

Sonogashira cross-coupling reaction with 4-[¹⁸F]fluoroiodobenzene for rapid ¹⁸F-labelling of peptides

SUPPLEMENTARY INFORMATION

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Experimental Section

Materials and Instrumentation

All chemicals used, with the exception of water were obtained from Sigma-Aldrich®, with the quality of some reagents as follows: copper(I) iodide of trace metal grade and the acetonitrile (CH₃CN) over molecular sieves (H₂O ≤0.01%), ≥99.8% (GC). Water was obtained from a Barnstead Nanopure water filtration system (Barnstead Diamond Nanopure pack organic free RO/DIS). High performance liquid chromatography (HPLC) purification and analysis of the ¹⁸F-radiolabelled products were performed using a Phenomenex LUNA®C18(2) column (100 Å, 250 x 10 mm, 10 µm) using gradient elution specific to the given compound (Gilson 321 pump, 171 diode array detector, Berthold Technologies Herm LC). Quality control analysis were performed on a PhenomenexLuna®10u C18(2) column (100 Å, 250 x 4.60 mm) (Shimadzu DGU-20A, LC-20AT, SPD-M20A, CBM-20A, SIL-20A HT, Raytest Ramona Star). Radio-TLC were performed using either EMD Merck F254 silica gel 60 aluminum backed thin layer chromatography (TLC) plates or Analtech RP18 with UV254 aluminum backed TLC plates (Bioscan AR-2000). Quantification of radioactive samples during chemistry was achieved using a Biodex ATOMLAB™ 400 dose calibrator.

Centrifugation of non-radioactive samples was achieved with a Hettich Zentrifugen Rotina 35R, whereas ¹⁸F-radiolabelled samples were centrifuged on a Fisher Scientific Mini Centrifuge. Reactions parameters were screened using an Eppendorf Thermomixer R and an IKAMAG® Ret-G Stir plate with an oil bath. Final formulation of the final ¹⁸F-radiolabelled products for animal injection were done using glassware kept in an Isotemp Vacuum Oven Model 285A and a rotary evaporator of a Buchi HB 140 Rotavapor-M with a Fisher Maxima C Plus Model M8C pump. High resolution Mass Spectroscopy was achieved with an Agilent Technologies 6220 oaTOF.

General protocol for resin synthesis of propargylglycine-bombesin derivative (1). Peptide **1** was prepared as per Richter *et al.*¹ with the N-terminus of the peptide containing the L-propargylglycine moiety. This peptide coupling was performed using solid-phase peptide synthesis (SPPS) on a Syro I (MultiSynTech/Biotage).

General protocol for resin synthesis of cysteine-containing peptide derivative (3). Peptide **3** was prepared as per Richter *et al.*¹, with the N-terminus of the peptide containing the L-propargylglycine moiety and the cysteine moiety. This peptide coupling was performed using solid-phase peptide synthesis (SPPS) on a Syro I (MultiSynTech/Biotage).

General protocol for the manual in-solution synthesis of [¹⁹F]2. To an Eppendorf Lobind 2.0 mL eppendorf tube, propargylglycine modified bombesin derivative **1** (3 mg) was added along with copper(I) iodide (3 mg), triethylamine (300 µL) and PdCl₂(PPh₃)₂ (3 mg). Next, water (400 µL) and DMF (300 µL) were added along with a commercially available [¹⁹F]FIB (10 µL). The mixture was then allowed to react in a thermoshaker for 60 min at 55 °C. Upon reaction completion, 2N hydrochloric acid (HCl, 1 mL) was added and the reaction mixture was centrifuged to remove all precipitates. The product was purified by Beckmann HPLC and the collected product fractions are evaporated to dryness by rotary evaporation to yield the desired compound. Yield: 0.5 mg (9 %) as a fluffy white powder. HR-MS m/z (ESI): C₅₇H₈₀FN₁₄O₁₀ ([M+H]⁺) calcd. 1139.616, found 1139.6151, ([M+Na]⁺) calcd. 1161.598, found 1161.5971. HPLC-analysis (Luna 10u C18(2)) (100 Å, 250 x 10 mm, 10 µm), gradient elution with (A: water; B: CH₃CN; 0 min 15 % B, 1.20 min 15 % B, 58.20 min 100 % B, 60.20 min 100% B, 3 mL/min): t_R = 19.5 min.

