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A novel 2-cyanobenzothiazole-based ^{18}F prosthetic group for conjugation to 1,2-aminothiol-bearing targeting vectors†

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†Electronic supplementary information (ESI) available: Experimental details for compounds **4**, **6**, **7** and **15**; UPLC co-injections of [$^{18/19}$F]-**2** and [$^{18/19}$F]-**16**.

ABSTRACT

In a bid to find efficient means to radiolabel biomolecules under mild conditions for PET imaging, a bifunctional ^{18}F prosthetic molecule has been developed. The compound, dubbed [^{18}F]FPyPEGCBT, consists of a 2-substituted pyridine moiety for [^{18}F]F$^-$ incorporation and a 2-cyanobenzothiazole moiety for coupling to terminal cysteine residues. The two functionalities are separated by a mini-PEG chain. [^{18}F]FPyPEGCBT could be prepared from its corresponding 2-trimethylammonium triflate precursor (100 °C, 15 min., MeCN) in preparative yields of 11 %± 2 (decay corrected, *n* = 3) after HPLC purification. However, because the primary radiochemical impurity of the fluorination reaction will not interact with 1,2-aminothiol functionalities, the ^{18}F prosthetic could be prepared for bioconjugation reactions by way of partial purification on a molecularly imprinted polymer solid-phase extraction cartridge. [^{18}F]FPyPEGCBT was used to ^{18}F-label a *cyclo*-(RGDfK) analogue which was modified with a terminal cysteine residue (TCEP·HCl, DIPEA, 30 min, 43 °C, DMF). Final decay-corrected yields of ^{18}F peptide were 7 % ± 1 (*n* = 9) from end-of-bombardment. This novel integrin-imaging agent is currently being studied in murine models of cancer. We argue that [^{18}F]FPyPEGCBT holds significant promise owing to its straightforward preparation, 'click'-like ease of use, and hydrophilic character. Indeed, the water-tolerant radio-bioconjugation protocol reported herein requires only one HPLC step for ^{18}F peptide purification and can be carried out remotely using a single automated synthesis unit over 124-132 min.

INTRODUCTION

Positron emission tomography (PET) is a non-invasive nuclear molecular imaging[1] technique which permits the visualization and quantification of biological and pharmacological processes in living systems. Successful PET research and diagnosis requires the development of selective and bio-available targeting molecules which are labelled with positron-emitting radioisotopes. Among the available PET isotopes (*e.g.* ^{11}C, ^{64}Cu, ^{68}Ga), ^{18}F stands out, owing to its attractive nuclear properties ($t_{1/2}$ = 109.8 min; E_{max} = 635 keV; 97% positron abundance) and ease of production on medical cyclotrons *via* the ^{18}O(p,n)^{18}F reaction.

The exquisite affinity and selectivity of certain peptides, proteins and oligonucleotides for specific bio-molecular targets has made them attractive PET targeting vectors. However, many biological targeting agents will not tolerate the high temperatures and basic conditions typically required to permit the direct incorporation of [^{18}F]F$^-$. Thus, the design of easy-to-^{18}F label small

bifunctional prosthetic groups which can be selectively coupled to sensitive biomolecules remains an active area of research.

2-Substituted pyridines are known to efficiently incorporate [^{18}F]F$^-$ under 'classical' S_NAr fluorination conditions.[2, 3] Unlike their C6 arene counterparts, they do not require an additional election-withdrawing functionality to elicit high reaction yields. As such, a number of 2-[^{18}F]fluropyridine prosthetic compounds have been prepared (Figure 1). These include [^{18}F]FPyME and [^{18}F]FPyBrA for conjugation to free thiol groups.[4, 5, 6] Unfortunately, the radiosynthetic protocols used to furnish bromoacetamide- and maleimide-bearing compounds such as [^{18}F]FPyME and [^{18}F]FPyBrA are typically complex because the functionalities used for bioconjugation are incompatible with standard nucleophilic [^{18}F]fluorination conditions (K[^{18}F]F-$K_{2.2.2.}$/K_2CO_3). Therefore, the conjugating moiety must be introduced after the [^{18}F]fluorination step, to the detriment of the overall radiosynthetic procedure. [^{18}]FPyKYNE,[7] [^{18}F]FPy5yne,[8] and PEG-[^{18}F]FPyKYNE[9] have been introduced for copper-catalyzed azide-alkyne cycloaddition (CuAAC) to azide-modified targeting agents (Figure 1). Such ^{18}F compounds are highly amenable to Cu-irrelevant systems, but are not good choices when potential outcomes include Cu binding, toxicity or metal-catalyzed degradation.

Figure 1. Some 2-[^{18}F]fluoropyridine-bearing prosthetic groups.

The coupling of 2-cyanobenzothiazole (CBT) and 1,2-aminothiols *via* chemoselective condensation chemistry adheres to 'click' criteria.[10] The CBT moiety reacts reversibly with free thiol groups but condenses rapidly and specifically with *N*-terminal cysteine residues.[11] Both aqueous buffer and mixtures of buffer and organic co-solvent may be used as reaction matrices. This ligation strategy has been used to label a diverse field of bioactive compounds with fluorescent tags, including luciferase protein, cyan florescent protein expressed on the surface of live cells, and a 90-mer DNA sequence.[11, 12] In 2012, an ^{18}F-bearing CBT derivative ([^{18}F]-**1**) was introduced as a prosthetic group for PET applications (Figure 2).[13] In this case, nucleophilic aliphatic [^{18}F]fluorination of a tosylated precursor afforded [^{18}F]-**1**, which was subsequently used to ^{18}F label a terminal cysteine- modified dimeric c(RGD) peptide (*vide infra*) and bioluminescent *Renilla* luciferase.

Figure 2. ^{18}F-bearing benzothiazoles described in Jeon *et al.*[13] ([^{18}F]-**1**) and this work ([^{18}F]-**2** and [^{18}F]-**3**).

We envisioned a '2nd generation' CBT-based ^{18}F prosthetic that marries the rapid, chemoselective bioconjugation potential of this functionality with the [^{18}F]fluorination potential of a 2-substituted pyridine. The following details our attempts to synthesize such a bifunctional molecule, dubbed [^{18}F]FPyPEGCBT ([^{18}F]-**2**). Peptides bearing the arginine-glycine-aspartic acid (RGD) motif may serve as ligands for certain cancer-associated integrin receptors.[14, 15] Integrin cell surface receptors play a role in the regulation of carcinogenesis, and these receptors are found upregulated in a variety of tumour types.[16] As such, a variety of ^{18}F- labelled RGD analogues have

been designed and tested *in vivo* as potential integrin imaging agents. An incomplete list of ^{18}F-RGD preparation strategies includes: oxime couplings with 4-[^{18}F]fluorobenzaldehyde[17] and [^{18}F]FDG;[18] acylation couplings with [^{18}F]SFB[19] and 4-nitrophenyl 2-^{18}F-fluoropropionate ([^{18}F]NFP);[20] CuAAC conjugations with [^{18}F]FPyKYNE,[21] (*N*-(4-[^{18}F]fluorophenyl)pent-4-ynamide,[22] and [^{18}F]F-pentyne;[23] chelation of Al[^{18}F]F;[24] and tetrazine-*trans*-cyclooctene ligation.[25] We describe herein the synthesis of a new ^{18}F peptide ligand of this class, which can be prepared *via* conjugation of [^{18}F]FPyPEGCBT to a 1,2-aminothiol-modified *cyclo*-(RGDfK) peptide.

