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Radiosynthesis and 'click' conjugation of ethynyl-4-[18F]fluorobenzene — an improved [18F]synthon for indirect radiolabeling‡

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Reproducible methods for [18F]radiolabeling of biological vectors are essential for the development of new [18F] radiopharmaceuticals. Molecules such as carbohydrates, peptides and proteins are challenging substrates that often require multi-step indirect radiolabeling methods. With the goal of developing more robust, time saving, and less expensive procedures for indirect [18F]radiolabeling of such molecules, our group has synthesized ethynyl-4-[18F]fluorobenzene ([18F]2, [18F]EYFB) in a single step (14 ± 2% non-decay corrected radiochemical yield (ndc RCY)) from a readily synthesized, shelf stable, inexpensive precursor. The alkyne-functionalized synthon [18F]2 was then conjugated to two azido-functionalized vector molecules via CuAAC reactions. The first 'proof of principle' conjugation of [18F]2 to 1-azido-1-deoxy-β-D-glucopyranoside (3) gave the desired radiolabeled product [18F]4 in excellent radiochemical yield (76 ± 4% ndc RCY (11% overall)). As a second example, the conjugation of [18F]2 to matrix-metalloproteinase inhibitor (5), which has potential in tumor imaging, gave the radiolabeled product [18F]6 in very good radiochemical yield (56 ± 12% ndc RCY (8% overall)). Total preparation time for [18F]4 and [18F]6 including [18F]F⁻ drying, two-step reaction (nucleophilic substitution and CuAAC conjugation), two HPLC purifications, and two solid phase extractions did not exceed 70 min. The radiochemical purity of synthon [18F]2 and the conjugated products, [18F]4 and [18F]6, were all greater than 98%. The specific activities of [18F]2 and [18F]6 were low, 5.97 and 0.17 MBq nmol⁻¹, respectively.

Keywords: [18F]Fluorine; click chemistry; radiochemistry; [18F]synthons; matrix metalloproteinase inhibitors (MMPIs); positron emission tomography (PET)

Introduction

Positron emission tomography (PET) provides detailed three-dimensional information on functional processes *in vivo* and has important diagnostic applications in oncology, neurology, and cardiology. Although numerous radioisotopes (copper-64, gallium-68, zirconium-89, and iodine-124) are available for PET, fluorine-18 remains very popular because of a unique combination of physical and chemical properties. These include low positron emission energy (0.202 MeV), excellent decay profile (97% β+ emission), advantageous half-life (109 min), availability in nucleophilic (F^-) or electrophilic (F^+) form, and similar steric and electronic properties to the hydroxyl group. The use of nucleophilic [18F]fluoride is considerably more widespread than electrophilic [18F]fluorine because of higher specific activity products, superior radiolabeling selectivity, and ready availability. However, direct radiolabeling with [18F]fluoride often employs harsh conditions including higher temperatures (>100 °C) that are often incompatible with large biomolecules.[1]

Biomolecules are typically radiolabeled via indirect methods where [18F]fluoride is first incorporated into a [18F]radiolabeled 'synthon' containing a functional group that reacts with the biomolecule under relatively mild conditions.[2] A number of [18F] synthons are available for indirect radiolabeling such as, but not limited to, *N*-succinimidyl-4-[18F]fluorobenzoate ([18F]SFB) and *p*-nitrophenyl-2-[18F]fluoropropionate ([18F]NFP) (Figure 1). The

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‡*Additional supporting information may be found in the online version of this article at the publisher's web-site.*

Abbreviations: *Acetonitrile, (CH_3CN); 4-azidophenacyl-[18F]fluoride, ([18F]APF); decay corrected, (dc); dichloromethane, (CH_2Cl_2); N, N-diisopropylethylamine, (DIPEA); dimethylformamide, (DMF); dimethylsulfoxide, (DMSO); equivalent, (eq.); ethyl acetate, (EtOAc); [18F]fluoroethylazide, ([18F]FEA); high performance liquid chromatography, (HPLC); high-resolution mass spectrometry, (HRMS); hour, (h); low-resolution mass spectrometry, (LRMS); metalloproteinase inhibitors, (MMPIs); p-nitrophenyl-2-[18F]fluoropropionate, ([18F]NFP); non-decay corrected, (ndc); nuclear magnetic resonance, (NMR); positron emission tomography, (PET); radiochemical yield, (RCY); retention time, (t_R); room temperature, (rt); solid phase extraction, (SPE); N-succinimidyl-4-[18F]fluorobenzoate, ([18F]SFB); trifluoroacetic acid, (TFA).*

