

¹⁸F-Trifluoromethanesulfinate Enables Direct C–H ¹⁸F-Trifluoromethylation of Native Aromatic Residues in Peptides

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Supporting Information

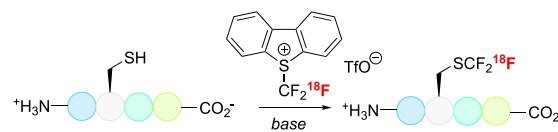
ABSTRACT: ¹⁸F labeling strategies for unmodified peptides with [¹⁸F]fluoride require ¹⁸F-labeled prosthetics for bioconjugation more often with cysteine thiols or lysine amines. Here we explore selective radical chemistry to target aromatic residues applying C–H ¹⁸F-trifluoromethylation. We report a one-step route to [¹⁸F]CF₃SO₂NH₄ from [¹⁸F]fluoride and its application to direct [¹⁸F]CF₃ incorporation at tryptophan or tyrosine residues using unmodified peptides as complex as recombinant human insulin. The fully automated radiosynthesis of octreotide[Trp(2-CF₂¹⁸F)] enables *in vivo* positron emission tomography 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, ¹⁸F is a widely used and clinically relevant radionuclide.² Because of its short half-life (*t*_{1/2} = 109.7 min), ¹⁸F 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 the fact that cyclotron-produced ¹⁸F sources are limited to ¹⁸F-fluoride and [¹⁸F]F₂. These constraints are stringent for biomolecules.

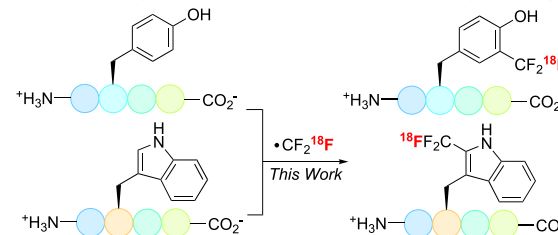
¹⁸F-radiolabeled peptides can be used to measure the distribution and pharmacokinetics of peptide-based therapeutics and serve as imaging biomarkers for therapy.^{6,7} These benefits have encouraged the development of methods for tagging peptides with radioactive functional groups.^{8–10} Fluorine-18 is incorporated into prefunctionalized peptides via direct C–¹⁸F, B–¹⁸F, and Si–¹⁸F bond formation or chelation with Al–¹⁸F.^{11–14} Alternatively, an ¹⁸F-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 thiols²⁰ or lysine amines²¹ to attach the ¹⁸F-prosthetic group. Although the structural alteration imposed by the ¹⁸F-prosthetic group is typically tolerated, it could alter the efficacy and/or function.^{1c} Therefore, innovative methods that employ [¹⁸F]fluoride and target native residues in unmodified peptides with ¹⁸F²² or a minimally sized ¹⁸F-prosthetic (e.g., [¹⁸F]CF₃) are of considerable value.

We reported the ¹⁸F-trifluoromethylation of native peptides with 5-¹⁸F-(trifluoromethyl)dibenzothiophenium trifluoromethanesulfonate, a method modifying cysteine thiols (Figure 1A).²³ We also applied tuned radical chemistry to program C–H ¹⁸F-trifluoromethylation of aromatic residues in proteins.^{24a} Sodium trifluoromethanesulfinate (NaTFMS, Langlois' re-

A. ¹⁸F-Radiolabeling of unmodified peptides at cysteine (2018)²³



B. ¹⁸F-Radiolabeling of unmodified peptides at tyrosine and tryptophan (this work)



•CF₂¹⁸F from CF₂¹⁸FSO₂NH₄⁺ (one step automated radiosynthesis)

- ✓ new fit-for-purpose reagent for radical C–H ¹⁸F-trifluoromethylation
- ✓ C–H ¹⁸F-trifluoromethylation of unmodified peptides
- ✓ highly chemoselective for tryptophan and tyrosine
- ✓ process amenable to automation and *in vivo* imaging

