MANGANESE CATALYZED FLUORINATION AND FLUORINE-18 LABELING

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Abstract

Fluorinated organic molecules are important in modern chemistry, affording pharmaceuticals, agrochemicals, materials and radioactive tracers for PET imaging. Nevertheless, approaches to incorporate fluorine into organic molecules are limited either in nature or in chemist’s toolbox. Moreover, few of them have been further translated to practical use for PET tracer production. Though enzymatic fluorination via a metal-oxo intermediate has yet been found, other non-oxygen transfer processes have inspired us to explore fluorination via such an oxidative strategy in synthetic models. In the past few years, several fluorination methods catalyzed by manganese catalysts have been discovered in our group. The use of simple fluoride ion in these reactions facilitated their adaption to $^{18}\text{F}$ chemistry using $[^{18}\text{F}]$fluoride. In this dissertation, this manganese-catalyzed strategy has been further developed, poised to the clinical implementation of $^{18}\text{F}$ PET.

In chapter 2, We describe a successful translation of manganese catalyzed decarboxylative $^{18}\text{F}$-fluorination to a radioactive scalable and automated robotic procedure for the preparation of clinical doses. This protocol provides access to tertiary carbon-$[^{18}\text{F}]$fluorine bonds that are hard to synthesize via conventional methods. It also offers a more efficient approach to prepare tracer molecules containing a $^{18}\text{F}$-fluoromethoxy group than current strategies. Two tracer candidates have been synthesized in this way, showing the utility of this method in drug development and PET imaging.

Inspired by a redirected heteroatom rebound process in SyrB2 enzyme, in chapter 3, we have evaluated the possibility of evolving an oxidative fluorination strategy in a non-heme coordination scaffold. A cis-difluoromanganese(III) complex has been synthesized, characterized and demonstrated fluorine transfer activity to alkyl radicals. Oxo-
manganese(IV) intermediates have been accessed. Additionally, fluorinated products from simple alkanes was observed in the catalytic conditions. These results have shown the mechanistic feasibility of this manganese catalyst platform to fluorination.

In the last chapter, we have explored the reaction further using $^{18}$F fluoride and presented a novel, late-stage C-H $^{18}$F labeling chemistry. A variety of building blocks and bioactive molecules have been successfully labeled with moderate radiochemical conversions. Operational simplicity of this labeling method will empower its application in automated radio-syntheses and high throughput screening of radiotracer candidates.
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Chapter I

Developing Novel Fluorination Reactions Inspired from Biological C-H Functionalizations via High-Valent Iron-Oxo Intermediates

1.1 Importance of organofluorine molecules and their application in PET imaging

Fluorinated natural products are rare, despite the relatively high abundance of fluorine on Earth. Only thirty fluorinated metabolites have been found, accounting for less than 1% of known halogen-containing natural products.\(^1\) The paucity of fluorinated molecules may be a consequence of the lack of soluble fluorine in nature due to the high electronegativity of the fluorine atom. Furthermore, the extremely high oxidative potential makes fluoride incompatible with the redox pathway.\(^2\)\(^-\)\(^3\) To date, fluorinases are the only enzymes known to incorporate fluoride into organic molecules via nucleophilic fluoride displacement at the 5'-position of S-adenosylmethionine.\(^4\)\(^-\)\(^5\)

![Figure 1.1 Examples of fluorine-containing molecules.](image-url)
Current interest in fluorination chemistry largely results from the widespread usage in the fields of pharmaceutical and agrochemical products and material sciences (Figure 1.1). Around 30% of pharmaceuticals and 30% of agrochemicals on the market are fluorinated. Many top-selling drugs, such as Lipitor, Advair and Crestor contain fluorine. One of the most widely used materials, polytetrafluoroethylene (PTFE), and its derivatives are highly fluorinated.

(a) Increased metabolic stability of Ezetimibe

(b) Increased drug activity of Mifentidine by altering the pK$_a$

**Figure 1.2** Introduction of fluorine into drug molecules can (a) enhance metabolic stability and (b) increase drug activity by altering the pK$_a$. 
The popularity of fluorinated organic molecules is attributed to the unique properties of fluorine atom. Because of the similar van der Waals radius, fluorine (1.47 Å) is a good isostere for hydrogen (1.20 Å) in medicinal chemistry to improve the metabolic stability of drug molecules via blocking undesired oxidative metabolism by cytochrome P450. Ezetimibe, a drug used to lower cholesterol level, contains fluorine on the metabolically labile sites and therefore, is more potent than the lead compound (SCH48461) without fluorine (Figure 1.2, a). Fluorine is the most electronegative element. Its incorporation into a molecule changes the molecule’s physicochemical properties such as lipophilicity and acidity. For instance, fluorine substitution of Mifentidine can enhance H2-antagonist affinity by decreasing the basicity of the formamidino group and thus increasing the concentration of the active form (Figure 1.2, b). In addition, it contributes to the high strength of the carbon-fluorine bond, which increases the thermal and oxidative stability of materials.

One of the important applications of fluorine-containing molecules is the use of radiotracers for positron emission tomography (PET). PET is a premier imaging modality that enables visualization of in vivo metabolic processes at the molecular level. It is also a useful tool to investigate the biodistribution and the target interaction of a drug candidate. This technique is based on the detection of gamma rays when positrons are emitted from radionuclides and annihilate with electrons. After the tracer containing a position-emitting radioisotope is injected into a vein, a PET detector captures emitted gamma rays and a software constructs a three-dimensional image showing the distribution of tracer molecules in the body. Of all the positron-emitting radionuclides including carbon-11, oxygen-15 and nitrogen-15, fluorine-18 is the most favorable one in clinical use because of its unique
Compared with other PET radioisotopes, fluorine-18 has a relatively long half-life (110 min), allowing more complicated radiosynthesis, longer time for transportation and \textit{in vivo} studies. Its weak positron energy and short annihilation range result in PET images with high resolution. The best PET radio tracer in clinical use is a $^{18}$F labeled radiotracer; 2-$[^{18}\text{F}]$-fluoro-deoxy-D-glucose ([$^{18}$F]FDG) is used for probing glucose metabolism in the brain, kidney and in cancer cells.$^{17}$

1.2 Methods for carbon-fluorine bond formation and their adaptation into radiofluorination reactions

Compared with the popularity of fluorine-containing organic molecules in chemicals industry, methods for fluorination are still far from general and practical. Conventional fluorination methods use either fluorine gas (F$_2$) or fluoride ion (F$^-$) as the fluoride electrophilic or nucleophilic sources. Though they have been well-established for industrial use (such as the Balz-Schiemann reaction and the Halex process), harsh reaction conditions diminish the functional group compatibility of fluorination reactions.$^{18}$ As a result, a building-block approach, in which introduction of fluorine into a simple building block occurs before additional synthetic steps, is often employed to build up a fluorine-containing complex molecule.$^{19}$ However, for many applications, especially the synthesis of $^{18}$F PET tracers, it is more desirable to install fluorine in the last step of the synthetic route, which is termed late-stage fluorination.$^{20}$ Therefore, many organic chemists have been working to develop reactions that can readily and selectively incorporate fluorine into complicated molecules.
There are two types of methods for carbon-fluorine bond formation, depending on which fluorine source is used. One type of reaction uses electrophilic fluorine sources, which are mostly derived from fluorine gas and transfer an equivalent of “F⁺⁺” in reactions (Scheme 1.1, a). Electrophilic fluorine sources used in early work include F₂, XeF₂, CF₃OF, HOF and CsSO₄F. These species are highly oxidizing, challenging to handle and therefore have limited applicability. The later discovery of crystalline and bench-stable N-fluoro reagents was a breakthrough in fluorine chemistry and these reagents have been widely used in fluorination of different types of substrates (Scheme 1.1, b). Additionally, these electrophilic fluorinating reagents have relatively weak nitrogen-
fluorine bonds (BDE = 65.5 kcal/mol for N-fluorosultam). Recently, it has been demonstrated that they can behave as “fluorine radical” sources and react with carbon radicals.

