Published in final edited form as:

Bioconj Chem. 2017 February 15; 28(2): 583–589. doi:10.1021/acs.bioconjchem.6b00678.

Automated, Resin-Based Method to Enhance the Specific Activity of Fluorine-18 Clicked PET Radiotracers

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Abstract

Radiolabeling of substrates with 2-¹⁸F-fluoroethylazide exploits the rapid kinetics, chemical selectivity, and mild conditions of the copper-catalyzed azide–alkyne cycloaddition reaction. While this methodology has proven to result in near-quantitative labeling of alkyne-tagged precursors, the relatively small size of the fluoroethylazide group makes separation of the ¹⁸F-labeled radiotracer and the unreacted precursor challenging, particularly with precursors >500 Da (e.g., peptides). We have developed an inexpensive azide-functionalized resin to rapidly remove unreacted alkyne precursor following the fluoroethylazide labeling reaction and integrated it into a fully automated radiosynthesis platform. We have carried out 2-¹⁸F-fluoroethylazide labeling of four different alkynes ranging from <300 Da to >1700 Da and found that >98% of the unreacted alkyne was removed in less than 20 min at room temperature to afford the final radiotracers at >99% radiochemical purity with specific activities up to >200 GBq/μmol. We have applied this technique to label a novel cyclic peptide previously evolved to bind the Her2 receptor with high affinity, and demonstrated tumor-specific uptake and low nonspecific background by PET/CT.

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconj-chem.6b00678. Additional experimental procedures, synthetic data, and Figures S1–S5 (PDF)

Notes

The authors declare the following competing financial interest(s): S.F., T.T., A.H., R.R., and S.M. hold equity positions in EvoRx Technologies.

This resin-based methodology is automated, rapid, mild, and general allowing peptide-based fluorine-18 radiotracers to be obtained with clinically relevant specific activities without chromatographic separation and with only a minimal increase in total synthesis time.

Graphical Abstract

INTRODUCTION

The copper catalyzed azide–alkyne cycloaddition (CuAAC) reaction is an extremely versatile example of “click chemistry” owing mainly to its high yield, chemical orthogonality, water compatibility, and rapid kinetics.^{1,2} In recent years, click chemistry has found extensive use in the field of fluorine-18 academic radiochemistry where fast reaction kinetics, high yields, and mild reaction conditions are highly desirable.^{3,4} The most popular application of fluorine-18 click chemistry involves the radiosynthesis of the prosthetic group 2-[¹⁸F]-fluoroethylazide (FEA) followed by its distillation and subsequent reaction with an alkyne precursor (mainly small molecules or peptides).⁴ While this reaction is rapid and highly efficient, separation of the unreacted precursor from the desired fluorine-18 labeled product can be very difficult through conventional methods such as chromatography—mainly due to the small size difference resulting from conjugation of the fluoroethylazide moiety. As the size of the precursor increases, this separation becomes progressively more difficult, if not impossible on the time scale of fluorine-18 radiochemistry. This poses a challenge for the radiosynthesis of fluorine-18 labeled macromolecules (e.g., peptides and small proteins) with high apparent specific activity (SA) for in vivo receptor imaging.

The specific activity for a radiopharmaceutical is a measurement of radioactivity per mass of cold and radioactive pharmaceutical. For fluorine-18 labeled radiopharmaceuticals, this often becomes a measurement of any coeluting cold impurity, particularly the precursor compound, which often competes with the radiopharmaceutical for binding to the biological target.⁵ This value is also referred to as apparent specific activity, usually expressed in GBq/μmol, and it is particularly relevant when the imaging target is a saturatable system, such as a cell surface receptor. This metric takes on additional importance when the radiopharmaceutical is synthesized by prosthetic group radiochemistry (e.g., fluoroethylazide), where the difference in structure between precursor and labeled species is subtle and the prosthetic group lies outside the binding site of the target.^{6,7} Low apparent specific activities, therefore, can result in poor tracer uptake and low signal-to-noise in tissues that express the target of interest.^{8,9}