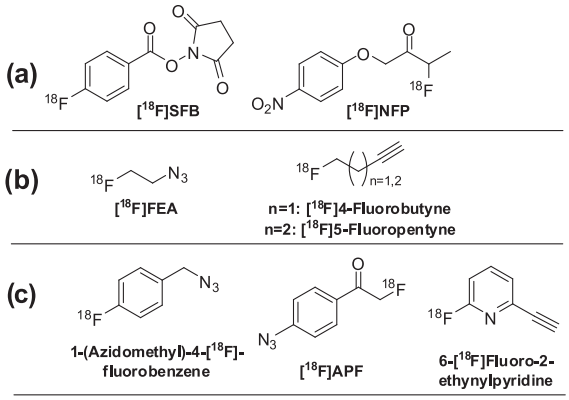


Figure 1. Examples of [^{18}F]synthons for radiolabeling, conjugated via: (a) acylation, (b) aliphatic 'click' and (c) aromatic/heteroaromatic 'click' reaction.

radiosynthesis of the aforementioned [^{18}F]synthons, and many others, often require multiple synthesis and purification steps[3] followed by conjugation to the molecule of interest through acylation/alkylation reactions.[4] However, in more recent times, the further development of [^{18}F]synthons has produced more efficient radiosyntheses. For example, the radiosynthesis of [^{18}F]NFP has recently been reported in 26% yield from one-step in 45 min, followed by conjugation to RGD peptides[5] as well as the radiosynthesis of [^{18}F]F-Py-TFP (6-[^{18}F]fluoronicotinic acid 2,3,5,6-tetrafluorophenyl ester), that was prepared in 60–70% yield from a single 10 min reaction, before being easily purified by Sep-Pak and also conjugated to a RGD peptide.[6]

A popular alternative strategy for indirect [^{18}F]radiolabeling is the CuAAC or 'click' reaction, and the growing applications of 'click chemistry' in PET studies has been recently reviewed.[4,7] The CuAAC reaction can be performed under aqueous conditions, is highly chemoselective, and provides conjugates in high radiochemical yield and purity. The triazole nitrogen atoms formed during the CuAAC reaction are also weak hydrogen bond acceptors, which increases hydrophilicity of the products.[8] To date, the alkyl-based synthons, such as, [^{18}F]fluoroethylazide ([^{18}F]FEA), [^{18}F]4-fluorobutyne and [^{18}F]5-fluoropentyne (Figure 1)[9] have been reported; however, their high volatility, lack of UV absorption, challenging purification, and susceptibility to defluorination limit their use as [^{18}F]synthons. More recently, some research groups are shifting focus from alkyl-based synthons toward aromatic [^{18}F]fluorine-based synthons that are less susceptible to *in vivo* radiodefluorination.[10] Thonon *et al.*[11] reported the radiosynthesis of the azido-aromatic synthon 1-(azidomethyl)-4-[^{18}F]-fluorobenzene, via a four-step 75-min synthesis (34% decay corrected (dc) radiochemical yield (RCY)), which was then successfully conjugated to an alkyne-functionalized amino acid and an alkyne-functionalized neuropeptide. Wester *et al.*[12] reported the azido-aromatic synthon 4-azidophenacyl-[^{18}F]fluoride ([^{18}F]APF) in up to 70% yield from a single 15-min reaction for subsequent protein conjugation. Furthermore, Daumar *et al.*[13] reported the radiosynthesis of the novel aromatic synthon, 6-[^{18}F]fluoro-2-ethynylpyridine, obtaining a 28% dc RCY in a single-step 10-min synthesis, followed by click conjugation to an azido-functionalized peptide (Figure 1).

Given the aforementioned challenges with alkyl-[^{18}F]synthons and promising aromatic [^{18}F]synthons, our group has developed radiosynthon [^{18}F]**2** and performed two proof of concept CuAAC experiments. [^{18}F]**2** and its precursor were designed with the following features to enable practical, straightforward use of this new [^{18}F]synthon. These include:

(1) An aromatic ring for improved UV detection and increased stability *in vivo*,
(2) An ammonium cation leaving group for straightforward purification, preferably by SPE for time reduction,
(3) An alkyne functionality that acts both as weakly activating group (electron withdrawing group) and functional group for subsequent 'click' conjugation reactions,
(4) Low volatility to avoid loss during heating, hence maximize radiochemical yield, and
(5) An inexpensive radiolabeling precursor that is prepared in a single step and isolated by filtration from commercially available 4-ethynyl-*N*,*N*-dimethylaniline.

The results herein describe the successful radiosynthesis and conjugation of [^{18}F]**2** to an azido-functionalized carbohydrate **3** (1-azido-1-deoxy-β-D-glucopyranoside) and a matrix metalloproteinase inhibitor **5** (3-(hydroxylcarbamoyl)-3(*S*)-hydroxy-2(*R*)-(cyclopentylmethyl)propionyl-L-*tert*-leucine-*N*-2-azidoethylamide) that has potential for development as a tumor PET imaging agent. A comparison of the efficiency of radiolabeling the matrix metalloproteinase inhibitor **5** to that achieved by Doan *et al.*[14] is also described.