Scheme 1.2 (a) Examples of nucleophilic fluorinating reagents. (b) Representative fluorination reactions using nucleophilic fluorinating reagents.

Another type of fluorine source is nucleophilic and is regarded as an “F−” equivalent (Scheme 1.2, a). Nucleophilic fluorination is difficult because strong hydrogen bonding between fluoride and the hydrogen-bond donor diminishes the nucleophilicity of fluoride. Alkali-metal fluorides are desirable reagents due to their low cost and low toxicity, but challenging in application because of their weak nucleophilicity and poor solubility in
organic solvents. Currently, numerous nucleophilic fluorinating reagents, which show increasing reactivity in organic solvent, have been employed in different reactions (Scheme 1.2, b).  

Fluorination reactions using fluoride sources are much more desirable than using “F⁺⁺” reagents if we consider their application in PET imaging. In ¹⁸F chemistry, [¹⁸F]fluoride is a favorable ¹⁸F source over [¹⁸F]F₂, not only because it is easier to prepare and to handle, but also due to its higher specific activity and maximum achievable radiochemical yield (RCY). The production of [¹⁸F]F₂ proceeds via the bombardment of a target gas containing enriched ¹⁸O₂ or neon gas and a small amount of ¹⁹F₂ as the carrier. A [¹⁸F]F₂ sample contains ¹⁹F₂ gas and only one of the fluorine atoms in [¹⁸F]F₂ is ¹⁸F-labeled, therefore has a specific activity less than 0.4 GBq/µmol. As a result, ¹⁸F-labeled molecules obtained via reactions using electrophilic fluorinating reagents, which are prepared from [¹⁸F]F₂ gas, do not have a high enough specific activity for a PET study. In contrast, [¹⁸F]fluoride is produced directly in enriched water (H₂₁⁸O) with much higher specific activity and therefore is the ¹⁸F source of choice. [¹⁸F]F-L-DOPA, a PET tracer for probing cerebral dopamine metabolism, can be synthesized using either [¹⁸F]fluoride or [¹⁸F]SelectFluor derived from [¹⁸F]F₂ gas (Scheme 1.3). However, the method using [¹⁸F]fluoride is preferred due to its higher specific activity and RCY, despite a longer synthetic protocol. Recently, a number of fluorination reactions, both aromatic and aliphatic, have been successfully translated into ¹⁸F chemistry using [¹⁸F]fluoride and some of them have further demonstrated the practicality in PET imaging, expanding the toolbox of radiochemistry.
1.3 Enzymatic C-H activation via high-valent iron-oxo intermediates

More than one billion years ago, the development of new metabolic pathways began for the adaption of life to an aerobic environment. Families of enzymes participate in different stages of O$_2$ reduction processes, also termed O$_2$ activation. Many of these enzymes such as cytochrome c oxidases, superoxide reductases and peroxidases, contain redox-active metal cofactors and can mediate numerous oxidative transformations. Coincidentally, these transformations share similarities in mechanism: a metal center reacts with O$_2$ or its derived species and forms various metal-oxygen intermediates like a metal-oxo species or a metal-superoxo and -(hydro)peroxo, and these active intermediates are subsequently engaged in different functionalizations.$^{44-46}$
A well-known example is the heme-containing monooxygenases, cytochrome P450 (CYP), which can perform selective C-H bond activation in drug metabolism and the biosynthesis of natural products.\textsuperscript{47} The mechanism of P450 catalyzed C-H hydroxylation, also termed the oxygen rebound mechanism, was first proposed by Groves in the 1970s.\textsuperscript{48-49} The key intermediates involved in this mechanism are a reactive oxo-iron(IV) porphyrin radical cation (compound I) that performs hydrogen abstraction from the substrate to generate a carbon radical, and a hydroxo-iron(IV) intermediate (compound II) that is involved in the subsequent rebound step of the carbon radical (\textbf{Scheme 1.4}).\textsuperscript{50-51}

\begin{center}
\begin{tikzpicture}
\node [align=center] at (0,0) {A well-known example is the heme-containing monooxygenases, cytochrome P450 (CYP), which can perform selective C-H bond activation in drug metabolism and the biosynthesis of natural products.};
\end{tikzpicture}
\end{center}

\textbf{Scheme 1.4} Oxygen rebound mechanism of P450-catalyzed C-H hydroxylation.

While compound I of chloroperoxidase (CPO) has been characterized and studied for a long time,\textsuperscript{52-53} it has been difficult to capture compound I of P450 (P450-I) due to its high reactivity.\textsuperscript{54} Previous studies of P450-I mainly relied on theoretical calculations and comparisons with CPO-I that is also thiolate-ligated.\textsuperscript{55-56} Since CPO-I can only activate C-H bonds with BDE around 89 kcal/mol,\textsuperscript{57} other active intermediates were proposed for C-H activation.\textsuperscript{58-59} The puzzle was solved in 2010 when Green and Rittle successfully prepared compound I of CYP119, a thermophilic cytochrome P450, in high yield.\textsuperscript{60} The
spectroscopic characterization data indicated that CYP119-I is an oxo-iron(IV) porphyrin cation radical that is antiferromagnetically coupled. Furthermore, CYP119-I was found to hydroxylate a unactivated C-H bond (BDE = 100 kcal/mol) in lauric acid with an apparent second-order rate constant at $\sim 10^7 \text{ M}^{-1} \cdot \text{s}^{-1}$. Since this report, other reactive compound I species such as AaeAPO-I and SeCYP119-I, have also been spectroscopically and kinetically characterized.\(^{61-62}\)

Another key intermediate in the oxygen rebound mechanism is the hydroxoiron(IV) intermediate (compound II). The basicity of compound II influences the reactivity of compound I for C-H activation. Mayer indicated that the reactivity of oxo-metal intermediates for hydrogen abstraction is determined by the free energy difference between the formed O-H bond in the metal complex and the broken C-H bond in the substrate.\(^{63-64}\) According to the Bordwell equation, the O-H bond strength of compound II is related to the $pK_a$ of compound II and the reduction potential of compound I (see Scheme 1.5).\(^{65}\) A more basic compound II results in a stronger O-H bond of compound II, therefore leading to increased reactivity of compound I.\(^{66}\) This rationale is supported by the fact that CPO and AaeAPO have smaller $pK_a$ values of compound II and weaker reactivity for C-H activation than P450.\(^{67-69}\) Green proposed that the high basicity of P450-II is attributed to the “push effect” of the ligated cysteine thiolate, which translates the electron density to the ferryl oxygen.\(^{66, 70}\) In CPO, however, such an effect is weakened by the longer Fe-S bond due to the hydrogen bonding in the “cys-pocket”.\(^{71}\)
Scheme 1.5 The calculation of hydrogen-abstracting activity of Compound I via the Bordwell equation.

