

[¹⁸F]Difluorocarbene for Positron Emission Tomography

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Summary Paragraph

The advent of total-body Positron Emission Tomography (PET) has vastly broadened the range of research and clinical applications of this powerful molecular imaging technology.¹ Such possibilities have accelerated progress in ¹⁸F-radiochemistry with numerous methods available to ¹⁸F-label (hetero)arenes and alkanes.² However, access to ¹⁸F-difluoromethylated molecules in high molar activity (A_m) is largely an unsolved problem, despite the indispensability of the difluoromethyl group for pharmaceutical drug discovery.³ We report herein a general solution by introducing carbene chemistry to the field of nuclear imaging with a [¹⁸F]difluorocarbene reagent capable of a myriad of ¹⁸F-difluoromethylation processes. In contrast to the tens of known difluorocarbene reagents, this ¹⁸F-reagent is carefully designed for facile accessibility, high molar activity and versatility. The issue of A_m is solved using an assay examining the likelihood of isotopic dilution upon variation of the electronics of the difluorocarbene precursor. Versatility is demonstrated with multiple [¹⁸F]difluorocarbene based reactions including O–H, S–H and N–H insertions, and cross-couplings that harness the reactivity of ubiquitous functional groups such as (thio)phenols, *N*-heteroarenes, and aryl boronic acids that are easy to install. Impact is illustrated with the labelling of highly complex and functionalised biologically relevant molecules and radiotracers.

Introduction

Since the pioneering work of Curtius⁴ and Staudinger,⁵ carbenes have played an important role in organic and organometallic chemistry,⁶ and have found applications in biological,⁷ medicinal⁸ and material sciences.⁹ One area yet to embrace the synthetic power of carbenes in radiolabelled form is nuclear science, and more specifically, Positron Emission Tomography (PET) (Fig. 1a). This molecular imaging technology, routinely used for clinical diagnosis and drug development, requires radiotracers for *in vivo* tracking of complex biological processes.¹⁰ Radiolabelling is possible with a range of positron-emitting radionuclides including ¹⁸F that displays outstanding properties (97% β^+ decay, 109.8 min half-life, 635 KeV positron energy).¹¹ A vital criterion for broad utility is high molar activity (A_m), which is best achieved using cyclotron-produced [¹⁸F]fluoride.¹² Despite recent advances in ¹⁸F-radiochemistry, access to ¹⁸F-labelled polyfluoroalkylated molecules is largely an unsolved problem, a considerable drawback considering the omnipresence of these motifs in drug discovery.^{13,3} A significant obstruction to progress in ¹⁸F-polyfluoroalkylation is low A_m caused by ¹⁹F-fluoride leaching from the fluorinated precursors used in ¹⁸F-labelling. The most fruitful efforts have focused on ¹⁸F-trifluoromethylation with metal-mediated and radical strategies using [¹⁸F]fluoride.^{14–16} Strikingly, molecules featuring a difluorinated motif found to be vital for drug efficacy, are either not within reach as ¹⁸F-isotopologues, only accessible in prohibitively low A_m , or from precursors that require lengthy syntheses.^{17–22} In a unique approach, a [¹⁸F]CF₂H radical precursor was generated from [¹⁸F]fluoride, but the more nucleophilic character of \cdot CF₂H with respect to \cdot CF₃ limits its utility to a prohibitively narrow pool of heteroarenes.²³ With this current state of play, the distinct advantages of CF₂H routinely embraced in drug discovery programs,³ including reduced lipophilicity compared to CF₃ and hydrogen bond donor ability, are not within reach for PET ligand discovery. Difluorocarbene chemistry can offer a general solution.^{3,24} Tens of reagents have been invented that can be activated under various conditions

enabling the released difluorocarbene (DFC) to participate in insertions,^{25,26} cycloadditions,^{24,27,28} and cross-coupling reactions^{29–32} leading to complex molecules substituted with (X)CF₂H/R (X = Csp³, Csp², O, N, S). In stark contrast, studies on [¹⁸F]difluorocarbene ([¹⁸F]DFC) are almost non-existent. In 1970, energetic ¹⁸F atoms from nuclear recoil were found to react with CF₂H₂ or CF₄ to release [¹⁸F]DFC upon substitution followed by elimination.³³ This study on the mechanism of atomic exchange reactions is neither practical nor suitable for PET ligand discovery. Here, we describe the merits of a bespoke [¹⁸F]DFC reagent prepared in high A_m. Its broad reactivity profile enabling O–H, S–H and N–H insertions, and cross-coupling reactions offers exciting opportunities in ¹⁸F-radiolabelling for PET ligand discovery and more generally for nuclear medicine.