RESULTS

Non-radioactive small molecule synthesis

As 2-nitro and 2-trimethylammonium triflate moieties are known to serve as a good leaving group in nucleophilic aromatic [^{18}F]fluorination reactions, we undertook the non-radioactive synthesis of precursors **12** and **14** (Scheme 1). First, ethylene glycol was activated toward nucleophilic substitution at both ends by conversion to the tosylated di-ester (**4**).[26, 27] Base-mediated displacement of one tosyl group by either 2-nitro-3-hydroxypyridine (**5**) or 2-dimethylamino-3-hydroxypyridine[8] (**7**) at elevated temperature yielded compounds **8** and **10** respectively. A satisfactory yield of 2-nitro- bearing compound **8** could not be achieved by this method. A second coupling reaction with 6'-hydroxy-2-cyanobenzothiazole (**11**) under similar conditions installed the second functionality. Nearly identical chemistry was used to furnish ^{19}F standard **2**, starting from 2-fluoro-3-hydroxypyridine[28] (**6**; Scheme 1). An additional methylation step was required to prepare trimethylammonium triflate salt **14** from 2-dimethylamino pyridine **13**.

Scheme 1. Synthesis of ^{18}F- labelling precursors **12** and **14**, along with ^{19}F standard **2**.

Non-radioactive synthesis of peptides

The peptide chosen for radiolabelling with [^{18}F]-**2** consisted of a *cyclo*-(RGDfK) sequence for integrin targeting which was modified with a terminal cysteine residue [Cys-PEG-c(RGDfK); **15**]. The two components are separated by a PEG2 tethering chain. The synthesis of peptide **15** is described in the Supporting Information section. Non-radioactive labelling of **15** with **2** (2 equiv.) to afford ^{19}F peptide standard **16** was carried out by mixing of the two compounds in DMSO at room temperature (3 h sonication, 25 h standing), followed by semi-preparative HPLC purification (*LC A,* Program 1).

*Optimizing the radiochemical yield of [^{18}F]-**2***

A series of test reactions were carried out in an attempt to find optimized conditions for the preparative synthesis of [^{18}F]-**2**. Crude reaction mixtures were assayed by radio-TLC to determine relative amounts of polar radio-impurities, including [^{18}F]F$^-$; desired product [^{18}F]-**2**; and non-reactive carboxamide side product [^{18}F]-**3** (Figure 3). A description of the general method can be found in the Supporting Information section. Results are summarized in Table 1. See Figure 3 for a representative radio-TLC trace.

Table 1, Entry 1 represents a starting point for our investigations, using trimethylammonium triflate precursor **14** (30 μmol K_2CO_3, 90 °C, 10 min). During the synthesis of [^{18}F]-**1**, researchers reported that non-basic crown ether 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Cr-6) was a superior alternative to commonly-used Kryptofix® 2.2.2. ($K_{2.2.2.}$).[13] Therefore, we chose to use this phase transfer catalyst as well. [^{18}F]F^- was eluted from Sep-pak light QMA anion exchange cartridges using mixtures of 18-Cr-6 in acetonitrile (2 mL) and *aqueous* K_2CO_3 (0.2 mL).

Table 1. Radio-TLC assessment of trial reactions for the optimization of [^{18}F]-**2**.

Entry	Precursor	PTC	Base (μmol)	*T* (°C)	Time (min)	[^{18}F]-2 (%)	[^{18}F]-3 (%)	[^{18}F]-2/[^{18}F]-3
1	**14**	18-Cr-6	30	90	10	20	10	2.1
2[a]	**14**	18-Cr-6	30	90	10	15	18	0.9
3[b]	**14**	18-Cr-6	30	90	10	20	45	0.5
4	**14**	18-Cr-6	30	120	10	19	22	0.9
5	**14**	18-Cr-6	8	90	10	45	24	1.9
6	**14**	DB18-Cr-6	8	90	10	23	9	2.6
7[c]	**14**	18-Cr-6	8	90	10	42	15	2.8
8	**14**	18-Cr-6	15	90	10	22	8	2.8
9	**14**	$K_{2.2.2.}$	15	90	10	8	70	0.1
10	**14**	18-Cr-6	15	90	15	48	28	1.7
11	**14**	18-Cr-6	15	100	15	49	15	3.3
12	**12**	18-Cr-6	14	100	15	1	1	0.5
13[a]	**12**	18-Cr-6	15	100	15	3	17	0.2

Reactions are in MeCN (1 mL) and utilize 3.6-4.0 μmol precursor, 64 μmol 18-Cr-6 as phase transfer catalyst (PTC), and K_2CO_3 as base, unless noted. [a]$KClO_4$ used as base. [b]Reaction in DMSO (1 mL). [c]Precursor = 7.3 μmol.

When perchlorate anion was used to strip [^{18}F]F^- from the QMA cartridge, yields of [^{18}F]-**2** by radio-TLC decreased slightly (Entry 2 vs. Entry 1). Employing DMSO as solvent did not improve yields, and was accompanied by a 2-fold relative increase in impurity **3** (Entry 3 vs. Entry 1). Also under these conditions, a significant increase in temperature (90°C→120°C) was not found to be beneficial (Entry 4 vs. Entry 1). A decrease in base (K_2CO_3) from 30 to 8 μmol was accompanied by a significant increase in product yield, along with a mitigation of **3** (Entry 5 vs. Entry 1). An alternative crown ether was also tested (2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene; DB18-Cr-6), with a decrease in [^{18}F]-**2** yield observed (Entry 6 vs. Entry 5). Increasing the concentration of precursor did not appear to have a significant effect on reaction yield (Entry 7 vs. Entry 5). When the amount of K_2CO_3 employed was increased from 8 to 15 μmol, a significant decrease in [^{18}F]-**2** yield was observed (Entry 8 vs. Entry 5); however, this change was deemed essential as [^{18}F]F^- could not be efficiently removed from QMA sorbent using the lower concentration of K_2CO_3.

Figure 3. Representative radio-TLC of crude [^{18}F]FPyPEGCBT ([^{18}F]-**2**) reaction mixture. Peak 1: [^{18}F]F^-, R_F = 0. Peak 3: [^{18}F]-**3**, R_F = 0.5. Peak 4:[^{18}F]-**2**, R_F = 0.6.

As expected, the replacement of $K_{2.2.2.}$ for 18-Cr-6 as phase transfer catalyst resulted in the formation of carboxamide impurity [^{18}F]-**3** as the major product (Entry 9 vs. Entry 8). Fortunately, the radiochemical yield of [^{18}F]-**2** in reactions containing 15 μmol K_2CO_3 could be improved by increasing the reaction time from 10 to 15 min (Entry 10 vs. Entry 8). In the end, the highest radiochemical yield and highest ratio of [^{18}F]-**2**/[^{18}F]-**3** was observed when both temperature and time were increased (Entry 11; 15 μmol K_2CO_3, 100 °C, 15 min). Attempts were also made to ^{18}F-label 2-nitro pyridine precursor **12** under these optimized conditions in both MeCN and DMSO (Entries 12 & 13 respectively). In both cases, labelling yields were very low. In light of these results, and because the separation of **12** and [^{18}F]-**2** cannot presumably be achieved by solid phase extraction methods (*vide infra*), precursor **12** was not employed for preparative radiosyntheses.

