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2-Fluoropyridine prosthetic compounds for the ^{18}F labeling of bombesin analogues

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ABSTRACT

Acetylene-bearing 2-[^{18}F]fluoropyridines [^{18}F]FPy5yne and PEG-[^{18}F]FPyKYNE were prepared via efficient nucleophilic heteroaromatic [^{18}F]fluorination of their corresponding 2-trimethylammoniumpyrdinyl precursors. The prosthetic groups were conjugated to azide- and PEG_3-modified bombesin(6–14) analogues via copper-catalyzed azide–alkyne cycloaddition couplings to yield mono- and di-mini-PEGylated ligands for PET imaging of the gastrin- releasing peptide receptor. The PEG_3- and PEG_2/PEG_3-bearing ^{18}F peptides showed decreased lipophilicity relative to an analogous non-mini-PEGylated ^{18}F peptide. Assessment of water-soluble peptide pharmacokinetics and tumour-targeting capabilities in a mouse model of prostate cancer is currently underway.

Positron emission tomography (PET) is a nuclear diagnostic technique in which radiolabeled probes are used to non-invasively image disease-associated molecular targets in vivo. ^{18}F ($t_{1/2}$ = 109.8 min) is widely considered the optimal radioisotope for PET;[1] however, the challenges associated with the synthesis of ^{18}F tracers are numerous, particularly when the targeting agent is a sensitive, functionally complex biomolecule. The basic, anhydrous, high temperature reaction conditions typically required to label with [^{18}F]F$^-$ usually necessitates the use of a small molecule prosthetic group, which is radio-synthesized prior to bioconjugation (i.e., pre-labeling). We previously described the ^{18}F-labeling of model azide-modified peptide[2] and oligonucleotide[3] sequences with prosthetic [^{18}F]FPy5yne ([^{18}F]-**1**, Fig. 1) by way of an efficient Cu^I-catalyzed azide–alkyne cycloaddition (CuAAC)[4,5] reaction. This chemoselective variant of the Huisgen 1,3-dipolar cycloaddition reaction has become synonymous with the 'click chemistry' concept,[6] owing to its facility, regioselectivity, and tolerance of water. Marik and Sutcliffe were the first to utilize CuAAC chemistry for the preparation of ^{18}F peptides.[7] Since then, a number of [^{18}F]fluoroaromatic[8,9] and [^{18}F]fluoroaliphatic[10–14] prosthetic groups bearing azide or acetylene moieties have been developed for this purpose. The primary technical advantage of [^{19}F]-**1**- and closely related analogue [^{18}F]FPyKYNE[15] ([^{18}F]-**2**)- relative to many other 'click'-able ^{18}F prosthetic groups lies in the outstanding capacity of the 2-substituted pyridine system to incorporate [^{18}F]F$^-$ by way of nucleophilic heteroaromatic substitution.[16,17] Furthermore, [^{18}F]-**1** and [^{18}F]-**2** can be prepared in a single radiochemical step and, like other *ortho*-[^{18}F]fluoropyridines,[18] are likely resistant to in vivo defluorination.

Bombesin (BBN)[19] is an amphibious 14- amino acid analogue of the gastrin-releasing peptide (GRP). BBN shares a homologous C terminus with GRP [Trp-Ala-Val-Gly-His-Leu-Met(NH_2)], and the two peptides display similar biologic function.[20] As such, bombesin and bombesin fragments show high affinity for the gastrin-releasing peptide receptor (GRPr).[4] GRPr (otherwise known as BB2r) is one of four receptor subtypes which make up the bombesin receptor superfamily (BB1r–BB4r)[21] and is distributed predominantly throughout the mammalian brain and gut. Apart from a host of normal physiological functions, GRP acts as a mitogen, morphogen, and proangiogenic factor of receptive tissues,[22] and as such GRP receptors are found overexpressed in a variety of cancer types.[23] In particular, Reubi and co-workers found GRPr expressed in 65% of a total of 77 excised breast tumors[24] as well as 100% (30:30) of a population of invasive prostatic neoplasms.[25]

Early attempts at exploiting the BBN/GRPr ligand/receptor pair for molecular SPECT imaging utilized [^{125}I]Tyr-modified BBN analogues.[26,27] A BBN(7–13) bioconjugate labeled with an

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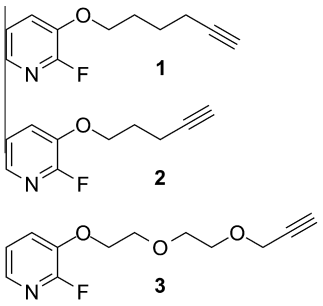


Figure 1. Acetylene-bearing 2-[^{18}F]fluoropyridines discussed in this work. F = ^{19}F or ^{18}F.

