^{18}F-Trifluoromethanesulfinate enables Direct C–H ^{18}F-Trifluoromethylation of Native Aromatic Residues in Peptides

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Supporting Information Placeholder

ABSTRACT: ^{18}F-Labeling strategies for unmodified peptides with [^{18}F]fluoride require ^{18}F-prosthetics for bioconjugation more often with cysteine thiols or lysine amines. Here, we explore selective radical chemistry to target aromatic residues applying C–H ^{18}F-trifluoromethylation. We report a one-step route to [^{18}F]$CF_3SO_2NH_4$ from [^{18}F]fluoride, and its application to direct [^{18}F]CF_3-incorporation at tryptophan or tyrosine residues using unmodified peptides as complex as recombinant human insulin. The fully automated radiosynthesis of octreotide[Trp(2-$CF_2{}^{18}F$)] enables *in vivo* PET imaging.

Positron Emission Tomography (PET) is a powerful molecular imaging modality for diagnosis, monitoring disease progression, studying biological processes *in vivo*, and investigating the efficacy of drugs.[1–3] Among the radioisotopes employed for the preparation of PET probes, ^{18}F is a widely used and clinically relevant radionuclide.[2] Due to its short half-life ($t_{1/2}$ = 109.7 min), ^{18}F must be incorporated into tracer molecules at a late stage of the synthetic process.[4,5] Additional challenges imposed by radiochemistry include low reaction concentration, solvent compatibility, and cyclotron-produced ^{18}F sources being limited to ^{18}F-fluoride and [^{18}F]F_2. These constraints are stringent for biomolecules.

^{18}F-Radiolabeled peptides can be used to measure the distribution and pharmacokinetics of peptide-based therapeutics, and serve as imaging biomarkers for therapy.[6,7] These benefits have encouraged the development of methods for tagging peptides with radioactive functional groups.[8-10] Fluorine-18 is incorporated into pre-functionalized peptides *via* direct C–^{18}F, B–^{18}F and Si–^{18}F bond formation, or chelation with Al–^{18}F.[11-14] Alternatively, an ^{18}F-labeled prosthetic group is prepared prior to bioconjugation. To preserve function, this latter conjugation ideally proceeds under mild reaction conditions.[15-19] Such strategies require handles with unique reactivity either by e.g., prior installation of unnatural amino acids or by taking advantage of the inherent reactivity of natural amino acids. To date, the latter has almost exclusively exploited the nucleophilicity of cysteine thiols[20] or lysine amines[21] to attach the ^{18}F-prosthetic group. Although the structural alteration imposed by the ^{18}F-prosthetic group is typically tolerated, it could alter efficacy and/or function.[1c] As such, innovative methods that employ [^{18}F]fluoride, and target native residues in unmodified peptides with ^{18}F ,[22] or a minimally-sized ^{18}F-prosthetic (e.g. [^{18}F]CF_3) are of considerable value.

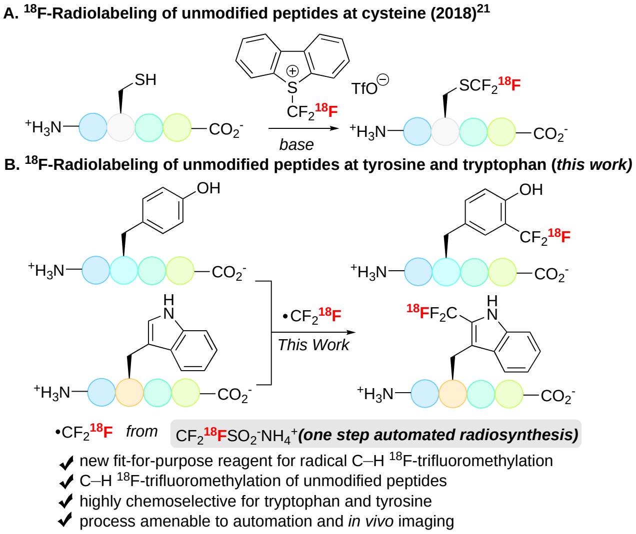
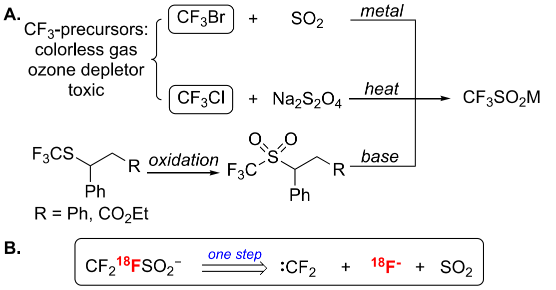