Conventional methods to increase the apparent SA involve either (1) the downscaling of the precursor used in the radiosynthesis which may adversely affect reaction yield and kinetics

or (2) time-consuming HPLC purification to reduce the precursor concentration in the final formulation. The latter is the preferred method in fluorine-18 radiochemistry, where substantial quantities of precursor are often needed to drive labeling quickly to completion. As discussed above, HPLC-based purifications often fail as the molecular weight of the precursor increases. In addition to adding significant time to the radiosynthesis, chromatography also introduces additives and solvents (e.g., acetonitrile, TFA) which must be removed in subsequent steps and specifically accounted for during product quality control and Good Manufacturing Practice (GMP) production.^{10–12}

To address these challenges, we have developed a TentaGel-based azide resin to rapidly remove unreacted alkyne precursor from 2-[¹⁸F]fluoroethylazide radiolabeling reactions leading to dramatically increased specific activities. We have investigated its usefulness on a series of model compounds with increasing molecular weight, from a small molecule, *N*-propargylphthalimide, to SUPR4, a cyclic nonapeptide with mid-nanomolar affinity for the Her2 receptor recently obtained by directed evolution of cyclic peptide libraries containing unnatural amino acids.¹³ Despite differences in chemical composition and molecular weight, the azide resin removed >98% of the unreacted alkyne precursors in under 20 min at room temperature. The final ¹⁸F-labeled products were determined to have specific activities between 12 and 222 GBq/μmol and radiochemical purities >99%. All radiosyntheses were carried out on the GE Tracerlab automatic synthesis module and did not require manual manipulation or HPLC purification. To demonstrate the utility of generating radiotracers with high apparent specific activities using this method, we carried out PET/CT imaging using ¹⁸F-labeled SUPR4 to visualize Her2 expression in mouse models of breast cancer. We conclude that this is a general technique to dramatically enhance the specific activity of large molecule (>500 Da) ¹⁸F-radiotracers on automated platforms under mild conditions with minimal extension of synthesis time.

RESULTS AND DISCUSSION

We hypothesized that an azide-derivatized solid support would act as an efficient scavenger of unreacted alkynes in the click labeling reaction and eliminate the need for HPLC purification. We chose the PEGylated TentaGel resin as the solid support because of its biocompatibility as well as its hydrophilicity and ease of chemical modification.¹⁴ Reaction of amine-derivatized TentaGel (TentaGel-NH₂) with 2-azidoacetic acid (Scheme 1) resulted in a highly derivatized resin with up to 96% of the reactive amines capped. The resin was found to be highly stable and could be stored up to 6 months at 4 °C in DMF with only 3% loss of reactivity (Figure S1).

To test the utility of our resin for preclinical and clinical applications, we adapted the well-established 2-[¹⁸F]-fluoroethylazide method for implementation on the GE Tracerlab automated radiosynthesis platform.⁴ In this approach, aqueous [¹⁸F]fluoride is trapped on an anion-exchange resin and eluted into a reaction vessel containing tosyl ethylazide where [¹⁸F]FEA is formed by displacement of the tosyl group in the presence of Kryptofix. The resulting [¹⁸F]FEA is distilled into a secondary reaction vessel where labeling of the alkyne precursor is carried out in the presence of copper. While many radiosyntheses involve the automated production of [¹⁸F]FEA, the click step is often carried out manually. We have

implemented the entire radiosynthesis of [^{18}F]FEA and subsequent click step on the automated module GE Tracerlab where the scavenging unit can be incorporated as part of the purification workflow in between the click labeling step and the final formulation unit. [^{18}F] fluoride was trapped into a QMA cartridge, released, and dried in the presence of K_2CO_3 and Kryptofix 2,2,2. Production of the prosthetic group [^{18}F]FEA from tosyl ethyl azide occurred in the Tracerlab main reactor in 15 min at 80 °C, followed by distillation of [^{18}F]FEA under flow of nitrogen into a receiving vial containing the alkyne precursor (830 nmol) and a mixture of catalysts and ligands (Scheme 2). Yields for the production of the [^{18}F]FEA on Tracerlab were up to 35% radiochemical yield (non-decay-corrected [mean = 29.5% $\pm$ 2.9%, n = 7]) relative to the input F-18 aqueous fluoride activity. After proceeding for 20 min at room temperature, the click reaction was transferred to the azide resin slurry (64 mg) which was preactivated with the same reagents immediately prior to the start of synthesis. The stripping reaction was allowed to proceed at room temperature with nitrogen agitation followed by dilution into 0.085% H_3PO_4 , purification on a C18 cartridge, and elution with PBS:EtOH for injection. The total synthesis time is approximately 80 min. A schematic of the Tracerlab setup and flow path is shown in Figure S2.