High-valent oxo-iron intermediates do not only occur in P450 enzymes. There are other notable examples such as methane monooxygenases (MMOs) with a diiron active site and mononuclear non-heme iron enzymes that feature a 2-his-1-carboxylate facial triad.\textsuperscript{72-73} The active site of the latter group of enzymes consists of three protein-based ligands (two histidines and a glutamate or an aspartate that has a carboxylate on the side chain) facially anchoring the iron(II) center. There are labile coordination sites on the opposite face available for external ligands like dioxygen, the cofactor, or the substrate molecule. Such a structural motif is distinct from the active site of P450 enzymes, in which two open coordination positions are arranged on the opposite side of the planar porphyrin; however, this structure still facilitates a broad range of reactions such as hydroxylation, desaturation, halogenation and cyclization via C-H activations.\textsuperscript{73}
Generally, nonheme iron enzymes can be classified according to whether they require a cofactor (or co-substrate) in dioxygen activation. The most common cofactor is α-ketoglutarate (α-KG), which is attacked by bound dioxygen, loses a molecule of CO$_2$ and generates an oxo-iron(IV) species.$^{74}$ TauD is a well-studied example of α-KG dependent enzymes and mediates C-H hydroxylation in taurine, which further decomposes to aminoacetaldehyde and sulfite.$^{75}$ This enzyme abstracts hydrogen from the C-H bond next to the sulfonate group, followed by the similar oxygen rebound step to P450-mediated C-H hydroxylation (Scheme 1.6). The highly active oxo-iron(IV) intermediate was captured in the reaction of the TauD·α-KG-taurine complex with dioxygen by a rapid freeze-quench technique and then characterized by Mössbauer, Raman and EXAFS spectroscopies.$^{76-79}$

Scheme 1.6 The mechanism of TauD-catalyzed C-H hydroxylation of taurine and the active site of TauD (PDB: 1GQW).

Different from α-KG, cofactors such as tetrahydrobiopterin (pterin or THB) serve as the two-electron sources used for O$_2$ reduction. PheH is one of the pterin-dependent hydroxylases, converting phenylalanine to tyrosine.$^{80}$ In the proposed mechanism, an Fe(II)-peroxy-THB intermediate is formed, followed by the generation an oxo-iron(IV)
species via an O-O bond cleavage. The aromatic ring of phenylalanine is attacked by this oxo-iron(IV) species, leading to the generation of a cationic intermediate. This proposal was supported by an inverse kinetic isotope effect and a 1,2-hydride shift when using a substrate with a site-specifically deuterated aromatic ring (Scheme 1.7).

**Scheme 1.7** The mechanism of PheH-catalyzed C-H hydroxylation of phenylalanine and the active site of PheH (PDB: 1GQW).

Nonheme iron enzymes without the need for a cofactor, such as 4-hydroxymandelate synthase (HMS), use “high-energy” substrates that contain an α-ketoacid scaffold, to facilitate the generation of oxo-iron(IV) intermediate. The oxo-iron(IV) species in HMS is formed by the mechanism similar to that in α-KG dependent enzymes, and then it is proposed to initiate a P450-like hydrogen abstraction-rebound process on the benzylic position of the substrate (Scheme 1.8).
Another group of these enzymes oxidize thiolate-containing substrates, which binds to the metal center via a highly covalent iron-sulfur bond to facilitate dioxygen activation.\textsuperscript{85} The most renown case is isopenicillin N synthase (IPNS), converting $\delta$-(l-$\alpha$-Aminoadipoyl)-l-cysteinyl-d-valine (ACV) to isopenicillin N (IPN), a $\beta$-lactam antibiotic.\textsuperscript{88} Baldwin and coworkers found that two oxidative cyclization steps via hydrogen abstraction are involved in this reaction, installing a $\beta$-lactam and then a thiazolidine ring in the IPN product, respectively.\textsuperscript{89-90} Recent efforts by Bollinger and Krebs indicated that the iron(III)-superoxo and the oxo-iron(IV) complexes are the C-H cleaving intermediates (Scheme 1.9).\textsuperscript{91-93}

**Scheme 1.8** The mechanism of HMS-catalyzed C-H hydroxylation and the active site of HMS (PDB: 3ZGJ).

**Scheme 1.9** Proposed mechanism IPNS and the active site of IPNS (PDB: 1BK0).
1.4 C-H activation mediated by iron or manganese model complexes

In the past four decades, the highly reactive oxo-iron(IV) complexes in nature have inspired the exploration of synthetic models that can mimic the enzymatic chemistry. For example, synthetic metalloporphyrins are well-studied models for P450. The earliest case to be reported was by Groves in 1979 where Fe(III)(TPP)Cl, an iron porphyrin catalyst, was found to hydroxylate simple alkanes and epoxidate simple alkenes. At the same time, an oxo-iron(IV) porphyrin and an oxo-iron(IV) porphyrin cation radical species were prepared. The oxo-iron porphyrin(IV) cation radical species was found to transfer oxygen to olefins and hydrocarbons, affording epoxides and alcohols, respectively. Since then, metalloporphyrin-catalyzed oxygenation has been extensively studied with numerous significant advances.

![Figure 1.3 Representative non-heme ligands.](image-url)
Exploring synthetic metal complexes with non-porphyrin ligands (shown in Figure 1.3), which mimic non-heme iron enzymes, are also of great interest. The first non-heme mononuclear oxo-iron(IV) complex \([\text{Fe}^\text{IV}(\text{O})(\text{cyclam-acetate})]^+\) (cyclam-acetate = 1-carboxymethyl-1,4,8,11-tetraazaacyclotetradecane) was observed by Wieghardt and coworkers using Mössbauer spectroscopy in 2000. Subsequently, another non-heme oxo-iron(IV) complex \([\text{Fe}^\text{IV}(\text{O})(\text{TMC})(\text{NCCH}_3)](\text{OTf})_2\) (TMC = 1,4,8,11-tetramethyl-1,4,8,11-tetraazaacyclotetradecane) was prepared and characterized. An Fe-O distance of 1.646(3) Å was observed in the high-resolution crystal structure, indicating the presence of a terminal oxo-iron(IV) bond. However, the oxidative reactivity of this complex was suppressed by the TMC ligand. Since then, a variety of non-heme oxo-iron(IV) species have been successfully synthesized, characterized and well-studied, some of which could activate strong C-H bonds. For example, \([\text{Fe}^\text{IV}(\text{O})(\text{TQA})]^2+\) (TQA = tris(2-quinolylmethyl)amine), \([\text{Fe}^\text{IV}(\text{O})(\text{Me}_3\text{NTB})]^2+\) (Me$_3$NTB = tris((N-methyl-benzimidazol-2-yl)methyl)amine) and \([\text{Fe}^\text{IV}(\text{O})(\text{TMCO})(\text{OTf})]^+\) (TMCO = 4,8,12-trimethyl-1-oxa-4,8,12-triazacyclotetradecane) could cleave a C-H bond in cyclohexane (BDE = 99.5 kcal/mol) at -40 °C with a second order rate constant of 0.37 M$^{-1}$s$^{-1}$, 0.25 M$^{-1}$s$^{-1}$ and 0.01 M$^{-1}$s$^{-1}$, respectively.

Early efforts by Que and coworkers showed that the Fe$^\text{II}$(TPA) complex and its analogues could oxidize C-H bonds in cyclohexane. Another key finding of these works was that the reactivity and selectivity of the iron complex could be controlled by modulating the steric and electronic properties of the ligand. Since then, non-heme iron(II) complexes with N-based ligands have been extensively used in C-H hydroxylation reactions. Most of these complexes such as (TPA, MEP, MCP, PyTACN) contain a
tetradeate $N$-based ligand, leaving two $cis$-labile coordination sites for the interaction with the oxidant. A breakthrough in this field came in 2007 when White and coworkers reported a predictably site-selective C-H oxidation of complex molecules. The non-heme iron catalyst employed in this reaction bears a more rigid ligand PDP ($N,N'$-bis(2-pyridyldimethyl)-2,2'-bipyrrrolidine). The selectivity could be achieved based on the steric and electronic environment of the C-H bonds. Later, the authors achieved site-selective oxidations of a large substrate scope including different nitrogen-containing molecules via a substrate and catalyst-controlled strategy (Scheme 1.10).

**Scheme 1.10** Representative non-heme iron catalyzed C-H hydroxylations developed by White’s group.
Synthetic manganese complexes are of particular interest, since several manganese containing enzymes in nature have been known to activate dioxygen. Both synthetic porphyrin and non-heme manganese complexes have shown high efficiency in the oxidation of hydrocarbons. Early in 1980, Groves and coworkers published the first C-H hydroxylation mediated by the manganese porphyrin, Mn(TPP)Cl, resulting in oxidation of cyclohexane to cyclohexanol (50% yield). For manganese porphyrins, oxo-Mn(V) species have been generally regarded as the active intermediates for the hydrogen abstraction. They have been generated by the reaction of Mn(III) porphyrins with oxidants and then characterized by various techniques such as $^1$H-NMR, UV-Vis, IR and Raman spectroscopies.