Figure 1. Direct ^{18}F-trifluoromethylation of native residues in unmodified peptides.

We reported the ^{18}F-trifluoromethylation of native peptides with 5-^{18}F-(trifluoromethyl)dibenzothiophenium trifluoromethanesulfonate, a method modifying cysteine thiols (Fig. 1A).[23] We also applied tuned radical chemistry to program C–H ^{19}F-trifluoromethylation of aromatic residues in proteins.[24a] Sodium trifluoromethanesulfinate (NaTFMS, Langlois' reagent) displayed selective reactivity for tryptophan under redox initiation. Recently, Krska *et al.* have demonstrated that Zn(TFMS)$_2$ (Baran's reagent), when activated with a stoichiometric oxidant or *via* visible photoredox catalysis, enabled trifluoromethylation of tyrosine in peptides that do not contain tryptophan residues.[25]

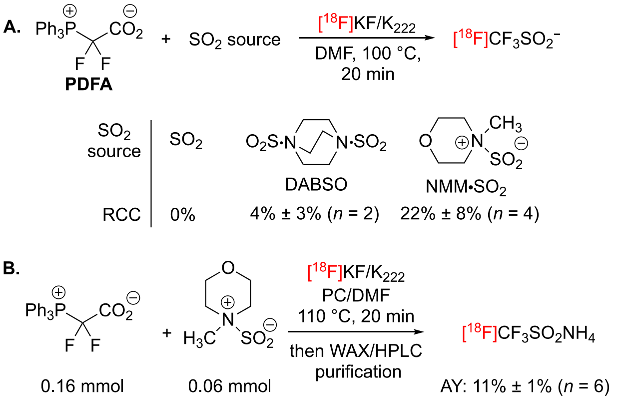
These precedents encouraged us to produce ^{18}F-trifluoromethanesulfinate for selective C–H ^{18}F-trifluoromethylation of these aromatic amino acid residues within unmodified peptides. This approach would generate non-canonical [^{18}F]CF_3-tryptophan and -tyrosine residues, a transformation unmatched by alternative ^{18}F-labeling methods (Fig. 1B).

Routes towards trifluoromethanesulfinic acid salts include metal or electro-reduction of a mixture of SO_2 and CF_3Br in DMF,[26] the treatment of CF_3Cl with $Na_2S_2O_4$,[27] or multistep syntheses from trifluoromethylsulfone precursors (Scheme 1A).[28] For ^{18}F-radiochemistry, these approaches would require a route towards the [^{18}F]CF_3-precursor, and one or more reactions post-labeling. Our design plan was to construct [^{18}F]$CF_3SO_2^-$ in one step applying a multi-component approach that combines ^{18}F-fluoride, a difluorocarbene source, and SO_2. The formation of [^{18}F]CF_3^- from difluorocarbene and [^{18}F]F^- is known,[29-31] so the challenge was to validate a protocol that couples *in situ* generated [^{18}F]CF_3^- with SO_2 (or a surrogate of this gaseous and toxic reagent) (Scheme 1B).



Scheme 1. A. Multi-step syntheses towards trifluoromethanesulfinic acid salts, M = metal. B. Proposed one-step radiosynthesis towards ^{18}F-trifluoromethanesulfinate.