*Preparative synthesis of prosthetic group [^{18}F]FPyPEGCBT ([^{18}F]-**2**).*

The preparative syntheses of [^{18}F]-**2** and ^{18}F peptide [^{18}F]-**16** were carried out remotely using a Tracerlab FXFN automated synthesis unit. [^{18}F]FPyPEGCBT was synthesized according to the conditions as described in Table 1, Entry 11, with some modifications (Scheme 2). In a bid to further improve the efficiency of [^{18}F]F^- extraction from anion exchange sorbent without increasing K_2CO_3 mass, QMA light anion exchange columns were replaced with ORTG '^{18}F trap-and-release' columns, which can be eluted with less eluent due to their smaller size (12.6 mg resin vs. 130 mg for QMA).[29] The cartridge was extracted with *aqueous* K_2CO_3 alone (0.5 mL), while the phase transfer catalyst in MeCN (2 mL) was added directly to the reactor. The addition and distillation of a second portion of MeCN (2 mL) was employed to further facilitate anhydrous conditions.

Scheme 2. Preparative synthesis of [^{18}F]FPyPEGCBT ([^{18}F]-**2**).

[^{18}F]FPyPEGCBT could be obtained chemically and radiochemically pure (>98% by radio-TLC) after HPLC purification (*LC C*, Program 3). Product identity was verified by co-injection of ^{18}F prosthetic group with non-radioactive standard (see Supporting Information). Decay-corrected, collected yield was 11% ± 2 (n = 3). Apart from intrinsic losses due to HPLC, low preparative yields were also attributed to:

a) a loss of activity in the form of H[^{18}F]F during [^{18}F]F concentration steps, as a result of minimal added base and the use of suboptimal phase transfer catalyst.

b) the hydrolysis of [^{18}F]-**2** or precursor **14** to their carboxamide side-products under the conditions required to facilitate [^{18}F]fluorination.

For full radio-bioconjugate syntheses, HPLC purification of [^{18}F]-**2** was abandoned in favour of solid-phase extraction on AffiniMIP SPE ^{18}F molecularly imprinted polymer cartridges. This sorbent has been validated for the separation of 4-fluorobenzaldehyde and ethyl 4-[^{18}F]fluorobenzoate from their respective dimethylamino and phenolic side products, as well as $K_{2.2.2.}$ and free [^{18}F]F$^-$.[30] To our knowledge, this sorbent has not been previously reported for the purification of trimethylammonium triflate-bearing pyridines. Product application notes describe the effective elution of AffiniMIP® SPE ^{18}F cartridges with MeCN. This solvent is not, unfortunately, amenable to many bioconjugation reactions, including this one. However, we observed no evidence of precursor **14** in samples analyzed by UPLC-MS when a '0.7 mL' size column was eluted with 1 mL DMF. Extraction efficiency of radioactivity was 60 % (decay-corrected), which is consistent with the removal of all non-polar ^{18}F species from the cartridge, as estimated by radio-TLC. The advantages realized through the use of AffiniMIP® SPE ^{18}F technology are significant in this case-namely, the obviation of a time-consuming HPLC step and a significant simplification of the overall radiochemical protocol.

*Preparative synthesis of ^{18}F peptide [^{18}F]-**16**.*

^{18}F peptide [^{18}F]-**16** was obtained *via* mixture of ^{18}F prosthetic group [^{18}F]-**2** and precursor peptide Cys-PEG-c(RGDfK) (**15**) in DMF in the presence of TCEP·HCl (2 equiv.) and DIPEA (12 equiv.). Choice of base, solvent and reducing agent were based on a non-radioactive 2-cyanobenzothiazole/1,2-aminothiol condensation reaction reported earlier.[31] Non-automated, trial reactions at room temperature with 1 mg/mL precursor peptide **15** afforded near-total bioconjugation yields (30 min, sonication). The assumption is this case is that the major radiochemical impurity ([^{18}F]-**3**), which does not contain a 2-cyano moiety, will not interfere with the coupling of [^{18}F]-**2** and **15**. Indeed, analytical radio-UPLC of such a reaction mixture before and after addition of peptide precursor suggest that [^{18}F]-**3** and other radio-impurities are chemically irrelevant under these conditions (Figure 4).

Figure 4. Radio-UPLC traces of partially purified [^{18}F]-**2** before (top) and after (bottom) incubation with **15** (1 mg/mL of peptide, sonication, 30 min). *LC B*, Program 2. [^{18}F]-**2** = 4.4 min. [^{18}F]-**3** = 3.6 min. [^{18}F]-**16** = 3.3 min. Percent yield by HPLC of [^{18}F]-**16** relative to intact ^{18}F prosthetic is 97 % (63 % relative to total radioactivity).

In a bid to minimize the use of costly peptide precursor and use techniques more amenable to automated synthesis, preparative syntheses were carried out with 0.6 mg of **15** in 1.4 mL DMF (0.43 mg/mL) of at 43 °C (Scheme 3). Mixing was accomplished *via* intermittent bubbling with argon. However, under these conditions, bioconjugation yields were not quantitative (Figure 5). Nevertheless, [^{18}F]-**16** could be easily separated from precursor **15** and radioactive impurities using semi-preparative HPLC (*LC C*, Program 4). Immobilization and elution from tC18 sorbent afforded the product peptide in a final formulation of 10% EtOH in isotonic saline. Product identity was verified by co-injection of the ^{18}F peptide with non-radioactive standard (see Supporting Information). Full automated synthesis of [^{18}F]-**16** was reproducibly achieved in a decay-corrected

yield of 7 % ± 1 % (n = 9) from end-of-bombardment (EOB). Total synthesis time was 124-132 min from EOB. The effective specific activity of [^{18}F]-**16** prepared at our site was estimated to be 4-12 GBq/μmol (n = 8) based on the amount of UV-absorbing material (320 nm) co-eluting with radio-product as determined by mass standard curve. It is hypothesized that effective specific activities and radio-bioconjugation yields could be improved by decreasing the mass of precursor **14** used for the preparative synthesis of [^{18}F]FPyPEGCBT (6 mg in 2 mL MeCN), as this would presumably decrease the amount of 2-dimethylamino- and 2-hydroxy-pyridine impurities generated during radio-fluorinations.

Scheme 3. Radiosynthesis of *cyclo*-(RGDfK) peptide analogue [^{18}F]-**16**.

Figure 5. Radio-HPLC trace of [^{18}F]-**16** reaction mixture. *LC C*, Program 4. [^{18}F]-**16** = 22.27 min. [^{18}F]-**3** = 25.12 min. [^{18}F]-**2** = 27.34 min. % yield by HPLC of [^{18}F]-**16** relative to intact [^{18}F]FPyPEGCBT is 74 % (40 % relative to total radioactivity).

Lipophilicity is thought to have a profound effect on radiotracer biodistribution and uptake *in vivo*; in some cases, the introduction of a non-polar tag can enhance clearance through the undesirable hepatobiliary pathway.[32] In the hopes of mitigating this effect, [^{18}F]-**16** was designed with two short ethylene glycol (mini-PEG) chains, a modification which has been reported to reduce overall bio-tracer lipophilicity and improve biodistribution profiles for other RGD-based ^{18}F imaging agents.[33] The distribution coefficient (log $D_{7.4}$) of [^{18}F]-**16** in 1-octanol and PBS (pH 7.4) was found to be -1.22 ± 0.02 (n = 4), suggesting that the ^{18}F peptide is relatively hydrophilic.