For non-heme manganese complexes, C-H hydroxylation with high regio-, stereo- and chemoselectivity could be achieved. Recently, Costas and coworkers found that a sterically bulky nonheme manganese complex can catalyze highly regio- and enantioselective oxidation of N-cyclohexylalkanamides. In addition, taking advantage of solvent hydrogen bonding, they showed successful examples of site- and chemoselective C-H oxidation (Scheme 1.11).

**Scheme 1.11** Representative non-heme manganese catalyzed C-H hydroxylations developed by Costas’ group.
Compared with non-heme iron systems that have been extensively studied for decades, there is much less information on the mechanisms and the active intermediates of non-heme manganese-catalyzed oxidations. Several synthetic oxo-Mn(IV) complexes bearing non-heme ligands have been reported (shown in Figure 1.4). For example, Nam and coworkers have synthesized and characterized a highly reactive nonheme oxo-Mn(IV) complex \([\text{Mn}^{IV}(O)(\text{Bn-TPEN})]^2+\) (Bn-TPEN = \(N\)-benzyl-\(N\),\(N\)'-tris(2-pyridylmethyl)-1,2-diamoethane), which can abstract a hydrogen atom from cyclohexane.\(^{118}\) However, it is generally regarded that nonheme oxo-Mn(IV) complexes are much weaker hydrogen-abstraction agents than oxo-Fe(IV) complexes, as most of them are only capable of reacting with substrates (xanthene, 9,10-dihydroanthracene, fluorene) containing weak C-H bonds (BDE \(\sim 75-80 \text{ kcal/mol}\)).\(^{119-123}\) Oxo-Mn(V) species are possible active intermediates but need to be further investigated.\(^{124}\)

**Figure 1.4** Representative examples of non-heme oxo-manganese(IV) complexes.
1.5 Modulating the radical rebound to construct carbon-fluorine bonds

In addition to oxygen rebound, which results in carbon-oxygen bond formation, nature has also developed approaches to achieve heteroatom-transfer reactions by modulating the radical rebound step. For instance, in SyrB2 enzyme, an αKG-dependent halogenase, chlorine rebound rather than oxygen rebound occurs after a substrate radical is generated via the hydrogen abstraction of an oxo-iron(IV) intermediate (Scheme 1.12, a). Another example is IPNS where after the second C-H activation by the oxo-iron(IV) species, the iron-bound sulfur recombines with the alkyl radical, building up the thiazolidine ring in IPN (Scheme 1.12, b).

a. SyrB2

b. IPNS

Scheme 1.12 Mechanisms of (a) carbon-chlorine bond formation in SyrB2 enzyme and (b) carbon-sulfur bond formation in IPNS enzyme.

Such enzymatic procedures led us to consider whether we could redirect the oxygen rebound step to a heteroatom-transfer process in synthetic systems, in which a substrate radical does not react with a metal-bound hydroxyl complex but recombine to a (metal-bound) heteroatom to form a carbon-heteroatom bond. Constructing carbon-fluorine bonds
is more desirable, as to date, no carbon-fluorine bond formation via an oxidative pathway has been found in nature. Early in 1980, when our group found the first C-H hydroxylation reaction catalyzed by manganese porphyrin complexes, they also observed cyclohexyl chloride product formation in the oxidation of cyclohexane with Mn(TPP)Cl and iodosylbenzene.\textsuperscript{111} A similar result was reported by Hill and coworkers in the same year, in which cyclohexyl-X (X = Cl, Br, I and N\textsubscript{3}) was produced when changing the axial ligand of the Mn porphyrin catalyst.\textsuperscript{128} Notably, the oxidation of norcarane gave significant amounts of unrearranged (U) and rearranged (R) chlorinated products with a U/R ratio of 0.21, indicating a long lifetime (24 ns) for the carbon radical intermediate, while the U/R ratio of oxygenated products was 2.4 corresponding to a radical lifetime of 2 ns (Scheme \textbf{1.13}, a).\textsuperscript{129} This result suggested that the chlorinated product was obtained via the transfer of chlorine from the manganese complex to the carbon radical likely escaping from the solvent cage. Recently, Groves and Goddard reported that the oxygen rebound rate is significantly influenced by the axial ligand.\textsuperscript{130} When the manganese porphyrin with bound fluoride was used, the oxidation reaction of norcarane afforded dramatically increased amounts of rearranged products, and the degree of stereoretention significantly reduced in the oxidation of \textit{cis}-decalin (Scheme \textbf{1.13}, b). These data indicated that fluoride can slow down the oxygen rebound step and thus increase the lifetime of the carbon radical intermediate. This axial ligand effect was further supported by DFT calculations. The calculated rebound barrier increases by 3.3 kcal/mol when the axial ligand changes from water to fluoride. Such results are promising, as the slow-down of the oxygen rebound step by using a fluoride ligand may be an approach to divert the carbon radical from the fast oxygen rebound to the fluorine rebound pathway.
Inspired by the axial ligand effect of fluoride in the radical rebound process, our group has developed a new strategy for carbon-fluorine bond formation. We proposed that the carbon radicals formed by the hydrogen abstraction of a high valent oxo-manganese complex may recombine to Mn-F species, therefore affording fluorinated products using fluoride sources. Though fluorination of carbon radicals in the presence of electrophilic fluorine reagents has been reported, such a C-H fluorination protocol would be much more advantageous.
more desirable especially if we consider its application in $^{18}$F labeling and PET imaging.

In 2012, our group discovered a manganese porphyrin, Mn(TMP)Cl, catalyzed aliphatic C-H fluorination.$^{134}$ This method used iodosylbenzene as the oxidant and the combination of silver fluoride (AgF) and tetrabutylammonium fluoride trihydrate (TBAF·3H$_2$O) as the fluoride source (Scheme 1.14). A group of simple alkyl substrates (cycloalkanes and substituted cyclic compounds) could be monofluorinated in modest to good yields. This protocol could also fluorinate complex molecules with high regioselectivity. For example, fluorination of 5α-androstan-17-one using this method gave only methylene-fluorinated products on the C2 and C3 positions in ring A. Fluorination of norcarane formed the unrearranged fluorinated product and the rearranged fluorinated product in a ratio of 2:1, corresponding to a radical lifetime of 2.5 ns. This U/R ratio is similar to the one observed in the study of the axial ligand effect of fluoride on the oxygen rebound process (see in session 1.6). It was proposed that an oxo-Mn(V) complex, Mn$^V$(O)(TMP)F, is involved in the hydrogen abstraction step, forming a carbon radical and a Mn$^IV$(TMP)(OH)F complex. Then the carbon radical recombines to a \textit{trans}-difluoro-Mn(IV) complex, Mn$^IV$(TMP)F$_2$, which is generated by the reaction between silver fluoride and Mn$^IV$(TMP)(OH)F. Mn$^IV$(TMP)F$_2$, which was independently synthesized and structurally characterized, was capable of trapping the carbon radicals and delivering the fluorine atom. DFT calculations indicated that the energy barrier of fluorine transfer from a \textit{trans}-difluoromanganese(IV) complex, Mn$^IV$(THP)F$_2$, to an equatorial cyclohexyl radical is lower than the same process from Mn$^IV$(THP)(OH)F by 3.4 kcal/mol.
Later, our group found that the substrate scope of this fluorination reaction could be further expanded by modulating the ligand of the manganese catalyst. When manganese porphyrin Mn(TMP)Cl was used as the catalyst to fluorinate benzylic C-H bonds, significant amounts of oxygenated products was observed. It is possible that a fast radical rebound to a Mn^{IV}-OH species occurs due to the low ionization potential of the benzylic radical. Changing the porphyrin ligand to a salen ligand gave efficient benzylic C-H fluorination with high selectivity over oxygenation. The new fluorination method could fluorinate complicated biomolecules such as a vitamin E analogue (δ-tocopherol acetate), affording monofluorinated products selectively on benzylic positions. Very recently, a bulky and electron-deficient manganese porphyrin, Mn(TPFPP)Cl, could efficiently catalyze C-H fluorination of electron-deficient and strained substrates, which are extremely challenging to be activated using conventional methods. Also, several lactones and a lactam were regio- and stereo-selectively fluorinated in moderate yield.