Exploratory studies performed with ^{19}F-fluoride provided useful information.[32] The difluorocarbene and SO_2 sources were found to be critical in enabling the construction of $CF_3SO_2^-$. The reaction of 2,2,-difluoro-2-(triphenylphosphonio)acetate (PDFA) with either 1,4-diazabicyclo[2.2.2]-octane *bis*(SO_2) adduct (DABSO)[33] or *N*-methyl-morpholine·SO_2 (NMM·SO_2) in the presence of KF/K_{222} in DMF at 100 °C afforded the ammonium salt of $CF_3SO_2^-$ in 31% and 44% yields after isolation by semi-preparative HPLC, respectively. A saturated solution of SO_2 in DMF did not lead to product formation, whilst ClF_2CCO_2Me in combination with PPh_3 was the only alternative difluorocarbene source found suitable for this process. For ^{18}F-labeling, PDFA was elected as the optimal reagent. In contrast to experiments carried out with fluoride, DABSO and PDFA afforded [^{18}F]CF_3SO_2K in trace amounts (Scheme 2A). However, the combination of PDFA, NMM·SO_2 and [^{18}F]KF/K_{222} gave [^{18}F]CF_3SO_2K in 22% radiochemical conversion (RCC). These results encouraged the development of a manual protocol to prepare, purify and isolate this novel ^{18}F-reagent for subsequent use (Scheme 2B). PDFA is thermally unstable and poorly soluble in DMF, so a mixture of this reagent and NMM·SO_2 was added as a suspension in a suitable solvent to azeotropically-dried ^{18}F-fluoride. Amongst all solvents tested, propylene carbonate (PC) was best when used with DMF.[34] Additional optimization tuning reagents, ratios of various components, and concentrations proved beneficial. The optimal process consisted of reacting PDFA (0.16 mmol) and NMM·SO_2 (0.06 mmol) with [^{18}F]KF/K_{222} (up to 10 GBq) in 350 μL PC/DMF mixture at 110 °C. Initial purification of [^{18}F]$CF_3SO_2^-$ using a weak anion exchange cartridge (WAX) removed most of the unreacted [^{18}F]fluoride and organic by-products. Elution with a solution of ~0.4 M ammonium hydroxide in EtOH followed by reverse phase HPLC purification afforded [^{18}F]$CF_3SO_2NH_4$ in >99% radiochemical purity. This protocol furnished up to 900 MBq of [^{18}F]$CF_3SO_2NH_4$ from 10 GBq of [^{18}F]fluoride. The overall nondecay corrected activity yield (AY) of isolated [^{18}F]$CF_3SO_2NH_4$ calculated from [^{18}F]fluoride was 11% ± 1% (n = 6, synthesis time = 70 mins). The identity of [^{18}F]$CF_3SO_2NH_4$ was established by HPLC and ESI-MS analysis (m/z) calc. for [^{19}F]$CF_3SO_2^-$: 133.0; found: 133.1).[32]