We obtained HPLC-purified [^{18}F]FPyPEGCBT in preparative decay-corrected yields which are inferior to prosthetic group [^{18}F]-**1** (11 % vs. ~20 % from EOB respectively). It should be noted however that such a comparison may not be representative of the overall utility of [^{18}F]-**2**, as this labelling agent can be obtained in functionally useful form after rapid AffiniMIP® SPE purification. By other metrics, [^{18}F]-**2** offers advantages over [^{18}F]-**1,** including a fully automated labelling protocol on a single synthesis unit that includes only one HPLC purification. Finally, we anticipate that the low lipophilicity of [^{18}F]FPyPEGCBT might favourably ameliorate biodistribution outcomes of ^{18}F bioconjugates prepared by way of CBT/1,2-aminothiol condensation reactions. When Jeon *et al.* used [^{18}F]-**1** to radiolabel an RGD-based dipeptide ([^{18}F]CBTRGD$_2$) for murine PET imaging of human glioblastoma tumours (U87MG), they found increased levels of convoluting radioactivity in non-target organs relative to an analogous ^{18}F peptide labelled with [^{18}F]NFP.[13] The authors suggest that the lipophilic nature of the CBT moiety was responsible for this effect.

CONCLUSION

2-Cyanobenzothiazole-bearing [^{18}F]FPyPEGCBT was invented for the ^{18}F-labelling of potential biological PET imaging agents by way of a mild, water-compatible 'click' conjugation reaction with 1,2-aminothiol groups. This novel ^{18}F prosthetic was prepared in a single radiochemical step *via* [^{18}F]fluorination of a 2-trimethylammonium pyridine-bearing precursor. Unfortunately, the 2-cyanobenzothiazole moiety is sensitive to hydrolytic degradation and necessitates the need to use suboptimal [^{18}F]fluorination conditions, to the detriment of preparative radiochemical yields. However, this disadvantage can be partially overcome through the use of molecularly imprinted polymer cartridges to partially purify [^{18}F]FPyPEGCBT prior to bioconjugation in an efficient fashion. In this way, a terminal cysteine-modified c(RGDfK) analogue was labelled with ^{18}F for integrin-based PET imaging. The bioconjugate exhibited favourable hydrophilicity as required for *in vivo* imaging applications. *In vitro* receptor binding affinity assays, integrin specificity assays, and μPET evaluation of this new radiotracer using brain and ovarian cancer cell lines are currently underway. As this protocol requires no manual handling, it can be easily scaled up to produce larger quantities of ^{18}F radiopharmaceutical if required. The production of proteins and oligonucleotides bearing terminal cysteine residues has been well established, in large part because such constructs can be used in native chemical ligation reactions.[34, 35] Thus [^{18}F]FPyPEGCBT could prove useful for the ^{18}F labelling of these PET agent classes as well.

EXPERIMENTAL

Chemicals and Media

Unless otherwise noted, reagents and solvents were purchased from Sigma-Aldrich (Basel, Switzerland) or VWR (Nyon, Switzerland) and were used without further purification. Silica gel (40-63 μm) for flash chromatography was obtained from Silicycle (Quebec City, Canada). '^{18}F trap-and-release' SPE anion-exchange cartridges and QMA Plus light anion exchange cartridges were obtained from ORTG (Oakdale, USA) and Waters (Baden-Dättwil, Switzerland) respectively. tC18 light cartridges were purchased from Waters. AffiniMIP® SPE ^{18}F cartridges ('0.7 mL' size) were obtained from PolyIntell (Val-de-Reuil, France).

Chromatography

TLC was performed on pre-coated silica gel 60F_{254} aluminum sheets from VWR. The compounds were visualized under ultraviolet light at 254 nm or 365 nm, or by brief immersion in ninhydrin-collidine, followed by heating. A Cyclone Plus Phosphor Imager (PerkinElmer, Waltham, USA) with OptiQuant software was used for radioactive detection.

Liquid Chromatography (LC) A: HPLC for semi-preparative use. UltiMate 3000 Rapid Separation LC system (Dionex, Basel, Switzerland). The UV detector was set at 220 nm, 254 nm, and 320 nm. The column used was a Phenomenex Luna 5μm C_{18} PFP(2) 100Å (250 × 10 mm). Program 1: flow rate= 3 mL/min. Gradient elution: 5 % MeCN in water containing 0.1 % trifluoroacetic acid (TFA) to 65 % MeCN in water containing 0.1 % TFA over 20 min, then 100 % MeCN for 10 min.

LC B: Ultra performance liquid chromatography (UPLC) for liquid chromatography-mass spectroscopy (LC-MS). ACQUITY® UPLC, H Class (Waters, Baden-Dättwil, Switzerland). The UV detector was set at 220 nm, 254 nm, and 320 nm. Radioactivity was detected with a Flow-Ram Radio-HPLC detector (LabLogic, Sheffield, UK). In-line low resolution electrospray ionization mass spectroscopy (ESI-MS) was obtained using a Waters ACQUITY® TQ detector. The UPLC column used was an ACQUITY UPLC HSS T3 1.8 µm (2.1 × 500 mm). Program 2: flow rate= 0.4 mL/min. Gradient elution: 5 % MeCN in water containing 0.1 % formic acid to 100 % MeCN containing 0.1 % formic acid over 5 min, then 100 % MeCN containing 0.1 % formic acid for 5 min.

LC C: Radio-HPLC for preparative use. PU-2089 Plus Quaternary Pump (JASCO, Schlieren, Switzerland). The UV detector (Knauer WellChrom K-2001 Filter Photometer; Basel, Switzerland) was set to 254 nm. The native TracerLab FXFN NaI detector and software was used. The column used was a Phenomenex Luna 5µm C_{18} 100Å (250 × 10 mm). Program 3: flow rate: 3 mL/min. Isocratic elution: 65:35 MeCN-H_2O. Program 4: flow rate: 3 mL/min. Gradient elution: 5 % MeCN in water containing 0.1 % TFA to 65 % MeCN in water containing 0.1 % TFA over 20 min, then 100 % MeCN for 10 min.

NMR

NMR spectra were recorded with a Varian Gemini 2000 NMR Spectrometer with a 300 MHz Oxford magnet (Oxfordshire, UK). NMR solvents were obtained from Cambridge Isotope Laboratories (Burgdorf, Switzerland). Chemical shifts (δ) are reported in ppm relative to the hydrogenated residue of the deuterated solvents.

Mass Spectroscopy (MS)

Low-resolution MS was carried out on the UPLC-MS apparatus described above (see *LC B*). High resolution MS spectra were obtained on an ESI/nanoESI-IT Esquire 3000 plus instrument (Bruker; Fällanden, Switzerland).