Scheme 1.14 Manganese-catalyzed fluorination of aliphatic C-H bonds.
Scheme 1.15 (a) Benzylic C-H fluorination catalyzed by manganese salen. (b) C-H fluorination of lactones and a lactam catalyzed by manganese pentafluorophenyl porphyrin.

In addition to C-H activation, radical fluorinations using fluoride could be achieved by the combination of other radical generation strategies and the fluorine transfer process by fluoro-Mn(IV) complexes. Our group found a complementary manganese-catalyzed decarboxylative fluorination reaction using carboxylic acids as the radical precursor (Scheme 1.16). This fluorination method could fluorinate a wide range of molecules including tertiary, secondary, primary, benzylic and aryloxy carboxylic acids. Oxidation-sensitive functional groups such as alkenes, alkynes and thiophenes, which would be activated in the oxidative C-H fluorination protocols, could be tolerated. Mechanistic studies indicated that an iodine(III) carboxylate is formed and further reacts with the
manganese(III) porphyrin to generate a carbon radical. Fluorinated products are produced by the reaction between the carbon radicals and a manganese-bound fluoride intermediate.

**Scheme 1.16** Manganese-catalyzed decarboxylative fluorination.

One of the compelling features of these fluorination methods is the use of fluoride ion as the fluorine source, which facilitates the translation of these reactions to $^{18}\text{F}$ chemistry with $[^{18}\text{F}]$fluoride. Indeed, our C-H fluorination protocols have been readily adapted to the late-stage radiofluorination by simply employing no-carrier-added $[^{18}\text{F}]$fluoride (**Scheme 1.17**). Typically, a Mn(TPFPP)$^{18}\text{F}$ or a Mn(Salen)$^{18}\text{F}$ complex is generated by the ligand exchange of the manganese-bound tosyl anion. Such a Mn(III)$^{18}\text{F}$ complex could be oxidized by iodosylbenzene to an oxo-Mn(V) intermediate, which then abstracts a hydrogen atom from the substrate. Though there is an extremely low concentration of the Mn(IV)$^{18}\text{F}$ intermediate in the reaction solution, $[^{18}\text{F}]$fluorine could be efficiently transferred to the substrate radical, affording the $^{18}\text{F}$-labeled product. A variety of existing drug molecules and amino acid based PET tracers could be selectively $^{18}\text{F}$-labeled with moderate to high radiochemical conversions. Moreover, a procedure that
could radiosynthesize molecules on a clinical scale has been successfully developed using a dry-down-free protocol, in which $[^{18}F]$fluoride could be directly eluted from an anion exchange column by an acetonitrile/acetone solution of the Mn catalyst. Protected ACPC (Boc-ACPC-OMe, boc-protected 1-amino-cyclopentane-carboxylic acid methyl ester) could be readily labeled in 10 minutes and 11.7 mCi labeled product, 3-$[^{18}F]$-FACPC, was isolated, with a 60% non-decay-corrected chemical yield. In addition, the potential of the Mn-catalyzed decarboxylative fluorination method for the application of PET imaging has also been demonstrated by several examples of $^{18}F$-labeled molecules using $[^{18}F]$fluoride.

Scheme 1.17 Manganese-catalyzed C-H $^{18}F$ labeling.

A “prefunctionalization” strategy is still prevailing among current methods to incorporate $^{18}F$ into organic molecules, including some recent advances. In these approaches, a highly reactive leaving group needs to be installed at the targeted labeling position of a precursor, dramatically increasing the complexity of the precursor
preparation. As harsh reaction conditions are required, these methods often produce side products and even shut down in the presence of many biologically relevant functional groups. Therefore, developing new $^{18}$F labeling reactions that can circumvent these drawbacks is highly desirable for the continued advancement of PET imaging, as it could provide access to tracer candidates that are hard to synthesize by conventional labeling methods.

Manganese-catalyzed fluorination reactions recently developed by our group can be solutions to issues related to the “prefunctionalization” strategy. These methods directly replace an sp$^3$ hydrogen atom or a ubiquitous sp$^3$ carboxylic acid to a fluorine, without the need for a tedious precursor synthesis. The radical nature of these reactions could potentially overcome the shortcomings of conventional $^{18}$F-substitution. Furthermore, they employ fluoride as the fluorine source, which would facilitate their translation to $^{18}$F labeling. Currently, fluoride is the only practical and widely used $^{18}$F source for the preparation of PET tracers with high specific activity.

While the decarboxylation strategy allows the specific replacement of an aliphatic carboxylic acid with fluorine-18, the regioselectivity of C-H [$^{18}$F]fluorination is not only determined by the electronic, steric and stereoelectronic properties of C-H bonds. It can also be modulated by changing the approaching direction of certain C-H bonds to the metal-oxo via a catalyst control strategy. The metabolic stability of the $^{18}$F-labeled products would be potentially increased, as the labeling sites using these methods are likely the positions attacked by cytochrome P450 enzymes. In addition to the highly site-selective hydrogen abstraction, the radical trapping step could enhance the stereoselectivity of $^{18}$F labeling reactions; this generally depends on the steric environment
where the substrate radical recombines to the metal-bound fluoride. Based on previous successful examples of high enantioselective oxidation reactions, we believe that $^{[18}\text{F}]$fluorination with high enantioselectivity facilitated by catalyst bearing a suitable chiral ligand would be possible. Considering the significance of stereochemistry in the biological activity of PET tracers, the preparation of a single stereoisomer without time-consuming chiral HPLC separation will be much more favorable.

**1.7 Conclusions**

Fluorinated organic molecules are of great importance due to their applications in pharmaceuticals, agrochemicals, material sciences and PET imaging. However, compared to the desired number of fluorine utilities, the chemist’s toolkit to install fluorine, especially radioactive fluorine-18, into organic molecules is still limited. Metal-oxo systems have been widely used in nature for C-H functionalization. Though enzymatic fluorination via such an oxidative strategy has yet to be discovered, other non-oxygen transfer processes such as chlorine and sulfur transfer have inspired us to explore fluorination via C-H activation by metal-oxo intermediates in synthetic models. Recently, our group has developed several fluorination methods catalyzed by manganese porphyrin and manganese salen complexes. The use of simple fluoride ion in these reactions facilitated their adaptation to $^{18}\text{F}$ chemistry using no-carrier-added $^{[18}\text{F}]$fluoride. In this dissertation, a manganese-catalyzed decarboxylative $^{18}\text{F}$ labeling approach has been further translated to a practical protocol for radiotracer production, showing the value of manganese-catalyzed methodology in clinical implementation. In addition, a new aliphatic C-H $^{18}\text{F}$ labeling mediated by a non-heme manganese complex has been developed with high applicability.
We envision that these approaches will have a meaningful impact in the PET clinical field by expanding the radiochemist’s toolbox and increasing the diversity of potential tracer candidates.