^{125}I-*m*-iodobenzoate functionality was introduced with increased stability and improved uptake into ovarian tumors relative to [^{125}I]Tyr^4-bombesin.[28] A variety of BBN derivatives have been labeled with ^{99m}Tc for SPECT,[29–34] with attempted optimizations focusing on the diminution of the hepatobiliary clearance that is common to ^{99m}Tc-labeled peptides. For PET, a sizable number of ^{64}Cu- labeled BBN analogues have been introduced. Most derivatives reported thus far have employed DOTA as the chelating agent,[35–38] although the observed decupration of this complex in vivo has prompted the investigation of alternative chelates.[39,40] Recently, the synthesis and pre-clinical evaluation of some ^{64}Cu-NOTA-bearing BBN antagonist ligands was described.[41] In addition, DOTA-modified [β-Ala^{11}, Thi^{13}, Nle^{14}]BBN(6–14) peptide has been labeled with ^{68}Ga (^{68}Ga-BZH3)[42] and evaluated for the delineation of gastrointestinal stromal tumors in humans.[43] Of interest to this work (vide infra), the chelator was tethered to the targeting agent by way of a PEG_2 chain.

An ^{18}F BBN derivative was first prepared through the use of acylating prosthetic *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB); tumour visualization was achieved in small animal PET experiments, but uptake was moderate and the clearance occurred primarily through the undesirable hepatobiliary route.[44] Subsequently, BBN–RGD heterodimers were labeled with [^{18}F]SFB and used to prove the viability of multi-peptide receptor PET imaging.[45] Schirrmacher et al. prepared a formylated silicon-fluoride acceptor (SiFA)-based prosthetic [*p*-(di-*tert*-butyl[^{18}F]fluorosilyl)benzaldehyde] and coupled it to a *N*-aminooxy-derivatized BBN analogue.[46] Finally, a dibenzocyclooctyne moiety was conjugated to [Lys^3]BBN to serve as reactive dipolarophile for the copper-free CuAAC coupling of a series of azide- bearing ^{18}F prosthetics (including N_3-$(CH_2)_4$-PEG_3-[^{18}F]F).[47]

Apart from pre-labeling approaches, the relatively robust nature of many modified BBN fragments has permitted the exploitation of direct ^{18}F-labeling technologies. Trimethylammonium triflate (TMA)-bearing benzonitrile pendant moieties have been used to ^{18}F-label BBN-based precursors. Additional lysine[48] and L-cysteric acid[49,50] residues were introduced in an attempt to diminish peptide lipophilicity and thus favorably modulate tracer biodistribution. In addition, BBN derivatives modified with SiFA (di-*tert*-butylarylsilyl) moieties were directly ^{18}F-labeled in acidic DMSO solutions.[51] The resulting di-*tert*-butyl[^{18}F]fluorosilyl group is stable in PBS and plasma over 2 h. An ^{18}F BBN analogue of this type (bearing an isolated lysine residue to improve water-solubility) showed poor and non-specific uptake into PC3 tumor xenografts but high and specific uptake into GPRr-rich murine pancreatic tissue. Notably, the log *D* value of the peptide was high (1.3 ± 0.1, $n = 5$), which suggests that the ligand was still rather lipophilic. The primary advantage of these post-labeling technologies lies in their remarkable radiosynthetic simplicity. However, it should be noted that both employ traditional $K_{2.2.2.}$/M[^{18}F]F/M_2CO_3 conditions at elevated temperatures, and thus do not represent general approaches for the direct ^{18}F-labeling of biological molecules.

The following work describes the synthesis of [^{18}F]-**1**- labeled BBN analogues with the potential to be used for the detection and assessment of GRPr-expressing lesions with PET. In addition, straightforward protocols for the radiosynthesis and CuAAC conjugation of a hydrosoluble derivative of [^{18}F]-**1** and [^{18}F]-**2** (PEG-[^{18}F]FPyKYNE; [^{18}F]-**3**)[52] are described here in detail for the first time. These methods will be of general interest to those seeking to ^{18}F-label azide-modified biomolecules.

The potential GRPr-targeting vectors utilized were based on BBN(6–14) [Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met(NH_2)] (Fig. 2). Truncation at the N-terminus of native bombesin yields a less potent peptide than the native form, but additional modifications can restore binding affinity for GRPr.[53] For this work, the following alterations relative to bombesin were introduced: D-Tyr^6, βAla^{11}, Thi^{13} and Nle^{14}. It is assumed that these changes also serve to enhance the stability of the peptide in vivo. We prepared this sequence on a continuous flow peptide synthesizer using standard Fmoc chemistry (see Supplementary data). An N_3–$CH_2C(O)$-residue was introduced to the terminus for CuAAC conjugation with acetylene-bearing pendant groups (**N_3-BBN**; Fig. 2). ^{19}F standard **1** and the radiosynthetic precursor of [^{18}F]-**1** (**4**; see Scheme 2) were prepared via separate syntheses as reported previously.[2] The coupling of **N_3-BBN** and **1** under straightforward CuAAC conditions [$Cu(CH_3CN)_4PF_6$, TBTA, DMSO, 1.5–2 h] yielded ^{19}F peptide standard **F-ALK-BBN** (see Supplementary data).