General protocol for the on-resin synthesis of [¹⁹F]2. With peptide **1**, synthesized on resin according to an amino acid loading of 0.6 mmol/g of starting resin (125 mg), cross coupling reaction was carried out manually on resin through the addition of the following reagents to the peptide synthesis tube: [¹⁹F]FIB (43.3 µL), CuI (5.7 mg), PdCl₂(PPh₃)₂ (10.5 mg), triethylamine (2 mL), and 1,4-dioxane (4 mL). The resin reaction was allowed to react for 72 hours at room temperature and once completed the peptide was cleaved off the resin. A cocktail (4 mL) solution containing 88% trifluoroacetic acid, 5% thioanisole, 5% water, and 2% ethane dithiol, was half (2 mL) used for the cleavage reaction of 3 hours at room temperature, followed by a subsequent wash with the remaining (2 mL) of solution made. Once cleaved the solution was dried under a stream of nitrogen and diethyl ether was used to precipitate the compound of interest. The [¹⁹F]FBpBBN was isolated by filtration and purified using Gilson HPLC and the collected fractions lyophilized to yield 13.2 mg (15 %) purified peptide as a white powder. LR-MS m/z (ESI): C₅₇H₈₀FN₁₄O₁₀ ([M+H]⁺) calcd. 1139.6, found 1139.6, ([M+Na]⁺) calcd. 1161.6, found 1161.6. HPLC-analysis (Jupiter 10u Proteo) (90 Å, 250 x 10 mm, 10 µm), gradient elution with (A: water; B: CH₃CN; 0 min 20 % B, 10 min 50 % B, 30 min 70 % B, 40 min 90 B, 3 mL/min): t_R = 18.1 min.

General protocol for the on resin synthesis of [¹⁹F]3. To peptide **3**, synthesized on resin according to an amino acid loading of 0.8 mmol/g of starting resin (25 mg). The cross coupling reaction was carried out manually on resin through the addition of the following reagents to the peptide synthesis tube: [¹⁹F]FIB (46.1 μL), CuI (7.6 mg), Pd(OAc)₂ (9.0 mg), tppts (22.7 mg), triethylamine (0.6 mL), PBS buffer (1.0 mL) and CH₃CN (0.5 mL). The resin reaction was allowed to react for 50 minutes at room temperature and once completed the peptide was cleaved off the resin. A cocktail (4 mL) solution containing 88% trifluoroacetic acid, 5% thioanisole, 5% water, and 2% ethane dithiol, was half (2 mL) used for the cleavage reaction of 3 hours at room temperature, followed by a subsequent wash with the remaining (2 mL) of solution made. Once cleaved the solution was dried under a stream of nitrogen and diethyl ether was used to precipitate the compound of interest. The [¹⁹F]FBpcBBN was isolated by filtration and purified using Gilson HPLC and the collected fractions lyophilized to yield 25.4 mg (13 %) purified peptide as a white powder. LR-MS m/z (MALDI): C₆₀H₈₄FN₁₅O₁₁S ([M+H]⁺) calcd. 1242.6, found 1242.7, HPLC-analysis (Jupiter 10u Proteo) (90 Å, 250 x 10 mm, 10 μm), gradient elution with (A: water; B: CH₃CN; 0 min 20 % B, 10 min 40 % B, 30 min 80 % B, 40 min 80 B, 3 mL/min): t_R = 21.9 min.

Protocol for the synthesis of 4-[¹⁸F]fluoroiodobenzene ([¹⁸F]FIB). 4-[¹⁸F]Fluoroiodobenzene ([¹⁸F]FIB) were prepared according to literature procedure².

Formulation of 4-[¹⁸F]fluoroiodobenzene ([¹⁸F]FIB) for use in cross-coupling reactions. The collected product of [¹⁸F]FIB from the HPLC (6 mL) was diluted into water (50 mL) and trapped onto a Waters Sep-Pak[®] C18 plus light cartridge (300 mg). Elution from the cartridge took place in the solvent of interest, such as DMF (1.5 mL), CH₃CN (3.0 mL), toluene (3.0 mL), acetone (3.0 mL), and tetrahydrofuran (THF, 3.0 mL). Purified [¹⁸F]FIB was used accordingly in the different cross-coupling reactions.