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

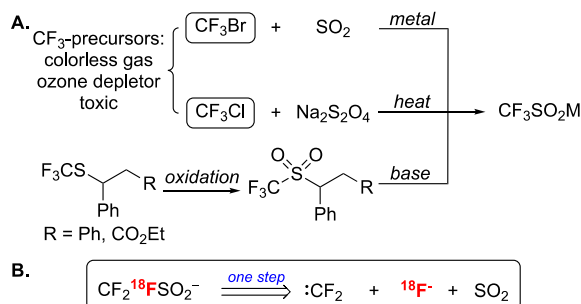
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agent) displayed selective reactivity for tryptophan under redox initiation. Recently, Krska et al. demonstrated that Zn(TFMS)₂ (Baran's reagent), when activated with a stoichiometric oxidant or via visible-light photoredox catalysis, enabled trifluoromethylation of tyrosine in peptides that do not contain tryptophan residues.²⁵ These precedents encouraged us to produce ¹⁸F-trifluoromethanesulfinate for selective C–H ¹⁸F-trifluoromethylation of these aromatic amino acid residues within unmodified peptides. This approach would generate noncanonical [¹⁸F]CF₃-tryptophan and -tyrosine residues, a transformation unmatched by alternative ¹⁸F labeling methods (Figure 1B).

Routes toward trifluoromethanesulfonic acid salts include metal or electroreduction of a mixture of SO₂ and CF₃Br in *N,N*-dimethylformamide (DMF),²⁶ treatment of CF₃Cl with Na₂S₂O₄,²⁷ or multistep syntheses from trifluoromethylsulfone precursors (Scheme 1A).²⁸ For ¹⁸F radiochemistry, these

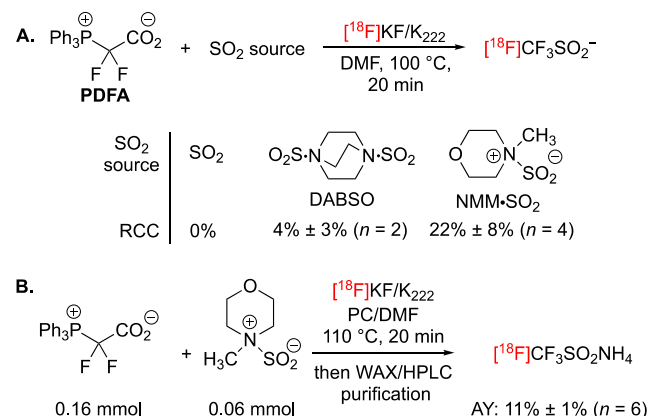
Scheme 1. (A) Multistep Syntheses toward Trifluoromethanesulfonic Acid Salts (M = Metal); (B) Proposed One-Step Radiosynthesis toward ¹⁸F-Trifluoromethanesulfinate



approaches would require a route toward the [¹⁸F]CF₃ precursor and one or more reactions postlabeling. Our design plan was to construct [¹⁸F]CF₃SO₂⁻ in one step by applying a multicomponent approach that combines ¹⁸F-fluoride, a difluorocarbene source, and SO₂. The formation of [¹⁸F]CF₃⁻ from difluorocarbene and [¹⁸F]F⁻ is known,^{29–31} so the challenge was to validate a protocol that couples in situ-generated [¹⁸F]CF₃⁻ with SO₂ (or a surrogate of this gaseous and toxic reagent) (Scheme 1B).

Exploratory studies performed with ¹⁹F-fluoride provided useful information.³² The difluorocarbene and SO₂ sources were found to be critical in enabling the construction of CF₃SO₂⁻. The reaction of 2,2-difluoro-2-(triphenylphosphonio)acetate (PDFA) with either 1,4-diazabicyclo[2.2.2]octane bis(SO₂) adduct (DABSO)³³ or *N*-methylmorpholine-SO₂ (NMM·SO₂) in the presence of KF/K₂₂₂ in DMF at 100 °C afforded the ammonium salt of CF₃SO₂⁻ in 31% or 44% yield, respectively, after isolation by semipreparative HPLC. A saturated solution of SO₂ in DMF did not lead to product formation, while ClF₂CCO₂Me in combination with PPh₃ was the only alternative difluorocarbene source found to be suitable for this process. For ¹⁸F labeling, PDFA was elected as the optimal reagent. In contrast to experiments carried out with fluoride, DABSO and PDFA afforded [¹⁸F]CF₃SO₂K in trace amounts (Scheme 2A). However, the combination of PDFA, NMM·SO₂ and [¹⁸F]KF/K₂₂₂ gave [¹⁸F]CF₃SO₂K in 22% radiochemical conversion (RCC). These results encouraged the development of a manual protocol to prepare, purify, and isolate this novel

