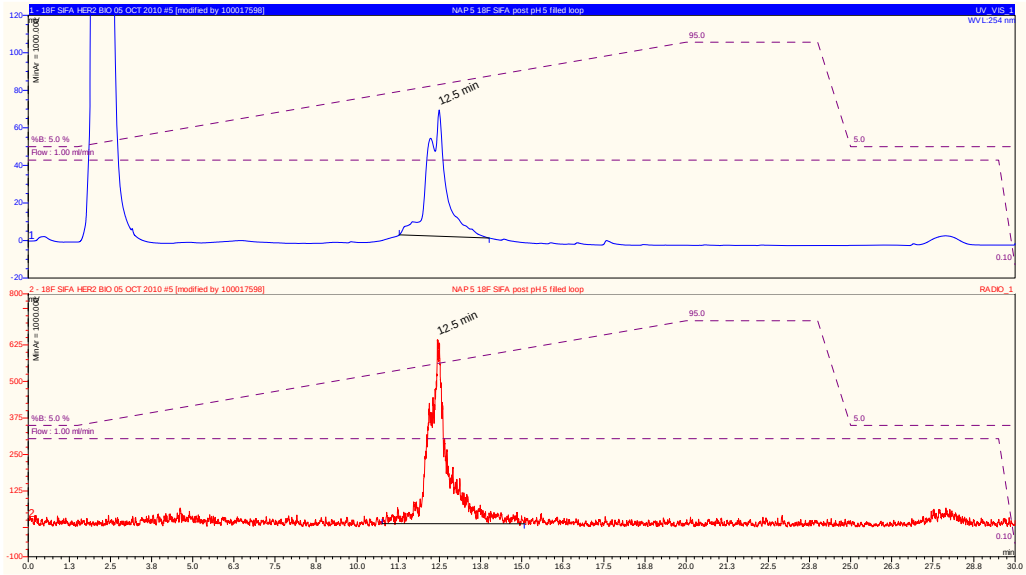
Supplemental Table 1. List of components of a FASTlab cassette to produce [^{18}F]FBA.

Manifold Position	Cassette Component
1	Silicone tubing (14 cm) to target recovery vessel
2	Eluent vial containing Kryptofix (8.25 mg, 21.9 µmol), K_2CO_3 (1.16 mg, 8.4 µmol), acetonitrile (660 µL), water (165 µL)
3	1 mL syringe (part of manifold)
4	QMA cartridge SepPak Light (Waters Ltd)
5	Silicone tubing (14 cm) to QMA cartridge at position 4
6	^{18}F-Fluoride inlet reservoir (part of manifold)
7	Silicone tubing (14 cm) to reactor vessel left hand side port
8	Silicone tubing (14 cm) to reactor vessel central port
9	MCX cartridge SepPak Light (Waters Ltd)
10	Silicone tubing (14 cm) to MCX cartridge at position 9
11	5 mL syringe (pert of manifold)
12	Reagent vial containing trimethylammonium benzaldehyde triflate (6.3 mg, 20 µmol) and anhydrous DMSO (2.1 mL)
15	Water bag spike
16	Ammonia reagent vial (4 mL, 4 % v/v)
17	Silicon tubing (42 cm) to ethanol solvent vial (30 mL)
19	Silicon tubing (42 cm) to product collection vial (10 mL)
24	5 mL syringe (part of manifold)
25	Silicone tubing (42 cm) to reactor vessel right hand side port