General procedure for the synthesis of [¹⁸F]2

Peptide **1** (0.1 mg) was added with CuI (1 mg), triethylamine (25 μL), Pd(OAc)₂ (0.1 mg) and TPPTS (1 mg) into an 1.5 mL eppendorf tube (Eppendorf Lobind). Next, PBS buffer (900 μL) was added along with HPLC purified [¹⁸F]FIB (100 μL) in the respective solvent. The mixture was then allowed to react in a thermoshaker for 10 min at 25 °C. Upon completion, 2 N HCl (0.7 mL) was added and the reaction mixture was centrifuged. This product mixture was removed from the eppendorf with a syringe. Compound [¹⁸F]2 was purified using semi-preparative HPLC.

General protocol for radio-TLC analysis of [¹⁸F]2

An EMD Merck F254 silica gel 60 aluminum backed TLC plate was spotted on the baseline and the plate was developed (63:37, CH₃CN: 0.2 % TFA in H₂O). There were three possible spots, one of the any degradation and possible [¹⁸F]2 compound which appeared on the baseline (R_f=0.0), any Pd-bound [¹⁸F]FIB which appeared in the middle of the TLC plate (R_f=0.54), and the starting material of [¹⁸F]FIB

which appeared close to the solvent front ($R_f=0.81$). To show that this product peak was not fully degradation, a secondary TLC plate was run to demonstrate movement of the product of interest. An Analtech RP18 with UV254 aluminum backed TLC plates was spotted on the baseline and the plate was developed (7:3, $\text{CH}_3\text{CN}:\text{TEA}$) Here only two possible spots, one of the degradation products which appeared on the baseline ($R_f=0.0$) and the product of [^{18}F]2 plus any unreacted [^{18}F]FIB and palladium-bound [^{18}F]FIB ($R_f=0.65$).

General protocol for the HPLC purification of [^{18}F]2. Gilson HPLC purification of the crude [^{18}F]2 was performed using a gradient elution as follows: (Luna 10u C18(2)) (100 Å, 250 x 10 mm, 10µm), (A: water; B: CH_3CN ; 0 min 15% B, 58 min 100% B, 60 min 100% B). The flow rate of the system was 3 mL/min, which gave a retention time of 23.2 minutes for the [^{18}F]2 product as confirmed with the use of a reference compound. The radiochemical purity was determined by the area under the peak of interest compared to the rest of the radio-chromatogram; as well the specific activity of [^{18}F]2 was calculated against a standard curve.

References:

- (1) S. Richter, M. Wuest, S. S. Krieger, B. E. Rogers, M. Friebe, R. Bergmann, F. Wuest, *Nucl. Med. Biol.* **2013**, *40*, 1025-1034.
- (2) J. D. Way, F. Wuest, *J. Label. Compds. Radiopharm.* **2014**, *57*, 104-109.

Figure S1: Colorimetric ligand substitution of $\text{Pd}(\text{OAc})_2$ with tppts.