We predicted that radioactive **[^{18}F]F-ALK-BBN** might exhibit unfavourable pharmacokinetic behaviour in vivo due to its high lipophilicity. Polyethylene glycol (PEG) spacers can serve as a means to enhance the hydrophilicity of peptide-based molecular imaging agents which might otherwise undergo undesirable hepatobiliary clearance as a result of chemical modification (e.g., truncation, amino acid replacement, and/or introduction of pendant groups). Thus alternative water-soluble ^{18}F analogues of BBN(6–14) were envisioned in which mini-PEG moieties could be introduced both through further modification of the N_3 peptide precursor, as well as through conjugation to a mini-PEGylated ^{18}F prosthetic. The latter strategy offers an opportunity to introduce two beneficial characteristics at the same time (i.e., ^{18}F and hydrophilicity). This will be of general utility when a potential targeting agent cannot be easily modified- or exhibits diminished bioactivity- with two pendant groups. To this end, we explored the use of a '2nd generation' [^{18}F]FPy5yne derivative (PEG-[^{18}F]FPyKYNE; [^{18}F]-**3**; Fig. 1), which bears a PEG_2 spacer between its [^{18}F]fluorinated and conjugating functionalities. An abstract reporting the radiosynthesis of [^{18}F]-**3** was reported in 2009.[52] The authors ^{18}F-labeled 2-bromo and 2-nitro precursors in radiochemical yields of ⩽50% by radio-TLC (165 °C, 5 min). The TMA moiety is the premier leaving group for the K[^{18}F]/$K_{2.2.2.}$ fluorination of homo- and hetero- aromatic systems, both in terms of [^{18}F]F^- incorporation[16] and ease of precursor removal.[54] Therefore, we agreed with the original inventors of [^{18}F]-**3** that TMA- bearing precursor **5** (Scheme 1) might offer a superior route to this ^{18}F prosthesis.

The synthesis of precursor **5** and non-radioactive PEG-FPyKYNE (**3**) is shown in Scheme 1. Mitsunobu coupling of 2-fluoro-3-hydroxypyridine[55] (**6**) and alcohol **7** was used to afford ^{19}F standard **3**. Then, a nucleophilic 2-F for 2-NMe_2 substitution under aqueous conditions was employed to furnish 2-dimethylaminopyridine **8** from **3**. This approach obviated the need to synthesize 2-dimethylamino-3-hydroxypyridine, which we have yet to prepare in yields higher than 52%.[2,15] Finally, the mixing of **8** with one equivalent of methyl triflate yielded PEG-NMe_3-KYNE (**5**) precursor salt.

PEG_3-bearing **N_3-BBN** derivative **N_3-BBN-PEG** was prepared by way of similar solid-phase synthetic techniques (see Supplementary data). In addition to the azide functionality, a pendant 15-amino-4,7,10,13-tetraoxapentadecaamide moiety was affixed