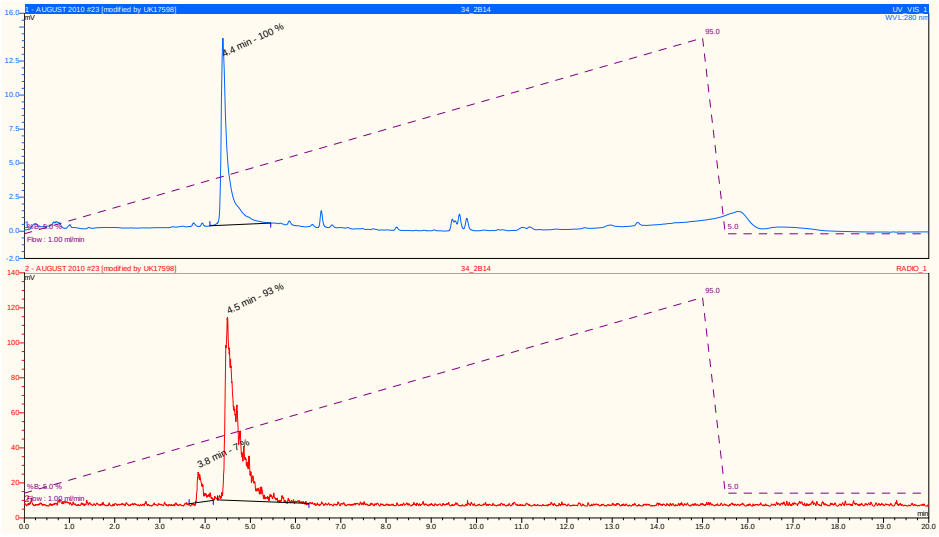
3 Analytical HPLC Data



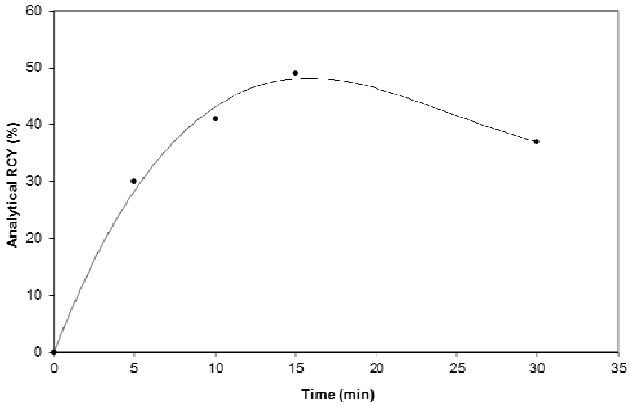
Supplemental Figure 2. Analytical HPLC of formulated [^{18}F]**11** [top: UV channel at 280 nm showing ascorbate (0.5 min) and peptide-related peaks (4.5, 5.2, 6.4 min); bottom: radioactivity channel showing [^{18}F]**11**, 5.1 min (95 %) and a decomposition product at 4.6 min (5 %)].



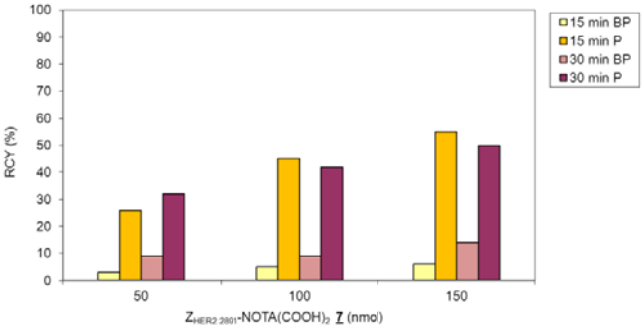
Supplemental Figure 3 . Analytical HPLC of formulated [^{18}F]**5** [top: UV channel at 254 nm showing ascorbate (1.6-3.4 min) and peptide-related peaks (cluster at 12.5 min); bottom: radioactivity channel showing [^{18}F]**5**, 12.5 min].



Supplemental Figure 4 . Analytical HPLC of formulated [^{18}F]**12** [top: UV channel at 280 nm; bottom: radioactivity channel showing [^{18}F]**12**, 4.5 min].