We chose to start with a commercially available alkyne-containing phthalimide to demonstrate the feasibility of our methodology on the GE Tracerlab automated synthesis platform. The ^{18}F -labeled product [^{18}F]1 (Figure 1) was readily obtained in 24% radiochemical yield (decay-corrected from [^{18}F]FEA) in 80 min. Analysis of the formulated product by radio-HPLC showed >99% removal of the unreacted alkyne and a radiochemical purity >99% (Figure S3). We do not observe any residual 2- ^{18}F fluoroethyl azide in the final product which is consistent with its quantitative removal during the C18 SPE step. The specific activity of the final product was 12 GBq/ μmol starting with as little as 0.72 GBq of aqueous fluoride (Table 1). Analysis of the azide resin following the synthesis showed that approximately 10% of the eluted activity was nonspecifically trapped indicating relatively low nonspecific binding.

While we did not explicitly measure concentration of residual copper in the final formulated product, we did observe in a separate experiment that the C18 SPE cartridge was able to remove >99% of Cu^{2+} from a concentrated CuSO_4 solution and that the concentration of Cu^{2+} in the PBS:EtOH eluent was below the limit of detection (LOD) of the spectrophotometric assay. From this, we estimate the concentration of copper in the final formulated product is no more than 50 $\mu\text{g}/\text{mL}$, and may be substantially lower. For reference, the Permitted Daily Exposure (PDE) for copper (parenteral) in humans is 300 $\mu\text{g}/\text{day}$.¹⁵ While our estimate suggests that the amount of copper remaining in the final product is within the margin of safety, precise quantification of Cu^+ and Cu^{2+} , either by atomic absorption spectroscopy or ion chromatography, is necessary prior to human translation.

Having demonstrated the methodology's feasibility using the phthalimide model compound, we next carried out a similar radiosynthesis with an N-terminal capped dipeptide alkyne to generate the labeled product [^{18}F]2 with 25% dcy and a specific activity >200 GBq/ μmol (Figure S4). This result was particularly encouraging given the presence of an unprotected carboxylic acid in close proximity to the alkyne group which appears to lower the click

labeling efficiency by a factor of 2 relative to [^{18}F]**1** but has no appreciable effect on the stripping efficiency. This is likely due to the stoichiometric excess of azide groups present on the resin (>30-fold molar excess) and potentially the enhanced reactivity of polymer-bound azides in the copper-catalyzed cycloaddition.¹⁶

While compounds [^{18}F]**1** and [^{18}F]**2** can be resolved from their unreacted alkyne precursors by HPLC, larger peptides frequently present a much more daunting chromatographic challenge. We sought to determine if our methodology could be applied to large peptides with molecular weights >1000 Da. We chose the model compound **3** due to its high molecular weight and diverse side-chain functionalities including the presence of two carboxylic acids and a carboxamide in close proximity to the reactive alkyne. As seen in Table 1, the click labeling reaction was quite poor (2%) but the resin stripping efficiency was >99% by analytical HPLC (Figure S5). This result suggested that our methodology was compatible with the production of high-specific activity ^{18}F -labeled peptides following optimization of the [^{18}F]FEA labeling reaction.