Experimental procedures

General

All reagents were purchased from Sigma-Aldrich and used without further purification. Aqueous [^{18}F]HF was produced on an IBA Cyclone® 18 twin cyclotron (ANSTO, Camperdown, Australia) using the ^{18}O(p, n)^{18}F nuclear reaction. C18 Sep-Pak® Plus & Lite SPE cartridges were purchased from Waters (Rydalmere, NSW, Australia), activated with ethanol (2 mL), then washed with H_2O (20 mL) prior to use; C18 Strata® X SPE cartridges were purchased from Phenomenex (Lane Cove, NSW, Australia), activated with ethanol (2 mL), and washed with H_2O (20 mL); Affinimp® SPE cartridges were purchased from Polyintell Intelligent Polymers(Chaussée du Vexin, Val-de-Reuil, France), activated with CH_3CN (4 mL). Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance DPX-400 spectrometer (LabX, Midland, ON, Canada) operating at 400.13 MHz for ^{1}H NMR and at 100.61 MHz for ^{13}C NMR. Low-resolution mass spectrometry was performed on a Micromass ZQ quadrupole mass spectrometer (LabX), and high-resolution mass spectrometry was performed at the University of Wollongong, Australia using a Bruker Daltonics BioApex-II 7 T FTICR spectrometer (LabX) equipped with an off-axis analytical electron spray ionization source. HPLC QC (quality control) percentage purity was calculated by integrating the area under the peak using EMPOWER software (Waters); details are available in Supporting Information. The radiosynthesis of [^{18}F]**2**, [^{18}F]**4,** and [^{18}F]**6** were confirmed by co-injection with non-radioactive standards (**2**, **4,** and **6**, respectively) using HPLC analysis. Radioactivity was measured with a Capintec R15C dose calibrator (Capintec, Ramsey, NJ, USA).

Chemistry

*4-Ethynyl-N,N,N-trimethylbenzenaminium trifluoromethanesulfonate (**1**)*

Methyl trifluoromethanesulfonate (0.95 mL, 8.61 mmol, 1.25 eq.) was added to 4-ethynyl-*N*,*N*-dimethylaniline (1.00 g, 6.89 mmol, 1.00 eq.) in CH_2Cl_2 (3 mL), and the solution stirred at rt for 2 h. The precipitate formed was collected by filtration and washed with CH_2Cl_2 (3 × 1 mL) to obtain the product as a white powder (1.61 g, 77%). ^{1}H NMR (400.13 MHz, $(CD_3)_2CO$): δ (ppm) 8.13 (d, J = 9.20 Hz, 2H), 7.74 (d, J = 9.20 Hz, 2H), 3.92 (s, 1H), 3.87 (s, 9H); ^{13}C NMR (100.61 MHz, $(CD_3)_2CO$): δ (ppm) 148.10, 134.43, 125.35, 121.82, 82.05, 57.65; LRMS (ES^+) *m/z* 160.08 $[M]^+$; LRMS

(ES^-) *m/z* 148.88 $[CF_3O_3S]^-$; HRMS (TOF MS AP^+) *m/z* $[M]^+$ $C_{11}H_{14}N$ calculated 160.1126; found 160.1118; HPLC QC purity 96.25%.

*(2R,3R,4R,5R)-2-(4-(4-Fluorophenyl)-1H-1,2,3-triazol-1-yl)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol (**4**)*

1-Ethynyl-4-fluorobenzene (71 mg, 0.59 mmol, 1.2 eq.) was dissolved in *n*-butanol (6 mL) and degassed using nitrogen gas. In a separate reaction vessel **3** (100 mg, 0.49 mmol, 1 eq.), sodium ascorbate (190 mg, 0.98 mmol, 2 eq.) and copper (II) sulfate (120 mg, 0.49 mmol, 1 eq.) were dissolved in H_2O (3 mL). The vessel was degassed using nitrogen gas and then the two reaction vessels were combined and stirred at room temperature for 16 h under a nitrogen atmosphere. H_2O (15 mL) was added to the reaction mixture and the precipitate formed was removed by filtration. The filtrate was purified directly using automated flash chromatography (Grace Reveleris 12 g C18 column; flow rate 24 mL/min; solvent system 1/99% CH_3CN/H_2O, increased to 80/20%). The colorless fractions containing the product (eluted at 45/55% CH_3CN/H_2O) were evaporated under reduced pressure then lyophilized to afford a white solid (90 mg, 56%). ^1H NMR (400.13 MHz, (CD_3OD): δ (ppm) 8.54 (s, 1H), 7.86 (m, 2H), 7.18 (m, 2H), 5.66 (d, 1H, $J = 9.20$ Hz), 3.93 (m, 2H), 3.74 (m, 1H), 3.59 (m, 3H); ^{13}C NMR (100.61 MHz, (CD_3OD): δ (ppm) 162.81 (d, $J_{F\text{-}C} = 246.37$ Hz), 146.58, 127.31 (d, $J_{F\text{-}C} = 8.23$ Hz), 126.64 (d, $J_{F\text{-}C} = 3.38$ Hz), 119.91, 115.42 (d, $J_{F\text{-}C} = 22.01$ Hz), 88.35, 79.77, 77.07, 72.69, 69.51, 60.99; LRMS (ES^+, 40 V) *m/z* $[M + Na]^+$ $C_{14}H_{16}FN_3NaO_5$: calculated 348.10; found 347.98; HRMS (TOF MS AP^+) *m/z* $[M + Na]^+$ $C_{14}H_{17}FN_3NaO_5$: calculated 348.0972, found 348.0989; HPLC QC purity 99.25%.