Non-Radioactive Synthesis

2-(2-((2-Nitropyridin-3-yl)oxy)ethoxy)ethyl tosylate (8). 2-Nitro-3-hydroxypyridine (**7**; 499 mg, 3.56 mmol), diethylene glycol di(p-toluenesulfonate) (**4**; 2.96 g, 7.14 mmol), and powdered K_2CO_3 (500 mg, 3.62 mmol) were added to a dry round-bottomed flask as powders. Dry MeCN (40 mL) was added, and the resulting slurry was refluxed under Ar for 1.5 h. After cooling to RT, the reaction mixture was poured into 0.05 M HCl (100 mL) and extracted three times into ethyl acetate. The organic portions were washed once with brine (30 mL), dried over Na_2SO_4, and concentrated. The product, an oil, was partially extracted from insoluble powders by addition of 6:4 ethyl acetate-hexanes. After removal and concentration of the solvent phase, the crude residue was purified by silica gel flash chromatography (6:4 ethyl acetate-hexanes) to yield 253 mg (19 %) of **8**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (s, 3H), 3.58 – 3.65 (m, 2H), 3.65 – 3.73 (m, 2H), 4.06 – 4.15 (m, 2H), 4.24 – 4.33 (m, 2H), 7.38 – 7.48 (m, 2H), 7.71-7.80 (m, 3H), 7.95 (dd, J = 8.5, 1.1 Hz, 1H), 8.11 (dd, J = 4.5, 1.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.09 [CH_3], 68.07 [CH_2], 68.42 [CH_2], 69.22 [CH_2], 69.91 [CH_2], 125.42 [CH], 127.61 [2 × CH], 129.42 [CH], 130.09 [2 × CH], 132.34 [C], 139.34 [CH],

144.89 [C], 146.07 [C], 148.52 [C]. HR-MS calcd. for $C_{16}H_{19}N_2O_7S$: 383.0908 $[M+H]^+$. Found: 383.0909.

6-(2-(2-((2-nitropyridin-3-yl)oxy)ethoxy)ethoxy)benzo[*d*]thiazole-2-carbonitrile (12). Tosylated compound **8** (232 mg, 0.607 mmol) and 2-cyano-6-hydroxybenzothiazole (**11**, 129 mg, 0.733 mmol) were added together in a RBF and partially dissolved in anhydrous MeCN (25 mL). To this mixture was added K_2CO_3 (169 mg, 1.22 mmol). A condenser was affixed and the reaction was heated to reflux, under argon. After 3 h, the reaction was cooled to RT and diluted with ethyl acetate (50 mL). The reaction mixture was filtered and the filtered solids were washed generously with ethyl acetate. The filtrate was concentrated and the residue purified on a flash column of silica (7:3 ethyl acetate-hexanes) to afford **12** (184 mg, 78 %) as an off-white powder. M.P. = 111-112 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.78 – 3.91 (m, 4H), 4.16 – 4.26 (m, 2H), 4.34 – 4.44 (m, 2H), 7.29 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.73 (dd, *J* = 8.5, 4.5 Hz, 1H), 7.86 (d, *J* = 2.5 Hz, 1H), 7.98 (dd, *J* = 8.5, 1.2 Hz, 1H), 8.09 (dd, *J* = 4.5, 1.2 Hz, 1H), 8.12 (d, *J* = 9.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 68.02 [CH_2], 68.61 [CH_2], 68.83 [CH_2], 69.35 [CH_2], 105.13 [CH], 113.62 [C], 118.80 [CH], 125.28 [CH], 125.46 [CH], 129.39 [CH], 133.67 [C], 137.54 [C], 139.31 [CH], 146.11 [C], 146.22 [C], 148.58 [C], 159.03 [C]. HR-MS calcd. for $C_{17}H_{15}N_4O_5S$: 387.0758 $[M+H]^+$. Found: 387.0755.

2-(2-((2-Fluoropyridin-3-yl)oxy)ethoxy)ethyl tosylate (9). 2-Fluoro-3-hydroxypyridine (**6**; 1.01 g, 8.90 mmol) and diethylene glycol di(p-toluenesulfonate) (**4;** 3.66 g, 8.83 mmol) were added to a dry flask as powders, under Ar. The compounds were dissolved in dry MeCN (50 mL) and powdered K_2CO_3 (2.42 g, 17.5 mmol) was added. The mixture was refluxed under Ar for 2.5 h, then cooled to RT and filtered. The filtrate was concentrated and purified on a flash column of silica gel (9:1 CH_2Cl_2-ethyl acetate, then 4:1 CH_2Cl_2-ethyl acetate) to afford 1.62 g (52 %) of **9** as an oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (s, 3H), 3.65 (dd, *J* = 5.2, 3.6 Hz, 2H), 3.70 (dd, *J* = 5.2, 3.6 Hz, 2H), 4.20 – 4.09 (m, 4H), 7.28 (ddd, *J* = 8.0, 4.8, 0.8 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.63 (ddd, *J* = 10.6, 8.0, 1.5 Hz, 1H), 7.75 – 7.71 (m, 1H), 7.80 – 7.75 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.09 [CH_3], 68.02 [CH_2], 68.23 [CH_2], 68.55 [CH_2], 69.95 [CH_2], 122.68 [d, *J* = 3.8 Hz, CH], 123.71 [d, *J* = 4.2 Hz, CH], 127.63 [2 × CH], 130.10 [2 × CH], 132.35 [C], 136.87 [d, *J* = 13.5 Hz, CH], 141.55 [d, *J* = 25.4 Hz, C], 144.89 [C], 152.68 [d, *J* = 235.3 Hz, C]). HR-MS calcd. for $C_{16}H_{19}FNO_5S$: 356.0963 $[M+H]^+$. Found: 356.0965.

6-(2-(2-((2-fluoropyridin-3-yl)oxy)ethoxy)ethoxy)benzo[*d*]thiazole-2-carbonitrile (2). 6-Hydroxybenzothiazole-2-carbonitrile (**11**, 936 mg, 5.31 mmol) was added to a round-bottomed flask containing 2-(2-((2-fluoropyridin-3-yl)oxy)ethoxy)ethyl tosylate (**9**; 1.57 g, 4.41 mmol) and the reagents were partially dissolved in MeCN (80 mL). The flask was charged with argon and powdered K_2CO_3 (1.22 g, 8.80 mmol) was added. The flask was affixed with a condenser and the reaction mixture was heated to reflux, under argon, for 3 h. The reaction was cooled to RT and filtered, washing generously with MeCN. The concentrated residue was purified on a flash column of silica gel (6:4 ethyl acetate-hexanes) to afford **2** (1.26 g, 79 %) as a white powder. M.P. = 112-114 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.92 – 3.82 (m, 4H), 4.30 – 4.19 (m, 4H), 7.26 (ddd, *J* = 8.0, 4.8, 0.6 Hz, 1H), 7.31 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.65 (ddd, *J* = 10.5, 8.0, 1.5 Hz, 1H), 7.71 (dt, *J* = 4.8, 1.5 Hz, 1H), 7.87 (d, *J* = 2.5 Hz, 1H), 8.11 (d, *J* = 9.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 68.02 [CH_2], 68.38 [CH_2], 68.77 [CH_2], 68.81 [CH_2], 105.16 [CH], 113.61 [C], 118.78 [CH], 122.64 [d, *J* = 4.2 Hz,

CH], 123.71 [d, *J* = 4.3 Hz, CH], 125.30 [d, *J* = 3.2 Hz, CH], 133.66 [C], 136.83 [d, *J* = 13.3 Hz, CH], 137.54 [C], 141.59 [d, *J* = 25.6 Hz, C], 146.21 [C], 152.70 [d, *J* = 235.2 Hz, C], 159.04 [C]. HR-MS calcd. for $C_{17}H_{15}FN_3O_3S$: 360.0813 $[M+H]^+$. Found: 360.0813.