Having established that this methodology was compatible with large peptide radiotracers, we applied the technique to the radiolabeling of SUPR4, a cyclic peptide containing unnatural amino acids (*N*-methyl norvaline) previously selected for binding to the Her2 receptor by mRNA display.¹³ SUPR4 was previously shown to have mid-nanomolar affinity for the Her2 receptor, rapid renal clearance from circulation, and high Her2-dependent tumor uptake by near-infrared (NIR) optical imaging. Preliminary radiochemical experiments showed that SUPR4 could be efficiently labeled using the fluoroethyl azide technique, but could not be resolved from the starting material by HPLC (Figure S6). Given the modest affinity and rapid clearance of this peptide, it was critical to increase the specific activity in order to obtain measurable tumor uptake above background by PET/CT imaging. Following brief optimization, we were able to obtain a 40% labeling efficiency of SUPR4 on the Tracerlab and >98% removal of precursor yielding a 99% radiochemically pure product with a specific activity of up to 40 GBq/ μmol (Figure 2A).

Analysis of the final formulated product showed very low levels of contamination in the UV HPLC trace although we do observe additional low intensity peaks in the region of the product. Previous studies of copper-mediated oxidative damage of methionine, tyrosine, tryptophan, histidine, and cysteine under CuAAC conditions suggested one possible source for these contaminants.¹⁷ However, analysis of a control radiosynthesis, where no SUPR4 precursor is added, showed the same peaks in the chromatogram (Figure S6B). From this, we conclude that these contaminants result from the components of the click reaction itself, and do not represent peptide-based side products. Furthermore, synthesis of the ^{19}F -SUPR4 “cold” standard was carried out under essentially identical conditions in an overnight reaction at 60% yield (see Experimental Procedures), further supporting the stability of the SUPR peptide to oxidative modification under CuAAC conditions. We speculate that the use of the TBTA ligand in conjunction with the inert atmosphere of the Tracerlab system could be attenuating these unwanted side reactions,¹⁷ although more work is necessary to conclusively rule out oxidative damage during labeling and purification.

The ^{18}F -labeled SUPR4 showed Her2-dependent binding in cell-based assays (Figure 2B), which was efficiently blocked by both the “cold” version as well as Cy5- and DOTA-conjugated versions of the same peptide (Figure 2C). Interestingly, these data suggest that the pendant functionality at the C-terminus may play a significant role in binding of SUPR4 to the Her2 receptor. PET/CT imaging of subcutaneous SKOV3 Her2-positive and MDA-MB-231 (Her2-negative) mouse models showed Her2-dependent uptake of the [^{18}F]SUPR4 radiotracer with relatively low background uptake in nontumor tissues (Figure 3A–C). In addition to uptake in the tumor, we observed significant signal in the gallbladder and kidneys consistent with rapid renal clearance with minor hepatobiliary excretion. Post-imaging analysis of tumor:muscle (T:M) uptake ratios showed a modest but statistically significant increase in SKOV3 tumors relative to MDA-MB-231 tumors which showed the expected T:M of 1 indicating no specific probe uptake in the absence of the Her2 receptor (Figure 3C). Serum analysis by radio-HPLC at 1 h post-injection showed >98% of the radiotracer was intact by comparison to an uninjected sample of the radiotracer, again in agreement with previous in vitro serum stability analysis (Figure 3D).

CONCLUSIONS

We have validated a simple, inexpensive, and automated radiochemical methodology for dramatically enhancing the specific activity of peptides and small molecules by integrating well-established 2- [^{18}F]fluoroethylazide labeling protocols with a scavenging azide resin. Previous efforts to enhance specific activity in click-based radiosyntheses have relied on either post-labeling derivatization to increase chromatographic separation of the derivatized precursor¹⁸ or sequestration of unreacted strained dibenzocyclooctyne precursors using a conceptually similar azide resin.^{19,20} In contrast to the first strategy, our method does not require a chromatography step and represents a significant improvement in both synthesis time and downstream quality control. In contrast to the second strategy, our method has been fully automated on the Tracerlab platform and is suitable for the processing of curie-level input activity. Our method has the advantage of generality—all four alkyne precursors in this study were removed with near-quantitative efficiency despite significant differences in size and chemical composition. While the strain-promoted click reaction is advantageous for mild, biocompatible labeling, the obligate use of large hydrophobic ring systems may adversely affect the affinity, solubility, and/or biodistribution of the resulting ligand. In contrast, the conjugation of a propargylglycine-modified precursor peptide (compounds **3** and **4**) and the low-molecular-weight 2- [^{18}F]fluoroethylazide synthon results in only a modest change in the structure of the labeled peptide, which minimizes the probability of altered affinity, selectivity, or biodistribution. Finally, the strain-promoted methodology relies on preparative HPLC to purify the ^{18}F -labeled azide precursor prior to the click labeling reaction. In contrast, our method takes advantage of the rapid and facile purification of 2- [^{18}F]fluoroethylazide by distillation, eliminating the need for chromatographic purification and rendering it highly suitable for adaptation to clinical-scale automated radiotracer production.