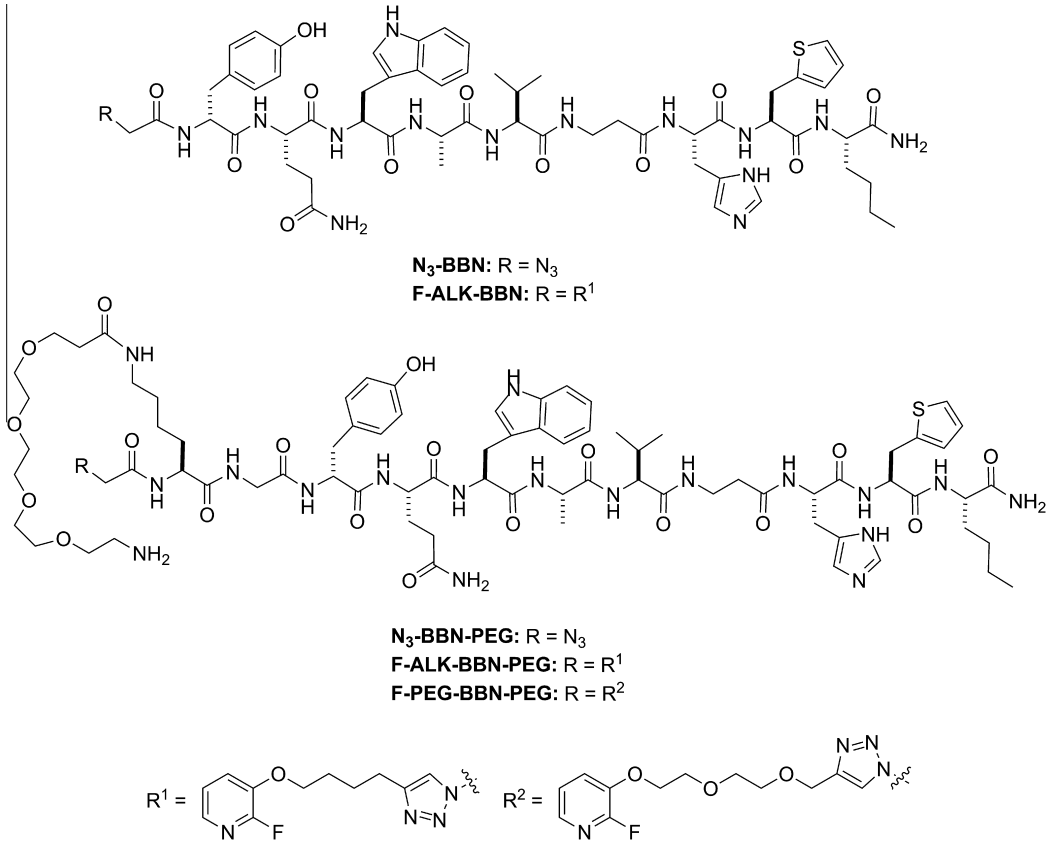


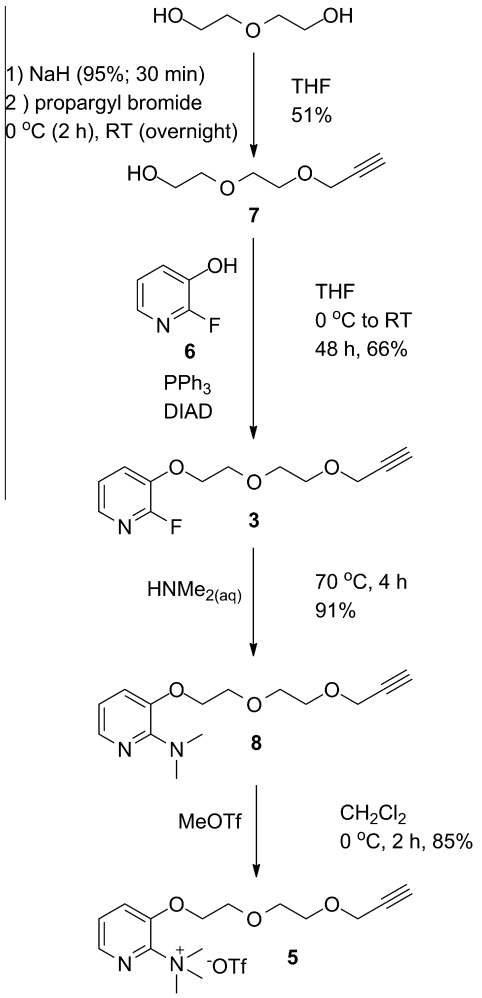
Figure 2. Peptides referenced in this work. F = ^{19}F and ^{18}F.

to an extraneous Lys residue using EDC-promoted acylation chemistry. Subsequent CuAAC coupling of the free peptide with **1** and **3** yielded non-radioactive peptide standards **F-ALK-BBN-PEG** and **F-PEG-BBN-PEG** respectively (see Supplementary data). Only a small difference in retention time was observed between **F-PEG-BBN-PEG** and **N$_3$-BBN-PEG** upon semi-preparative HPLC separation (ΔR_t = 0.3 min). Nevertheless, **F-PEG-BBN-PEG** could be reliably obtained in chemically pure form after careful HPLC purification as determined by HPLC and MALDI-TOF.

Radiosyntheses of [^{18}F]FPy5yne ([^{18}F]-**1**) followed a protocol that was introduced[2] and later improved[3] by us previously. Efficient incorporation of anhydrous [^{18}F]F$^-$ was achieved by way of a nucleophilic heteroaromatic substitution reaction from a 2-TMA triflate precursor (**4**) in DMSO (0.5 mL), in the presence of K_2CO_3 and $K_{2.2.2.}$ phase transfer catalyst at 110 °C (Scheme 2). We have now further optimized the isolation of [^{18}F]-**1**. First, it was established that extraction of [^{18}F]-**1** from DMSO reaction solvent (0.5 mL) was not required prior to HPLC purification. Instead, the reaction mixture was diluted with an equal amount of water and injected directly onto a semi-preparative HPLC column. Under these conditions, successful HPLC separations could only be achieved if the reaction mixture was transferred from the original vessel to another one before dilution with H_2O. We hypothesize that this step serves to remove any basic material ($K_{2.2.2.}/K_2CO_3$/[$^{18/19}$F]F$^-$) affixed to the reaction vessel, which would otherwise be completely solubilized if placed in contact with water. Second, after HPLC purification and immobilization of [^{18}F]-**1** onto a tC_{18} solid-phase extraction (SPE) cartridge (Sep-pak®, Waters), the radio-compound was eluted directly into a microcentrifuge tube containing N_3-peptide precursor using bioconjugation solvent (DMF or DMSO). In this fashion, losses associated with the volatilization of [^{18}F]-**1** during evaporation of HPLC eluent were obviated. Average decay corrected (DC) yield of [^{18}F]-**1** was 47 ± 6% (n = 5) from start-of-synthesis.