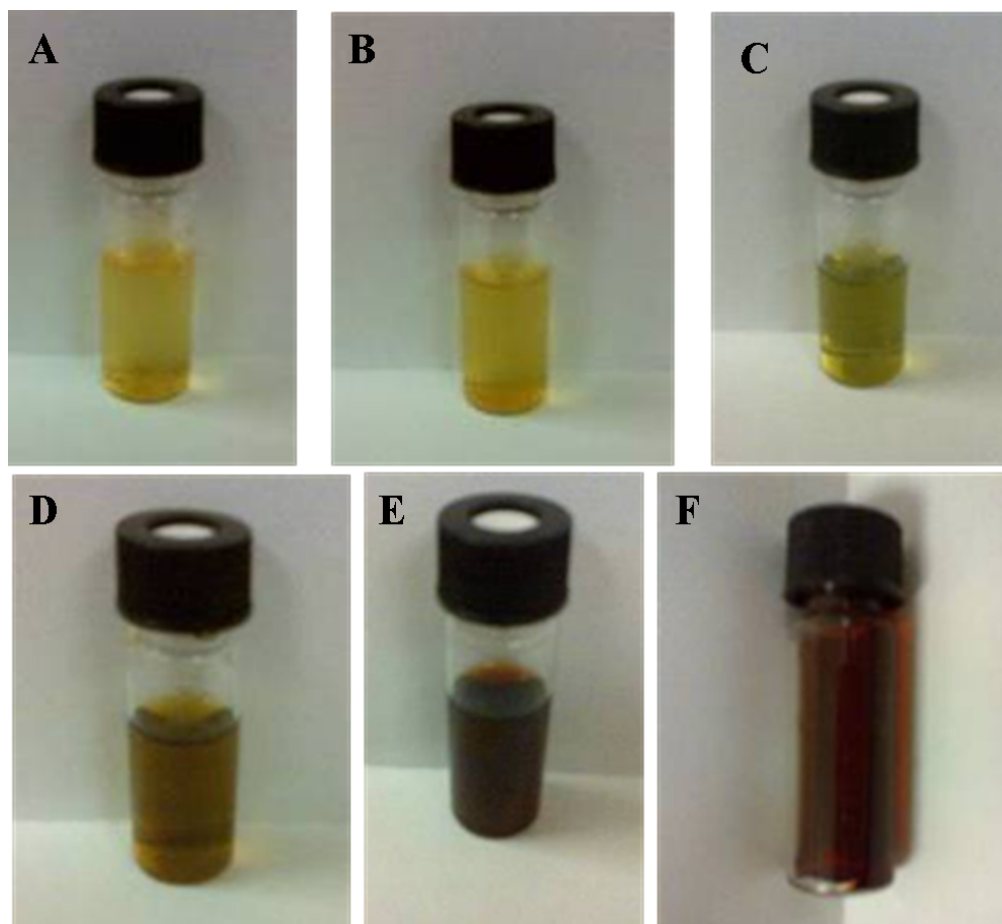


Figure S2. HPLC trace of reaction mixture (radioactivity trace: blue; UV trace: red) (top) and HPLC traces of purified [^{18}F]**2** (bottom) and co-injected reference compound [^{19}F]**2** (middle).

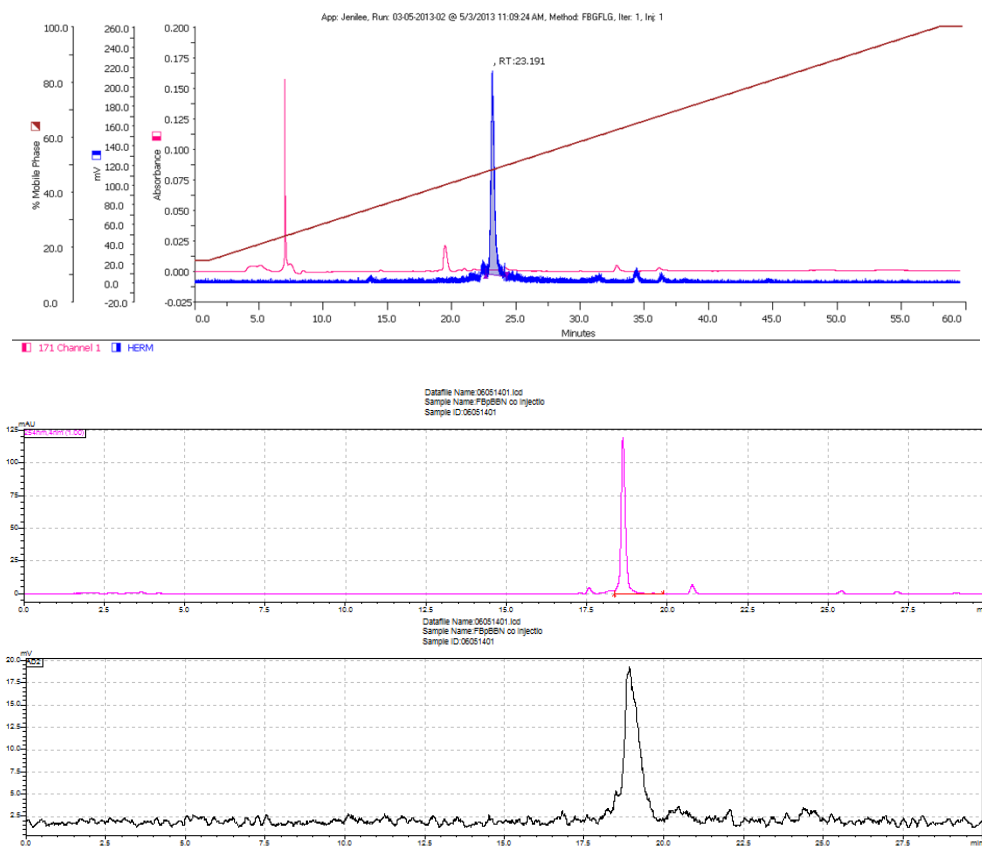


Figure S3: Structures of cysteine-containing peptides **3** and [^{18}F]**3**.