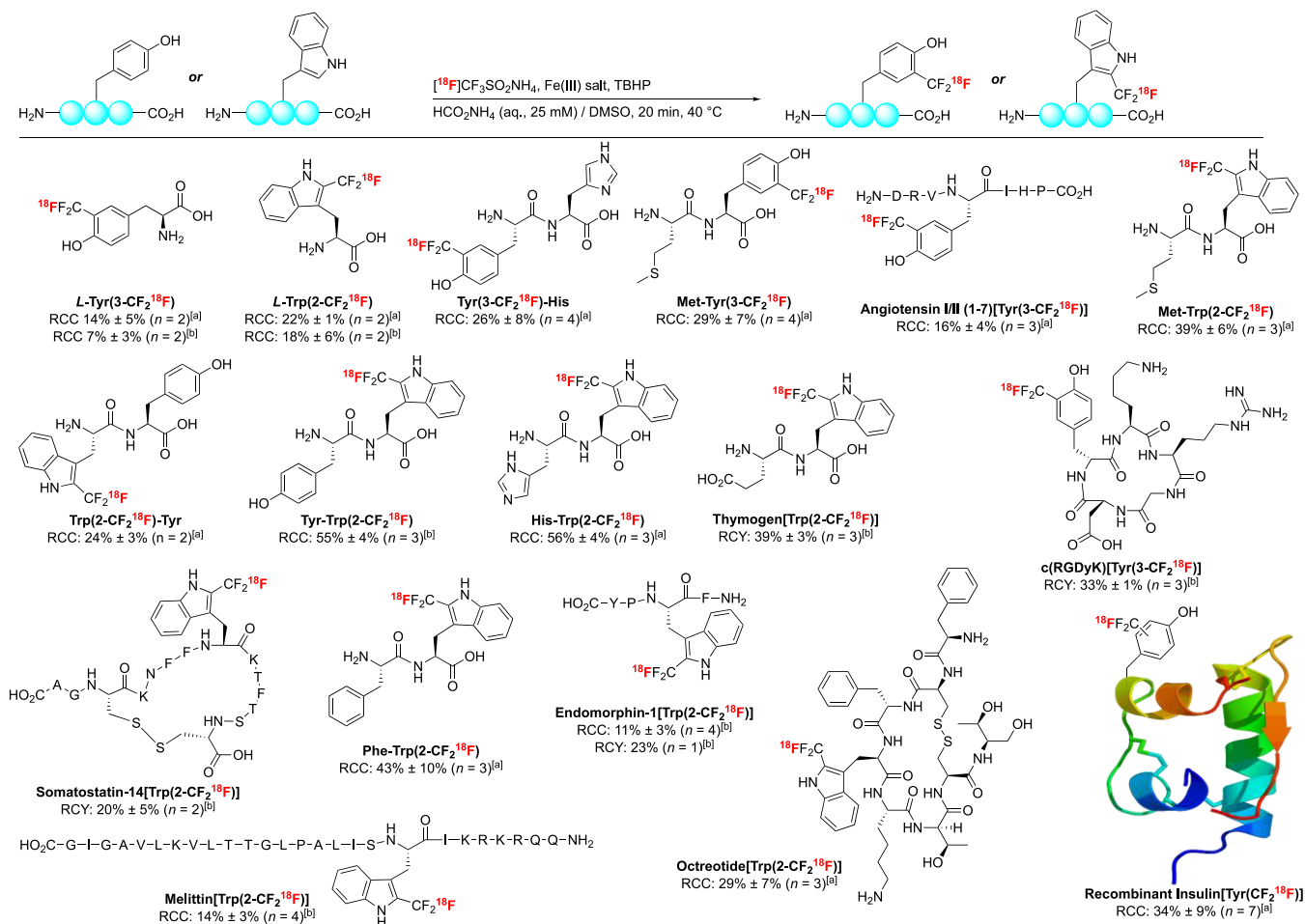
Scheme 2. (A) Initial Studies toward the One-Step Synthesis of [¹⁸F]CF₃SO₂⁻; (B) Radiosynthesis, Purification, and Isolation of [¹⁸F]CF₃SO₂NH₄