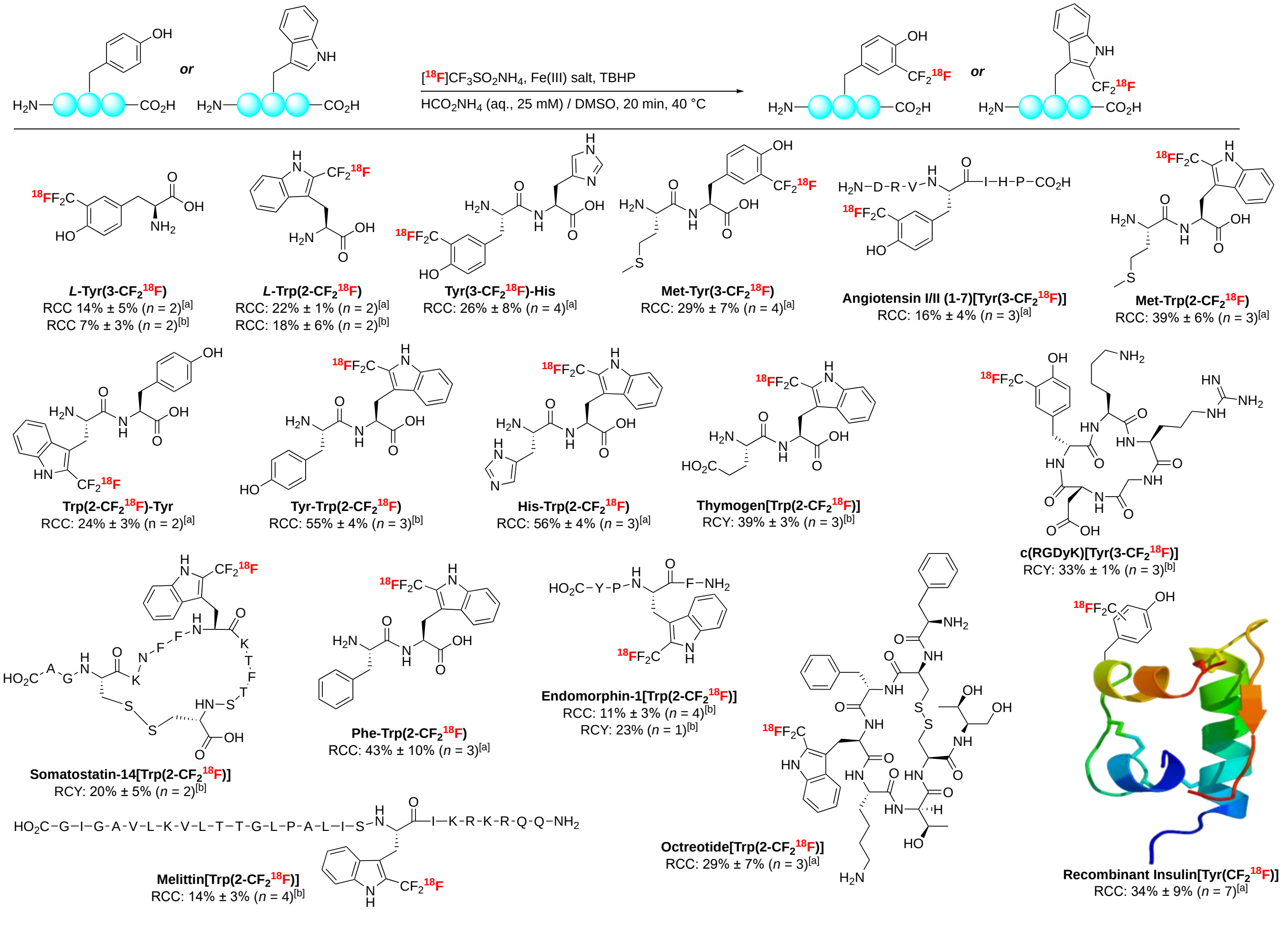


Scheme 2. A. Initial studies towards one step synthesis of [^{18}F]$CF_3SO_2^-$. B. Radiosynthesis, purification and isolation of [^{18}F]$CF_3SO_2NH_4$.

Next, we studied the C–H ^{18}F-trifluoromethylation of model peptides containing L-tryptophan and/or L-tyrosine residues using [^{18}F]$CF_3SO_2NH_4$ and *t*-butyl hydroperoxide (TBHP) as the oxidant. In ^{19}F-mode, CF_3SO_2Na is added in large excess (up to ~ 200 equiv) to enable C–H trifluoromethylation of peptides and proteins.[24,35] These conditions are not compatible with ^{18}F-radiochemisty due to inherent constraints on concentrations for both large peptides and [^{18}F]$CF_3SO_2NH_4$, the latter being by far the limiting reagent. An additional complication was competitive oxidation of [^{18}F]$CF_3SO_2^-$ into [^{18}F]$CF_3SO_3^-$ with the initiation oxidant. For ^{19}F-trifluoromethylation, this issue is solved using an excess of CF_3SO_2Na with respect to TBHP, or *via* slow addition of TBHP to the reaction mixture.[36] These solutions are not suitable for ^{18}F-labeling because [^{18}F]$CF_3SO_2NH_4$ is the limiting reagent, and operational simplicity is paramount for ^{18}F-radiochemistry.