1.8 References


Chapter II

Radiosynthesis of PET Tracers Using Manganese Porphyrin Catalyzed Decarboxylative $^{18}$F-Fluorination

2.1 Introduction

Conventional methods for $^{18}$F labeling on aliphatic positions mainly rely on nucleophilic $^{18}$F substitution. This approach uses $[^{18}\text{F}]$fluoride as the $^{18}$F source and can be performed at a relatively late stage of a synthetic sequence.\textsuperscript{1} Despite its popularity, there are several limitations of this strategy.\textsuperscript{2} One is the narrow substrate scope because of the nature of the substitution reaction. A large amount of current aliphatic $^{18}$F tracers are labeled on the primary position of an alkyl chain (see Figure 2.1), which is the most active position for an S\textsubscript{N}2 reaction.\textsuperscript{3} Labeling tertiary carbons is challenging, as an S\textsubscript{N}2 reaction is not feasible on tertiary carbons and an S\textsubscript{N}1 reaction suffers from side reactions such as rearrangement or elimination.

![Figure 2.1 Examples of alkyl $^{18}$F PET tracers.](image_url)
A multistep synthesis is required either for the preparation of a precursor with an active leaving group on the labeling site, or for the radiolabeling.\textsuperscript{4-6} For instance, $[^{18}\text{F}]-2$-FACPC, a non-natural amino acid PET tracer for detecting brain tumors, is radiosynthesized from the cyclic sulfamidate precursor by nucleophilic substitution using $[^{18}\text{F}]$fluoride.\textsuperscript{7} The preparation of the cyclic sulfamidate precursor is labor-intensive and time-consuming, which needs eight steps from the commercially available starting material dimethyl glutarate (Scheme 2.1, a). Another example is the building block approach to synthesize tracers containing an $^{18}\text{F}$-labeled alkyl chain. In this method, introduction of $^{18}\text{F}$ into a building block by substitution using $[^{18}\text{F}]$fluoride occurs before additional one or more steps to reach the final tracer molecule.\textsuperscript{5} Many $^{18}\text{F}$-labeled aliphatic building blocks are gaseous and therefore, difficult to purify and handle (Scheme 2.1, b).\textsuperscript{7}

\textbf{a. Synthesis of the cyclic sulfamidate precursor and radiosynthesis of $[^{18}\text{F}]-2$-FACPC}

\textbf{b. Radiosynthesis of $[^{18}\text{F}]$FDDNP}

\textbf{Scheme 2.1} (a) Synthesis of the cyclic sulfamidate precursor and radiosynthesis of $[^{18}\text{F}]-2$-FACPC. (b) Radiosynthesis of $[^{18}\text{F}]$FDDNP.
A new $^{18}$F labeling reaction is desirable if it can target previously inaccessible functional groups. This can increase the diversity in potential tracer candidates or improve existing labelling technologies of known tracer molecules. Recently, many advances have occurred addressing radiofluorination on aliphatic positions. For example, decatungstate-catalyzed and manganese-catalyzed $[^{18}\text{F}]$fluorination reactions can selectively replace the unactivated C-H bond with a C-$^{18}$F bond, providing access to tertiary-labeled amino acids that are more difficult to synthesize using nucleophilic substitution (Scheme 2.2, a). Moreover, the manganese-mediated method can be scaled up and used to radiosynthesize $[^{18}\text{F}]$-3-ACPC from a precursor that can be easily prepared from a commercially available starting material (Scheme 2.2, b).

\[
\begin{align*}
\text{C–H} & \quad \rightarrow \quad \text{C–$^{18}\text{F}$} \\
\text{Scheme 2.2} & \quad \text{Examples of $^{18}\text{F}$-labeled molecules using (a) decatungstate-catalyzed $[^{18}\text{F}]$fluorination and (b) manganese-catalyzed $[^{18}\text{F}]$fluorination.}
\end{align*}
\]

To achieve clinical success of a newly developed $^{18}$F labeling reaction, several challenges need to be addressed, including automation, scale-up to a clinical dose, and
proof-of-concept imaging studies. Although there has been a recent abundance of radiofluorination methodologies, few new reactions have been further optimized for clinical research due to limitations of these methods in practice.\textsuperscript{10} Herein, we describe a successful implementation of a recently developed manganese-catalyzed decarboxylative (radio)fluorination for radiosyntheses of PET tracers.

There are several features that set this manganese-catalyzed decarboxylative \(^{18}\text{F}\) labeling reaction apart from other radiofluorination techniques. Firstly, it employs no-carrier-added \(^{18}\text{F}\) fluoride, which is the preferred \(^{18}\text{F}\) source due to its high specific activity. It is a radical-based fluorination, potentially circumventing drawbacks of nucleophilic \(^{18}\text{F}\)-substitution. Furthermore, \(^{18}\text{F}\)-labeled products can be easily separated from carboxylic acid precursors because of the large difference in polarity.

\begin{center}
\begin{tabular}{c c c c}
\textbf{R-COOH} & \textbf{Mn[TMP]Cl, \(^{18}\text{F}\)F}, PhIO & \textbf{CH\(_3\)CN, 50 °C, under air, 10 min} & \textbf{R-\(^{18}\text{F}\)} \\
\hline
\includegraphics[width=0.2\textwidth]{manganese_fluorination}

\end{tabular}
\end{center}

\textbf{Scheme 2.3} Small-scale manganese catalyzed decarboxylative \(^{18}\text{F}\) labeling. Radiochemical conversions (RCCs) are averaged over \(n\) experiments.

Previously, this radiochemistry was conducted using hand manipulations under a small scale of radioactivity (<100 \(\mu\text{Ci}\)); >1 mCi of final radiolabeled product is required in a typical PET tracer production process, which is generally prepared from a Ci-scale
starting amount of $[^{18}\text{F}]$fluoride. For safety considerations, a large-scale radiolabeling reaction needs to be conducted via an automated synthetic protocol, which has yet to be developed. In addition, only several simple molecules have been presented and additional effort is needed to expand the substrate scope of this radiochemistry.\textsuperscript{11} In this chapter, we further optimized this $^{18}\text{F}$-labeling method for large-scale radiolabeling and automated synthesis using a commercially available platform. Finally, and most importantly, we have successfully applied this optimized protocol to radiosyntheses of two potential PET imaging agents, demonstrating its practicality for real clinical research.

2.2 Results and Discussions

2.2.1 Optimization of Mn-catalyzed decarboxylative $^{18}\text{F}$-fluorination for automated synthesis and large-scale radiolabeling

Before optimizing the reaction for large-scale radiolabeling using an automated synthesizer, we first wanted to test whether our previous reported method\textsuperscript{11} could be smoothly transferred to the radiolabeling facility at Merck. 2,4-Dichlorophenoxyacetic acid (precursor of 1) and 1-(3-chloro-4-fluorobenzoyl)-4-methylpiperidine-4-carboxylic acid (precursor of 2) were chosen as model substrates. $[^{18}\text{F}]$Fluoride (~50 mCi) was eluted by a K$_2$CO$_3$ aqueous solution and ~400 µCi of the eluted solution was further diluted by acetonitrile for each reaction (Figure 2.2, a). We found that both model substrates could be readily labeled and the decay-corrected radiochemical conversions (RCCs) were determined by radio-HPLC (Scheme 2.4, red numbers).
Figure 2.2 (a) Preparation of K$_{\text{18}}$F aqueous solution for small-scale radiolabeling. (b) Dry down free approach without the azeotropic drying step.