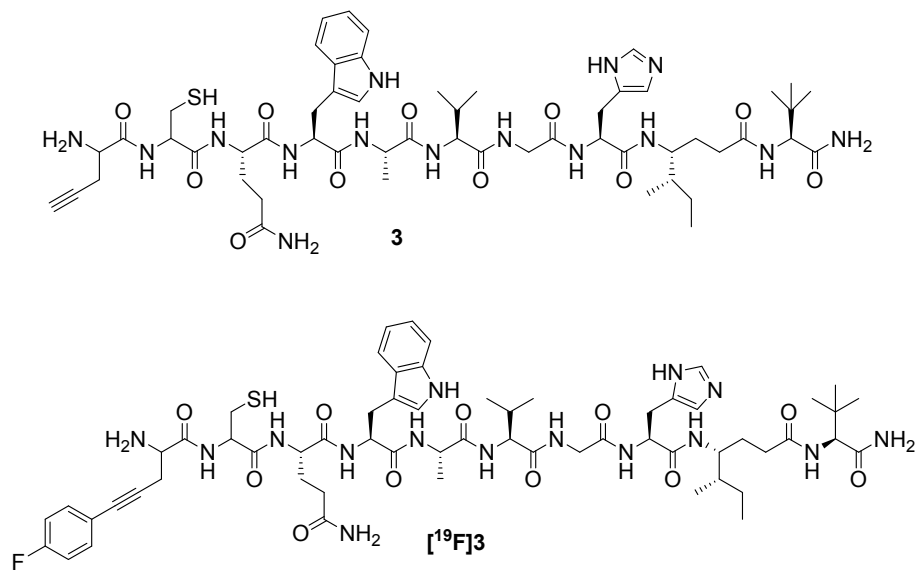


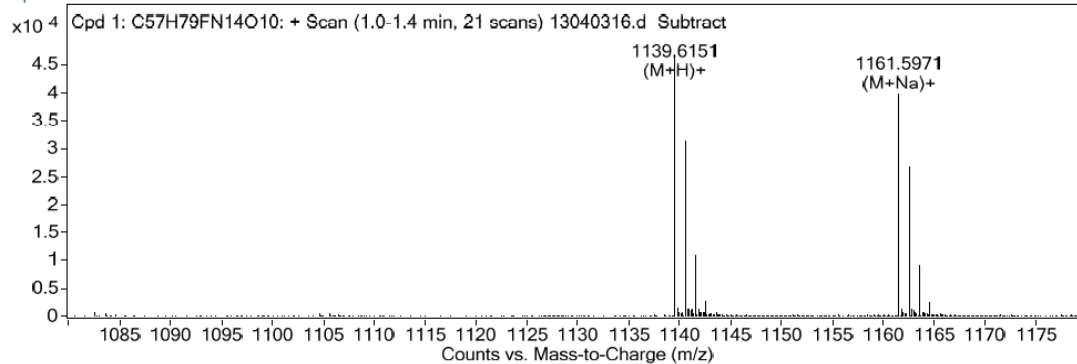
Figure S4: HR-MS for [¹⁹F]2.

Qualitative Compound Report

Comment	J. Way, Wuest, Oncology	Sample Name	fbpbno 413 p2
Data File	13040316.d	Instrument Name	oaTOF6220
Position	-1	Operator	ami
Acq Method		DA Method	da ami low mass.m

Compound Table

MS Spectrum



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
1139.6151	1139.616	-0.78		46917	C57 H80 F N14 O10	(M+H)+
1161.5971	1161.598	-0.75	1	39775	C57 H79 F N14 Na O10	(M+Na)+

--- End Of Report ---

Figure S5: LR-MS for [¹⁹F]2.

