

Review Article

Radiodifluoromethylation of well-functionalized molecules

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Received March 25, 2024; Accepted April 2, 2024; Epub April 25, 2024; Published April 30, 2024

Abstract: The strategic installation of a [^{18}F]fluorine atom at the specific position of the lead molecule is a never-ending challenge for radiochemists in their endeavour to develop novel positron emission tomography (PET) imaging applications. Although the radiosynthesis of [^{18}F]CF $_2$ H-containing molecules has been explored in the past decade, more methods need to be explored for various well-functionalized compounds. Recently, two novel strategies of radiodifluoromethylation were reported, namely the utilization of [^{18}F]difluorocarbene building block and frustrated Lewis pair-mediated C- ^{18}F bond formation, respectively. These methods provide an efficient radiofunctionalization of complex CF $_2$ H-containing molecules for drug discovery and PET ligand development.

Keywords: [^{18}F]difluoromethylation, [^{18}F]difluorocarbene, frustrated Lewis pair, positron emission tomography

Introduction

PET has evolved as a functional and noninvasive imaging modality, playing a pivotal role in diagnosing diseases, monitoring disease progression, and aiding drug development. The cornerstone of this technique lies in the development of PET tracers - biologically targeted small or large molecules labeled with positron-emitting radionuclides [1]. Among the PET nuclides, fluorine-18 (^{18}F) stands out as the preferred radioisotope due to its advantageous attributes, including a practical half-life ($t_{1/2} = 109.8$ min), a favorable positron emission profile (97%), relatively low positron energy ($E = 0.635$ keV) and high availability. Within the realm of various fluorinated moieties, the difluoromethyl (CF $_2$ H) group, considered a lipophilic bioisostere of conventional hydrogen-bond donor groups such as hydroxy (-OH) and thiol (-SH) groups [2], has garnered significant attention in drug discovery [3]. A plethora of bioactive molecules bearing the CF $_2$ H motif have been discovered, including thiazopyr, pantoprazole, and ZSTK474 etc. (Figure 1).

As various methodologies for the synthesis of CF $_2$ H-containing molecules have emerged, there have been continuous efforts in the exploration of radiosynthesis methods involving [^{18}F]CF $_2$ H (Figure 2). Gouverneur and coworkers developed a silver(I)-mediated [^{18}F]fluorodecarboxylation method, using electrophilic [^{18}F]fluorinating reagents to convert α -fluorarylacetic acids (Figure 2A) [4]. They also introduced an $^{18}\text{F}/\text{Cl}$ exchange reaction involving Ar-CHFCI and nucleophilic [^{18}F]fluoride (Figure 2A) [5]. Similarly, Shen, Gouverneur and coworkers disclosed the radiosynthesis of [^{18}F]Ar-SCF $_2$ H by an Ag-mediated $^{18}\text{F}/\text{Cl}$ exchange strategy [6]. Furthermore, this strategy was implemented for the radiolabeling of ethyl α -bromo- α -fluoro(aryloxy)acetates to generate [^{18}F] α,α -difluoroaryloxy acetic acid, which could be further transformed into

[^{18}F]Ar-OCF $_2$ H [7]. In 2019, Gouverneur's work established a Mn-mediated [^{18}F]fluorodecarboxylation process, enabling access to [^{18}F]Ar-CF $_2$ H and [^{18}F]ArO-CF $_2$ H moieties (Figure 2A) [8]. Ritter, Vasdev, Liang and coworkers disclosed a cascade method for preparing [^{18}F](het)Ar-CF $_2$ H, which involved the activation of the benzoyl auxiliary, followed by sequential benzylic bromination and $^{18}\text{F}/\text{Br}$ exchange (Figure 2A) [9]. Subsequently, the Liang group reported a two-step strategy that included the nucleophilic ^{18}F -fluorination of benzyl (pseudo)halides, followed by oxidative benzylic C-H fluorination (Figure 2B) [10]. Genicot and coworkers described a photoredox flow approach for direct radical C-H ^{18}F -difluoromethylation of *N*-heteroarenes (Figure 2C) [11]. In addition, some multi-step syntheses were reported for obtaining [^{18}F]CF $_2$ H-containing compounds. For example, these syntheses involve Ag-mediated desulfurative [^{18}F]fluorination [12] and [^{18}F]fluorination of iodonium salt [13]. In spite of these outstanding results, there is still an unmet need to develop new methods to introduce [^{18}F]CF $_2$ H group on well functionalized drug-like scaffolds. In this highlight, we focus on two recent advances of radiodifluoromethylation using a new type of [^{18}F]difluorocarbene building block and frustrated Lewis pair-activated C- ^{18}F bond formation, respectively. These new methodologies may provide a new strategy and 'retro-radiosynthetic' approach to design ^{18}F -labeled difluoromethyl groups beyond conventional methods and enable novel functionalities to be explored in PET pharmaceuticals.