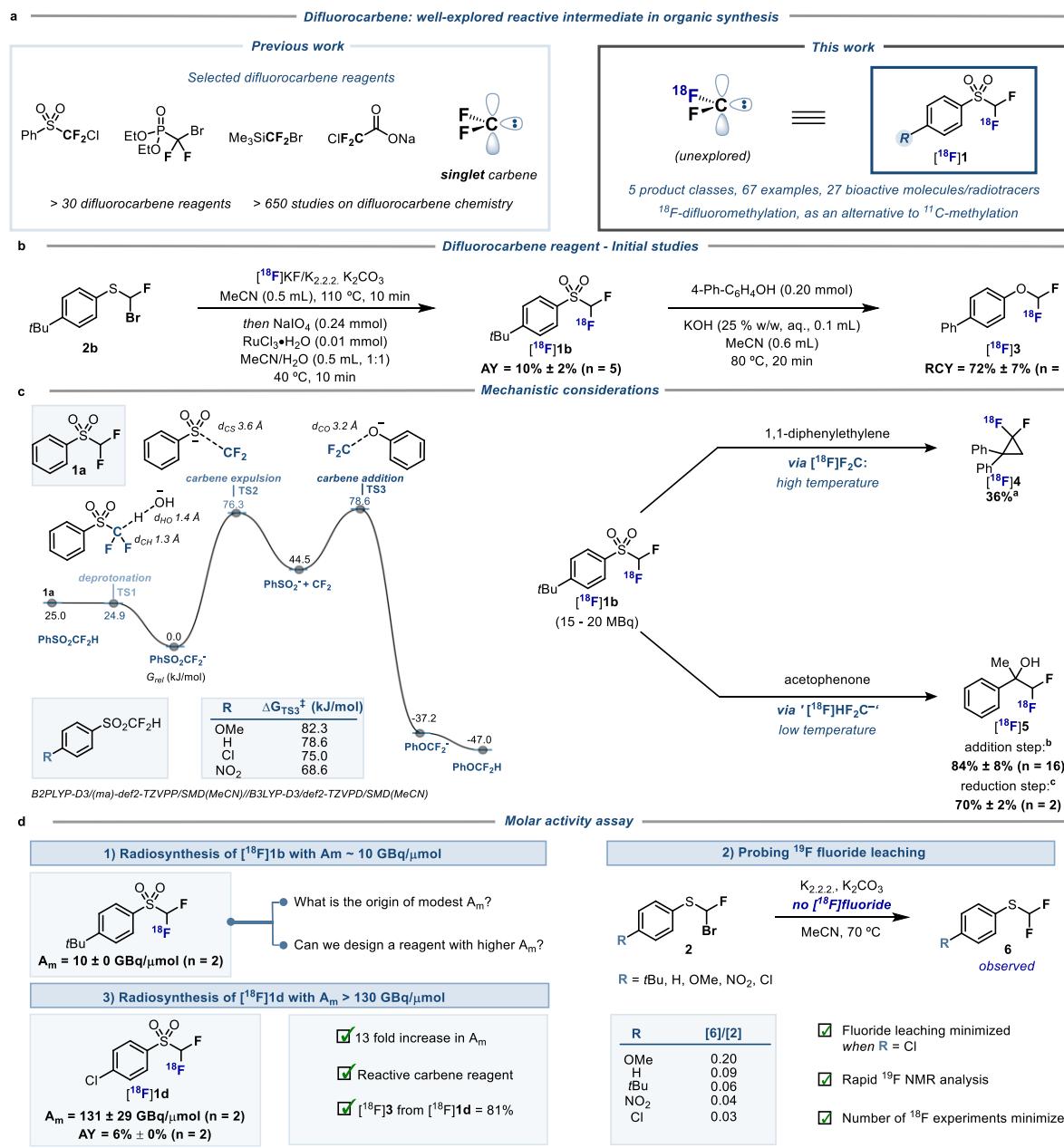


Fig. 1|Difluorocarbene chemistry, radiosynthesis and divergent reactivity of $^{18}\text{F}\text{1}$.

a. Development of the ^{18}F -difluorocarbene reagent **1** offering new opportunities in PET radiotracer discovery. **b.** Two-step one-pot radiosynthesis of **1b** and subsequent ^{18}F -difluoromethylation of [1,1'-biphenyl]-4-ol. **c.** Stepwise mechanism for difluorocarbene release from **1a**. Carbene and nucleophilic reactivity of **1b**. RCY determined by radioHPLC. ^a1,1-Diphenyl-ethylene (0.10 mmol), NaOH (0.05 mmol), propylene carbonate (0.3 mL), 200 °C. ^bAcetophenone (0.04 mmol), LiHMDS (0.10 mmol), THF/DMI (9:1), -78 °C, 20 min. ^cMg (0.80

mmol), DMF/AcOH (9:1), rt, 20 min. **d.** ^{19}F NMR fluoride leaching assay. Ratio [**6**]/[**2**] measured at 1 h (Supplementary Fig. 14).

Results and Discussion

Initial experiments indicated that a strategy based on $^{19}\text{F}/^{18}\text{F}$ exchange of in situ generated DFC had a poor prognosis, encouraging the implementation of an ^{18}F -labelled reagent-based approach (Supplementary Fig. 26).³⁴ We ruled out volatile candidates (e.g., HCF_3 , ClCF_2H) or salts (e.g., $\text{ClF}_2\text{CO}_2\text{Na}$, $\text{Ph}_3\text{P}^+\text{CF}_2\text{CO}_2^-$) that would be difficult to handle, purify or characterise, as well as reagents that preferentially require fluoride for DFC release in order to avoid isotopic dilution (e.g., TMSCF_2Br). Considering the demand for methods enabling direct ^{18}F -difluoromethylation of oxygen, sulfur and nitrogen nucleophiles, we opted for a reagent releasing [^{18}F]DFC under basic conditions to allow simultaneous activation of the heteroatom nucleophile for insertion reactions. Although not used as a DFC precursor, ((difluoromethyl)sulfonyl)benzene (**1a**) stood out as an attractive candidate for labelling. In 1960, Hine and Porter reported that **1a** reacts with sodium methoxide in methanol to provide difluoromethyl ether at a rate faster than expected for an $\text{S}_{\text{N}}2$ reaction, an observation suggesting a mechanism involving DFC formation;³⁵ no further studies ensued likely due to the invention of more efficient DFC reagents than **1a**. From a radiochemistry perspective, however, radiochemical yield does not necessarily correlate with chemical yield, and in our judgement, **1a** could offer clear advantages over newer reagents (Supplementary Fig. 27, Supplementary Table 7). Specifically, [^{18}F]**1a** could be within reach using cyclotron-produced [^{18}F]fluoride by applying a protocol that minimizes radiosynthesis time and therefore decay, and critically offers opportunities to solve the A_m problem by tuning the electronic properties of the aryl group. In initial experiments, we opted to label 1-(*tert*-butyl)-4-((difluoromethyl)sulfonyl)benzene (**1b**) considering volatility and precursor stability. Nucleophilic substitution of (bromofluoromethyl)-(4-(*tert*-butyl)phenyl)sulfane (**2b**) with [^{18}F]fluoride followed by

$\text{RuCl}_3/\text{NaIO}_4$ oxidation afforded $[\text{F}^{18}]1\mathbf{b}$ isolated in $10\% \pm 2\%$ ($n = 5$) non-decay corrected (n.d.c.) activity yield (AY) (Fig. 1b). Gratifyingly, the reaction of $[\text{F}^{18}]1\mathbf{b}$, a model phenol and KOH gave $[\text{F}^{18}]3$ in 72% radiochemical yield (RCY), suggesting that $[\text{F}^{18}]$ DFC release and O–H insertion took place under these conditions. The radiosynthesis was automated on the cassette-based TRASIS AllinOne platform, and $[\text{F}^{18}]1\mathbf{b}$ was obtained in 72 mins overall synthesis time in a n.d.c activity yield (AY) of up to 6%, and A_m of $10 \pm 0 \text{ GBq}/\mu\text{mol}$ ($n = 2$, decay corrected (d.c.) to the end of synthesis) from 148 GBq of starting activity (Supplementary Fig. 33). At this stage, further studies focused on mechanism and improving the A_m .