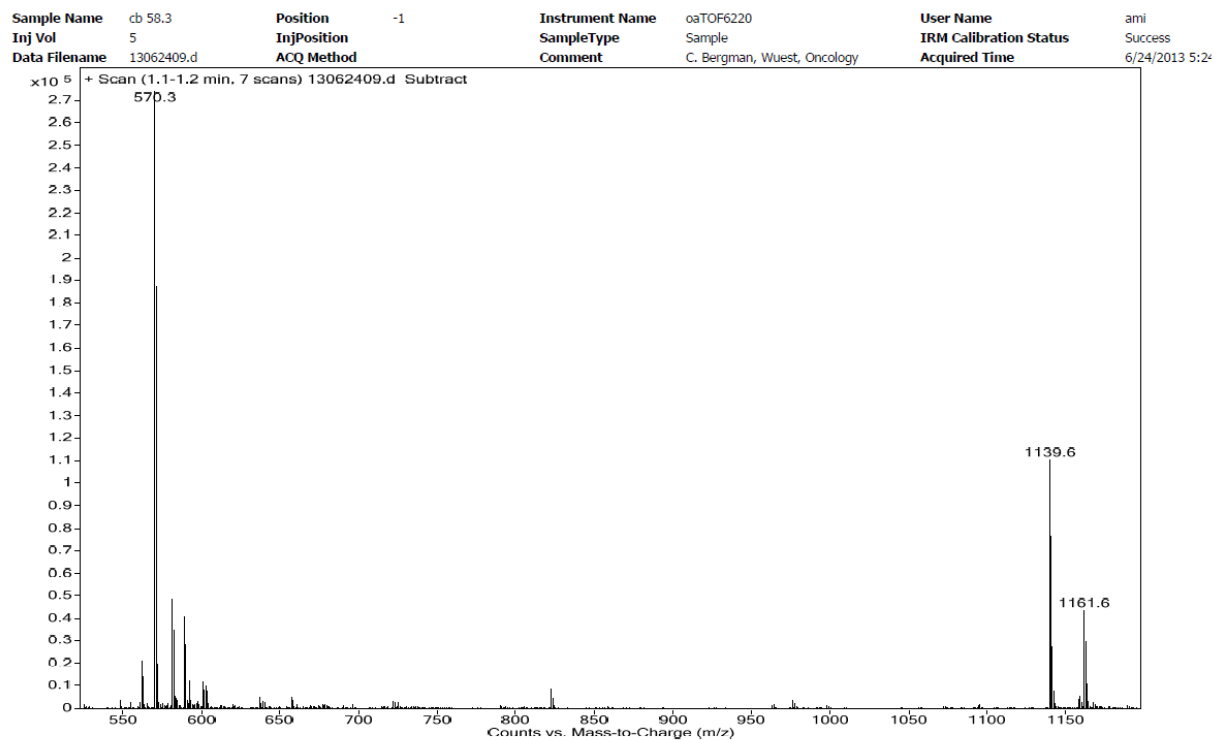


Figure S6: HPLC purification for [¹⁹F]2 on resin coupling.