Literature highlight

Direct [^{18}F]difluoromethylation from [^{18}F]difluorocarbene

The Gouverneur group introduced a novel method for [^{18}F]difluoromethylation using [^{18}F]difluorocarbene ([^{18}F]DFC) to generate a variety of (hetero)arenes bearing [^{18}F]CF $_2$ H

$[^{18}\text{F}]\text{CF}_2\text{H}$ labeling

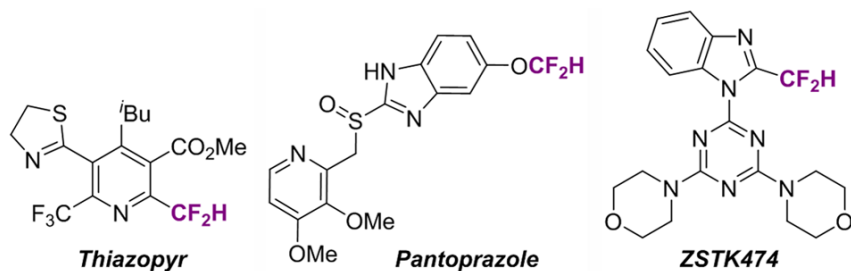


Figure 1. Representative CF_2H -containing bioactive molecules.

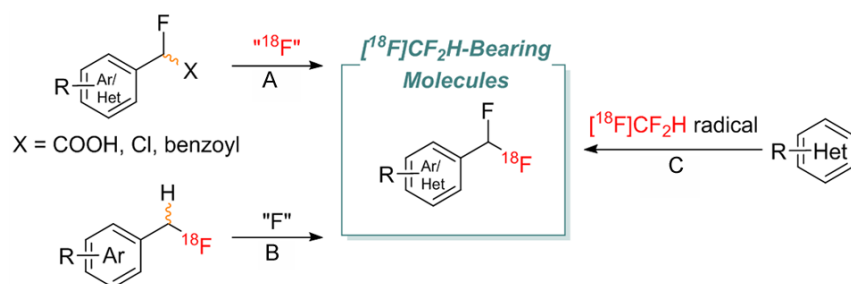


Figure 2. Previous approaches to access $[^{18}\text{F}]\text{CF}_2\text{H}$ -containing molecules.