¹⁸F reagent for subsequent use (Scheme 2B). PDFA is thermally unstable and poorly soluble in DMF, so a mixture of this reagent and NMM·SO₂ was added as a suspension in a suitable solvent to azeotropically dried ¹⁸F-fluoride. Among all solvents tested, propylene carbonate (PC) was best when used with DMF.³⁴ Additional optimization tuning reagents, ratios of various components, and concentrations proved to be beneficial. The optimal process consisted of reacting PDFA (0.16 mmol) and NMM·SO₂ (0.06 mmol) with [¹⁸F]KF/K₂₂₂ (up to 10 GBq) in 350 μL of PC/DMF mixture at 110 °C. Initial purification of [¹⁸F]CF₃SO₂⁻ using a weak anion exchange cartridge (WAX) removed most of the unreacted [¹⁸F]fluoride and organic byproducts. Elution with a solution of ~0.4 M ammonium hydroxide in EtOH followed by reversed-phase HPLC purification afforded [¹⁸F]CF₃SO₂NH₄ in >99% radiochemical purity. This protocol furnished up to 900 MBq of [¹⁸F]CF₃SO₂NH₄ from 10 GBq of [¹⁸F]fluoride. The overall non-decay-corrected activity yield (AY) of isolated [¹⁸F]CF₃SO₂NH₄ calculated from [¹⁸F]fluoride was 11% ± 1% (n = 6, synthesis time = 70 min). The identity of [¹⁸F]CF₃SO₂NH₄ was established by HPLC and ESI-MS analysis (*m/z* calcd for [¹⁹F]CF₃SO₂⁻, 133.0; found, 133.1).³²

Next, we studied the C–H ¹⁸F-trifluoromethylation of model peptides containing L-tryptophan and/or L-tyrosine residues using [¹⁸F]CF₃SO₂NH₄ and *tert*-butyl hydroperoxide (TBHP) as the oxidant. In ¹⁹F mode, CF₃SO₂Na 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 ¹⁸F radiochemistry because of the inherent constraints on concentrations for both large peptides and [¹⁸F]CF₃SO₂NH₄, the latter being by far the limiting reagent. An additional complication was competitive oxidation of [¹⁸F]CF₃SO₂⁻ to form [¹⁸F]CF₃SO₃⁻ with the initiation oxidant. For ¹⁹F-trifluoromethylation, this issue is solved using an excess of CF₃SO₂Na with respect to TBHP or via slow addition of TBHP to the reaction mixture.³⁶ These solutions are not suitable for ¹⁸F labeling because [¹⁸F]-CF₃SO₂NH₄ is the limiting reagent and operational simplicity is paramount for ¹⁸F radiochemistry.

The treatment of L-Tyr with [¹⁸F]CF₃SO₂NH₄ and TBHP in AcOH/aqueous ammonium formate did not lead to C–H ¹⁸F-trifluoromethylation after 20 min, even at 60 °C.³² Extensive optimization overcame the ¹⁸F labeling constraints and led to L-Tyr(3-CF₂¹⁸F) in 14% RCC when the reaction

Scheme 3. Substrate Scope of C–H ¹⁸F-Trifluoromethylation of Native Aromatic Residues of Peptides^a

^aReagents and conditions: peptide (0.03 mmol), TBHP (2 or 4 equiv), and [a] $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (2 equiv) or [b] FeCl_3 (2 equiv). The synthesis time for the ¹⁸F-labeled peptide from $[^{18}\text{F}]\text{CF}_3\text{SO}_2\text{NH}_4$ was 90 min.³²

was performed in the presence of TBHP and $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ³⁶ in DMSO/aqueous ammonium formate at 40 °C for 20 min (Scheme 3).³² ¹⁸F-trifluoromethylation at C2 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 $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, L-Tyr(3- CF_2^{18}F) was formed in 7% RCC. The C–H ¹⁸F-trifluoromethylation of L-Trp was also successful with $[^{18}\text{F}]\text{CF}_3\text{SO}_2\text{NH}_4$ upon activation by either $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ or FeCl_3 in the presence of TBHP. When these conditions were applied, L-Trp(2- CF_2^{18}F) was obtained in 22% and 18% RCC, respectively. Two additional regioisomers resulting from competitive ¹⁸F labeling at C4 and C7 were also formed, giving a combined RCC of 10% or 9% when $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ or FeCl_3 , respectively, was employed.³⁷

