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Communication

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

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ABSTRACT: ^{18}F labeling strategies for unmodified peptides with [^{18}F]fluoride require ^{18}F-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 ^{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 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, ^{18}F is a widely used and clinically relevant radionuclide.[2] Because of 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 the fact that cyclotron-produced ^{18}F sources are 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 prefunctionalized 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 the efficacy and/or function.[1c] Therefore, 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.

We reported the ^{18}F-trifluoromethylation of native peptides with 5-^{18}F-(trifluoromethyl)dibenzothiophenium trifluoromethanesulfonate, a method modifying cysteine thiols (Figure 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' re-

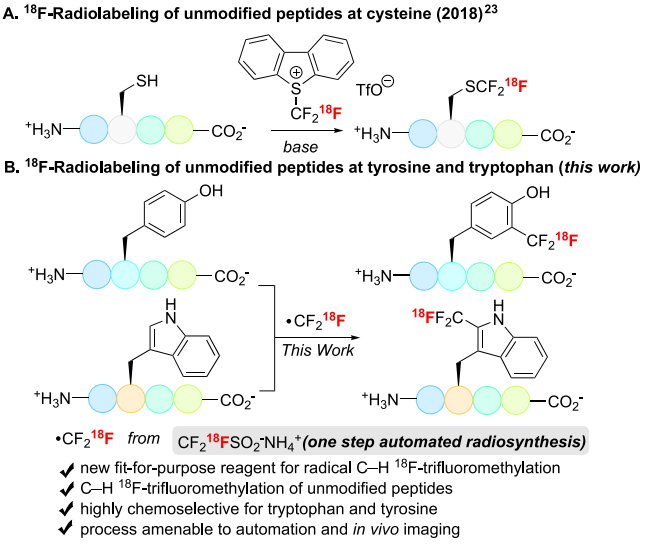

Figure 1. Direct ^{18}F-trifluoromethylation of native residues in unmodified peptides.

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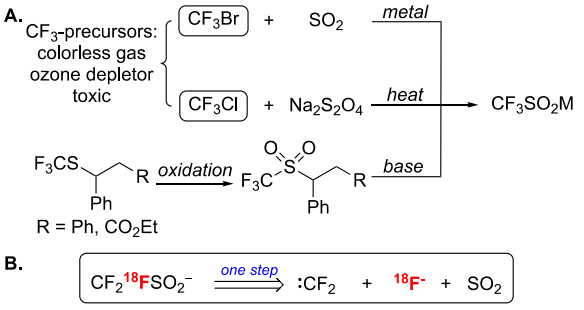
agent) displayed selective reactivity for tryptophan under redox initiation. Recently, Krska et al. demonstrated that $Zn(TFMS)_2$ (Baran's reagent), when activated with a stoichiometric oxidant or via visible-light photoredox catalysis, enabled trifluoromethylation of tyrosine in peptides that do not contain tryptophan residues.[25] These precedents encouraged us to produce ^{18}F-trifluoromethanesulfinate for selective C−H ^{18}F-trifluoromethylation of these aromatic amino acid residues within unmodified peptides. This approach would generate noncanonical [^{18}F]CF_3-tryptophan and -tyrosine residues, a transformation unmatched by alternative ^{18}F labeling methods (Figure 1B).

Routes toward trifluoromethanesulfinic acid salts include metal or electroreduction of a mixture of SO_2 and CF_3Br in *N*,*N*-dimethylformamide (DMF),[26] treatment of CF_3Cl with $Na_2S_2O_4$,[27] or multistep syntheses from trifluoromethylsulfone precursors (Scheme 1A).[28] For ^{18}F radiochemistry, these

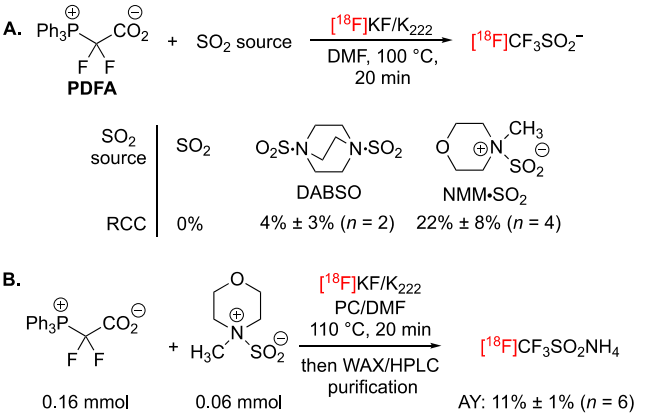
Scheme 1. (A) Multistep Syntheses toward Trifluoromethanesulfinic Acid Salts (M = Metal); (B) Proposed One-Step Radiosynthesis toward ^{18}F-Trifluoromethanesulfinate



approaches would require a route toward the [^{18}F]CF_3 precursor and one or more reactions postlabeling. Our design plan was to construct [^{18}F]$CF_3SO_2^-$ in one step by applying a multicomponent 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).