Quantum chemical studies, performed at the B2PLYP-D3/(ma)-def2-TZVPP//B3LYP-D3/def2-TZVPD level of theory with SMD acetonitrile, gave insight into the mechanism of DFC release under basic conditions, its reactivity with a phenol, and the effect of phenyl substitution of $[\text{F}^{18}]1$ (Fig. 1c) (Supplementary Table 8). DFC formation from $1\mathbf{a}$ occurs by stepwise α -elimination. Initial C–H deprotonation by hydroxide is facile and exergonic by 24.9 kJ/mol, while the subsequent C–S cleavage, which liberates free DFC, has a barrier of 76.3 kJ/mol and is endergonic by 44.5 kJ/mol. The overall barrier for phenol difluoromethylation, in which C–O formation between free DFC and the phenolate anion is rate-limiting, is 78.6 kJ/mol. Although recombination of DFC and the phenyl sulfinate anion is kinetically competitive, this will occur reversibly, while C–O formation is highly exergonic by 81.7 kJ/mol and therefore irreversible. The possibility of a concerted $S_{\text{N}}2$ -like carbene transfer was considered, however, with an activation barrier of 112.2 kJ/mol (Supplementary Fig. 67), this can be rejected in favour of the stepwise process illustrated (Fig. 1c). A series of synthetically accessible difluoromethyl para-substituted aryl sulfones were examined computationally. In each case, the C–O formation step with phenolate defines the overall activation barrier, and is consistent with experimental studies (Supplementary Table 8); more electron-deficient substituents were computationally predicted to show greater reactivity. Each reagent considered showed both

kinetic feasibility and thermodynamic irreversibility of C–O formation, suggesting flexibility with regard to the choice of the para-substituent for the optimisation of A_m . Experimentally, [^{18}F]**1b** with 1,1-diphenylethylene and NaOH underwent [2+1] cycloaddition to afford the *gem*-difluorinated cyclopropane product [^{18}F]**4** in 36% RCY, demonstrating that [^{18}F]**1b** releases [^{18}F]DFC under these conditions. We also prepared difluoromethylated alcohol [^{18}F]**5** by reacting acetophenone with [^{18}F]**1b** under base activation followed by reductive cleavage of the sulfone group. These results demonstrated the divergent reactivity profile of [^{18}F]**1** that can serve either as [^{18}F]DFC precursor or as a surrogate of the [^{18}F]CF₂H anion (Fig. 1c).

Under labelling conditions, a plausible scenario to account for the low A_m of [^{18}F]**1b** is nucleophilic substitution of **2b** with hydroxide, affording an α -fluorohemiacetal prone to ^{19}F -fluoride elimination, which then competes for reaction with **2b** (Supplementary Table 13). Computational studies predict that S_N2 displacement is more facile with a hydroxide nucleophile than fluoride, with standard activation barriers (i.e., neglecting differences in concentration) of 62.5 and 92.8 kJ/mol, respectively (Supplementary Fig. 69).

Experimentally, $^{19}\text{F}/^{18}\text{F}$ isotope exchange of sulfone **1b** was not observed under the conditions applied for ^{18}F -incorporation, but importantly, the reaction of **2b** with K₂₂₂/K₂CO₃ in the absence of external fluoride source gave (4-(*tert*-butyl)phenyl)(difluoromethyl)sulfane **6b**, as evidenced by ^{19}F NMR spectroscopy (Supplementary Fig. 14 and Supplementary Table 1). As varying aryl substitution may influence the degree of fluoride leaching and offer a pathway to improve A_m , five DFC precursors featuring -H (**2a**), *p*-*t*Bu (**2b**), *p*-NO₂ (**2c**) *p*-Cl (**2d**) and *p*-OMe (**2e**) substitution were reacted with K₂₂₂/K₂CO₃ in the absence of external fluoride. These all afforded the corresponding (aryl)(difluoromethyl)sulfanes (**6**), albeit in varying amounts (Fig. 1d). The results indicated that **6** was formed in increased amounts in the following order: Cl ~ NO₂ < *t*Bu < H < OMe, highlighting that chloro or nitro para-substitution may increase A_m by reducing ^{19}F -fluoride leaching. Experiments performed in the presence of [^{18}F]fluoride

(135–148 GBq of starting activity) informed that [¹⁸F]**1d** was obtained in the highest A_m (131 ± 29 GBq/μmol, n = 2, decay corrected to the end of synthesis), a result corroborating our ¹⁹F NMR assay.

With [¹⁸F]**1b** and [¹⁸F]**1d** in hand, our priority objective was to develop a versatile route to ¹⁸F-labelled ArOCF₂H, a motif increasingly encountered in medicinal chemistry.^{3,36} Currently, ¹⁸F-labelling requires over-engineered starting materials (ArOCHFCl or ArOCHFCO₂H) that are not readily accessible and afford [¹⁸F]ArOCF₂H in prohibitively low A_m (< 1 GBq/μmol).^{21,37} ¹⁸F-Difluoromethylation of alcohol precursors with [¹⁸F]DFC would represent a direct approach to [¹⁸F]ArOCF₂H, and complements ¹¹C-methylation protocols routinely applied in radioligand design.^{38,39} Building on our initial experiments with [1,1'-biphenyl]-4-ol and [¹⁸F]**1b** (Fig. 1b), we applied optimised reaction conditions consisting of reacting 0.20 mmol of [1,1'-biphenyl]-4-ol with [¹⁸F]**1b** or [¹⁸F]**1d** for 20 mins at 80 °C under aqueous KOH conditions (MeCN/H₂O = 6/1, v/v, 0.7 mL) to a range of phenols. A variety of functionalised electronically and sterically differentiated (hetero)aryl phenols found in pharmaceutical agents gave the desired labelled products. Specifically, the method tolerates alkoxy, ketone, alkyl, ester, halide, cyano, nitro, sulfonamide, aldehyde and basic amine functionalities ([¹⁸F]**3**, [¹⁸F]**6–23** (27% – 88% RCY)) (Fig. 2). Of note, [¹⁸F]DFC inserted site-selectively into the phenolic O–H of 4-hydroxybenzaldehyde (¹⁸F)**15**, 51%). A substrate with an ester prone to basic hydrolysis was labelled applying modified reaction conditions (NaH, DMF at 140 °C) (¹⁸F)**23**, 27% RCY). Even challenging benzylic alcohol (4-methoxyphenyl)methanol of higher pKa underwent [¹⁸F]DFC insertion, albeit with a lower RCY of 14% (¹⁸F)**24**). Next, we investigated [¹⁸F]DFC insertions into S–H and N–H bonds. These ¹⁸F-labeling reactions lead to motifs that currently either require multi-step precursor synthesis, limiting scope (¹⁸F)SCF₂H),²² or are not available in their ¹⁸F-labelled form (¹⁸F)NCF₂H). Electronically neutral, deficient, and rich