*(2S,3R)-2-(Cyclopentylmethyl)-N1-((R)-1-(2-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)ethylamino)-3,3-dimethyl-1-oxobutan-2-yl)-N4,3-dihydroxysuccinamide (**6**)*

1-Ethynyl-4-fluorobenzene (35 mg, 0.28 mmol, 1.2 eq.) and **5** (100 mg, 0.23 mmol, 1 eq.) was dissolved in *n*-butanol (6 mL) and degassed using nitrogen gas. In a separate reaction, sodium ascorbate (94 mg, 0.46 mmol, 2 eq.) and copper (II) sulfate (60 mg, 0.23 mmol, 1 eq.) were dissolved in H_2O (3 mL). This vessel was degassed and then the two reaction vessels were combined. The reaction was stirred at room temperature for 16 h under an atmosphere of nitrogen. H_2O (15 mL) was added and the resultant yellow precipitate was removed by filtration and the filtrate evaporated under reduced pressure. The yellow film was purified by preparative HPLC (isocratic 35/55/10, CH_3CN/H_2O/100 mM NH_4HCO_3, pH 8, 5 mL/min, T3 Atlantis 250 × 10 mm, t_R 19.12 min, λ 254 nm) then lyophilized to afford the product as a white solid (48 mg, 38%). ^1H NMR (400.13 MHz, $(CD_3)_2SO$): δ (ppm) 10.56 (s, 1H), 8.86, (s, 1H), 8.48 (s, 1H), 8.24 (bs, 1H), 7.86 (m, 2H), 7.49 (d, 1H, $J = 7.20$ Hz), 7.27 (m, 2H), 5.33 (d, 1H, $J = 6.40$ Hz), 4.44 (m, 2H), 4.14 (d, 1H, $J = 7.20$ Hz), 3.73 (t, 1H, $J = 6.40$ Hz), 3.53 (m, 2H), 2.65 (m, 1H), 1.71 (m, 1H), 1.59-0.85 (m, 10H), 0.80 (s, 9H); ^{13}C NMR (100.61 MHz, $(CD_3)_2SO$): δ (ppm) 173.12, 171.23, 169.32, 162.18 (d, $J_{F\text{-}C} = 195.38$ Hz), 145.91, 127.93, 127.57 (d, $J_{F\text{-}C} = 6.54$ Hz), 122.00, 116.27 (d, $J_{F\text{-}C} = 17.30$ Hz), 71.68, 60.54, 49.52, 49.12, 37.88, 35.04, 34.23, 33.06, 32.28, 27.02, 25.15; LRMS (ES^+, 40 V) *m/z* $[M + H]^+$ $C_{26}H_{38}FN_6O_5$: calculated 533.29; found 533.09; *m/z* $[M + Na]^+$ $C_{26}H_{37}FN_6NaO_5$: calculated 555.27; found 555.08; HRMS (TOF MS AP^+) *m/z* $[M + H]^+$ $C_{26}H_{38}FN_6O_5$: calculated 533.2888; found 533.2918; HPLC QC purity 98.51%.

Radiochemistry

*Ethynyl-4-[^{18}F]fluorobenzene ([^{18}F]**2**)*

An aqueous [^{18}F]HF solution (0.1-2 GBq) was added to a reaction vial containing a solution of potassium carbonate (4.5 mg in 45 μL H_2O, 32.3 μmol) and K_{222} (Kryptofix 2.2.2) (12.1 mg, in 121 μL CH_3CN, 32.3 μmol). The solvent was evaporated under a stream of nitrogen at 100 °C under vacuum, and the residue azeotropically dried three times by repeated addition and evaporation of anhydrous CH_3CN (3 × 1 mL). To the activated $K^{18}F.K_{222}$ complex was added 4-ethynyl-*N,N,N*-trimethylbenzenaminium trifluoromethanesulfonate **1** (5 mg, 16.15 μmol) in DMF (1 mL), and the mixture heated at 150 °C for 5 min. After cooling the reaction vessel in a slurry of iced H_2O (~60 s) and venting the mixture, mobile phase (1 mL, 50/50/0.1, CH_3CN/H_2O/TFA, v/v) was added and the vial shaken to aid washing down the walls of the reaction vial. The mixture was purified by HPLC (Luna C18 column 250 × 10 mm, 10 μm; isocratic CH_3CN/H_2O/TFA 50/50/0.1, v/v; 6.0 mL/min, t_R 13 min, λ 254 nm). HPLC collected [^{18}F]**2** was diluted with H_2O (12 mL), transferred to a C18 Strata X SPE cartridge (Phenomenex), washed with additional H_2O (10 mL) and remained cartridge trapped for the subsequent elution into the click conjugation reaction. [^{18}F]**2** was obtained in 14 ± 2% collected ndc RCY, $n = 3$ (RCY was calculated based on [^{18}F]radioactivity eluted from the cartridge into the subsequent reaction vessel).