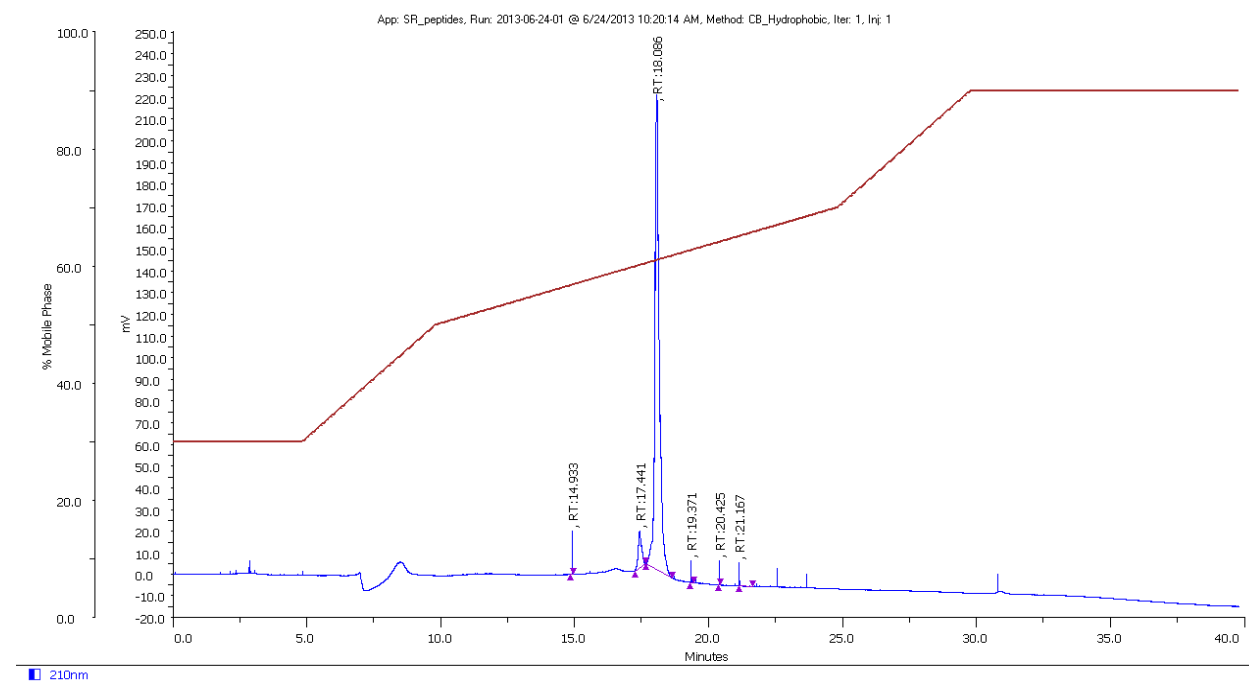


Figure S7: MALDI-TOF MS for **[¹⁹F]3** on resin coupling.

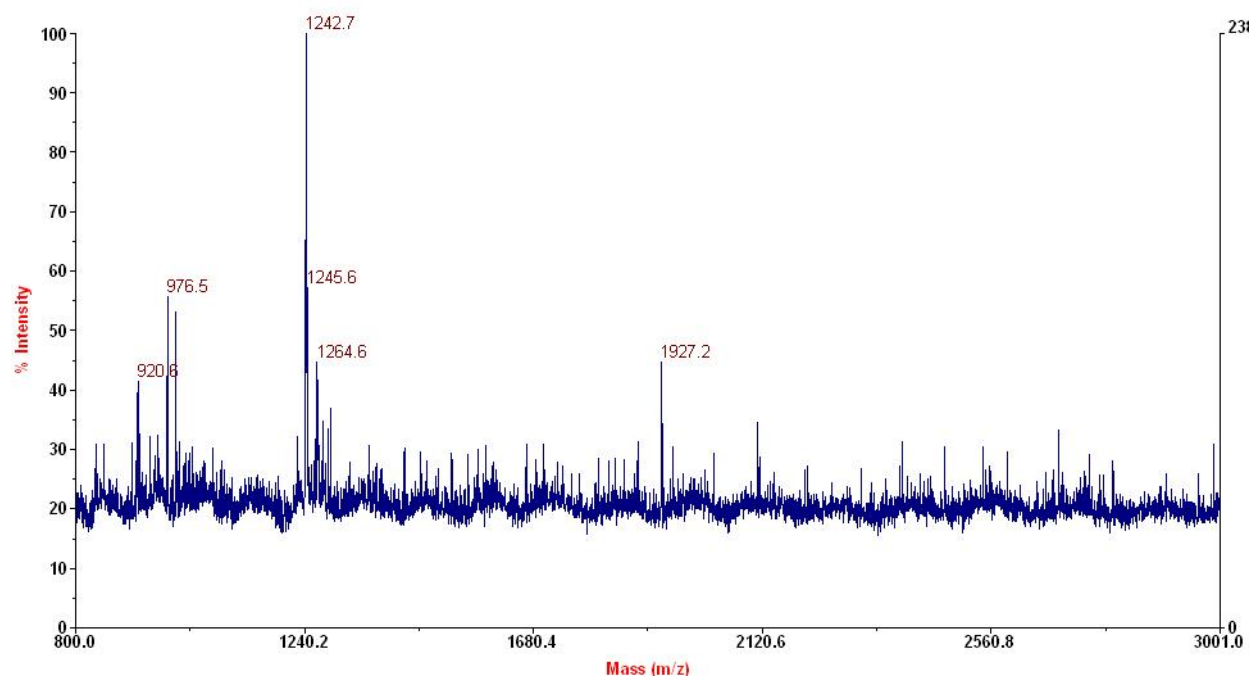


Figure S8: HPLC for [^{19}F]3 on resin coupling.