The reaction conditions used to prepare PEG-[^{18}F]FPyKYNE ([^{18}F]-**3**) were identical to those used for synthesis of [^{18}F]-**1** (Scheme 4). Incorporation of [^{18}F]F$^-$ during three separate experiments was achieved in yields of 80 ± 10% (3 traces from 3 experiments, n = 9 total), as determined by the gamma counting of fractionated radioTLC plates. HPLC purification of [^{18}F]-**3** was straightforward and collected yields were satisfactory and reproducible (45–64% DC, n = 4). However, in contrast to [^{18}F]-**1**, which could be efficiently extracted from HPLC eluent using tC_{18} SPE sorbent after dilution with 50 mL of water, [^{18}F]-**3** was not well-suited for this approach. The decay- corrected trapping efficiency was 27–42% (n = 4 experiments) when a small ('light') tC_{18} SPE cartridge was used.[a] Not surprisingly, final preparative radiochemical yields of [^{18}F]-**3** were only 16 ± 2% DC (n = 4). However, the efficiency of the extraction step was significantly improved by employing two full-size ('Plus') tC_{18} SPE columns. The radio-compound was eluted from the SPE cartridge with MeOH, then the solvent was evaporated (90 °C) under a stream of He (73 ± 2% efficiency, DC, n = 3). It is worth noting that this approach would likely not be possible for the radiosynthesis of [^{18}F]-**1** owing to its low boiling point (69–70 °C).[2] Solvent removal by evaporation resulted in longer protocol times, but higher collected yields (39 ± 9% DC, n = 4) versus tC_{18} 'light' extraction. An attempt to concentrate the solvent mixture at 110 °C resulted in significantly more product loss of [^{18}F]-**3** by

[a] Trapping the ^{18}F prosthetic molecule on a 'light' SPE column allowed for elution with a small volume of organic solvent (0.3 mL). In this way, direct elution into the bioconjugate reaction mixture was possible, without the need for a secondary concentration step.



Scheme 1. Synthesis of the radiosynthetic precursor (**5**; PEG-NMe_3-KYNE) to PEG-[^{18}F]FPyKYNE and its ^{19}F standard (**3**).

evaporation (41% efficiency, DC). Measured apparent specific activity at end-of-synthesis was 180 ± 9 GBq/μmol (4.9 ± 0.3 Ci/μmol; n = 3).

Earlier syntheses of ^{18}**F-ALK-BBN** focused on shortening and simplifying the general protocol for the preparation of [^{18}F]-**1**- labeled peptides as reported in Inkster et al.[2] In the aforementioned report, HPLC-purified ^{18}F peptide was concentrated by azeotropic distillation of the collected HPLC eluent (4–8 mL). However, this method typically takes between 25 and 40 min and does not assure complete removal of trifluoroacetic acid (TFA) from the final formulation. Thus, this drydown step was avoided in favor of immobilization of the ^{18}F peptide on tC_{18} sorbent (Scheme 2). Ideally the tracer could then be eluted from the SPE column with a solvent amendable for pre-clinical use. The choice of final formulation demanded significant attention because **[^{18}F]F-ALK-BBN** is only sparingly soluble in aqueous solutions. Benzoic acid (10%) in PBS resulted in acceptable levels of solvation (81% DC), but a signal associated with BzOH co-eluted with **[^{18}F]F-ALK-BBN**, thus convoluting HPLC assay of the final formulation. A summary of solvent systems tested can be found in the Supplementary data. In the end, a 9:1 mixture of (2-hydroxypropyl)-β-cyclodextrin[56,57] solution (10% HPβCD in PBS) and DMSO was employed (88% solvated, DC). The average preparative bioconjugate yield of **[^{18}F]F-ALK-BBN** was 34±17% DC (n = 8).

[^{18}F]F-ALK-BBN-PEG was synthesized in a manner similar to the synthesis of **[^{18}F]F-ALK-BBN** (Scheme 3). However, three important improvements were introduced. First, inclusion of zsodium ascorbate to the reaction mixture (15 equiv. relative to peptide) was accompanied by a marked increase in radiochemical yield as observed by HPLC, such that complete consumption of [^{18}F]-**1** starting material could be achieved under 30 min at room temperature. Second, the removal of the ^{18}F-labeled peptide from HPLC eluent was achieved by way of tC_{18} 'light' trapping, rather than evaporation of MeCN/0.1% TFA at 80 °C. Apart from improved radiosynthetic efficiency, the obviation of this evaporative step contributes to the general utility of this procedure because many biomolecules are sensitive to excessive heating. Third, as **[^{18}F]F-ALK-BBN-PEG** is soluble in most aqueous mixtures, this radiotracer could be formulated in 10% EtOH in isotonic saline, which is a well-established solvent system for peptide-based PET ligands. Average collected yield after bioconjugate synthesis was 31 ± 19% DC (n = 11). The apparent specific activity of **[^{18}F]F-ALK-BBN-PEG** in four separate experiments was found to be 42 ± 25 GBq/μmol (1.2 ± 0.7 Ci/μmol) at end-of-synthesis.[b]