Production of high-specific-activity radiotracers is essential for achieving sensitive imaging in tissues with low target density or activity.⁸ For example, the Isatin-based apoptosis radiotracer [^{18}F]ICMT-11 was originally prepared using the [^{18}F]FEA method for

preclinical testing.²¹ While the labeling reaction proceeded in high yield, the low specific activity of the labeled product (1.2 GBq/ μ mol) resulted in poor uptake in apoptotic cells in vitro and apoptotic tumors in vivo. Despite reaction optimization and preparative HPLC purification,²² the specific activity remained low (<25 GBq/ μ mol) and necessitated a switch to a more conventional tosyl displacement strategy to obtain the desired GMP radiotracer at clinically relevant specific activities (>685 GBq/ μ mol) on an automated platform.^{23,24} The methodology we describe in this manuscript offers the possibility of greatly enhancing the specific activities of [¹⁸F]FEA-labeled radiotracers where HPLC purification is undesirable or prohibitively difficult and increasing the translational potential of this radiochemical strategy.

EXPERIMENTAL PROCEDURES

Additional experimental procedures and synthetic data can be found in the Supporting Information.

Synthesis of Azide Scavenging Resin.

TentaGel S-NH₂ (1 g, 0.45 mmol/g NH₂, Sigma) was swollen for 1 h in DMF followed by extensive washing in DMF. 2-Azidoacetic acid (100 μ L, 1.3 mmol) and HBTU (500 mg, 1.3 mmol) were dissolved in DMF (7 mL) and added to the washed resin along with *N,N*-diisopropylethylamine (235 μ L, 1.4 mmol). The reaction was allowed to proceed for 1 h at room temperature after which the resin was filtered and the reaction was carried out again in the same conditions. After the second coupling, the resin was washed with DMF and DCM and dried under vacuum. A semiquantitative Kaiser test²⁵ was performed on three independently synthesized batches of resin and the azide loading was found to be between 96% and 86% (mean = 91%) based on residual reactive amino groups. For comparison, the loading efficiency of the same resin exhaustively acylated with acetic anhydride was found to be 93%.

Radiochemistry.

Radiosyntheses were performed with a Tracerlab FX (General Electric Healthcare, Munster, Germany) automatic module. F-18 was obtained as an aqueous solution from the MD Anderson Cyclotron Radiochemistry Facility (CRF). [¹⁸F]Fluoride was adsorbed on an ion exchange cartridge (Preconditioned Sep-PAK Light QMA Cartridge, ABX GmbH, Radeberg, Germany). [¹⁸F]Fluoride was flushed into the reaction vial with a potassium carbonate and Kryptofix 2.2.2. (K₂₂₂) water/CH₃CN solution (700 μ L; 52.8 mg of K₂CO₃, 240.1 mg of K₂₂₂, 4 mL of water, 16 mL of CH₃CN). The solution was dried under vacuum and under nitrogen flow at 80 °C for 2 min. 500 μ L of dry CH₃CN was added and then the mixture was azeotropically dried at 120 °C for an additional 3 min. Synthesis of 2-[¹⁸F]fluoroethylazide was carried out by adding 2-azidoethyltosylate precursor (5 mg) in CH₃CN (0.5 mL) to the dried [¹⁸F]fluoride and stirring at 80 °C for 15 min. Distillation of the volatile 2-[¹⁸F]fluoroethylazide was performed under N₂ flow for 2.5 min at 60 °C into a receiving vial containing CuSO₄ (50 μ L; 35 mg/mL in water), sodium ascorbate (50 μ L; 174 mg/mL in PBS), TBTA (13 μ L; 100 mg/mL in DMF), and piperidine (13 μ L; 20% in DMF) and alkyne precursor (830 nmol in 25 μ L of DMF). Click radiolabeling of the alkyne