The treatment of L-Tyr with [^{18}F]$CF_3SO_2NH_4$ and TBHP in AcOH/aq. ammonium formate did not lead to C–H ^{18}F-trifluoromethylation after 20 mins, even at 60 °C.[32] Extensive optimization overcame ^{18}F-labeling constraints, and led to L-Tyr(3-CF_2^{18}F) in 14% RCC when the reaction was performed in the presence of TBHP and $Fe(NO_3)_3·9H_2O$[36] in DMSO/aq. ammonium formate at 40 °C for 20 mins (Scheme 3).[32] ^{18}F-Trifluoromethylation on the C_2-position was detected in 2% RCC. These two regioisomers are separable by HPLC. The RCC of L-Tyr(3-CF_2^{18}F) increased to 53% when the reaction was performed at 60 °C. When $FeCl_3$ was used at 40 °C instead of $Fe(NO_3)_3·9H_2O$, L-Tyr(3-CF_2^{18}F) was formed in 7% RCC. The C–H ^{18}F-trifluoromethylation of L-Trp was also successful with [^{18}F]$CF_3SO_2NH_4$ when activated by either $Fe(NO_3)_3•9H_2O$ or $FeCl_3$ in the presence of TBHP. Applying these conditions, L-Trp(2-CF_2^{18}F) was obtained in 22% and 18% RCC, respectively. Two additional regioisomers resulting from competitive ^{18}F-labeling on the C_4- and C_7-positions were also formed giving a combined RCC of 10% or 9% when $Fe(NO_3)_3·9H_2O$ or $FeCl_3$ was employed, respectively.[37]

A series of dipeptides was evaluated focusing on feasibility and selectivity (Scheme 3).[32] For reactions leading to more than one ^{18}F-labeled product, identification was made by comparison of HPLC traces with fully characterized references prepared independently. Dipeptides Tyr-Trp and Trp-Tyr underwent [^{18}F]CF_3 incorporation exclusively at Trp, a result corroborating our previous studies.[24] For Tyr-Trp, ^{18}F-labeling experiments performed with $[^{18}F]CF_3SO_2NH_4$ and TBHP with either $Fe(NO_3)_3{\cdot}9H_2O$ or $FeCl_3$ gave 40% and 55% RCC, respectively. For dipeptide Phe-Trp, ^{18}F-trifluoromethylation occurred at Trp, affording Phe-Trp(2-$CF_2{}^{18}F$) in 43% RCC. $[^{18}F]CF_3$-incorporation on Trp occurred at the C_2-, C_4- and C_7-positions (C_2 major) while ^{18}F-labeling on Phe was not observed. ^{18}F-Labeling at His was not detected for either Tyr-His or His-Trp. Met oxidation was minimized for the ^{18}F-trifluoromethylation of Met-Trp or Met-Tyr by decreasing TBHP:Fe(III) ratio (1:1). Oxidative dimerization of cysteine by disulfide formation is unavoidable.[24,25] Next, we studied biologically relevant peptides of increasing complexity. The dipeptide immunomodulator thymogen (Oglufanide^{TM})[38] was ^{18}F-trifluoromethylated at Trp with an isolated radiochemical yield (RCY) calculated from $[^{18}F]CF_3SO_2NH_4$ of 39%. Endomorphin-1, a tetrapeptide associated with Alzheimer's disease,[39,40] underwent Trp-selective ^{18}F-labeling in 23% RCY, and somatostatin-14, a cyclic tetradecapeptidic hormone with broad inhibitory effect on endocrine secretion, was ^{18}F-labeled in 20% RCY.[41] The ^{18}F-trifluoromethylation of the 26-residue venom peptide melittin[42] and of octreotide,[43] an octapeptide that mimics natural somatostatin, was equally successful (14% RCC and 29% RCC, respectively). Tyrosine-containing peptides were examined next. Angiotensin fragment 1-7, a peptide with anti-inflammatory properties,[44,45] and c(RGDyK), a peptide ligand of integrin $\alpha_v\beta_3$ receptors,[46] both underwent ^{18}F-labeling at Tyr in 16% and 33% RCC, respectively. The C–H ^{18}F-trifluoromethylation of a much larger peptide was considered with recombinant human insulin (MW: 5808 Da).[47] This experiment was carried out with insulin (5.2 μmol), $Fe(NO_3)_3{\cdot}9H_2O$ (5.8 equiv) and TBHP (11.5 equiv) in DMSO/aq. ammonium formate affording $[^{18}F]CF_3$-insulin as a mixture of four products, resulting from $[^{18}F]CF_3$ incorporation at all tyrosine residues, in 34% overall RCC. The main site of ^{18}F-trifluoromethylation was at chain A residue Y19, a result consistent with the report of Krska *et al.*[25]