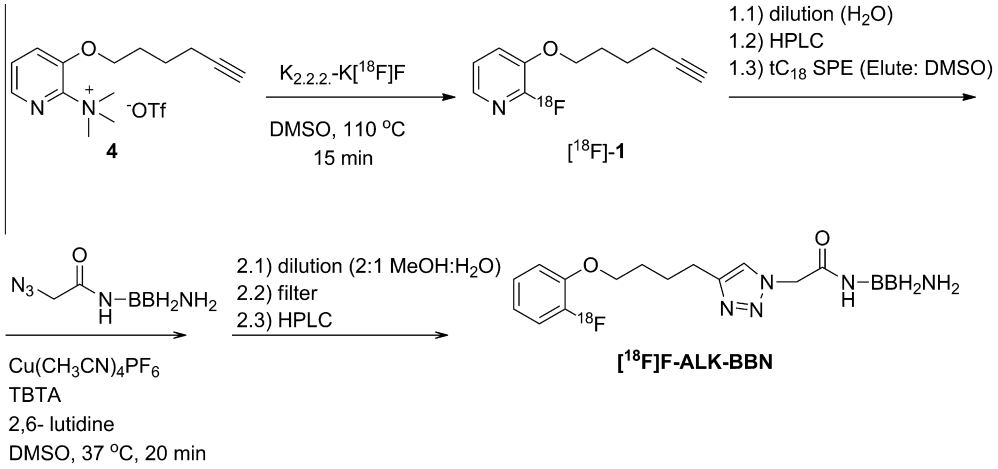
Based on our observations during the HPLC purification of non-radioactive **F-PEG-BBN-PEG**, it was expected that the clean separation of **N_3-BBN-PEG** and **[^{18}F]F-PEG-BBN-PEG** would be challenging. In a bid to minimize chemical and radiochemical impurities, straightforward CuAAC conditions were employed [$Cu(CH_3CN)_4PF_6$/TBTA, RT, DMSO, 30 min; Scheme 4]. Under these conditions, the radio-bioconjugation did not proceed to completion. Nevertheless, the collected yield from start of bioconjugation was 25 ± 13% DC (n = 4), and tracer was obtained in amounts amendable for in vivo study.[c] The apparent specific activity of **[^{18}F]F-PEG-BBN-PEG** at end-of-synthesis was found to be 78 ± 38 GBq/μmol (2.1 ± 1 Ci/μmol; n = 7).

During HPLC purification of **[^{18}F]F-PEG-BBN-PEG**, a radio-impurity was consistently observed that was notable in terms of amount (7–17% of total radioactivity) and polarity (Fig. 3, R_t = 12.3 min). In an effort to establish the nature of this chemical species, small molecule prosthetic group [^{18}F]-**3** was synthesized and incubated in a similar CuAAC reaction mixture, but without N_3 peptide (Fig. 4). The radio-impurity was observed in this case as well, although the percent yield by HPLC was smaller than expected (4%). It is hypothesized that this radio-peak represents Cu-chelated [^{18}F]-**3**. The variability in observed yield during ^{18}F peptide preparations may be the result of small but significant differences in free copper in each reaction mixture. Alternatively, the specific activity of [^{18}F]-**3** could be a factor, as the radiochemical yield of any association between Cu and ^{18}F small molecule should be negatively correlated with the amount of non-radioactive chelator present. Furthermore, a dependence on high specific activity conditions does much to explain why this species does not form in detectible quantities during non-radioactive syntheses of **F-PEG-BBN-PEG**.

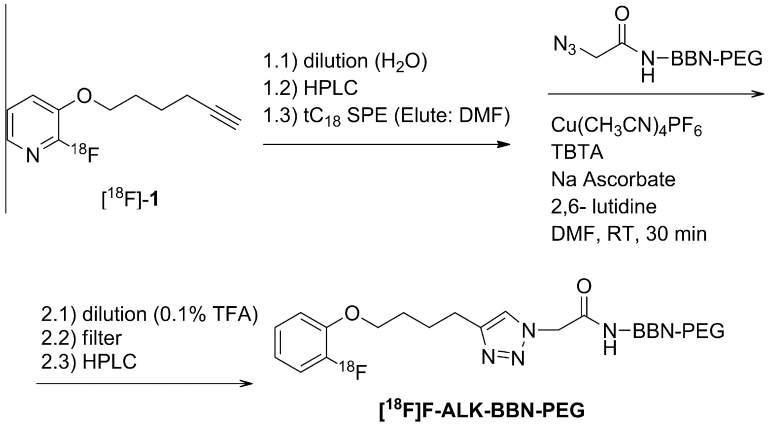
The lipophilicity of **[^{18}F]F-ALK-BBN** was assessed relative to its mono- and di-mini-PEGylated derivatives (**[^{18}F]F-ALK-BBN-PEG** and **[^{18}F]F-PEG-BBN-PEG**) by measuring their coefficients of distribution into equal parts octanol and sodium phosphate buffer (20 mM, pH 7.4). As expected, $\log D_{[7.4]}$ values decreased with an increase in ethylene oxide groups in the targeting vectors (1.40 ± 0.01, −0.92 ± 0.03 and −1.12 ± 0.01 respectively, n = 4). The relatively large $\log D_{[7.4]}$ found for **[^{18}F]F-ALK-BBN** (1.40 ± 0.01) is consistent with the qualitative observation that this modified peptide is not soluble in purely aqueous solutions. The difference in $\log D$ values between **[^{18}F]F-ALK-BBN-PEG** and **[^{18}F]F-PEG-BBN-PEG** (0.20) is similar to the observed shift in $\log D$

[b] The specific activities were determined to be 14, 39, 43, and 74 GBq/μmol. The relationship between these values and murine PET results (PC3 lesions) will be detailed in a later manuscript.