precursor was performed at room temperature for 20 min. The mixture was transferred into a plastic solid-phase scavenging reactor containing the azide resin (800 μ L; 80 mg/mL) preswollen in DMF, washed with a CuSO₄ and sodium ascorbate mixture (200 μ L), and loaded with CuSO₄ (50 μ L; 35 mg/mL in water), sodium ascorbate (50 μ L; 174 mg/mL in PBS), TBTA (13 μ L; 100 mg/mL in DMF), and piperidine (13 μ L; 20% in DMF). The resulting slurry was agitated for 20 min at room temperature after which the resin was filtered off and washed with DMF (500 μ L). The combined filtrate was transferred into a quenching vial containing 0.085% (v/v) H₃PO₄ in water (15 mL) before being loaded onto a light C18 cartridge (Sep-PAK Light, Waters, Milford, USA) prewashed with EtOH (3 mL) and water (6 mL). The cartridge was washed with 6 mL of water, dried under nitrogen and eluted with 1 mL of ethanol ([¹⁸F]**1**) or 1:1 ethanol/PBS ([¹⁸F]**2** – [¹⁸F]**4**). The overall synthesis time was approximately 80 min. Activity was determined by dose calibrator and a sample was taken for quality control (QC). Prior to injection, the product was diluted at least 5-fold in sterile saline to maintain a final ethanol concentration below 10%. Radiochemical purity was determined by dividing the product peak area by the sum of all product peak areas in the radioHPLC trace. Specific activity was determined by dividing the activity present in the final formulated product by the moles of starting material remaining in the formulated product after purification (determined by HPLC, see Supporting Information).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by an MDACC Moonshot Knowledge Gap Pilot Project grant (S.W.M.), HHSN261201300065C (S.W.M., S.V.F.), (R21) 1R21CA181994-01 (S.W.M.), 2R44CA206771-02 (S.W.M., S.V.F.), P50CA94056-15 Molecular Imaging Center (D.P.W.), the MDACC Odyssey Program (L.E.K.), and MDACC start-up funding (S.W.M.). Dr. Ornelas was supported by a cancer prevention educational award (R25T CA057730, Dr. Shine Chang, PI). The Nuclear Magnetic Resonance Facility and Small Animal Imaging Facility are supported by the MD Anderson Cancer Support Grant CA016672 (DePinho). The authors wish to thank the staff at the Cyclotron Radiochemistry Facility (CRF), particularly Julius Balatoni and Gregory Waligorski, for their technical assistance in Tracerlab setup. We also thank the staff at the MD Anderson Small Animal Imaging Facility (SAIF), particularly Jorge De la Cerda and Caterina Kaffes for their assistance with the animal imaging experiments and PET image reconstruction.

ABBREVIATIONS

CuAAC	Copper-catalyzed azide–alkyne cycloaddition
FEA	2-fluoroethylazide
PET/CT	Positron Emission Tomography/Computed Tomography
TFA	Trifluoroacetic acid
QMA	Quaternary methylammonium
SPE	Solid Phase Extraction
SUPR	Scanning Unnatural Protease Resistant

TBTA	Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine
Cy5	Cyanine 5
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid

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Figure 1.
 ^{18}F -Radiolabeled Compounds Synthesized in this Study.

Figure 2.