(hetero)aromatic thiophenols readily underwent [¹⁸F]DFC insertion, providing access to all [¹⁸F]SCF₂H products ([¹⁸F]25–29, 70%–90% RCY). Benzimidazole was successfully subjected to N–H insertion under either aqueous (KOH; MeCN/H₂O = 6/1), ([¹⁸F]30, 81% RCY) or anhydrous reaction conditions (NaH, DMF, 61% RCY). (1*H*-benzo[*d*]imidazol-2-yl)methanol underwent selective ¹⁸F-difluoromethylation at nitrogen affording [¹⁸F]31 (52% RCY). 1*H*-Indazole gave [¹⁸F]32 in good RCY (73%) (1:1 regioisomers). Similarly, 1*H*-benzo[*d*][1,2,3]triazole was a competent substrate ([¹⁸F]33, 71% RCY). Functional group tolerance investigated with a robustness screening is broad (Supplementary Figs. 15–17). With this information in hand, we considered the late-stage ¹⁸F-difluoromethylation of complex biologically active molecules (Fig. 3). The ¹⁸F isotopologue of the anti-inflammatory drug roflumilast [¹⁸F]35, along with eight [¹⁸F]OCF₂H and [¹⁸F]NCF₂H drug analogues (estrone [¹⁸F]34, 2'-hydroxy-3-phenylpropiophenone [¹⁸F]36, chloroxine [¹⁸F]37, PF-03774076 [¹⁸F]38, triclabendazole [¹⁸F]39 and thiabendazole [¹⁸F]40) were successfully labelled with RCYs reaching 82%. Moreover, the [¹⁸F]OCF₂H analogues of the PET radioligands P2X7R, PMB-protected PF-06809247, DASA-23, MPC-6827, DPA-714, and iloperidone were obtained in up to 98% RCY ([¹⁸F]41–[¹⁸F]46). As the proton source leading to OCF₂H originates from the solvent (Supplementary Figs. 3–4), [²H¹⁸F]OCF₂H radiotracers ([²H¹⁸F]41, 84% RCY, 94% D; [²H¹⁸F]46, 74% RCY, 96% D; [²H¹⁸F]34, 64% RCY, 96% D) were all within reach. This is significant because deuterium incorporation is a common strategy to increase metabolic stability of drugs and radiotracers.⁴⁰ For drug analogues (fenofibrate, FMTEB, *N*-Boc-fluoxetine, phenylalanine, flutamide and etoricoxib) that do not feature a phenol functionality, we developed a one-pot sequence from aryl boronic acids (Fig. 3b). Oxidation with urea hydrogen peroxide (0.20 mmol, 1.0 equiv.) in MeCN for 5 mins followed by [¹⁸F]DFC O–H insertion led to [¹⁸F]47, [¹⁸F]48, [¹⁸F]49, [¹⁸F]50, [¹⁸F]51 and [¹⁸F]52 in good RCYs (22% – 86%). For fenofibrate featuring a C(sp²)–Cl bond, we developed a one-pot

sequence consisting of borylation-oxidation-¹⁸F-difluoromethylation (Supplementary Fig. 45). With the knowledge that our ¹⁸F-difluoromethylation protocol is not detrimentally impacted by impurities, as evidenced by one-pot tandem procedures, a telescoped radiosynthesis of [¹⁸F]46 directly from [¹⁸F]fluoride was implemented to reduce the time required for labelling and purification. A one-pot ¹⁸F-fluorination-oxidation sequence to generate [¹⁸F]1b, followed by C-18 filtration, ¹⁸F-difluoromethylation, semi-preparative HPLC purification and reformulation afforded [¹⁸F]46 in 2% AY and >99% radiochemical purity (135 mins total synthesis time) (Supplementary Fig. 50). This result is comparable to AY obtained for clinically relevant [¹⁸F]UCB-J prepared directly from [¹⁸F]fluoride.⁴¹ An in vivo study using naive C57BL/6J mice was undertaken with FCH₂CH₂O-substituted [¹⁸F]DPA-714 and its OCF₂H analogue [¹⁸F]46.⁴²⁴³ The study started with the radiosynthesis of [¹⁸F]46 (680 MBq, A_m = 40 GBq/μmol) using [¹⁸F]1d and the TRASIS AllinOne platform. Extracts of mouse plasma and brain homogenates 5 minutes post-injection of [¹⁸F]46 were absent of metabolites based on radio-HPLC, this is in contrast to [¹⁸F]DPA-714 where radiolabelled metabolites were observed (Supplementary Figs. 62–65). These initial data corroborate expectations on the metabolic stability of OCF₂H.⁴⁴ To further demonstrate the utility of this [¹⁸F]DFC method in supporting (pre)clinical PET studies, [¹⁸F]46 was successfully used to image microglial activation in the striatum of a quinolinic acid lesion model of Huntington's disease (Supplementary Fig. 66).⁴⁵