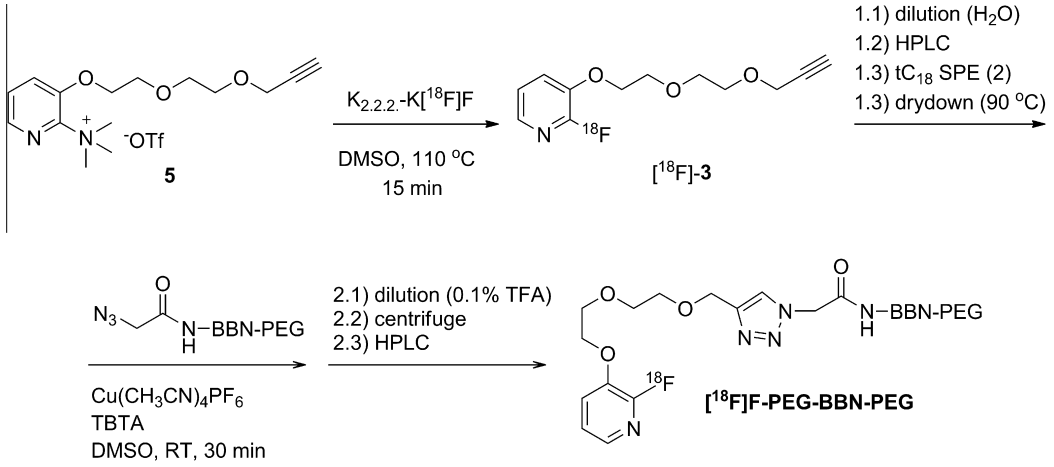
[c] A maximum of 19.9 mCi **[^{18}F]F-PEG-BBN-PEG** was prepared starting from a single batch of [^{18}F]F$^-$.



Scheme 2. Radiosynthesis of [^{18}F]-**1** and **[^{18}F]F-ALK-BBN-PEG**.



Scheme 3. Radiosynthesis of **[^{18}F]F-ALK-BBN-PEG**. [^{18}F]-**1** was prepared as in Scheme 2.



Scheme 4. Radiosynthesis of [^{18}F]-**3** and **[^{18}F]F-PEG-BBN-PEG**.

between two [^{18}F]SFB-modified $c(RGD)_2$ peptides prepared with and without PEG_2 tethering groups (0.18).[58]

The straightforward preparation of [^{18}F]-**1** as described here is a clear improvement on earlier protocols.[2,3] In particular, it was determined that [^{18}F]-**1** need not be extracted out of the reaction solvent prior to HPLC purification, and that the removal of HPLC eluent could be achieved by SPE extraction rather than evaporation. When compared with other pre-labeled ^{18}F prostheses in use today, the simplicity of this approach stands out; indeed, only two radiochemical transformations and two SPE concentration steps are required to obtain the final radio-bioconjugate. On the other hand, the need for two HPLC purifications to obtain ^{18}F peptide of high specific activity remains a significant downside to the use of [^{18}F]FPy5yne. However, others in our group have