For large-scale radiosyntheses, it is critical to remove residual water in order to obtain anhydrous [$^{18}$F]fluoride for the subsequent radiolabeling step. Generally, a dry-down approach is applied, which requires repeated azeotropic drying of aqueous [$^{18}$F]fluoride with acetonitrile at high temperatures (> 100 °C) under a nitrogen stream. The azeotropically dried $^{18}$F activity is introduced into the reaction solution with the aid of phase transfer reagents such as 18-crown-6 or Kryptofix 222 (K$_{\text{222}}$). We found that the tedious dry-down step could be avoided for this method by directly eluting [$^{18}$F]fluoride with the solution of Mn(TMP)Cl in acetonitrile (Figure 2.2, b). The eluted solution (~5 mCi) was used directly for the reaction. Using this protocol, both compounds 1 and 2 were obtained with high RCC values (Scheme 2.4, blue numbers).
Scheme 2.4 $^{18}$F labeling of compound 1 and 2 using the Mn-catalyzed decarboxylative protocol with reported radiochemical conversions (RCCs). RCCs are the average of $n$ runs and determined by radio-HPLC. Condition A: K$^{18}$F aqueous solution (~0.4 mCi) was used for reactions. Condition B: the solution of Mn(TMP)$^{18}$F in acetonitrile (~5 mCi) was used for reactions.

With this initial success, we set out to test the compatibility of our method to large-scale radiosyntheses using an automated synthesizer. For an automated synthesizer, a lower loading of reagents is highly desirable for the convenience of the purification of the $^{18}$F-labeled compounds. Additionally, the reaction needs to be conducted under microwave conditions. Using 5 mg of the precursor of 2 and 65 mCi of activity for the reaction, exploratory reaction conditions afforded 6.5 mCi labeled product after purification by semipreparative HPLC, with 10% decay-corrected radiochemical yield (RCY).

Scheme 2.5 Large-scale radiosynthesis of 2 using Mn-catalyzed decarboxylative method with reported radiochemical yield (RCY).
The overall efficiency of a radiosynthetic protocol is not only determined by the RCY value of the reaction, but also influenced by the elution efficiency of $[^{18}F]$fluoride from an anion exchange column. The elution efficiency was found to be dependent on the catalyst and the organic solvent being used (Table 2.1). Initial elution conditions using Mn(TMP)Cl in acetonitrile only recovered 10% of the activity. Changing the solvent to methanol boosted the elution efficiency, but destroyed the radiolabeling reaction, possibly because the reaction between methanol and PhIO suppressed the formation of the iodine(III) carboxylate compound formed by reaction with the substrate. Using manganese porphyrin complexes with more labile ligands such as triflate (OTf) or $p$-toluenesulfonate (OTs) afforded high elution efficiency (92% and 90%, respectively). Mn(TMP)OTf was found to catalyze a labeling reaction with high RCY (17%) as well.

Table 2.1 Elution of $[^{18}F]$fluoride using manganese porphyrin complexes in organic solvents.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>Elution efficiency (%)</th>
<th>RCY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mn(TMP)Cl</td>
<td>CH$_3$CN</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Mn(TMP)Cl</td>
<td>CH$_3$OH</td>
<td>95</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>Mn(TMP)Cl</td>
<td>Acetone</td>
<td>60</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>Mn(TMP)OTs</td>
<td>Acetone</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Mn(TMP)OTf</td>
<td>Acetone</td>
<td>90</td>
<td>17</td>
</tr>
</tbody>
</table>
2.2.2 18F labeling of tertiary carbons via Mn-catalyzed decarboxylation

Previously, our reported Mn-catalyzed decarboxylative [18F]-fluorination reactions mainly employed from benzylic and O-alkyl carboxyl acids.11 We posed the question: can this strategy be applied to the construction of tertiary C-18F bonds, which are hard to access using previous radiosynthetic methods?

![Figure 2.3 Scope of 18F labeling of tertiary carbons via Mn-catalyzed decarboxylation.](image)

Given the success of preparing compound 2 we were encouraged to expand the generality of this 18F-fluorination method to other substrates with carboxylic acids on the tertiary carbon position. PET imaging is a non-invasive approach to investigate molecule biodistribution, target interactions as well as pharmacodynamics in pharmacokinetics.12-14 Therefore a 18F labeling method is particularly useful in medicinal chemistry if it can efficiently radiolabeled existing drug molecules. To emphasize the practicality of this transformation, we sought to explore various simple tertiary carboxylic acids that feature
structural components of known pharmaceuticals. As shown in Figure 2.3, the selected compounds could be efficiently $^{18}$F-labeled via our Mn-catalyzed decarboxylative method with RCCs ranging from 33 to 76%. We were delighted to find that some of these compounds bearing sterically hindered carboxylic acids (4-7) could readily participate in this $^{18}$F-labeling, even though preparations of the analogous $^{19}$F homologues resulted in low yields. Notably, most of these molecules contain a piperidinyl or a pyrrolidinyl motif, which are among the most frequently used pharmaceutical core fragments in U.S. FDA approved drugs. This demonstrates the significant potential of this method to increase diversity in $^{18}$F-labeled molecules and furthermore, expand the clinically relevant radiochemical space.

2.2.3 Radiosynthesis of $[^{18}\text{F}]$F13640 analogue using Mn-catalyzed decarboxylative $^{18}$F-fluorination

To further demonstrate the value of this method in labeling new potential tracer candidates, we sought to apply the large-scale automated radiosynthesis protocol to a new potential radiotracer molecule, which is an analogue of the known PET tracer $[^{18}$F]-F13640. Befiradol (F13640) is a highly potent and selective 5-HT$_{1A}$ receptor agonist. 5-HT$_{1A}$ is a receptor of the neurotransmitter serotonin (5-HT), which is mainly in the central nervous system (CNS) of animals, including humans. It has been found to treat depressive disorders and other CNS disorders such as tardive dyskinesia and neuropathic pain. F13640 shows high binding affinity to the 5-HT$_{1A}$ receptor and gives more than a 1000-fold selectivity over a large range of other CNS targets. It is currently undergoing clinical
trials for the treatment of severe, chronic pain and Levodopa-induced dyskinesia in Parkinson’s disease, which was recently initiated by Neurolixis.\textsuperscript{19-22}

\[^{18}\text{F}]\text{F13640} (\text{Figure 2.4}, \text{left}) \text{ is a brain PET tracer and prefers to bind to the high-}
affinity state of the 5-HT\textsubscript{1A} receptor, which can be used to measure the functional receptors
in pathophysiological processes.\textsuperscript{23-24} F13640 is a particularly interesting target molecule,
as it comprises both an aromatic fluorine and a tertiary fluorine in the same molecule. The
current PET tracer \[^{18}\text{F}]\text{F13640} \text{ is } ^{18}\text{F}-labeled at the aromatic position. It would be highly
intriguing to prepare an analogue with an \(^{18}\text{F}\) atom labeled at the tertiary carbon position,
which was previously hard to access due to the lack of labeling methods. We could then
compare it with \[^{18}\text{F}]\text{F13640} \text{ in a PET imaging study.}

\textbf{Figure 2.4} Structure of \[^{18}\text{F}]\text{F13640} (left) and its analogue \[^{18}\text{F}]8 (right).

Encouraged by the previous success of labeling 2, which is a structural fragment of
F13640, using the Mn-catalyzed decarboxylative method, we set out to investigate if our
method could radiosynthesize \[^{18}\text{F}]8 (\text{Figure 2.4}, \text{right}). \[^{18}\text{F}]8 \text{ is an analogue of}
\[^{18}\text{F}]\text{F13640} \text{ with a tertiary } ^{18}\text{F} \text{ and a Boc-protecting group on the secondary amine that}
could potentially be readily removed after a labeling reaction. The precursor of 8 was
prepared by a seven-step synthetic protocol starting from 1-boc-4-(aminomethyl)piperidine-4-carboxylic acid ethyl ester. Radiosynthesis was achieved in 10
minutes using the previous optimized reaction conditions. The crude reaction mixture was
diluted with water and purified by semipreparative HPLC, affording 2.5 mCi of $^{18}$F$\text{[8]}$ with a decay-corrected RCY of 5%.

Scheme 2.6 Radiosynthesis of $^{18}$F$\text{[8]}$ using the Mn-catalyzed decarboxylative method.