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*-methylmorpholine·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% or 44% yield, respectively, after isolation by semipreparative HPLC. A saturated solution of SO_2 in DMF did not lead to product formation, while ClF_2CCO_2Me in combination with PPh_3 was the only alternative difluorocarbene source found to be 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

Scheme 2. (A) Initial Studies toward the One-Step Synthesis of [^{18}F]$CF_3SO_2^-$; (B) Radiosynthesis, Purification, and Isolation of [^{18}F]$CF_3SO_2NH_4$



^{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. 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·SO_2 (0.06 mmol) with [^{18}F]KF/K_{222} (up to 10 GBq) in 350 μL of 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 byproducts. Elution with a solution of ∼0.4 M ammonium hydroxide in EtOH followed by reversed-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 non-decay-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 min). The identity of [^{18}F]$CF_3SO_2NH_4$ was established by HPLC and ESI-MS analysis (m/z calcd for [^{19}F]$CF_3SO_2^-$, 133.0; found, 133.1).[32]

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 *tert*-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 because of the 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]$CF_3SO_2^-$ to form [^{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/aqueous ammonium formate did not lead to C−H ^{18}F-trifluoromethylation after 20 min, even at 60 °C.[32] Extensive optimization overcame the ^{18}F labeling constraints and led to L-Tyr(3-CF_2^{18}F) in 14% RCC when the reaction

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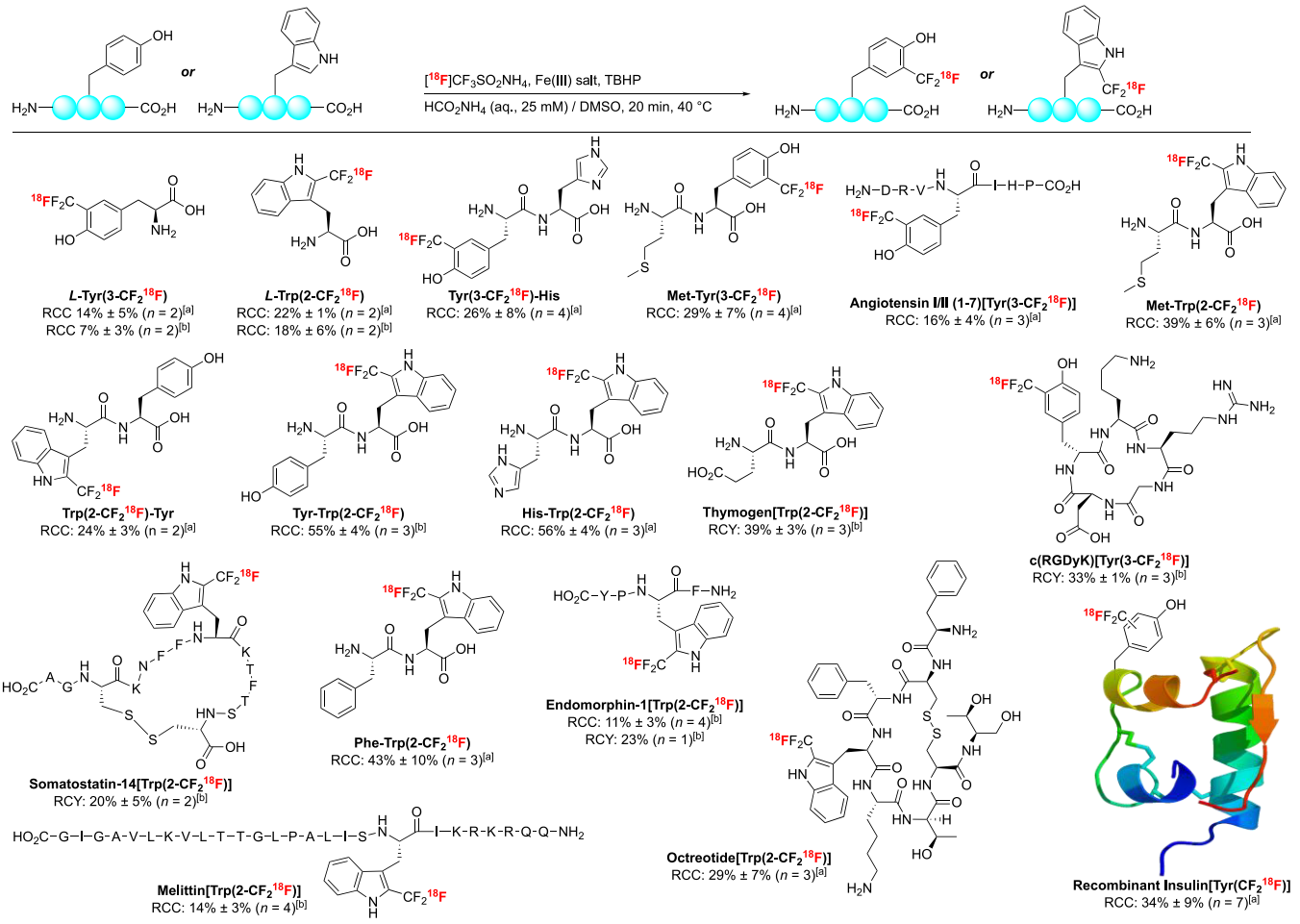
Scheme 3. Substrate Scope of C−H ^{18}F-Trifluoromethylation of Native Aromatic Residues of Peptides[a]