*(2R,3R,4R,5R)-2-(4-(4-[^{18}F]Fluorophenyl)-1H-1,2,3-triazol-1-yl)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol ([^{18}F]**4**)*

Compound **3** (1.1 mg, 5.36 μmol), sodium ascorbate (23.0 mg, 116 μmol), copper iodide (4 mg, 19.3 μmol), 2,6-lutidine (14 μL, 120.9 μmol) and H_2O (0.4 mL) were added to a reaction vial warmed at 80 °C for 1 min, or until the solution turned pale yellow. [^{18}F]**2** was eluted from the C18 Strata X SPE cartridge with CH_3CN (0.6 mL) into the reaction vial and stirred at 80 °C for 10 min. H_2O (1.5 mL) was added to the mixture, and it was filtered through a syringe filter before HPLC purification (Waters Atlantis Prep T3 C18 column, 250 × 10 mm, 5 μm, isocratic CH_3CN/H_2O/TFA, 50/50/0.1, v/v, 2.0 mL/min, t_R 15.5 min, λ 254 nm), obtaining 76 ± 4% collected ndc RCY, $n = 3$ (11% overall two step).

*(2S,3R)-2-(Cyclopentylmethyl)-N1-((R)-1-(2-(4-(4-[^{18}F]fluorophenyl)-1H-1,2,3-triazol-1-yl)ethylamino)-3,3-dimethyl-1-oxobutan-2-yl)-N4,3-dihydroxysuccinamide ([^{18}F]**6**)*

Compound **5** (2.2 mg, 5.31 μmol), sodium ascorbate (23 mg, 116.10 μmol), copper iodide (3.8 mg, 19.25 μmol), 2,6-lutidine (14 μL, 120.88 μmol) and H_2O (0.4 mL) were added to a reaction vial and warmed to 45 °C for 1 min, or until the solution turned pale yellow. [^{18}F]**2** was eluted from the C18 Strata X SPE cartridge with CH_3CN (0.6 mL) into the reaction vial and stirred at 45 °C for 10 min. H_2O (1.5 mL) was added to the mixture, that was then syringe filtered before HPLC purification (Waters XTerra Prep MS C18 column, 350 × 7.8 mm, 10 μm, isocratic CH_3CN/H_2O/TFA 35/65/0.1, v/v, 3.0 mL/min, t_R 14.5 min, λ 233 nm), obtaining 56 ± 12% collected ndc RCY, $n = 3$ (8% overall two step).

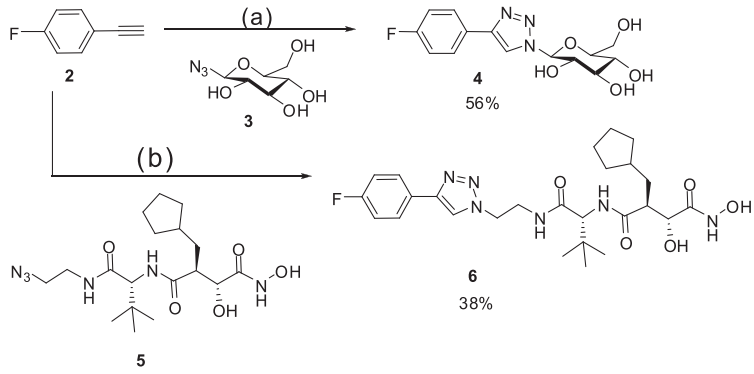
Purity, stability, and specific activity measurement

Radiochemical purity, stability, and specific activity measurements were determined by analytical HPLC QC (Supporting Information). The specific activity values were obtained by measurement of the radioactivity injected and the no-carrier added [^{19}F]UV absorbance associated with the corresponding [^{18}F]radioactive peak. The concentration was then obtained by comparison of the UV area under the curve to the non-radioactive standard concentration curve.

Results and discussion

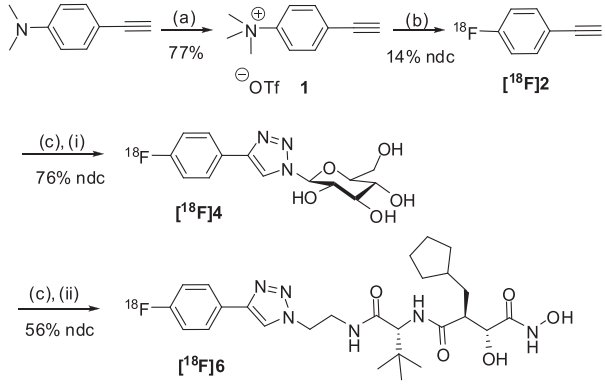
Chemistry

Synthesis of the non-radioactive standard **4** was achieved in a single step from commercially available starting materials. 1-Azido-1-deoxy-β-D-glucopyranoside **3** was dissolved in water and added to 1-ethynyl-4-fluorobenzene **2** in *n*-butanol. To this solution was added sodium ascorbate, 2,6-lutidine and copper (II) sulphate. The solution was stirred at room temperature for 16 h and then the crude reaction mixture purified directly by reverse phase flash chromatography to give the desired standard **4** in 56% yield (Scheme 1). The non-radioactive standard **6** was synthesized in a similar manner described previously, except precursor **5** was prepared via a nine-step synthesis (Supporting Information),[14]