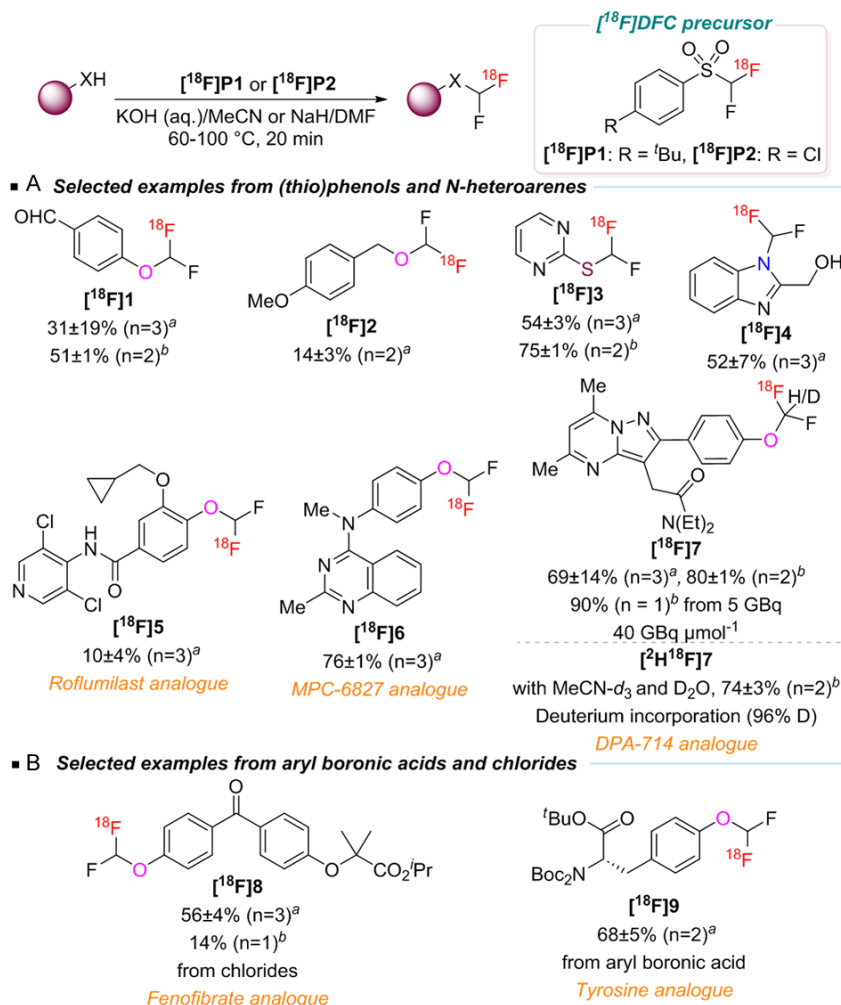


Figure 3. $[^{18}\text{F}]\text{DFC}$ insertion to form $[^{18}\text{F}]\text{CF}_2\text{H-X-Are}$ motifs (X = O, S and N). ^a $[^{18}\text{F}]\text{P1}$ was used. ^b $[^{18}\text{F}]\text{P2}$ was used.

motif [14]. The work entailed $[^{18}\text{F}]\text{DFC}$ insertion into X-H (X = O, S, and N) bonds, and cross-coupling reactions with palladium-DFC complexes.

A wide range of electronically and/or sterically diverse (hetero)aryl phenols, thiophenols, and N-heteroarenes were investigated, yielding the desired products featuring $[^{18}\text{F}]\text{OCF}_2\text{H}$, $[^{18}\text{F}]\text{SCF}_2\text{H}$, and $[^{18}\text{F}]\text{NCF}_2\text{H}$ moieties (Figure 3).

Notably, $[^{18}\text{F}]\text{DFC}$ selectively inserted into the phenolic O-H bond of 4-hydroxybenzaldehyde ($[^{18}\text{F}]\text{1}$, 51% radiochemical yield, RCY). Selective ^{18}F -difluoromethylation at the nitrogen atom was achieved in the case of (1H-benzo[d]imidazol-2-yl) methanol, yielding $[^{18}\text{F}]\text{4}$ with 52% RCY. Subsequently, late-stage ^{18}F -difluoromethylation of complex biologically active molecules was successfully demonstrated, including analogues of roflumilast ($[^{18}\text{F}]\text{5}$), MPC-6827 ($[^{18}\text{F}]\text{6}$) and DPA-714 ($[^{18}\text{F}]\text{7}$). Additionally, $[^2\text{H}^{18}\text{F}]\text{7}$ was obtained using deuterated solvents, such as CD_3CN and D_2O , which is significant because deuterium (D) incorporation is a common strategy to enhance the metabolic stability of drug molecules and radiotracers. An in vivo study was conducted with $[^{18}\text{F}]\text{7}$ in the application of imaging microglial activation of Huntington's disease. The authors also reported a one-pot tandem procedure, involving borylation, oxidation, and sequential ^{18}F -difluoromethylation, for producing labeled analogues of drugs and bioactive molecules (for example $[^{18}\text{F}]\text{8}$ and $[^{18}\text{F}]\text{9}$). One plausible reaction mechanism involves a series of cascade steps, namely the deprotonation of DFC precursor, DFC expulsion and addition, and protonation. The free DFC is favored as the predominant pathway for this DFC insertion transformation (Figure 4A). By contrast, the concerted $\text{S}_{\text{N}}2$ -like pathway is of higher energy barrier (Figure 4B).