[a]Reagents and conditions: peptide (0.03 mmol), TBHP (2 or 4 equiv), and [a] $Fe(NO_3)_3{\cdot}9H_2O$ (2 equiv) or [b] $FeCl_3$ (2 equiv). The synthesis time for the ^{18}F-labeled peptide from $[^{18}F]CF_3SO_2NH_4$ was 90 min.[32]

was performed in the presence of TBHP and $Fe(NO_3)_3{\cdot}9H_2O$[36] in DMSO/aqueous ammonium formate at 40 °C for 20 min (Scheme 3).[32] ^{18}F-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 $Fe(NO_3)_3{\cdot}9H_2O$, 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 $[^{18}F]CF_3SO_2NH_4$ upon activation by either $Fe(NO_3)_3{\cdot}9H_2O$ 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 ^{18}F labeling at C4 and C7 were also formed, giving a combined RCC of 10% or 9% when $Fe(NO_3)_3{\cdot}9H_2O$ 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 ^{18}F-labeled product, identification was made by comparison of HPLC traces with fully characterized references prepared independently. The dipeptides Tyr-Trp and Trp-Tyr underwent $[^{18}F]CF_3$ 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% or 55% RCC, respectively. For the 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 C2, C4, and C7 (C2 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 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 (oglufanide)[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 a broad inhibitory effect on endocrine secretion, was ^{18}F-labeled in 20% RCY.[41] The ^{18}F-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 $\alpha_v\beta_3$ receptors,[46] both underwent ^{18}F labeling at Tyr in 16% and 33% RCC, respectively. The C−H ^{18}F-

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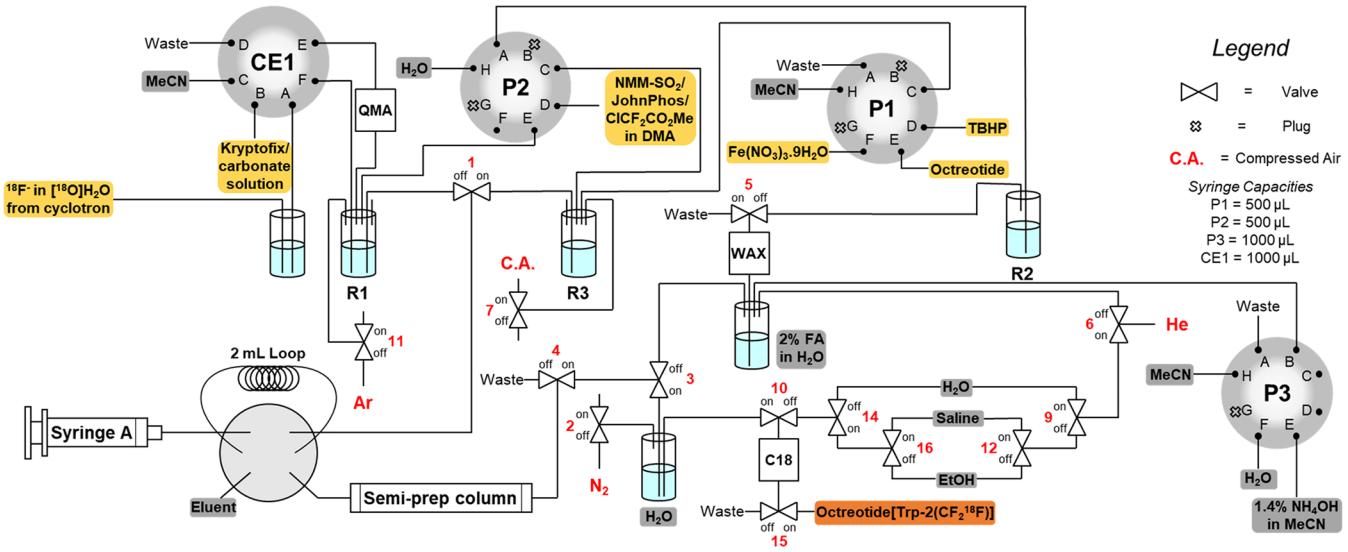

Figure 2. Automated radiosynthesis of octreotide[Trp(2-$CF_2{}^{18}F$)] from [^{18}F]fluoride on the Advion NanoTek microfluidic synthesis system.

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), $Fe(NO_3)_3 \cdot 9H_2O$ (5.8 equiv), and TBHP (11.5 equiv) in DMSO/aqueous ammonium formate and afforded [^{18}F]CF_3-insulin in 34% overall RCC as a mixture of four products resulting from [^{18}F]CF_3 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]$CF_3SO_2NH_4$ required optimization of selected steps. The addition of the suspension of PDFA and NMM·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·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 semipreparative HPLC (A_m = 1.13 GBq/μmol, synthesis time = 40 min). 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.

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 of 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 min. 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 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]$CF_3SO_2NH_4$, which was prepared in one step from [^{18}F]fluoride, a difluorocarbene reagent, and a source of SO_2. 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, 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]$CF_3SO_2NH_4$ 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 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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