Scheme 1. Synthesis of non-radioactive standards **4** and **6**, reagents: (a) **3**, or (b) **5**, $CuSO_4$, sodium ascorbate, 2,6-lutidine, n-butanol/H_2O 2/1, room temperature, 16 h.

and then purified by preparative HPLC to give the desired standard **6** in a 38% yield (Scheme 1). The synthesis of the 4-ethynyl-*N*,*N*,*N*-trimethylbenzenaminium trifluoromethanesulfonate radiolabeling precursor **1** was achieved as outlined in Scheme 2. 4-Ethynyl-*N*,*N*-dimethylaniline was dissolved in dichloromethane (CH_2Cl_2); methyl triflate was added, and the solution was stirred at room temperature for 2 h. The resulting precipitate was collected by filtration and washed with CH_2Cl_2 to give the desired radiolabeling precursor **1** in 77% yield.[15]



Scheme 2. Synthesis of 1, radiosynthesis of [^{18}F]**2** and click conjugated products [^{18}F]**4** and [^{18}F]**6**, reagents: (a) methyl triflate, CH_2Cl_2, room temperature, 2 h; (b) K^{18}F.K_{222} complex, DMF, 150 °C, 5 min; (c) (i) **3**, 80 °C, or (ii) **5**, 45 °C, sodium ascorbate, copper iodide, 2,6-lutidine, CH_3CN/H_2O 2/1, 10 min.

Radiochemistry

[^{18}F]**2** was synthesized under classical radiolabeling conditions by nucleophilic aromatic [^{18}F]fluorination, and subsequently was 'click' conjugated using a CuAAC reaction to **3** and **5** to produce [^{18}F]**4** and [^{18}F]**6,** respectively (Scheme 2).

Radiosynthesis of ethynyl-4-[^{18}F]fluorobenzene ([^{18}F]2)

Radiosynthesis of [^{18}F]**2** was performed with no-carrier-added [^{18}F]fluoride through the activated K^{18}F.K_{222} complex. Optimization of the radiosynthesis of [^{18}F]**2** (Figure 2) was performed by reacting **1** with K^{18}F.K_{222} complex under various reaction conditions including solvent (CH_3CN, DMF, and DMSO), temperature (100 and 150 °C), time (5, 10, and 15 min) and precursor quantity (10, 5, 2 mg).

All optimization experiments started with a precursor mass of 10 mg. At the specified time points, small aliquots (~100 μL) of the reaction mixture were analyzed by HPLC and the RCY compared (all radiochemical yields were calculated by collecting the HPLC product peak and measuring the amount of radioactivity collected). After the last time point, HPLC mobile phase was added to the remaining reaction mixture and shaken to aid washing the sides of the reaction vial, before the entire contents was also analyzed by HPLC and the RCY compared. Reaction conditions using DMF at 150 °C for 5 min was found to be optimal and was further developed to decrease the precursor **1** mass to 5 mg (14 ± 2% collected ndc RCY, $n = 3$).

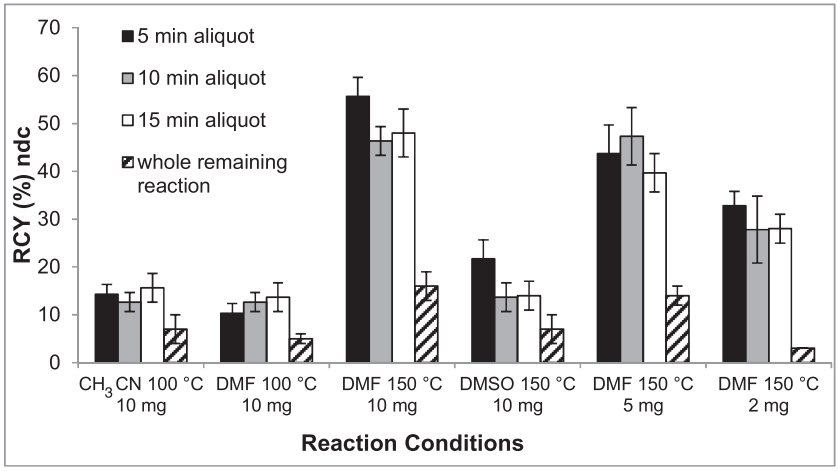


Figure 2. Summary of reaction yield optimization (ndc RCY), of synthon [^{18}F]**2**, based on aliquot samples (5–15 min) and total remaining reaction mixture by HPLC; the effects of varying solvent, temperature, time, and precursor mass, $n = 3$.