2-(2-((2-Dimethylaminopyridin-3-yl)oxy)ethoxy)ethyl tosylate (10). 2-Dimethylamino-3-hydroxypyridine (**5**; 426 mg, 3.08 mmol) and di ethylene glycol di(*p*-toluenesulfonate) (**4**; 2.56 g, 6.17 mmol) were added to a dry flask as powders, under Ar. The compounds were dissolved in dry MeCN (40 mL) and powdered K_2CO_3 (423 mg, 3.06 mmol) was added. The slurry was refluxed under Ar for 2 h 20 min, then cooled to RT and filtered through a short plug of Celite. The filtrate was concentrated and purified on a flash column of silica gel (7:3 CH_2Cl_2-EtOAc) to afford 698 mg (59 %) of **10**, as a clear oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (s, 3H), 2.85 (s, 6H), 3.67 – 3.59 (m, 2H), 3.75 – 3.67 (m, 2H), 4.05 – 3.96 (m, 2H), 4.17 – 4.08 (m, 2H), 6.75 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 2H), 7.81 – 7.71 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.09 [CH_3], 40.35 [2 × CH_3], 67.19 [CH_2], 67.93 [CH_2], 68.77 [CH_2], 69.99 [CH_2], 115.27 [CH], 118.88 [CH], 127.63 [2 × CH], 130.11 [2 × CH], 132.34 [C], 138.35 [CH], 144.83 [C], 144.90 [C], 152.31 [C]. HR-MS calcd. for $C_{18}H_{25}N_2O_5S$: 381.1479 $[M+H]^+$. Found: 381.1488.

6-(2-(2-((2-(Dimethylamino)pyridin-3-yl)oxy)ethoxy)ethoxy)benzo[*d*]thiazole-2-carbonitrile (13). 6-Hydroxybenzothiazole-2-carbonitrile (**11**, 399 mg, 2.27 mmol) was partially dissolved in a solution of 2-(2-((2-dimethylaminopyridin-3-yl)oxy)ethoxy)ethyl tosylate (**10**; 656 mg, 1.72 mmol) in dry MeCN (45 mL). The flask was charged with Ar and powdered K_2CO_3 (308 mg, 2.23 mmol) was added. The flask was affixed with a condenser and the reaction mixture was heated to reflux, under Ar. The reaction mixture became bright yellow and insolubles formed. After 4 h, the reaction was cooled to RT and filtered through a short plug of Celite, washing with MeCN (80 mL). The concentrated residue was purified on a flash column of silica gel (7:3 CH_2Cl_2-EtOAc) to afford **13** (628 mg, 95 %). ^{1}H NMR (300 MHz, CD_2Cl_2) δ 2.95 (s, 6H), 4.00 - 3.90 (m, 4H), 4.27 – 4.19 (m, 2H), 4.17 – 4.08 (m, 2H), 6.70 (dd, *J* = 7.8, 4.9 Hz, 1H), 7.01 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.25 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.40 (d, *J* = 2.4 Hz, 1H), 7.79 (dd, *J* = 4.9, 1.5 Hz, 1H), 8.07 (d, *J* = 9.2 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.37 [2 × CH_3], 67.37 [CH_2], 68.06 [CH_2], 68.69 [CH_2], 68.97 [CH_2], 105.14 [CH], 113.61 [C], 115.23 [CH], 118.77 [CH], 118.96 [CH], 125.31 [CH], 133.68 [C], 137.56 [C], 138.15 [CH], 144.88 [C], 146.21 [C], 152.22 [C], 159.08 [C]. HR-MS calcd. for $C_{19}H_{21}N_4O_3S$: 385.1329 $[M+H]^+$. Found: 385.1332.

3-(2-(2-((2-cyanobenzo[*d*]thiazol-6-yl)oxy)ethoxy)ethoxy)-*N,N,N*-trimethylpyridin-2-aminium trifluoromethanesulfonate (14). 6-(2-(2-((2-(Dimethylamino)pyridin-3-yl)oxy)ethoxy)ethoxy)benzo[*d*]thiazole-2-carbonitrile (**13**; 619 mg, 1.61 mmol) was dissolved in anhydrous toluene (5 mL) and the flask was charged with argon. The reaction was cooled to 0 °C, then methyl trifluoromethansulfonate (0.22 mL, 1.94 mmol) was added dropwise *via* syringe. After a few moments, a yellow gel precipitated out of solution. The reaction was stirred at 0 °C for 15 min total, after which stirring was stopped and the reaction solvent was removed by glass pipette. The round-bottomed flask was held at 0 °C while the precipitate was triturated with two additional portions of dry toluene (2 × 5 mL). Upon removal of solvent *in vacuo*, the product salt solidified (**14**; 812 mg, 92 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.62 (s, 9H), 3.93 – 3.81 (m, 2H), 4.02 – 3.93 (m, 2H), 4.28 – 4.19 (m, 2H), 4.52 – 4.43 (m, 2H), 7.30 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.73 (dd, *J* = 8.3, 4.5 Hz,

1H), 7.87 (d, *J* = 2.5 Hz, 1H), 7.97 (dd, *J* = 8.3, 1.1 Hz, 1H), 8.14 (d, *J* = 9.1 Hz, 1H), 8.17 (dd, *J* = 4.5, 1.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 53.33 [3 × CH_3], 68.02 [CH_2], 68.14 [CH_2], 68.46 [CH_2], 68.83 [CH_2], 105.13 [CH], 113.62 [C], 118.69 [CH], 125.38 [CH], 125.45 [CH], 128.37 [CH], 133.80 [C], 137.60 [C], 138.58 [CH], 142.89 [C], 146.25 [C], 146.98 [C], 158.99 [C]. HR-MS calcd. for $C_{20}H_{23}O_3N_4S$: 399.1485 $[M]^+$. Found: 399.1487.

Radiochemistry

No-carrier-added $[^{18}F]F^-$ was produced by irradiation (200-300 μA·min) of 2 mL of ^{18}O-enriched water (>97% pure; Marshall Isotopes; Tel-Aviv, Israel) using an IBA Cyclone 18 MeV cyclotron (Louvain-la-Neuve, Belgium). Total activity produced was ~12-18 GBq. Preparative syntheses were carried out remotely using a Tracerlab FXFN automated synthesis unit (GE Healthcare, Münster, Germany).

*Example of preparative radiosynthesis of [^{18}F]FPyPEGCBT ([^{18}F]-**2**)*

$[^{18}F]F^-$ was extracted from $[^{18}O]H_2O$ *via* immobilization on an '^{18}F trap-and-release' anion-exchange column, then eluted from the sorbent with a mixture of potassium carbonate (2.1 mg) in water (0.5 mL), into a reactor containing 18-Cr-6 (16.8 mg) in dry acetonitrile (2 mL; ABX, Radeberg, Germany). Solvent was removed by way of azeotropic distillation at 110 °C under an Ar stream. After 4 min, the reactor was cooled to 60 °C and an additional portion of dry acetonitrile (1 mL) was added. A second evaporation step was carried out at 110 °C for 3.5 min, then the temperature was briefly raised to 120 °C, followed by cooling to 40 °C. A solution of precursor **14** (6.4 mg, 11.7 μmol) in dry acetonitrile (2 mL) was added to the reactor and heated to 100 °C for 15 minutes. After cooling to 40°C, the reaction was quenched with 0.02 M HCl (10 mL) and passed through an AffinaMIP® SPE ^{18}F cartridge ('0.7 mL' size), which was activated previously with acetonitrile (2.5 mL). The cartridge was washed with an 8:2 mixture of H_2O-MeCN (2.5 mL), then [^{18}F]-**2** was eluted off with a solution of TCEP·HCl (1 mM) in DMF (1.3 mL) for further use.