Pd-catalyzed $[^{18}\text{F}]\text{difluoromethylation}$ of aryl boronic acids was also successfully achieved through the capture and transfer of $[^{18}\text{F}]\text{DFC}$ (Figure 5). This method was applied to a diverse array of aryl boronic acids, including those featuring heterocycles and vinyl groups. Importantly, $[^{18}\text{F}]\text{CF}_2\text{H}$ analogues of various drugs and bioactive molecules, such as Fenofibrate and protected phenylalanine,

■ **Proposed Mechanism**

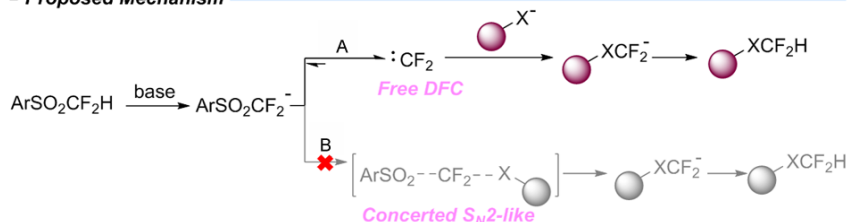


Figure 4. $[^{18}\text{F}]\text{DFC}$ insertion transformation ($X = \text{O}, \text{S}$ and N).

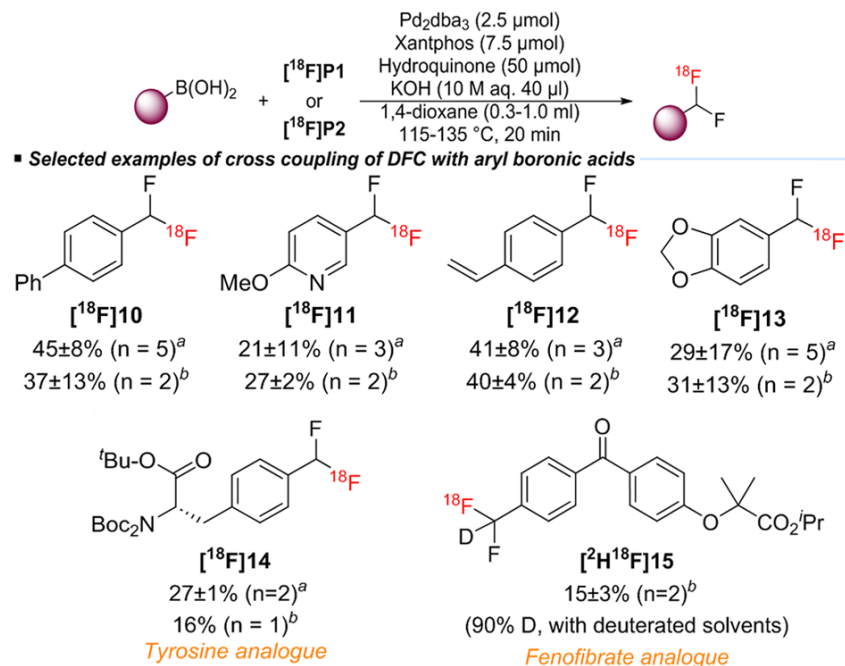


Figure 5. $[^{18}\text{F}]\text{DFC}$ -mediated cross coupling reactions to form $[^{18}\text{F}]\text{CF}_2\text{H}$ -Arene motifs. ^a $[^{18}\text{F}]\text{P1}$ was used. ^b $[^{18}\text{F}]\text{P2}$ was used.