The decreased precursor amount resulted in lower yields based on the 5–15 min aliquot HPLC injections, however comparable yields based on the overall remaining reaction mixture, which was a more accurate representative of the reaction. Across the range of experimental conditions tested, the HPLC yields of the whole remaining reaction mixture were unsurprisingly much lower than the small aliquot injections, as losses from adherence of radiochemical products to the wall of the reaction vessel were accounted for by the washing step.

The discrepancy between the aliquot and whole remaining reaction yields may also be due to the loss of volatile [^{18}F]methylfluoride that was not apparent in the small aliquot HPLC injections. The possibility of [^{18}F]methylfluoride formation was anticipated, but the predicted high stability of [^{18}F]**2** due to the ethynyl functional group being only weakly activating was considered more favorable than a higher RCY that would likely be obtained from a more activated ring system (e.g., 2- or 4-substituted nitrobenzene or pyridine).[16]

The superiority of the trimethylammonium group is demonstrated with the use of less activating substituents, such as bromine or iodine (Hammett constant ~0.23), as opposed to more activating substituents such as cyanide or nitrogen dioxide (Hammett constant ~0.65 and ~0.8, respectively).[1] However, it has been reported that in the presence of less activating substituents with low Hammett constants (σ) *para* to the trimethylammonium leaving group, aliphatic substitution undesirably occurs on the $N(CH_3)_3$ group, and [^{18}F]methylfluoride formation competes with the desired aromatic [^{18}F]radiolabeling.[1] The Hammett constant σ of the ethynyl group 0.23, similar to that of other less activating substituents such as bromine or iodine[17] gives rise to low RCYs of only 12–15% due to the aforementioned competing reaction, hence the RCY obtained herein is within the expected range.[1]

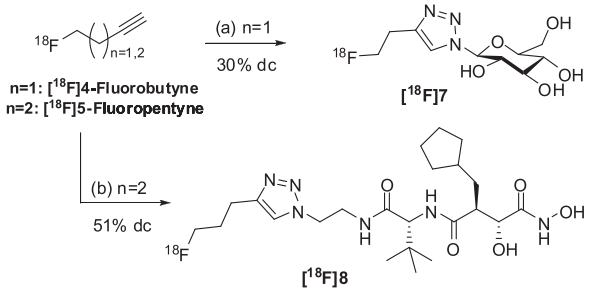
The suitability of the trimethylammonium triflate leaving group, as opposed to halogen groups, in the presence of the less activating ethynyl substituent was also assessed using the non-radioactive standard **2** to synthesize [^{18}F]**2** via ^{19}F/^{18}F halogen exchange. Using the same reaction conditions as before (DMF, 150 °C, 5 mg, 5 min), the radiosynthesis of [^{18}F]**2** was successful, however in a lower yield (RCY 3% ndc) compared to the trimethylammonium precursor **1** (RCY 14 ± 2% ndc). These results demonstrated that other less activating halogens (bromine, chlorine, or iodine) would most likely have not been suitable and concurs with the literature that the trimethylammonium leaving group is superior to halides, particularly in the presence of less activating substituents such as the ethynyl group in this case.[1,18]

In an attempt to circumvent time-consuming purification by HPLC, SPE (phenomenex C18 Strata™ X, Waters C18 Sep-Pak® (Plus & Lite), and Polyintell Affinimip®) was also investigated for ease and to decrease total synthesis time. However, the chemical and radiochemical purity obtained from the various SPE attempts were not satisfactory for general use and for the subsequent click conjugation, hence HPLC purification was utilized. Following HPLC purification, [^{18}F]**2** was transferred to a C18 Strata™ X SPE cartridge for concentration and solvent exchange required for subsequent elution into the click conjugation reaction.

Radiosynthesis of [^{18}F]4 and [^{18}F]6

To investigate the utility of synthon [^{18}F]**2**, it was first decided, as a proof of concept, to click conjugate it to a commercially available azido-functionalized carbohydrate **3**, producing [^{18}F]**4**. This could then be directly compared to a similar literature radiolabeling[19] where **3** was click conjugated to synthon [^{18}F]4-fluorobutyne to give [^{18}F]**7**, which was prepared in a three-step reaction in a 30% dc RCY (Scheme 3). [^{18}F]4-Fluorobutyne was reported not isolated because of its volatility and was used for conjugation immediately without purification. Hence, the RCY could not be compared directly to that of [^{18}F]**2**; however, [^{18}F]**2** required a shorter synthesis time, fewer synthetic steps, and was able to be isolated because of its higher boiling point. The subsequent click conjugation of [^{18}F]**2** and **3** was performed under similar conditions to those described in the literature (copper(I) iodide, sodium ascorbate, and 2,6-lutidine in CH_3CN/H_2O)[19] to produce [^{18}F]**4** in 76 ± 4% ndc RCY (11% overall two step). The main advantage of [^{18}F]**2** over [^{18}F]4-fluorobutyne was the elimination of a time-consuming and difficult distillation. Instead, [^{18}F]**2** was purified by HPLC, then easily trapped and eluted from a Strata X SPE cartridge for the subsequent conjugation step, resulting in a comparable overall RCY to that of [^{18}F]-4-fluorobutyne.