*Example of preparative radiosynthesis of ^{18}F peptide [^{18}F]-**16***

^{18}F prosthetic group [^{18}F]-**2** was prepared as described above and transferred to a 5 mL conical vial. *N,N*-diisopropylethyl amine (39 μM) in DMF (0.1 mL) was added and the reactor was heated to 43 °C for 30 min. *In lieu* of mechanical stirring, the reaction mixture was sparged with argon for 2 seconds every minute. The bioconjugation reaction was quenched with the addition 0.02 M HCl (3 mL) and transferred onto a semi-preparative HPLC column (*LC C*, Program 4). ^{18}F-labelled peptide analogue [^{18}F]-**16** was identified based on its radioactive HPLC signal (R_t = 22.2 min), and collected into a round-bottomed flask containing water (40 mL). The diluted radio-product was immobilized on a tC18 light solid-phase extraction column, which was activated previously with MeOH (3 mL) and water (6 mL). The cartridge was washed with water (3 mL), then [^{18}F]-**16** was eluted off the column with EtOH (0.5 mL). Upon dilution with isotonic saline (4.5 mL), the final formulation was obtained. Collected activity was 459.1 MBq (8 % decay-corrected from EOB). Total synthesis time was 132 min from EOB.

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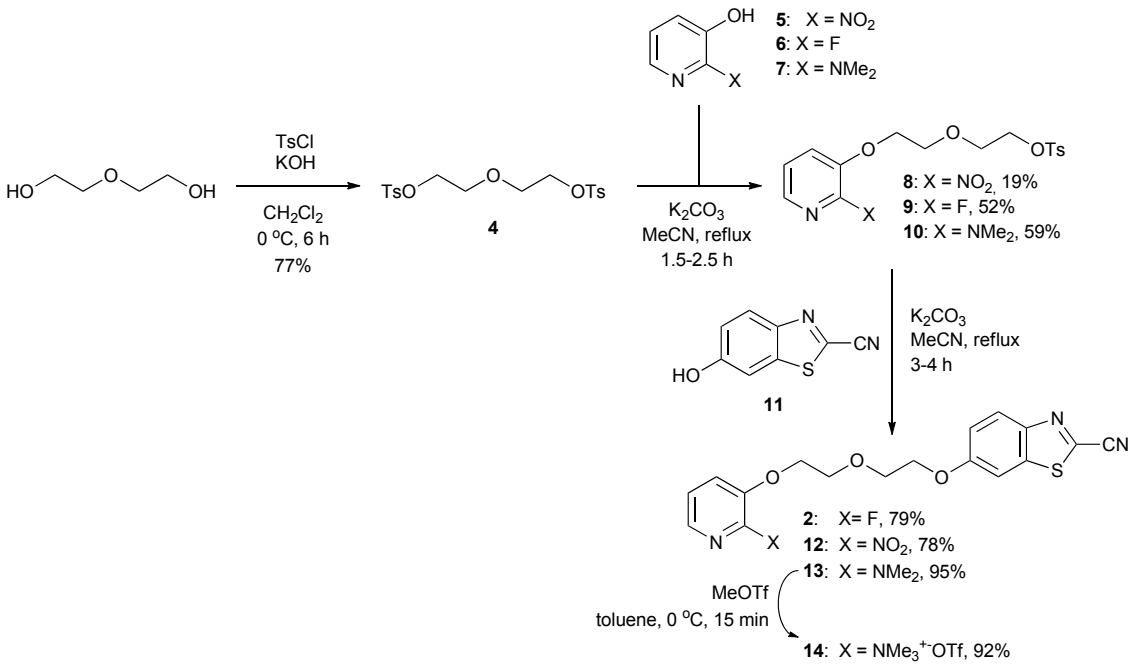
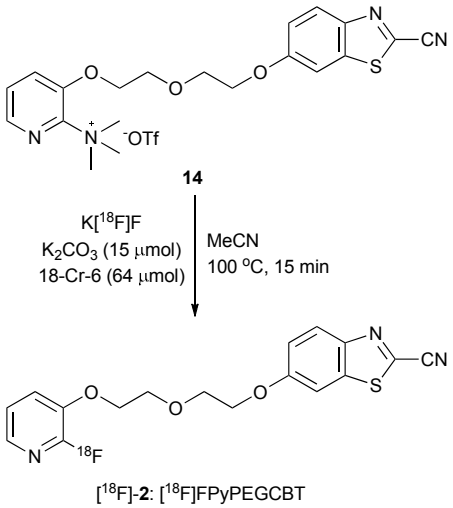
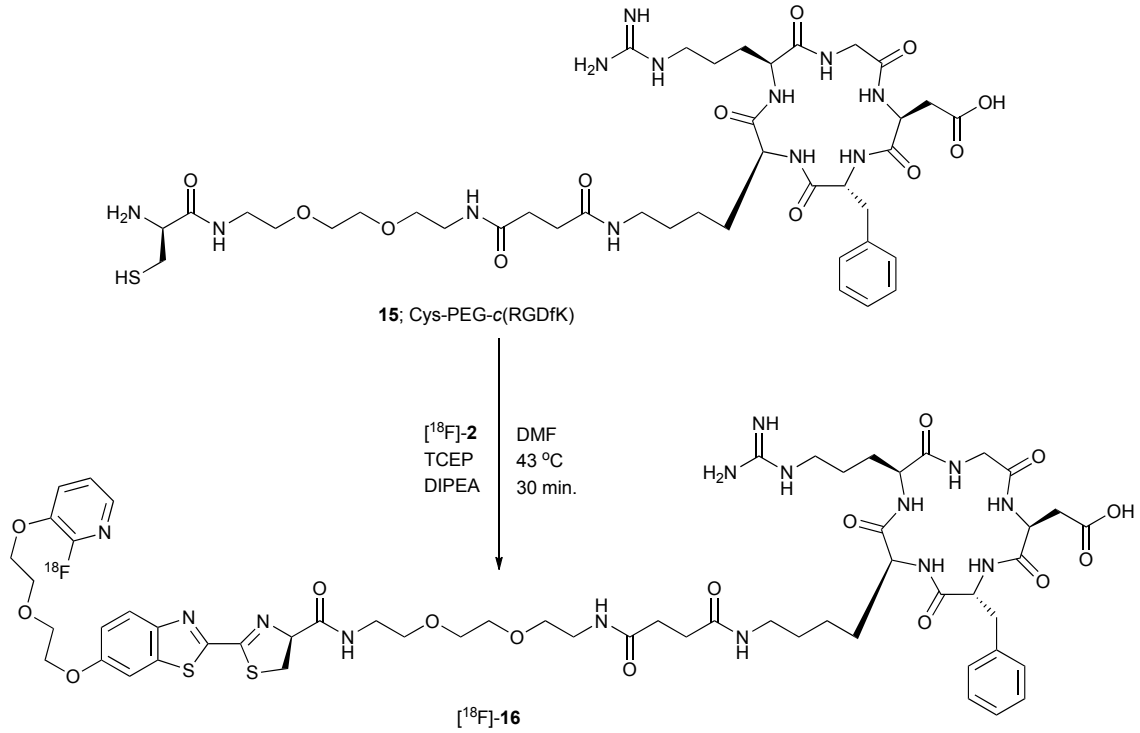