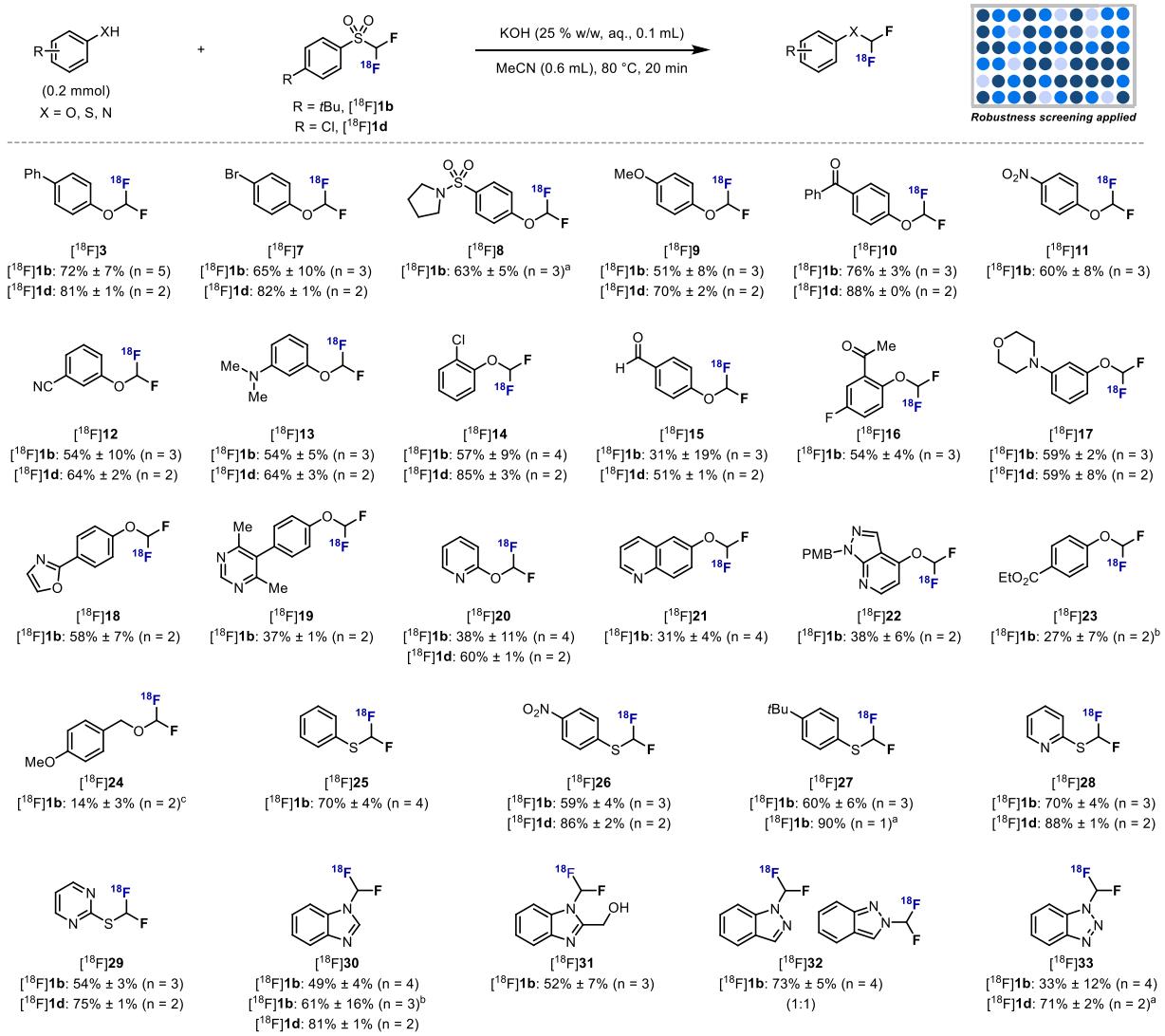


Fig. 2| Scope of $[^{18}\text{F}]$ OCF₂H, $[^{18}\text{F}]$ SCF₂H and $[^{18}\text{F}]$ NCF₂H from (thio)phenols and N-heterocycles.

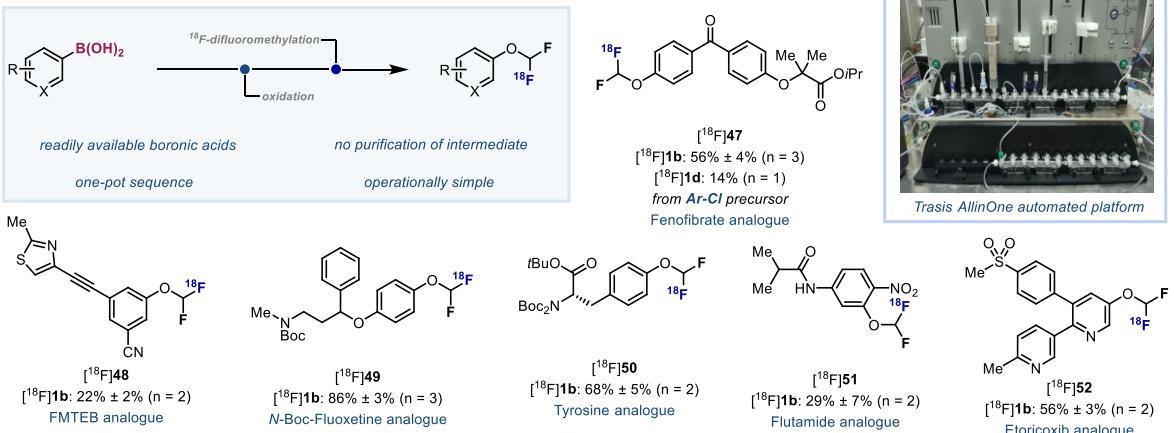
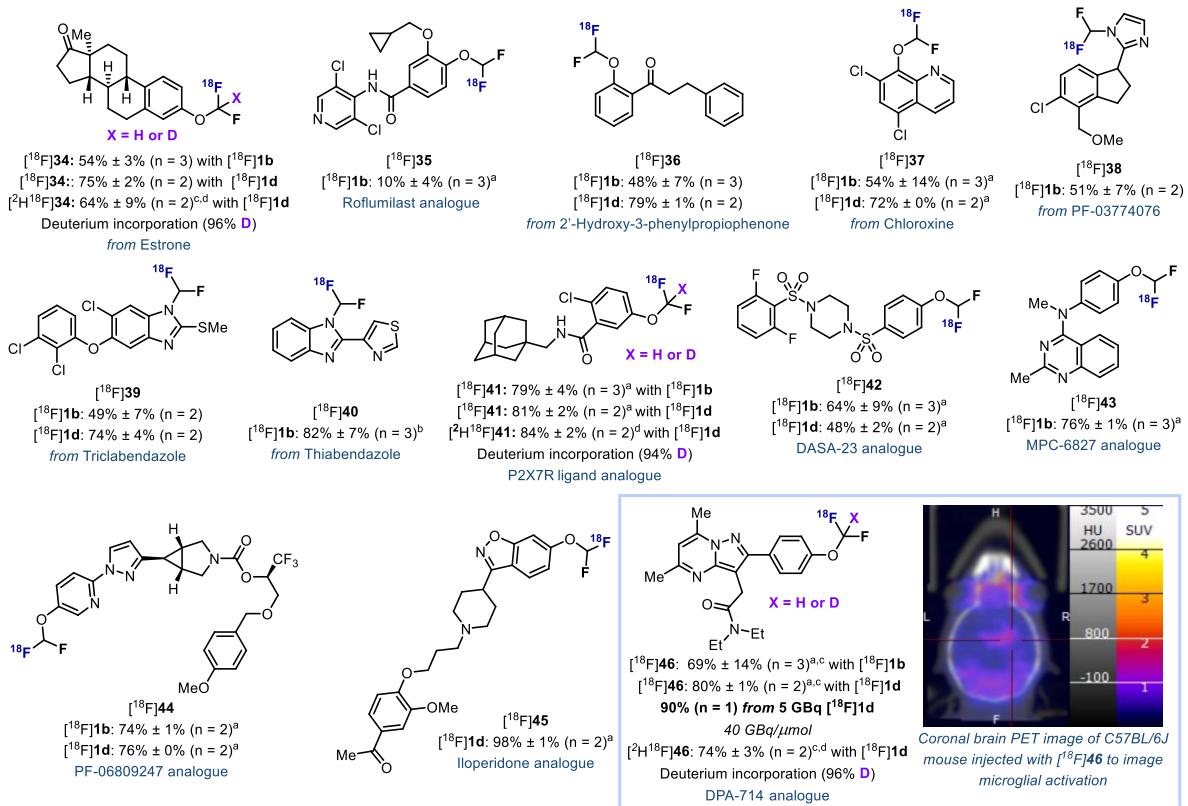


Fig. 3| Scope of [¹⁸F]OCF₂H, [¹⁸F]SCF₂H and [¹⁸F]NCF₂H. a, Biologically relevant molecules and radioligands scope of [¹⁸F]OCF₂H and [¹⁸F]NCF₂H from phenols and N-heterocycles. b, Scope of [¹⁸F]OCF₂H from boronic acids and aryl chlorides (Supplementary Figs. 44–45). RCY determined by radioHPLC. ^aReaction at 100 °C. ^bReaction in DMF (0.3 mL) at 140 °C with NaH (0.10 mmol) instead of KOH. ^cReaction on 0.10 mmol scale. ^dMeCN-*d*₃, D₂O, 100°C (Supplementary Fig. 43).