The optimized conditions for the radiolabeled carbohydrate [^{18}F]**4**, the procedure described by Kim *et al.*[19] for the radiosynthesis of [^{18}F]**7** and for [^{18}F]**8** described by Doan *et al.*[14] (Scheme 3) provided the starting point for the click conjugation of [^{18}F]**2** to the matrix metalloproteinase inhibitor **5**. Similar reaction conditions (copper iodide, sodium ascorbate, 2,6-lutidine in CH_3CN/H_2O for 10 min), except a lower reaction temperature (45 °C) and an alternate base diisopropylethylamine (DIPEA), were utilized for radiolabeling the matrix metalloproteinase inhibitor **5** to produce [^{18}F]**6**. The initial HPLC purification conditions employed were those described by Doan *et al.* for the purification of the alkyl-analog [^{18}F]**8** [14]; however, in our hands, the basic mobile phase conditions resulted in [^{18}F]**6** largely streaking and broadening over an extended period of time. This made collecting [^{18}F]**6** in a concentrated volume difficult and the losses on the HPLC column led to low RCYs (~5–10% ndc). It is difficult to explain this occurrence because the radiosynthons are similar in structure with the only difference being a small aliphatic chain ([^{18}F]**8**) versus an aromatic ring ([^{18}F]**6**). Nevertheless, optimization of the HPLC purification conditions showed that the use of an acidic mobile phase corrected the product peak broadening issue, enabling [^{18}F]**6** to be collected as one sharp peak over a short timeframe. This significantly improved the RCY (56 ± 12% ndc) (8% overall two step) such that it was comparable to that obtained by Doan *et al*. for [^{18}F]**8** (51% dc RCY).



Scheme 3. Radiosynthesis of literature click conjugated products [^{18}F]**7** and [^{18}F]**8**, reagents: (a) [^{18}F]-4-fluorobutyne, **3**, sodium ascorbate, copper iodide, 2,6-lutidine, CH_3CN/H_2O, 1/1, 90 °C, 10 min; (b) **5**, [^{18}F]-5-fluoropentyne 45 °C, sodium ascorbate, copper iodide, DIPEA, CH_3CN/H_2O 1/1, 10 min.

Purity, stability, and specific activity

The purity and stability of synthon [^{18}F]**2** was analyzed by HPLC QC at time points $t = 0$ and 180 min. No significant decrease in radiochemical purity was observed (99.4 to 99.3%) over 180 min. The radiochemical purity of the radiolabeled products [^{18}F]**4** and [^{18}F]**6** at was found to be 99.0 and >99.9%, respectively. Chemical purity determined by UV analysis of [^{18}F]**4** and [^{18}F]**6** revealed absence of the starting azide or reaction side products.

The specific activities of [^{18}F]**2** and [^{18}F]**6** in our initial conjugation attempts were low, 5.97 and 0.17 MBq nmol^{-1}, respectively; however, this is consistent with previously reported literature for a similar synthon.[13] The low specific activity may be due to the purity of the radiolabeling precursor **1** being 96.25%, increased purity of **1** may have resulted in higher specific activity. Further investigation into the development of higher specific activity conjugated product was not a priority for this preliminary work, given the synthon had higher potential applications in conjugation to large biomolecules, such as proteins, where the effective specific activity is predominantly based on the quantity of protein used. Detailed preparative and analytical (QC) HPLC conditions and chromatographs are available in Supporting Information.

Conclusions

A new radiolabeled synthon ethynyl-4-[^{18}F]fluorobenzene, [^{18}F]EYFB, [^{18}F]**2**, was prepared in a single step (14 ± 2% ndc RCY) from a shelf stable, inexpensive precursor. The precursor 4-ethynyl-*N*,*N*,*N*-trimethylbenzenaminium trifluoromethanesulfonate **1** was prepared in a single step from a commercially available aniline and is isolated by simple filtration. Two proof of concept CuAAC experiments including the indirect [^{18}F]radiolabeling of an azido-functionalized carbohydrate **3** ([^{18}F]**4**, 76 ± 4% ndc RCY (11% overall two step)) and an azido-functionalized matrix metalloproteinase inhibitor **5** ([^{18}F]**6**, 56 ± 12% ndc RCY (8% overall two step)) showed the conjugation of [^{18}F]**2** can be performed in the presence of primary and secondary hydroxyl groups, hydroxamic acids, and amide functionalities. As a synthon, [^{18}F]**2** has the advantage of bearing an ammonium cation leaving group for the potential of straight forward SPE purification, an aromatic ring for UV detection, an alkyne functionality for both leaving group activation and click conjugation, as well as an aromatic non-electron deficient C-^{18}F bond, which are known to have increased stability *in vivo*. Given this range of desirable characteristics, [^{18}F]**2** is a promising new [^{18}F]synthon and with further optimisation may be useful for radiolabeling small and large molecules for PET radiotracer/radiopharmaceutical development. We are currently utilizing and evaluating [^{18}F]**2** for indirect [^{18}F]radiolabeling of several biological vectors and the results from these studies will be reported in due course.

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Supporting information

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