HO
OH
TsCl
KOH
CH2Cl2
0 °C, 6 h
77%
TsO
OTs
4
OH
N
X
5: X = NO2
6: X = F
7: X = NMe2
K2CO3
MeCN, reflux
1.5-2.5 h
8: X = NO2, 19%
9: X = F, 52%
10: X = NMe2, 59%
HO
N
S
CN
11
K2CO3
MeCN, reflux
3-4 h
2: X= F, 79%
12: X = NO2, 78%
13: X = NMe2, 95%
MeOTf
toluene, 0 °C, 15 min
14: X = NMe3+-OTf, 92%

Scheme 1

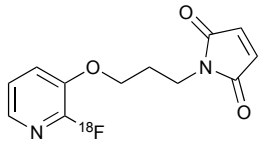


[^{18}F]-**2**: [^{18}F]FPyPEGCBT

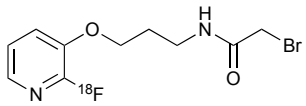
Scheme 2



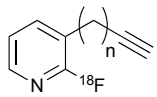
Scheme 3



[18F]FPyME

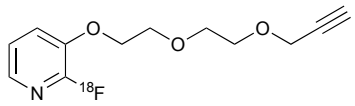


[18F]FPyBrA



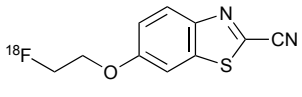
n = 3, [18F]FPyKYNE

n = 4, [18F]FPy5yne

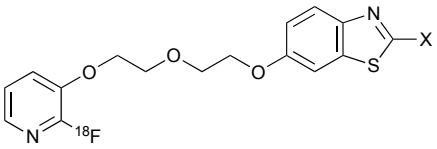


PEG-[18F]FPyKYNE

Fig. 1



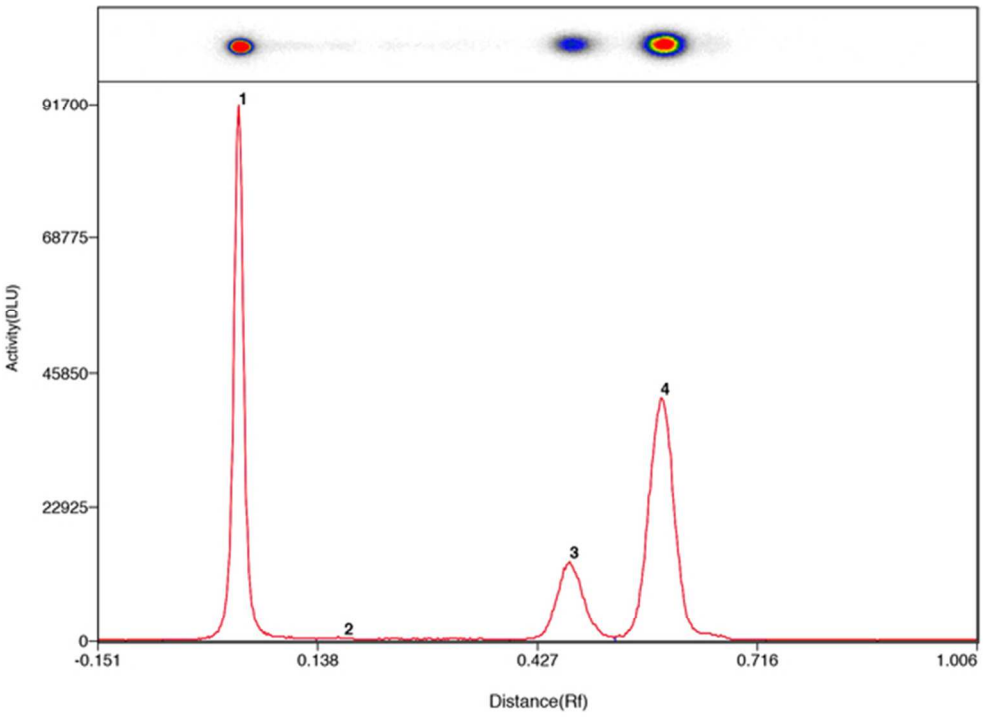
[^{18}F]-**1**



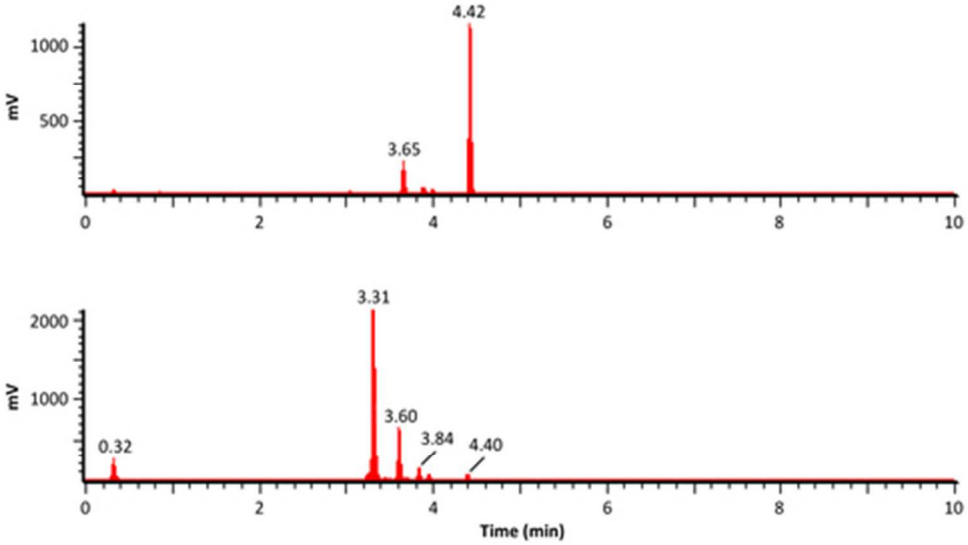
[^{18}F]-**2**, [^{18}F]FPyPEGCBT: X = CN

[^{18}F]-**3**: X = C(O)NH$_2$

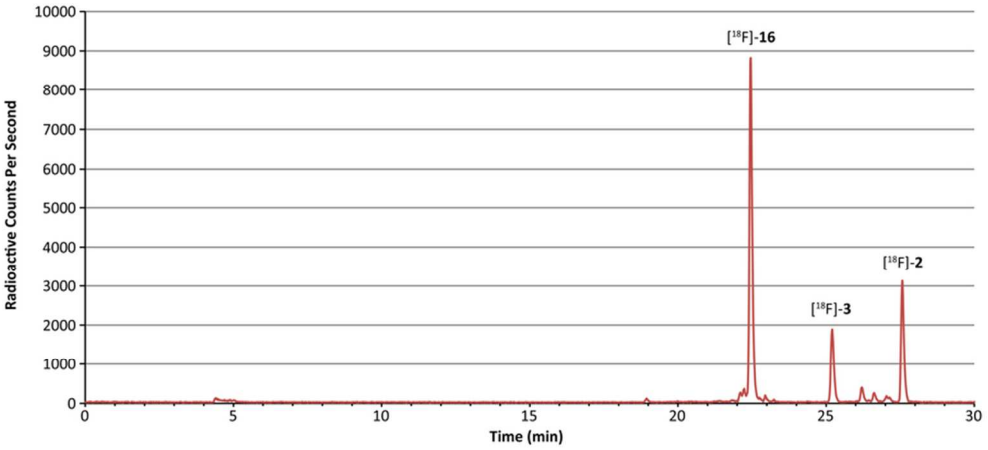
Fig. 2



54x39mm (300 x 300 DPI)



41x23mm (300 x 300 DPI)



77x35mm (300 x 300 DPI)