Next, we investigated the use of transition-metal complexes for the capture and transfer of [¹⁸F]DFC derived from [¹⁸F]1b and [¹⁸F]1d for site-selective aromatic ¹⁸F-difluoromethylation, an additional unsolved problem in ¹⁸F-radiochemistry.⁴⁶ Metal-DFC complexes known to convert aryl boronic acids into difluoromethylated arenes served as a starting point for investigations.^{29–32} 1a is amenable to Cu-mediated cross-coupling with aryl boronic acids leading to (phenylsulfonyl)difluoromethyl-substituted arenes, so the challenge at hand was to favour the formation of ¹⁸F-difluoromethylated arene products from [¹⁸F]1.⁴⁷ After extensive optimisation studies on 4-biphenyl boronic acid (0.20 mmol) (Supplementary Fig. 46 and Supplementary Table 6), [¹⁸F]4-(difluoromethyl)-1,1'-biphenyl ([¹⁸F]53) was formed in 45% RCY under basic conditions (40 µL KOH_(aq)) in the presence of Pd₂dba₃ (2.50 µmol), Xantphos (7.50 µmol) and hydroquinone (50 µmol) in 1,4-dioxane (1.0 mL) at 130 °C. In the absence of Pd or hydroquinone, [¹⁸F]53 was not formed, or in significantly decreased RCY (Supplementary Table 6). In contrast to Pd cross-coupling reactions that must be performed under anaerobic conditions, our F-18 protocol does not require the exclusion of moisture or air.^{29–32} These optimised conditions were applied to various aryl boronic acids (Fig. 4), including those featuring heterocycles commonly found in drug discovery pipelines, yielding [¹⁸F]56, [¹⁸F]58, [¹⁸F]60, [¹⁸F]61 in up to 45% RCY. (6-Methoxypyridin-3-yl)boronic acid underwent site-selective ¹⁸F-difluoromethylation at the 3-position in 27% RCY ([¹⁸F]56), thereby complementing radical processes favouring the 2- and 4-positions.²³ Substrates presenting functional groups such as alkenes ((4-vinylphenyl)boronic acid) and alkynes ((3-cyano-5-((2-methylthiazol-4-yl)ethynyl)phenyl)boronic acid) yielded [¹⁸F]62 and [¹⁸F]64 in 41% and 24% RCY, respectively. Aryl boronic acids can therefore be used either for ¹⁸F-difluoromethylation or ¹⁸F-difluoromethoxylation. Fenofibrate ([¹⁸F]63 and [¹⁸F]47), FMTEB ([¹⁸F]64 and [¹⁸F]48), N-Boc-fluoxetine ([¹⁸F]65 and [¹⁸F]49), protected phenylalanine ([¹⁸F]66 and [¹⁸F]50) were

subjected to cross-coupling or tandem oxidation ^{18}F -difluoromethylation conditions to afford either $[^{18}\text{F}]\text{CF}_2\text{H}$ or $[^{18}\text{F}]\text{OCF}_2\text{H}$ analogues from a common precursor (RCY = 20%–86%).

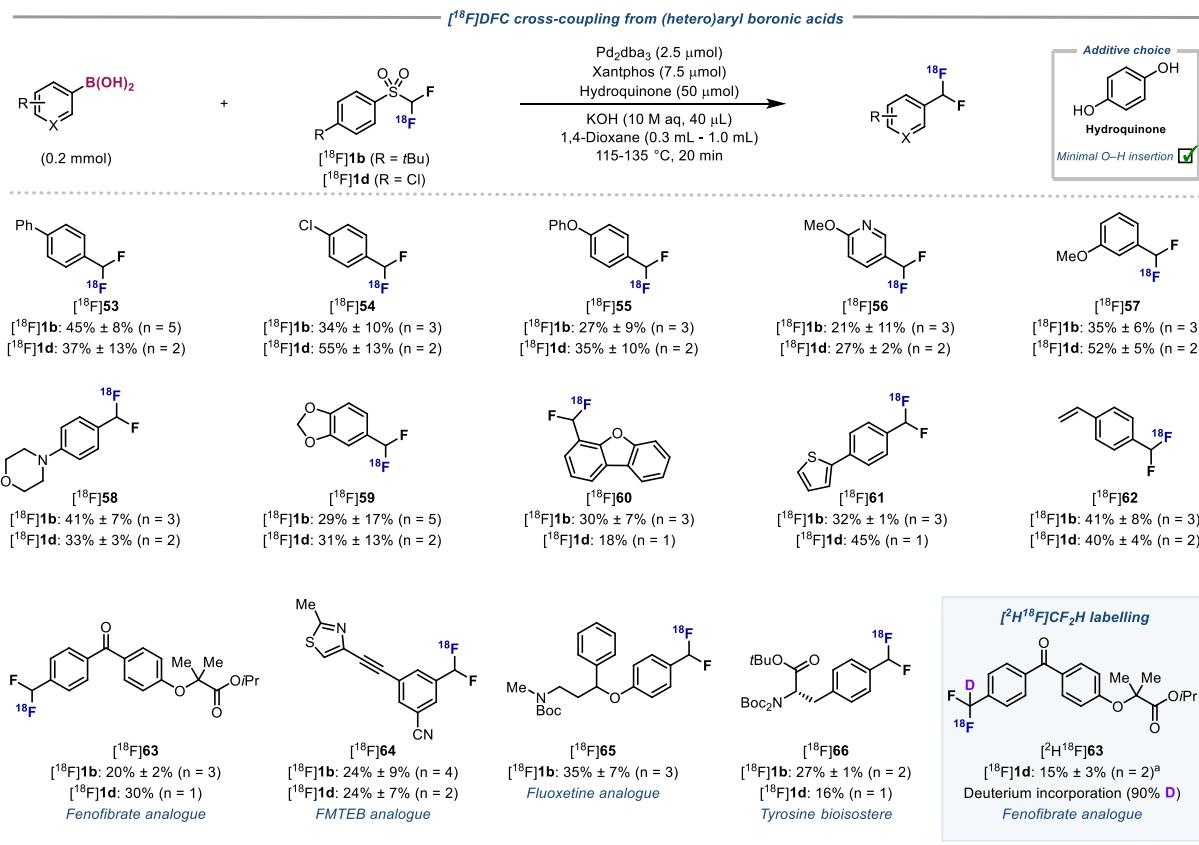


Fig. 4| Scope of $[^{18}\text{F}]\text{ArCF}_2\text{H}$. RCY determined by radioHPLC. Reactions performed with 1,4-dioxane (0.3–1.0 mL) at 115–135 °C. ^a1,4-dioxane-*d*₈ (1.0 mL), D₂O, hydroquinone-*d*₂, 120 °C (Supplementary Figs. 47 and 56).