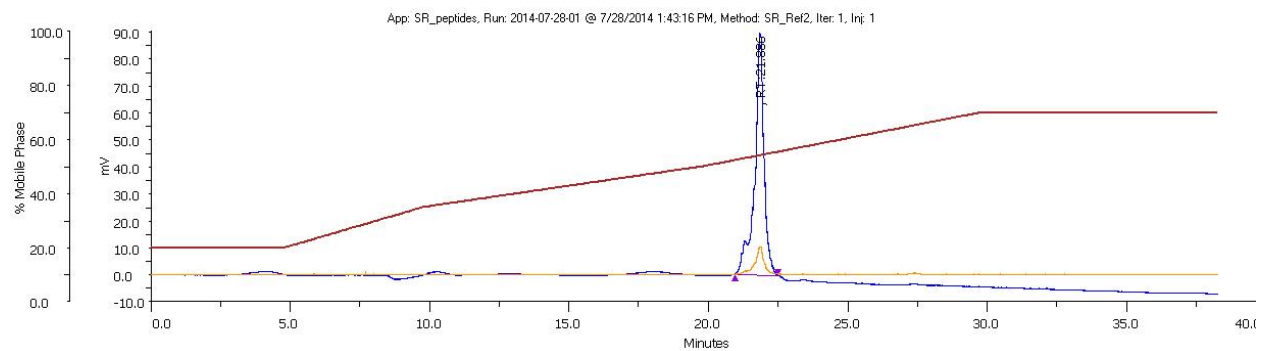
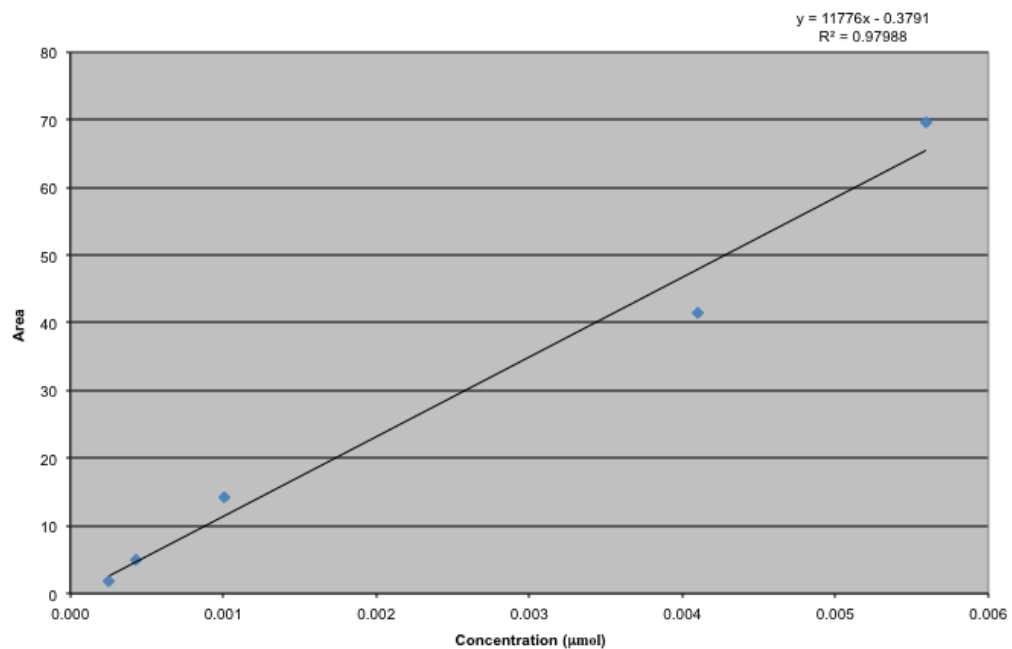


Figure S9: Specific activity calibration curve for [^{19}F]**2** and specific activity calculations for [^{18}F]**2**.



Run ID	Activity (GBq)	Area	Specific Activity (GBq/μmol)
11-03-2014-03	0.00316	0	98
10-03-2014-02	0.00774	0	240
17-03-2014-02	0.0273	0	848