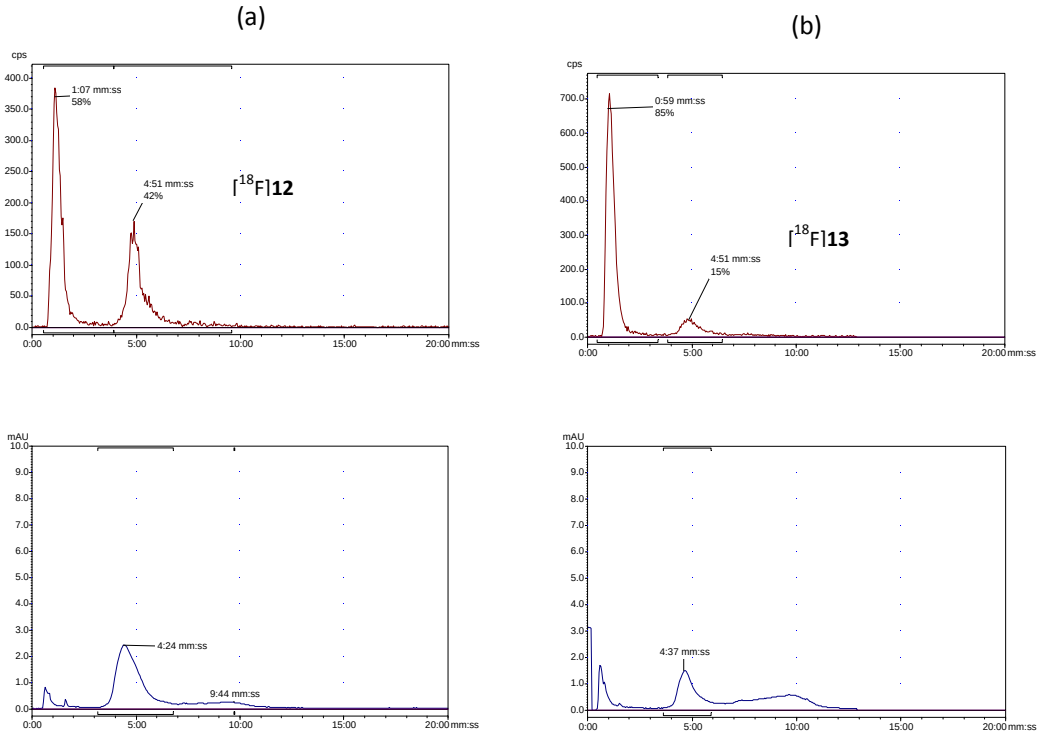
Supplemental Figure 5. Time course study for preparing [^{18}F]**12** showing labeling efficiencies measured by analytical radio HPLC. A ¼ ratio of $AlCl_3$/peptide was used.



Supplemental Figure 6. Impact of increasing the peptide/$AlCl_3$ concentration on the analytical RCY of [^{18}F]**12.** The reaction mixtures of increasing reagent concentrations were analysed after 15 min and 30 min. (BP: by-product, P: product).

Supplemental Table 2. Impact of lowering the reaction temperature (i.e. 70 °C vs. 100 °C) on analytical RCY of [^{18}F]AlF-NOTA(COOH)$_2$-Z$_{HER2:02891}$ [^{18}F]**12** using the reagent ratio ¼ of the protocol described in the main article.

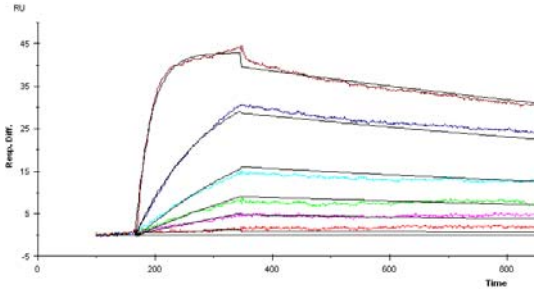
Time (min)	**Temperature (°C)**	**Product**	**Pre-peak**
5	70	-	-
15	70	1 %	-
30	70	5 %	-
60	70	12 %	-



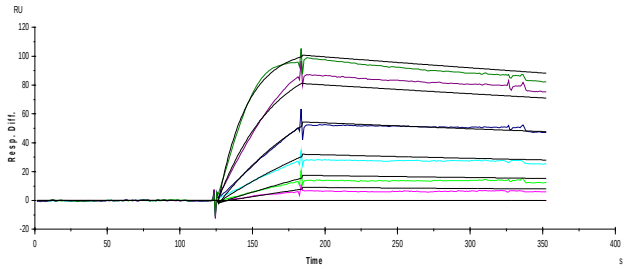
Supplemental Figure 7. HPLC analysis comparison of (a) [^{18}F]**12** and (b) [^{18}F]**13** preparations (50 nmol peptide in 50 µL total reaction volume, top: radioactivity channel, bottom UV signal at 280 nm).