Using hydroquinone-*d*₂ and 1,4-dioxane-*d*₈, the [²H¹⁸F]CF₂H analogue of fenofibrate [²H¹⁸F]63 was obtained in 15% RCY (90% D). Inductively coupled plasma mass spectrometry (ICP-MS) analysis of [¹⁸F]53 indicated a Ru and Pd content of 242.18 µg/L and 18.53 µg/L respectively, which is below the threshold recommended by ICH Q3D(R1) guidelines for in-human injection.⁴⁸

Conclusion

PET ligand discovery is at the forefront of molecular imaging and, similarly to pharmaceutical discovery, can immediately benefit from a diversity-oriented approach to radiolabelling. Although most practicing radiochemists are familiar with ¹⁸F-incorporation leading to aryl and alkyl fluoride, the ambition to consider ¹⁸F-polyfluoroalkyl substitution as a means to invent new PET ligands has been tempered by the likelihood of low A_m, and the discouraging requirement to synthesise over-engineered precursors only accessible after time-consuming multi-step syntheses. We now present [¹⁸F]difluorocarbene radiochemistry as a demonstration that the unique properties of the difluoromethyl group can now be exploited in PET ligand discovery programs without compromising on A_m and precursor availability. The simplicity of the protocol and the diverse range of molecules labelled in this study should encourage rapid adoption in PET centres that have access to cyclotron-produced [¹⁸F]fluoride, and more generally spark new programs to advance nuclear medicine imaging.

References and Notes

- 1) Reardon, S. Whole-body PET scanner produces 3D images in seconds. *Nature* **570**, 285–287 (2019).
- 2) Ajenjo, J., Destro, G., Cornelissen, B. & Gouverneur, V. Closing the gap between ¹⁹F and ¹⁸F chemistry. *EJNMMI radiopharm. chem.* **6**, 1–37 (2021).

- 3) Sap, J. B. *et al.* Late-stage difluoromethylation: concepts, developments and perspective. *Chem. Soc. Rev.* **50**, 8214–8247 (2021).
- 4) Buchner, E. & Curtius, T. Ueber die Einwirkung von Diazoessigäther auf aromatische Kohlenwasserstoffe. *Ber. Dtsch. Chem. Ges.* **18**, 2377–2379 (1885).
- 5) Staudinger, H. & Kupfer, O. Über reaktionen des methylens. iii. Diazomethan. *Ber. Dtsch. Chem. Ges.* **45**, 501–509 (1912).
- 6) Hopkinson, M. N., Richter, C., Schedler, M. & Glorius, F. An overview of N-heterocyclic carbenes. *Nature* **510**, 485–496 (2014).
- 7) Geri, J. B. *et al.* Microenvironment mapping via Dexter energy transfer on immune cells. *Science* **367**, 1091–1097 (2020).
- 8) Marinelli, M., Santini, C. & Pellei, M. Recent advances in medicinal applications of coinage-metal (Cu and Ag) N-heterocyclic carbene complexes. *Curr. Top. Med. Chem.* **16**, 2995–3017 (2016).
- 9) Smith, C. A. *et al.* N-heterocyclic carbenes in materials chemistry. *Chem. Rev.* **119**, 4986–5056 (2019).
- 10) Campbell, M. G. *et al.* Bridging the gaps in ¹⁸F PET tracer development. *Nat. Chem.* **9**, 1–3 (2017).
- 11) Deng, X. *et al.* Chemistry for positron emission tomography: recent advances in ¹¹C-, ¹⁸F-, ¹³N-, and ¹⁵O-labeling reactions. *Angew. Chem. Int. Ed.* **58**, 2580–2605 (2019).
- 12) McCluskey, S. P., Plisson, C., Rabiner, E. A. & Howes, O. Advances in CNS PET: the state-of-the-art for new imaging targets for pathophysiology and drug development. *Eur. J. Nucl. Med. Mol. Imaging* **47**, 451–489 (2020).
- 13) Prchalová, E., Štěpánek, O., Smrček, S. & Kotora, M. Medicinal applications of perfluoroalkylated chain-containing compounds. *Future Med. Chem.* **6**, 1201–1229 (2014).

- 14) Huiban, M. *et al.* A broadly applicable [¹⁸F]trifluoromethylation of aryl and heteroaryl iodides for PET imaging. *Nat. Chem.* **5**, 941–944 (2013).
- 15) Levin, M. D. *et al.* A catalytic fluoride-rebound mechanism for C(sp³)-CF₃ bond formation. *Science* **356**, 1272–1276 (2017).
- 16) van der Born, D. *et al.* A universal procedure for the [¹⁸F]trifluoromethylation of aryl iodides and aryl boronic acids with highly improved specific activity. *Angew. Chem. Int. Ed.* **53**, 11046–11050 (2014).
- 17) Mizuta, S. *et al.* Catalytic decarboxylative fluorination for the synthesis of tri- and difluoromethyl arenes. *Org. Lett.* **15**, 2648–2651 (2013).
- 18) Verhoog, S. *et al.* Silver-mediated ¹⁸F-labeling of aryl-CF₃ and aryl-CHF₂ with ¹⁸F-fluoride. *Synlett.* **27**, 25–28 (2016).
- 19) Shi, H. *et al.* Synthesis of ¹⁸F-Difluoromethylarenes from Aryl (Pseudo) Halides. *Angew. Chem. Int. Ed.* **55**, 10786–10790 (2016).
- 20) Yuan, G. *et al.* Metal-free ¹⁸F-labeling of aryl-CF₂H via nucleophilic radiofluorination and oxidative C–H activation. *Chem. Commun.* **53**, 126–129 (2017).
- 21) Sap, J. B. *et al.* Synthesis of ¹⁸F-difluoromethylarenes using aryl boronic acids, ethyl bromofluoroacetate and [¹⁸F]fluoride. *Chem. Sci.* **10**, 3237–3241 (2019).
- 22) Zhao, Q. *et al.* Radiosynthesis of [¹⁸F]ArylSCF₂H using aryl boronic acids, S-(chlorofluoromethyl) benzenesulfonothioate and [¹⁸F]fluoride. *CCS Chem.* **3**, 1921–1928 (2021).
- 23) Trump, L. *et al.* Late-Stage ¹⁸F-Difluoromethyl Labeling of N-Heteroaromatics with High Molar Activity for PET Imaging. *Angew. Chem. Int. Ed.* **58**, 13149–13154 (2019).
- 24) Ni, C. & Hu, J. Recent advances in the synthetic application of difluorocarbene. *Synthesis* **46**, 842–863 (2014).