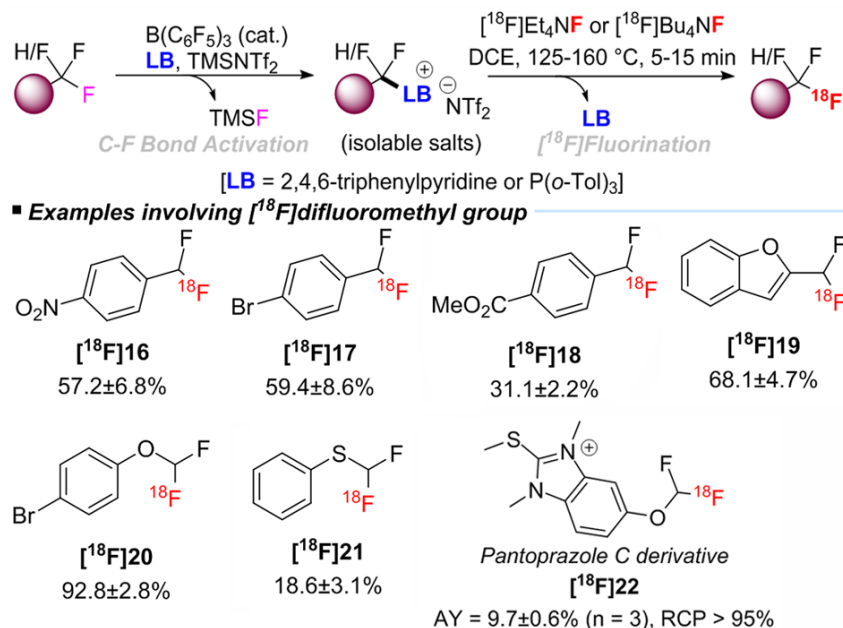


Figure 6. $[^{18}\text{F}]\text{Difluoromethylation}$ using frustrated Lewis pair method.

were successfully obtained under these conditions.

Frustrated Lewis pair-mediated C- ^{18}F bond formation

Young and coworkers have previously reported that the frustrated Lewis pair (FLP) can selectively activate C-F bonds within CF_3^- and CF_2H -containing compounds [15]. This activation leads to the formation of pyridinium and phosphonium salts in high yield. These resulting salts can be employed in a diverse range of transformations, involving Wittig coupling [15a], nucleophilic substitution, photoredox alkylation, nucleophilic transfer, and hydrogenation reactions [15b], to incorporate a wide array of functional groups into parent molecules. Building upon their pioneering work, Young and his team have now successfully applied this two-step approach to ^{18}F labeling of difluoromethyl and trifluoromethyl groups [16].

This FLP-mediated transformation process tolerates a variety of well functionalized groups (Figure 6). Difluoromethylarenes with electron-withdrawing nitro, bromo, and ester groups can be isotopically labeled, to generate the corresponding products in 31-59% radiochemical conversions. Furthermore, difluoromethylated heteroarenes, difluoromethoxide and difluoromethylsulfide substrates ($[^{18}\text{F}]\text{19}$ - $[^{18}\text{F}]\text{21}$) were also obtained in 19-93% conversions. As a proof-of-concept, a biologically relevant molecule $[^{18}\text{F}]\text{22}$, as a derivative of pantoprazole C, was successfully isolated with non-decay corrected yields of ca. 10%. Control experiments indicated that the cationic fragment of the salts likely served as a source of ^{18}F fluoride, which is unfortunately detrimental to obtain higher molar activity in the $[^{18}\text{F}]\text{fluorination}$ process.

Conclusion

Undoubtedly, these recent advances present novel opportunities and stimulate innovative ideas for the development of potential $[^{18}\text{F}]\text{CF}_2\text{H}$ -containing PET imaging probes. It is firmly anticipated that an expanding array of strategies for synthesizing compounds featuring the $[^{18}\text{F}]\text{CF}_2\text{H}$ group will be unveiled, thus contributing to the ongoing advancement

of drug discovery and PET applications across diverse scientific disciplines.

Acknowledgements

The authors acknowledge support in part from the NIH (AG073428, MH128705, AG079956, AG080262 and NS130128), Emory Radiology Chair Fund and Endowed Directorship.

Disclosure of conflict of interest

None.

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[¹⁸F]CF₂H labeling

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