4 Biacore Assay

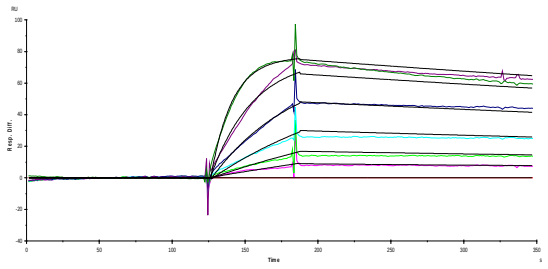
A



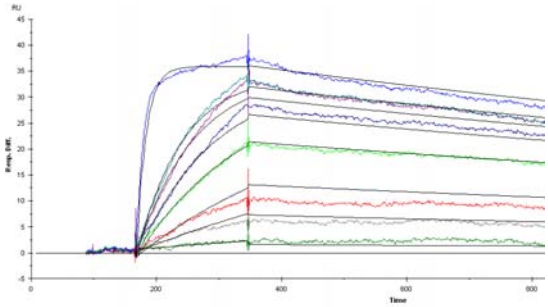
B



C



D



Supplemental Figure 8. Examples of binding curves towards human recombinant HER2 receptor fitted to a 1:1 binding model. A) compound **5**, replicate 2 (0.1, 0.5, 1, 2, 5 and 25 nM); B) compound **7**, replicate 1 (0.78, 1.56, 3.13, 6.25, 12.5 and 25 nM); C) compound **9**, replicate 1 (0.78, 1.56, 3.13, 6.25, 12.5 and 25 nM); D) compound **11**, replicate 1 (0.1, 0.5, 1, 2, 3, 4 and 5 nM).

5 HercepTest™ Immunohistochemistry

IHC for HER2/neu protein expression in excised tumors was performed using the HercepTest™ (DAKO, K5204) according to the protocol described in the manufacturer's guide. Formalin-fixed paraffin-embedded tumors (A431 and NCI-N87) were sectioned, deparaffinized in xylene and rehydrated through alcohols to distilled water. Sections were subjected to heat-induced epitope retrieval by immersing slides in DAKO Epitope Retrieval Solution (0.01 m citrate buffer; pH 6). Slides were incubated with the primary rabbit polyclonal antibody to the HER2/neu oncoprotein and the antibody was localized by incubation with the DAKO Visualization Reagent (dextran polymer conjugated with horseradish peroxidase and goat antirabbit immunoglobulins). Diaminobenzidine (DAB) was used as the chromogen, and the sections were counterstained with hematoxylin. A control slide consisting of three pelleted, formalin-fixed, paraffin-embedded human breast cell lines with staining intensity scores of 0, +1, and +3 (supplied in the HercepTest kit) and a negative control consisting of substituting normal rabbit serum (Dako Negative Control Reagent) for the HER2/neu primary antibody were included for the staining run.

6 Biodistribution Data

All data are presented as average from 3 animals ± SD.

6.1 Naïve CD1 nude mice

Supplemental Table 3. Naïve CD-1 nude mice biodistribution of [^{111}In]ABY-025.

	2 Minutes	90 Minutes	120 Minutes	180 Minutes
% Injected Dose				
Bone	4.82 ± 0.19	1.30 ± 0.18	1.15 ± 0.10	0.88 ± 0.05
Muscle	18.10 ± 0.36	4.64 ± 0.87	4.13 ± 0.66	3.44 ± 0.10
Blood	41.88 ± 2.29	1.52 ± 0.36	0.85 ± 0.05	0.28 ± 0.02
Kidneys	12.95 ±1.17	73.78 ± 2.97	76.66 ± 1.62	76.93 ± 0.71
Bladder/Urine	0.75 ± 0.57	4.60 ± 0.29	5.22 ± 0.40	6.33 ± 0.93
Lung	1.61 ± 0.09	0.19 ± 0.05	0.15 ± 0.01	0.11 ± 0.01
Liver	8.57 ± 0.53	3.06 ± 0.26	2.85 ± 0.13	3.09 ± 0.26
Spleen	0.68 ± 0.05	0.07 ± 0.03	0.07 ± 0.02	0.07 ± 0.02
Stomach	1.19 ± 0.16	0.24 ± 0.12	0.15 ± 0.01	0.13 ± 0.02
SI & LI	8.56 ± 1.27	1.43 ± 0.36	1.11 ± 0.07	1.28 ± 0.30
Skin	10.84 ± 1.77	5.82 ± 0.45	4.83 ± 0.84	5.00 ± 1.10
Heart	1.02 ± 0.08	0.08 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
% Injected Dose / Gramm				
%ID/G Bone	3.34 ± 0.43	0.97 ± 0.14	0.82 ± 0.12	0.65 ± 0.01

%ID/G Muscle	1.45 ±0.10	0.40 ± 0.08	0.34 ± 0.04	0.29 ± 0.02
%ID/G Blood	18.58 ± 2.28	0.73 ± 0.17	0.39 ± 0.03	0.13 ± 0.02
%ID/G Lung	7.66 ± 0.66	0.92 ± 0.13	0.67 ± 0.07	0.49 ± 0.05
%ID/G Liver	3.92 ± 0.84	1.71 ± 0.07	1.72 ± 0.17	1.80 ± 0.14
%ID/G Skin	2.48 ± 0.23	1.45 ± 0.03	1.15 ± 0.16	1.24 ± 0.34
%ID/G Kidney	30.68 ± 2.77	174.83 ± 7.05	181.67 ± 3.85	182.3 ± 1.69

Supplemental Table 4. Naïve CD-1 nude mice biodistribution of [^{18}F]**12**.