- 25) Fier, P. S. & Hartwig, J. F. Synthesis of difluoromethyl ethers with difluoromethyltriflate. *Angew. Chem. Int. Ed.* **52**, 2092–2095 (2013).
- 26) Xie, Q. *et al.* Efficient difluoromethylation of alcohols using TMSCF_2Br as a unique and practical difluorocarbene reagent under mild conditions. *Angew. Chem. Int. Ed.* **56**, 3206–3210 (2017).
- 27) Birchall, J. M., Haszeldine, R. N. & Roberts, D. W. Cyclopropane chemistry. Part II. Cyclopropanes as sources of difluorocarbene. *J. Chem. Soc. Perkin Trans. I* 1071–1078 (1973).
- 28) Jia, Y., Yuan, Y., Huang, J., Jiang, Z. & Yang, Z. Synthesis of Difluorinated Heterocyclics through Metal-Free [8+1] and [4+1] Cycloaddition of Difluorocarbene. *Org. Lett.* **23**, 2670–2675 (2021).
- 29) Feng, Z., Min, Q. & Zhang, X. Zhang, Access to difluoromethylated arenes by Pd-catalyzed reaction of arylboronic acids with bromodifluoroacetate. *Org. Lett.* **18**, 44–47 (2016).
- 30) Deng, X., Lin, J. & Xiao, J. Pd-catalyzed transfer of difluorocarbene. *Org. Lett.* **18**, 4384–4387 (2016).
- 31) Feng, Z., Min, Q., Fu, X., An, L. & Zhang, X. Chlorodifluoromethane-triggered formation of difluoromethylated arenes catalysed by palladium. *Nat. Chem.* **9**, 918–923 (2017).
- 32) Fu, X. *et al.* Controllable catalytic difluorocarbene transfer enables access to diversified fluoroalkylated arenes. *Nat. Chem.* **11**, 948–956 (2019).
- 33) Smail, T. & Rowland, F. S. Insertion reactions of mono-and difluorocarbene with hydrogen halides. *J. Phys. Chem.* **74**, 1866–1871 (1970).
- 34) Prakash, G. S. *et al.* Long-Lived Trifluoromethanide Anion: A Key Intermediate in Nucleophilic Trifluoromethylations. *Angew. Chem. Int. Ed.* **53**, 11575–11578 (2014).

- 35) Hine, J. & Porter, J. J. The Formation of Difluoromethylene from Difluoromethyl Phenyl Sulfone and Sodium Methoxide. *J. Am. Chem. Soc.* **82**, 6178–6181 (1960).
- 36) Xing, L. *et al.* Fluorine in drug design: a case study with fluoroanisoles. *ChemMedChem* **10**, 715–726 (2015).
- 37) Khotavivattana, T. *et al.* ¹⁸F-Labeling of Aryl-SCF₃, -OCF₃ and -OCHF₂ with [¹⁸F]Fluoride. *Angew. Chem. Int. Ed.* **54**, 9991–9995 (2015).
- 38) Dahl, K., Halldin, C. & Schou, M. New methodologies for the preparation of carbon-11 labeled radiopharmaceuticals. *Clin. Transl. Imaging* **5**, 275–289 (2017).
- 39) Pipal, R. W. *et al.* Metallaphotoredox aryl and alkyl radiomethylation for PET ligand discovery. *Nature* **589**, 542–547 (2021).
- 40) Mullard, A. Deuterated drugs draw heavier backing. *Nat. Rev. Drug Discov.* **15**, 219–222 (2016).
- 41) Cai, Z. *et al.* Synthesis and in vivo evaluation of [¹⁸F]UCB-J for PET imaging of synaptic vesicle glycoprotein 2A (SV₂A). *Eur. J. Nucl. Med. Mol. Imaging* **46**, 1952–1965 (2019).
- 42) Zheng, J., Winkeler, A., Peyronneau, M.-A., Dollé, F. & Boisgard, R. Evaluation of PET Imaging Performance of the TSPO Radioligand [¹⁸F]DPA-714 in Mouse and Rat Models of Cancer and Inflammation. *Mol. Imaging Biol.* **18**, 127–134 (2016).
- 43) Keller, T. *et al.* Radiosynthesis and Preclinical Evaluation of [¹⁸F]F-DPA, A Novel Pyrazolo[1,5a]pyrimidine Acetamide TSPO Radioligand, in Healthy Sprague Dawley Rats. *Mol. Imaging Biol.* **19**, 736–745 (2017).
- 44) Kuchar, M. & Mamat, C. Methods to increase the metabolic stability of ¹⁸F-radiotracers. *Molecules* **20**, 16186–16220 (2015).
- 45) Lelos, M. J. & Dunnett, S. B. Generating excitotoxic lesion models of Huntington's disease. 209–220 (Springer, 2018).

- 46) Zhou, W. *et al.* Transition-metal difluorocarbene complexes. *Chem. Commun.* **57**, 9316–9329 (2021).
- 47) Li, X. *et al.* Copper-mediated aerobic (phenylsulfonyl)difluoromethylation of arylboronic acids with difluoromethyl phenyl sulfone. *Chem. Commun.* **52**, 3657–3660 (2016).
- 48) *Quality Guidelines*. <https://www.ich.org/page/quality-guidelines> (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2019).

Data availability

Materials and methods, optimization studies, experimental procedures, mechanistic studies, ¹H NMR, ¹³C NMR and ¹⁹F NMR spectra, high-resolution mass spectrometry, infrared and HPLC data are available in the Supplementary Information.

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Authors contributions

J.B.I.S. and C.F.M. did label the *t*Bu-substituted difluorocarbene reagent and performed with this reagent all insertions and cycloadditions. J.B.I.S., C.F.M. and J.F. prepared substrates and performed all automation

experiments. J.B.I.S. and J.F. performed the cross-coupling reactions, one-pot procedures, the synthesis of the radiotracer for the imaging study, the radiosynthesis of all difluorocarbene reagents, the experiments with the Cl-substituted difluorocarbene reagent, and developed the NMR assay to probe isotopic dilution. J.B.I.S and N.J.W.S. performed preliminary studies for the radiosynthesis of the *t*Bu-substituted difluorocarbene reagent. A.B.D. and R.S.P. performed and analyzed the computational studies. T.A.M. and C.F.M. did an initial metabolic stability study. J.B.I.S., J.F, M.J.L. and S.J.P. performed all the *in vivo* experiments. S.M.H. prepared selected substrates. J.B.I.S, J.F and V.G. conducted the revisions. J.B.I.S., R.S.P., and V.G. wrote the manuscript. All authors read and commented on the paper. V.G. conceived and supervised the project.

Competing interests C.G. is an employee of UCB Pharma. C.W.A. is an employee of Pfizer Inc. A patent is being filed.

Additional information

Supplementary information is available for this paper.

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