Radiosynthesis and In Vitro Binding Analysis of [¹⁸F]-SUPR4. (A) Radiosynthesis of [¹⁸F]-SUPR4. The γ -counter radioHPLC trace of the final product (top) shows a single radiolabeled product at 23 min while the corresponding UV trace (middle) shows <2% of the precursor remaining. Injection of the ¹⁹F-SUPR4 standard (bottom) confirms the identity of the radiolabeled product at 23 min. (B) Cell lines with varying levels of Her2 expression (BT474 and SKOV3 (shCTRL): Her2 high; SKOV3 (shHER2) and MDA-MB-231: Her2 low) were incubated with 20 μ Ci [¹⁸F]-SUPR4 for 30 min followed by washout and analysis of cell-bound activity. Data was normalized to the activity bound to BT474 cells. (C) BT474 cells preblocked with 20 μ M “cold” [¹⁹F]-SUPR4, 20 μ M Cy5-SUPR4, or 20 μ M DOTA-SUPR4 for 15 min prior to incubation with 15 μ Ci of [¹⁸F]-SUPR4 for 30 min followed by washout and analysis of cell-bound activity. The results were normalized to the BT474 cell-bound activity. Data was analyzed using unpaired, two-tailed *t* tests in GraphPad Prism 6 (** *p* 0.01; *** *p* 0.001; **** *p* 0.0001).

Figure 3.

PET/CT with $[^{18}\text{F}]$ -SUPR4. (A) Mice with a Her2-positive SKOV3 subcutaneous tumor were intravenously injected with $\sim 100 \mu\text{Ci}$ of $[^{18}\text{F}]$ SUPR4. Images were acquired 30 min post-injection. Representative axial (left panel), coronal (middle panel), and sagittal (right panel) slices are shown. T = tumor, GB = gallbladder, K = Kidney, GI = gastrointestinal tract. (B) Maximal Intensity Projections (MIP) of a SKOV3 (left panel) and MDA-MB-231 mouse (right panel) imaged 30 min after injection with $[^{18}\text{F}]$ SUPR4. (C) Standard Uptake Values (SUVmean) for each tumor and contralateral muscle were obtained from PET/CT images and used to calculate the tumor:muscle ratio of $[^{18}\text{F}]$ SUPR4 uptake in the SKOV3 ($n = 6$) and MD-MB-231 ($n = 4$) tumors. (D) Mouse plasma collected at 1 h post-injection of $[^{18}\text{F}]$ SUPR4 and analyzed by radioHPLC (bottom panel). For reference, a radio-HPLC trace of the pre-injected $[^{18}\text{F}]$ SUPR4 is shown (top panel). Data was analyzed using unpaired t tests on GraphPad Prism 6 (***) $p < 0.001$.

Scheme 1.
Synthesis of Azide Resin

Scheme 2.

2-[¹⁸F]Fluoroethylazide Labeling and Scavenging During Radiosynthesis of Compound
[¹⁸F]1

Table 1.

Summary of Resin Stripping Efficiency and Radiochemical Results^a

compound	MW (Da)	resin stripping efficiency ^c (%) (%, <i>n</i> = 3)	click conversion ^d (%)	click RY (dc) ^e (%)	tracerlab stripping efficiency ^f (%)	apparent specific activity (GBq/μmol)	starting activity of aqueous F18 (GBq)
[¹⁸ F]1	288.1	98 ± 0.5	65	24	>99	12	0.72
[¹⁸ F]2	596.3	96.2 ± 0.9	30	25	>99	222	1
[¹⁸ F]3	1762.8	>99	2	-	>99%	-	3.7
[¹⁸ F]4 ^b	1343.7	98.7 ± 0.8	40.3 ± 2.0	20.3 ± 4.7	98.7 ± 0.8	2.8–40	3.7

^aPercentages are reported as average ± standard deviation.^b*n* = 3 repeats.^cResin stripping efficiency represents the extent of precursor removal immediately following reaction with the azide resin.^dClick conversion represents the fraction of [¹⁸F]FEA converted to labeled product immediately following the click reaction as assessed by radioHPLC.^eRadiochemical yield (decay corrected) is calculated by dividing the activity of the final formulated product by the total activity of [¹⁸F]FEA produced from F-18 aqueous fluoride input.^fTracerlab stripping efficiency represents the efficiency of removal of the precursor as calculated in the final formulated compound upon completion of the automated process.