	2 Minutes	90 Minutes	120 Minutes	180 Minutes
% Injected Dose				
Bone	5.03 ± 0.72	1.19 ± 0.22	1.07 ± 0.01	0.84 ± 0.10
Muscle	21.76 ± 1.42	4.18 ± 0.37	3.53 ± 0.18	2.34 ±0.83
Blood	36.01 ± 4.50	1.29 ± 0.19	0.70 ± 0.03	0.23 ± 0.04
Kidneys	21.55 ± 6.57	70.33 ± 1.26	73.92 ± 1.77	78.18 ± 4.51
Bladder/Urine	0.74 ± 0.66	9.16 ± 0.66	8.81 ± 0.90	9.06 ± 2.22
Lung	1.78 ± 0.37	0.20 ± 0.03	0.12 ± 0.01	0.08 ± 0.01
Liver	7.23 ± 1.04	3.19 ± 0.23	3.13 ± 0.18	2.61 ± 0.12
Spleen	0.55 ± 0.05	0.06 ± 0.02	0.07 ± 0.02	0.05 ± 0.01
Stomach	1.10 ± 0.30	0.19 ± 0.03	0.14 ± 0.03	0.13 ± 0.07
SI & LI	9.57 ± 1.33	1.39 ± 0.16	1.27 ± 0.05	1.08 ± 0.21
Skin	9.53 ± 0.33	6.74 ± 0.59	5.52 ± 0.10	4.07 ± 0.97
Heart	1.01 ± 0.29	0.07 ± 0.01	0.07 ± 0.01	0.04 ± 0.00
% Injected Dose / Gramm				
%ID/G Bone	3.32 ± 0.44	0.85 ± 0.17	0.72 ± 0.05	0.55 ± 0.04
%ID/G Muscle	1.67 ± 0.08	0.34 ± 0.02	0.28 ± 0.01	0.18 ± 0.06
%ID/G Blood	15.24 ± 1.57	0.59 ± 0.11	0.30 ± 0.01	0.10 ± 0.01
%ID/G Lung	7.59 ± 0.54	0.86 ± 0.13	0.57 ± 0.10	0.34 ± 0.03
%ID/G Liver	4.13 ± 0.59	1.89 ± 0.13	1.73 ± 0.18	1.37 ± 0.10
%ID/G Skin	2.10 ± 0.05	1.60 ± 0.18	1.24 ± 0.08	0.90 ± 0.21
%ID/G Kidney	351.06 ± 15.56	166.65 ± 2.99	175.16 ± 4.18	185.26 ± 10.69

Supplemental Table 5. Naïve CD-1 nude mice biodistribution of [^{18}F]**5**.

	2 Minutes	90 Minutes	120 Minutes	180 Minutes
% Injected Dose				
Bone	3.82 ± 0.36	11.36 ± 1.79	13.25 ± 2.76	15.66 ± 3.96
Muscle	11.70 ± 1.68	8.12 ± 1.64	6.44 ± 0.75	3.50 ± 0.77
Blood	82.92 ± 5.57	9.99 ± 0.89	7.55 ± 0.18	3.65 ± 0.43
Kidneys	5.04 ± 0.36	10.09 ± 0.67	8.65 ± 0.21	5.81 ± 0.51
Bladder/Urine	0.36 ± 0.16	8.31 ± 0.60	12.53 ± 2.19	15.35 ± 3.74
Lung	2.04 ± 0.50	0.59 ± 0.02	0.48 ± 0.08	0.25 ± 0.04
Liver	17.77 ± 2.14	11.59 ± 1.34	9.11 ± 0.79	4.60 ± 0.84