A series of dipeptides was evaluated, focusing on feasibility and selectivity (Scheme 3).³² For reactions leading to more than one ¹⁸F-labeled product, identification was made by comparison of HPLC traces with fully characterized references prepared independently. The dipeptides Tyr-Trp and Trp-Tyr underwent $[^{18}\text{F}]\text{CF}_3$ incorporation exclusively at Trp, a result corroborating our previous studies.²⁴ For Tyr-Trp, ¹⁸F labeling experiments performed with $[^{18}\text{F}]\text{CF}_3\text{SO}_2\text{NH}_4$ and TBHP with either $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ or FeCl_3 gave 40% or 55% RCC, respectively. For the dipeptide Phe-Trp, ¹⁸F-trifluoromethyla-

tion occurred at Trp, affording Phe-Trp(2- CF_2^{18}F) in 43% RCC. $[^{18}\text{F}]\text{CF}_3$ incorporation on Trp occurred at C2, C4, and C7 (C2 major), while ¹⁸F labeling on Phe was not observed. ¹⁸F labeling at His was not detected for either Tyr-His or His-Trp. Met oxidation was minimized for the ¹⁸F-trifluoromethylation of Met-Trp or Met-Tyr by decreasing the 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 (ogluflanide)³⁸ was ¹⁸F-trifluoromethylated at Trp with an isolated radiochemical yield (RCY) calculated from $[^{18}\text{F}]\text{CF}_3\text{SO}_2\text{NH}_4$ of 39%. Endomorphin-1, a tetrapeptide associated with Alzheimer's disease,^{39,40} underwent Trp-selective ¹⁸F labeling in 23% RCY, and somatostatin-14, a cyclic tetradecapeptidic hormone with a broad inhibitory effect on endocrine secretion, was ¹⁸F-labeled in 20% RCY.⁴¹ The ¹⁸F-trifluoromethylations of melittin,⁴² a 26-residue venom peptide, and octreotide,⁴³ an octapeptide that mimics natural somatostatin, were 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 $\alpha\beta_3$ receptors,⁴⁶ both underwent ¹⁸F labeling at Tyr in 16% and 33% RCC, respectively. The C–H ¹⁸F-