Scheme 3. Substrate scope for C–H ^{18}F-trifluoromethylation of native aromatic residues of peptides. Peptide (0.03 mmol). TBHP (2 or 4 equiv). [a] $Fe(NO_3)_3{\bullet}9H_2O$ (2 equiv). [b] $FeCl_3$ (2 equiv). Synthesis time for the ^{18}F-labeled peptide from $[^{18}F]CF_3SO_2NH_4$ = 90 mins.[32]

To date, automated radiosyntheses have focused on small molecules, rarely on peptides.[48] To demonstrate translational applicability, we developed a fully automated radiosynthesis of octreotide[Trp(2-$CF_2{}^{18}F$)] on the Advion NanoTek® microfluidic synthesis system (Figure 2).[32] The automated radiosynthesis of $[^{18}F]CF_3SO_2NH_4$ required optimization of selected steps. The addition of the suspension of PDFA and $NMM{\cdot}SO_2$ in PC/DMF to a vial containing $[^{18}F]KF$ was not compatible with automation. This issue was solved by changing the difluorocarbene source to ClF_2CCO_2Me, a reagent activated with (2-biphenyl)di-*tert*-butylphosphine (JohnPhos), and the solvent to DMA; no change was required for $NMM{\cdot}SO_2$. With these modifications, starting from up to 45 GBq of $[^{18}F]$fluoride, $[^{18}F]CF_3SO_2NH_4$ was produced in up to 6% ± 1% activity yield (non-decay corrected, *n* = 2), after semi-preparative HPLC (A_m = 1.13 GBq/μmol, synthesis time = 40 mins). Removal of HPLC solvents was necessary to afford dry $[^{18}F]CF_3SO_2NH_4$ required for peptide ^{18}F-labeling. This critical drying step also required extensive modification. For automation, $[^{18}F]CF_3SO_2NH_4$ was trapped on a WAX cartridge and subsequently eluted with NH_4OH in MeCN (1.4%) followed by evaporation.