Spleen	0.66 ± 0.17	0.14 ± 0.04	0.09 ± 0.02	0.07 ± 0.03
Stomach	0.89 ± 0.22	1.60 ± 0.83	1.66 ± 0.37	0.54 ± 0.25
SI & LI	5.05 ± 0.96	26.90 ± 4.43	29.60 ± 1.83	38.28 ± 1.51
Skin	5.07 ± 1.46	6.87 ± 1.10	5.28 ± 0.56	2.76 ± 0.33
Heart	1.09 ± 0.23	0.25 ± 0.04	0.19 ± 0.01	0.11 ± 0.01
% Injected Dose / Gramm				
%ID/G Bone	1.81 ± 0.38	5.29 ± 0.97	6.47 ± 1.88	7.56 ± 1.64
%ID/G Muscle	0.63 ± 0.04	0.44 ± 0.09	0.36 ± 0.05	0.20 ± 0.04
%ID/G Blood	24.89 ± 1.26	2.96 ± 0.05	2.34 ± 0.27	1.13 ± 0.11
%ID/G Lung	7.17 ± 0.81	2.00 ± 0.12	1.76 ± 0.10	0.88 ± 0.06
%ID/G Liver	7.56 ± 0.56	4.88 ± 0.25	4.02 ± 0.58	2.02 ± 0.36
%ID/G Skin	0.78 ± 0.15	1.06 ± 0.16	0.84 ± 0.01	0.45 ± 0.07
%ID/G Kidney	10.56 ± 0.74	21.15 ± 1.4	18.13 ± 0.45	12.18 ± 1.06

Supplemental Table 6. Naïve CD-1 nude mice biodistribution of [^{18}F]**11**.

	2 Minutes	90 Minutes	120 Minutes	180 Minutes
% Injected Dose				
Bone	5.02 ± 0.71	1.57 ± 0.36	1.34 ± 0.24	1.10 ± 0.04
Muscle	21.87 ± 0.90	3.60 ± 0.75	2.03 ± 0.40	2.86 ± 1.14
Blood	35.30 ± 3.53	2.16 ± 0.68	1.28 ± 0.20	1.03 ± 0.27
Kidneys	13.06 ± 1.97	4.81 ± 0.64	2.34 ± 0.23	1.38 ± 0.40
Bladder/Urine	0.42 ± 0.29	44.55 ± 7.77	40.74 ± 7.85	51.80 ± 15.18
Lung	1.57 ± 0.32	0.27 ± 0.08	0.17 ± 0.06	0.17 ± 0.02
Liver	7.72 ± 0.94	3.49 ± 0.36	3.38 ± 1.06	2.61 ± 0.70
Spleen	0.63 ± 0.08	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.02
Stomach	1.27 ± 0.28	0.64 ± 0.22	0.45 ± 0.28	0.35 ± 0.18
SI & LI	8.68 ± 0.26	33.96 ± 9.81	46.06 ± 7.70	37.12 ± 12.25
Skin	12.23 ± 0.71	3.07 ± 1.01	1.50 ± 0.09	1.16 ± 0.34
Heart	0.80 ± 0.03	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.03
% Injected Dose / Gramm				
%ID/G Bone	3.95 ± 0.36	1.23 ± 0.29	1.03 ± 0.19	0.89 ± 0.05
%ID/G Muscle	2.01 ± 0.18	0.33 ± 0.07	0.18 ± 0.04	0.27 ± 0.12
%ID/G Blood	17.89 ± 1.98	1.08 ± 0.33	0.63 ± 0.13	0.54 ± 0.16
%ID/G Lung	8.35 ± 0.75	1.15 ± 0.35	0.81 ± 0.24	0.84 ± 0.11
%ID/G Liver	5.00 ± 0.62	2.37 ± 0.31	2.31 ± 0.79	1.95 ± 0.62
%ID/G Skin	3.23 ± 0.27	0.80 ± 0.25	0.39 ± 0.03	0.32 ± 0.10
%ID/G Kidney	30.94 ± 4.66	11.39 ± 1.53	5.55 ± 0.56	3.26 ± 0.95

6.2 Xenograft Biodistribution Data

Supplemental Table 7. Key ratios from the NCI-N87 xenograft biodistribution of [^{111}In]ABY025, [^{18}F]**5**, [^{18}F]**11** and [^{18}F]**12**.