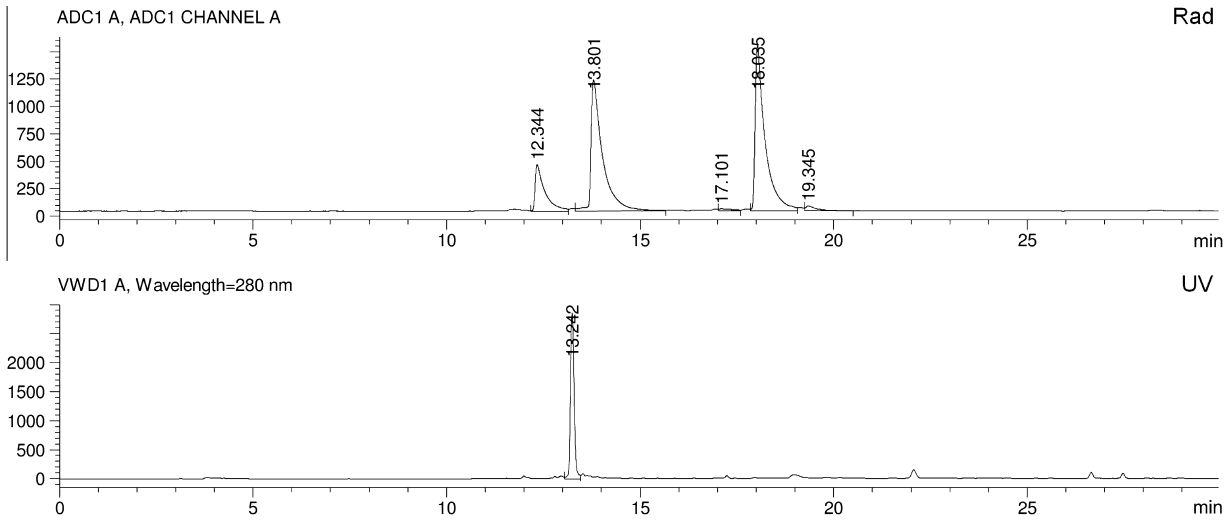


Figure 3. Preparative HPLC of **[^{18}F]F-PEG-BBN-PEG** synthesis. Top: radioactive detection (arbitrary units). Bottom: UV, 280 nm (absorbance units). R_t of **N_3-BBN-PEG** = 13.2 min. R_t of **[^{18}F]F-PEG-BBN-PEG** = 13.8 min. R_t of [^{18}F]-**3** = 18.1 min. Note the radiochemical impurity at 12.3 min. HPLC System: 7 (see Supplementary data).

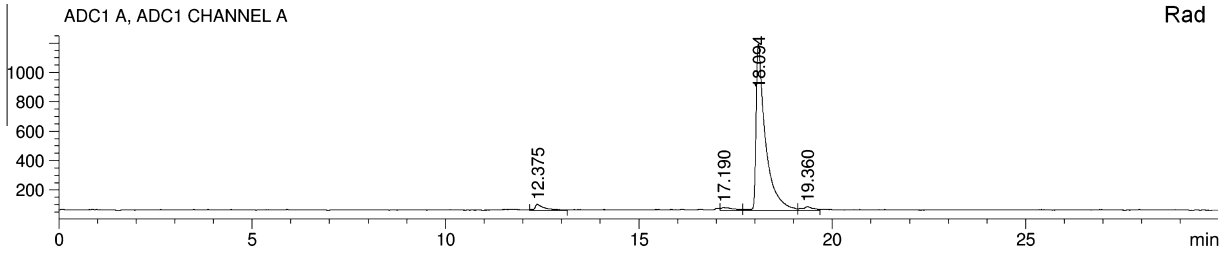


Figure 4. Radiotrace of purified [^{18}F]-**3** (R_t = 18.1 min) after incubation in $Cu(CH_3CN)_4PF_6$/TBTA in DMSO (RT, 30 min). Note the radiochemical impurity at 12.4 min. HPLC System: 7 (see Supplementary data).

recently established a protocol whereby [^{18}F]**-1** can be efficiently separated from precursor **4** and other interfering species using only C_{18} SPE methods.[59] This is possible because unusually small amounts of precursor are used during radiosyntheses (100 nmol). It is the excellent capacity of 2-substituted pyridines to incorporate [^{18}F]F^- that makes this approach viable.

Potential GRPr-based tumour-targeting agent **[^{18}F]F-ALK-BBN** was produced in preparative yields amendable for in vivo study via CuAAC coupling to [^{18}F]-**1**, but this peptide required a non-standard solvent system for formulation (1:1 10% HPβCD in PBS:DMSO). However, efforts to ^{18}F- label mini-PEGylated precursor peptide **N_3-BBN-PEG** with [^{18}F]-**1** resulted in a water- soluble tracer of enhanced hydrophilicity relative to **[^{18}F]F-ALK-BBN** (**[^{18}F]F-ALK-BBN-PEG**). The similarity of PEG-[^{18}F]FPyKYNE to [^{18}F]FPy5yne ensured that the radiosynthesis of this mini-PEGylated derivative was straightforward, although a large amount of SPE sorbent and an additional solvent evaporation step was required to obtain CuAAC-ready [^{18}F]-**3** after HPLC purification and SPE. In this regard, the reduced volatility of [^{18}F]-**3** relative to [^{18}F]-**1** proved advantageous. The coupling of [^{18}F]-**3** and **N_3-BBN-PEG** yielded di-mini-PEGylated ligand **[^{18}F]F-PEG-BBN-PEG** in radiochemical yields and specific activities sufficient for further biological assay. $\log D_{[7.4]}$ measurements suggests that this peptide is significantly more hydrophilic than **[^{18}F]F-ALK-BBN** and slightly more hydrophilic than **[^{18}F]F-ALK-BBN-PEG**.

The overall preparative yields of **[^{18}F]F-ALK-BBN-PEG** [14 ± 13% DC (n = 4) from EOB] and **[^{18}F]F-PEG-BBN-PEG** [10 ± 7% DC (n = 4) from EOB] compare reasonably well with yields obtained through the direct labeling of GRPr ligands which bear SiFA precursors [13 ± 3% DC (n = 4)][51] and TMA–benzonitrile groups (~15% DC).[50]

We have committed to repeated syntheses of **[^{18}F]F-ALK-BBN**, **[^{18}F]F-ALK-BBN-PEG**, and **[^{18}F]F-PEG-BBN-PEG** for the determination of certain key molecular imaging parameters of these radiopeptides in prostate cancer-bearing mice.[60,61] A complete summary of in vivo and ex vivo results will be reported in due course. In addition, the radio-protocols outlined here make up a viable general strategy for the [^{18}F]fluorination/post-synthetic PEGylation of other, less-established biological targeting vectors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.060.

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