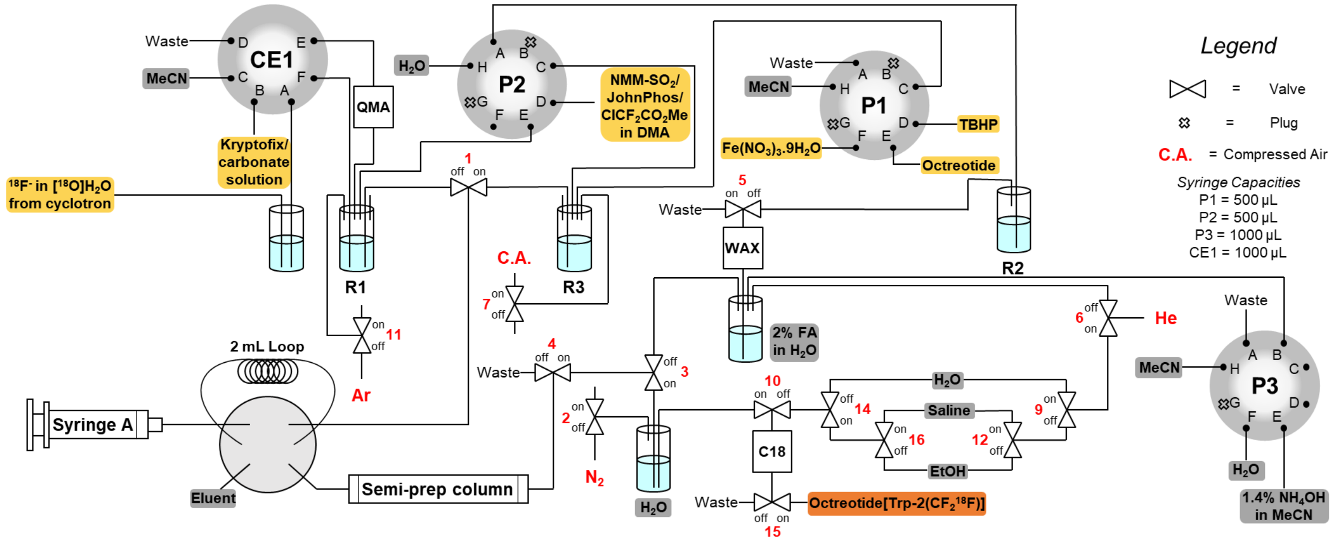


Figure 2. Automated radiosynthesis of octreotide[Trp(2-$CF_2{}^{18}F$)] from [^{18}F]fluoride on the Advion NanoTek® microfluidic synthesis system.

Successful C-H ^{18}F-trifluoromethylation in the presence of $Fe(NO_3)_3{\cdot}9H_2O$ (4 equiv) and TBHP (8 equiv) afforded up to 69 MBq octreotide[Trp(2-$CF_2{}^{18}F$)] (n = 3, A_m = 0.28 ± 0.08 GBq/µmol) after purification by HPLC. The total synthesis time from [^{18}F]fluoride to octreotide[Trp(2-$CF_2{}^{18}F$)] was 133 mins. This automated protocol enabled an *in vivo* PET imaging experiment with this [^{18}F]CF_3-peptide on naïve Sprague Dawley rats, a preliminary study suggesting excretion *via* the gastro-intestinal pathway and the kidneys.[32,49,50,51]

In conclusion, we report the first protocol enabling direct ^{18}F-labeling of unmodified peptides at tryptophan and tyrosine residues (high selectivity for tryptophan) applying direct C–H ^{18}F-trifluoromethylation. This method is a new tool to accelerate the discovery of ^{18}F-peptides as imaging agents as well as the development of peptide-based drugs. The strategy required the novel ^{18}F-reagent [^{18}F]$CF_3SO_2NH_4$ prepared in one step from [^{18}F]fluoride, a difluorocarbene reagent, and an SO_2 source. The iron salt was essential to overcome the difficulties arising from [^{18}F]$CF_3SO_2NH_4$ being the limiting reagent, thereby enabling C–H ^{18}F-trifluoromethylation of peptides as complex as insulin. The automated radiosynthesis of octreotide[Trp(2-$CF_2{}^{18}F$)] from [^{18}F]fluoride enabled *in vivo* PET imaging. This major milestone, unrivalled by known methods making use of minimally-sized labeled prosthetics,[23,52,53] sets the stage for an in-depth investigation on clinically relevant peptides. Considering the number of reactions relying on Langlois-type reagents, [^{18}F]$CF_3SO_2NH_4$ could expand considerably the radiochemical space for PET applications beyond the peptides described herein.

ASSOCIATED CONTENT

SUPPORTING INFORMATION

The Supporting Information is available free of charge on the ACS Publications website.
Detailed experimental procedures, characterization of new compounds, automation protocol, *in vivo* experiments.

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Notes
The authors declare no competing financial interests.

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