Ratio	Compound	Time post injection in min			
		2	**90**	**120**	**180**
Tumor/ blood	[^{111}In]ABY-025	0.12	13.74	21.24	75.41
	[^{18}F]**11**	0.11	6.74	12.68	12.88
	[^{18}F]**12**	0.14	8.26	19.64	39.91
	[^{18}F]**5**	0.04	1.01	1.08	2.65
Tumor/ muscle	[^{111}In]ABY-025	1.60	34.10	37.26	52.48
	[^{18}F]**11**	1.34	28.89	47.09	30.75
	[^{18}F]**12**	1.25	21.55	24.70	25.04
	[^{18}F]**5**	1.53	7.98	7.16	11.48
Tumor/ liver	[^{111}In]ABY-025	0.44	5.76	4.99	6.91
	[^{18}F]**11**	0.36	2.83	1.32	2.00
	[^{18}F]**12**	0.11	0.31	0.40	0.40
	[^{18}F]**5**	0.13	0.44	0.49	0.77

Supplemental Table 8. Key ratios from the A431 xenograft biodistribution of [^{111}In]ABY025, [^{18}F]**5**, [^{18}F]**11** and [^{18}F]**12**.

Ratio	Compound	Time post injection in min			
		2	**90**	**120**	**180**
Tumor/ blood	[^{111}In]ABY-025	0.06	3.11	4.24	12.06
	[^{18}F]**11**	0.10	3.17	2.94	5.04
	[^{18}F]**12**	0.12	3.12	5.11	13.19
	[^{18}F]**5**	0.03	0.58	0.96	1.65
Tumor/ muscle	[^{111}In]ABY-025	0.64	4.47	5.44	7.02
	[^{18}F]**11**	1.02	8.72	8.59	8.67
	[^{18}F]**12**	1.27	5.58	5.31	7.61
	[^{18}F]**5**	0.89	2.85	4.33	5.34
Tumor/ liver	[^{111}In]ABY-025	0.20	0.67	0.85	0.75
	[^{18}F]**11**	0.30	0.70	0.74	1.17
	[^{18}F]**12**	0.41	1.02	0.83	1.10
	[^{18}F]**5**	0.08	0.26	0.40	0.69

Supplemental Table 9. Tumor uptake values in %ID/g for NCI-N87 and A431 xenograft biodistribution of [^{111}In]ABY025, [^{18}F]**5**, [^{18}F]**11** and [^{18}F]**12**.

% ID/g	Compound	Time post injection in min			
		2	**90**	**120**	**180**
NCI-N87	[^{111}In]ABY-025	2.08 ± 0.74*	7.14 ± 2.67*	7.82 ± 2.12*	7.86 ± 2.38*
	[^{18}F]**11**	2.19 ± 0.84*	7.15 ± 0.69*	7.03 ± 0.96**	6.36 ± 0.29**
	[^{18}F]**12**	2.03 ± 0.65*	4.79 ± 1.26*	4.45 ± 0.27**	4.51 ± 1.01*
	[^{18}F]**5**	1.39 ± 0.36*	3.49 ± 0.74*	2.93 ± 1.05	2.70 ± 0.79*
A431	[^{111}In]ABY-025	1.08 ± 0.36	1.07 ± 0.22	1.38 ± 0.29	1.24 ± 0.12
	[^{18}F]**11**	1.55 ± 0.31	0.99 ± 0.73	0.91 ± 0.23	0.95 ± 0.27
	[^{18}F]**12**	2.26 ± 0.30	1.37 ± 0.32	1.40 ± 0.36	1.35 ± 0.49
	[^{18}F]**5**	0.81 ± 0.39	2.07 ± 0.46	2.38 ± 0.75	1.81 ± 0.47

* $p < 0.5$; ** $p < 0.01$ for NCI-N87 uptake value compared to A431 uptake value.

Supplemental Table 10. Key ratios from the dual tumor xenograft biodistribution of [^{18}F]**11**.

Ratio	Tumor	Time post injection in min					
		2	**30**	**60**	**90**	**120**	**180**
Tumor/ blood	A431	0.14	0.98	2.16	3.50	2.95	4.70
	NCI-N87	0.14	2.41	4.63	9.82	15.54	15.44
Tumor/ muscle	A431	1.47	3.62	6.73	8.19	7.78	11.23
	NCI-N87	1.39	8.97	14.71	25.36	42.63	42.88
Tumor/ liver	A431	0.47	0.56	0.79	0.99	0.63	1.57
	NCI-N87	0.45	1.39	1.70	2.98	3.26	5.01
Tumor/ kidney	A431	0.11	0.08	0.17	0.29	0.4	0.73
	NCI-N87	0.10	0.20	0.37	0.83	2.18	2.28

7 References

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