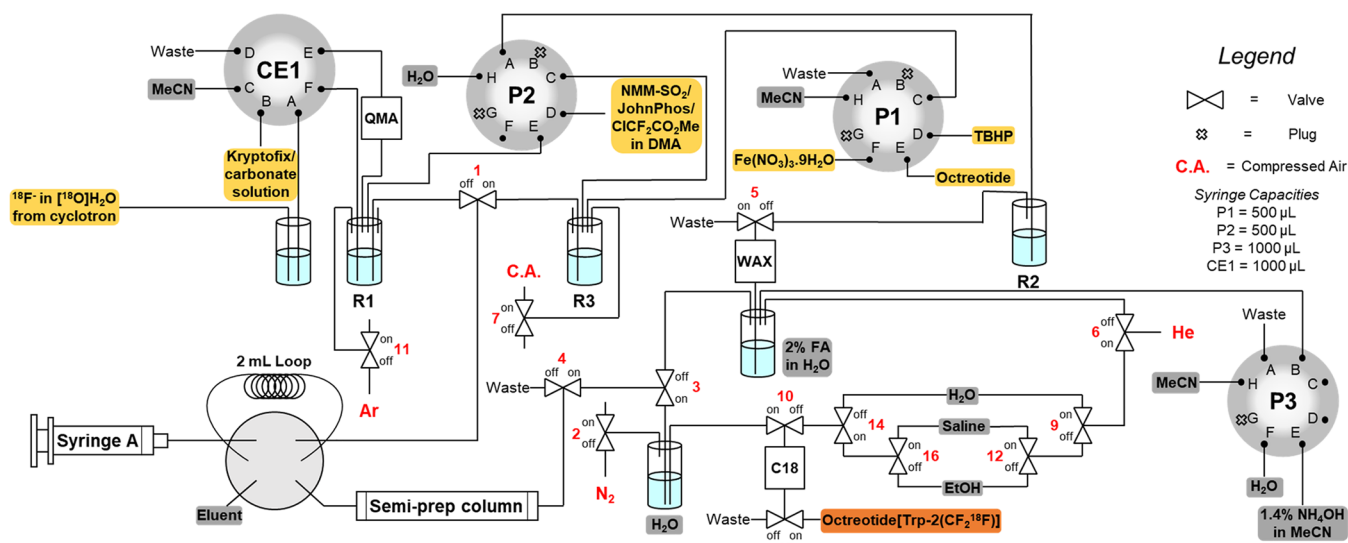


Figure 2. Automated radiosynthesis of octreotide[Trp(2- CF_2 - ^{18}F)] from [^{18}F]fluoride on the Advion NanoTek microfluidic synthesis system.

trifluoromethylation of a much larger peptide, recombinant human insulin (MW = 5808 Da), was also considered.⁴⁷ This experiment was carried out with insulin (5.2 μmol), $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (5.8 equiv), and TBHP (11.5 equiv) in DMSO/aqueous ammonium formate and afforded [^{18}F]CF₃-insulin in 34% overall RCC as a mixture of four products resulting from [^{18}F]CF₃ incorporation at all tyrosine residues. The main site of ^{18}F -trifluoromethylation was at chain A residue Y19, a result consistent with the report of Krska et al.²⁵

To date, automated radiosyntheses have focused on small molecules but 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]CF₃SO₂NH₄ required optimization of selected steps. The addition of the suspension of PDFA and NMM·SO₂ 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 $\text{ClF}_2\text{CCO}_2\text{Me}$, a reagent activated with (2-biphenyl)di-*tert*-butylphosphine (JohnPhos), and the solvent to DMA; no change was required for NMM·SO₂. With these modifications, starting from up to 45 GBq of [^{18}F]fluoride, [^{18}F]CF₃SO₂NH₄ was produced in up to 6% \pm 1% activity yield (non-decay-corrected, $n = 2$) after semipreparative HPLC ($A_m = 1.13 \text{ GBq}/\mu\text{mol}$, synthesis time = 40 min). Removal of HPLC solvents was necessary to afford dry [^{18}F]CF₃SO₂NH₄ required for peptide ^{18}F labeling. This critical drying step also required extensive modification. For automation, [^{18}F]CF₃SO₂NH₄ was trapped on a WAX cartridge and subsequently eluted with NH₄OH in MeCN (1.4%) followed by evaporation.

Successful C–H ^{18}F -trifluoromethylation in the presence of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (4 equiv) and TBHP (8 equiv) afforded up to 69 MBq of octreotide[Trp(2- CF_2 - ^{18}F)] ($n = 3$, $A_m = 0.28 \pm 0.08 \text{ GBq}/\mu\text{mol}$) after purification by HPLC. The total synthesis time from [^{18}F]fluoride to octreotide[Trp(2- CF_2 - ^{18}F)] was 133 min. This automated protocol enabled an in vivo PET imaging experiment with this [^{18}F]CF₃-peptide on naive Sprague–Dawley rats, a preliminary study suggesting excretion via the gastrointestinal pathway and the kidneys.^{32,49–51}

In conclusion, we have reported the first protocol enabling direct ^{18}F labeling of unmodified peptides at tryptophan and tyrosine residues (with high selectivity for tryptophan) via 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]CF₃SO₂NH₄, which was prepared in one step from [^{18}F]fluoride, a difluorocarbene reagent, and a source of SO₂. The iron salt was essential to overcome the difficulties arising from [^{18}F]CF₃SO₂NH₄ 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, unrivaled by known methods making use of minimally sized labeled prosthetics,^{23,52,53} sets the stage for in-depth investigations of clinically relevant peptides. In view of the number of reactions relying on Langlois-type reagents, [^{18}F]CF₃SO₂NH₄ 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 at <https://pubs.acs.org/doi/10.1021/jacs.9b11709>.

Detailed experimental procedures, characterization of new compounds, automation protocol, and in vivo experiments (PDF)

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Notes

The authors declare no competing financial interest.

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