To further confirm the $^{18}$F-labeled position in $\text{[8]}$ when using the Mn-catalyzed decarboxylative method, we also conducted the reaction under $^{19}$F conditions, in which triethylamine trihydrofluoride (Et$_3$N·3HF) was used as the fluoride source (Scheme 2.7). The fluorinated product after the reaction was detected by both high-resolution mass spectrophotometry and $^{19}$F NMR spectroscopy (see in section 2.5.12). In the $^{19}$F NMR spectrum, diagnostic peaks for the alkyl fluoride product appeared as singlet resonances at -161 and -163 ppm (two isomers). We observed the same characteristic peaks for authentic $^{19}$F$\text{[8]}$ indicating that this method fluorinates $\text{[8]}$ at the tertiary carbon position on the piperdine ring.

Scheme 2.7 Mn-catalyzed decarboxylative $^{19}$F-fluorination of $\text{[8]}$.
2.2.4 Radiosynthesis of the $[^{18}\text{F}]$MK-9470 analogue using Mn-catalyzed
decarboxylative $^{18}$F-fluorination

The second molecule under examination was the analogue of $[^{18}\text{F}]$MK-9470 (Figure 2.5). $[^{18}\text{F}]$MK-9470 is a selective and high-affinity inverse agonist for the
cannabinoid CB1 receptor (CB1R) and has been developed as a PET radiotracer for use in
human brain imaging. Cannabinoid receptors are among the most abundant G-protein-
coupled receptors in the CNS and their biological activities are found to be mediated by
the interaction with agonists or inverse agonists. CB1R is one of the two subtypes of
cannabinoid receptors mainly located in the CNS, while another subtype, CB2R is mainly
found in immune system cells. CB1R agonists, such as $\Delta^9$-tetrahydrocannabinol (a major
active component of marijuana) are known to be associated with the treatment of pain,
nausea and emesis, while CB1R inverse agonists, such as rimonabant, are used for the
treatment of obesity and smoking cessation. Previous studies showed that $[^{18}\text{F}]$MK-
9470 is a potent PET radiotracer to image the in vivo characterization of CB1R distribution
in a healthy human brain. Thus, it has the potential to be an in vivo study tool of CB1R
biology and pharmacology in related neurological and psychiatric diseases, and also allow
the demonstration of target engagement and dose-occupancy studies for potential dose
regimens.

![Figure 2.5 Structure of $[^{18}\text{F}]$MK-9470 (left) and its analogue $[^{18}\text{F}]$9 (right).]
\(^{18}\)F-fluoromethylethers and \(^{18}\)F-fluoroethylethers are important labeling targets due to the simple synthesis of phenolic precursors and the wide presence of phenolic functionalities in bioactive molecules. However, current methods to radiosynthesize these tracers often use volatile and synthetically tedious labeling agents such as \(^{18}\)FCH\(_2\)Br or \(^{18}\)FCH\(_2\)CH\(_2\)Br.\(^5\) Previous radiosynthesis of \(^{18}\)F\-[MK-9470 required the preparation of \(^{18}\)FCH\(_2\)CH\(_2\)Br and then distillation into a vial containing the phenol precursor for the subsequent radiolabeling.\(^26\) Our Mn-catalyzed decarboxylative approach, which can label \(O\)-alkyl groups, especially methoxy functionalities, could circumvent this drawback by avoiding the need for these labeling agents.

\[ \text{Scheme 2.8 Radiosynthesis of } ^{18}\text{F}[\text{MK-9470 using } ^{18}\text{F}[\text{FCH}_2\text{CH}_2\text{Br.}} \]

The target molecule under examination was the \(^{18}\)F-fluoromethoxy analogue of \(^{18}\)F\-[MK-9470. Previous studies showed that the replacement of FCH\(_2\)CH\(_2\) with FCH\(_2\) in \(^{19}\)F\-[9 resulted in an improvement in CB1R potency and selectivity, and a slightly lower lipophilicity over MK-9470, indicating \(9\) as a preferred PET tracer.

The use of the Mn-catalyzed decarboxylative method enabled the successful radiosynthesis of \(9\) in a one-pot, one-step automated protocol. The labeling precursor was readily prepared from the reaction of a phenol with ethyl bromoacetate, and the subsequent
deprotection of the obtained ether. After $[^{18}\text{F}]$fluoride (~200 mCi) was eluted via a dry-down free protocol, 93 mCi activity was used for the reaction. Radiosynthesis was achieved in 10 minutes using the previous optimized reaction conditions. The crude reaction mixture was diluted with water and purified by semipreparative HPLC, affording 10.3 mCi of 9 with a decay-corrected RCY of 11% and a radiochemical purity >99% (Scheme 2.9). The entire process from receiving an aqueous sample of $[^{18}\text{F}]$fluoride to obtaining the purified product could be accomplished in less than 45 minutes.

Scheme 2.9 Radiosynthesis of $[^{18}\text{F}]$9 using the Mn-catalyzed decarboxylative method.

To further confirm the $^{18}$F-labeled position in 9 when using the Mn-catalyzed decarboxylative method, we also conducted the reaction under $^{19}$F conditions. Using the previously reported reaction conditions with triethylamine trihydrofluorine ($\text{Et}_3\text{N} \cdot 3\text{HF}$) as the fluoride source, the $^{19}$F-product was detected by both high-resolution mass spectrometry and $^{19}$F NMR spectroscopy (Scheme 2.10). In the $^{19}$F NMR spectrum, the peak for the fluorinated product observed as a triplet resonance at -148.2 ppm ($^2J_{\text{HF}} = 54.6$ Hz). The same NMR features were observed for authentic compound $[^{19}\text{F}]$9, indicating that this method fluorinates compound 9 at the methoxy position.
2.3 Conclusions

In conclusion, we have developed a practical and robust method for aliphatic radiofluorination using a Mn-catalyzed decarboxylation strategy. This protocol is compatible with the automation and scale-up conditions required for tracer production for clinical use. The scope of this transformation has been further expanded to tertiary carbons that are challenging to label using established methods. We have successfully demonstrated the labeling of “drug-like” structures and the radiosynthesis of a radiotracer candidate. The value of this approach has been further highlighted by the radiosynthesis of an existing PET tracer via a simpler and more efficient pathway than previously possible. We are hoping that this new fluorination method could further expand the radiochemist’s toolbox and have a positive impact on $^{18}$F PET imaging.

2.4 Materials and Methods

2.4.1 General procedures

Precursors of compound 1, 3-7, starting materials for the synthesis of the precursors of compound 2, 8 and 9, and authentic samples were purchased from commercial sources. 5,10,15,20-Tetramesityl-porphyrinatomanganese(III) chloride [Mn$^{III}$(TMP)Cl] was prepared by metalation of tetramesitylporphyrin. Mn(TMP)OTs, Mn(TMP)OTf were
prepared from Mn(TMP)Cl with AgOTs and AgOTf, respectively. Iodosylbenzene (PhIO) was prepared by hydrolysis of iodobenzene diacetate with an aqueous sodium hydroxide solution. All solvents were purified according to the method of Grubbs.\textsuperscript{33} \textsuperscript{1}H NMR spectra were obtained on a Bruker NB 300 or a Bruker Avance-III (500 MHz) spectrometer and are reported in ppm using the residual protio solvent signal as an internal standard (CHCl\textsubscript{3} at δ 7.26, acetone at 2.04 or methylene chloride at 5.32). Data reported as: chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz); integrated intensity. \textsuperscript{13}C NMR spectra were recorded on a Bruker 500 (125 MHz) spectrometer and are reported in ppm using solvents as an internal standard (CDCl\textsubscript{3} at 77.15 ppm, acetone-\textit{d}\textsubscript{6} at 29.92 ppm or methylene chloride-\textit{d}\textsubscript{2} at 54.0 ppm). \textsuperscript{19}F NMR spectra (282 MHz) were obtained on a Bruker NB 300 spectrometer and were referenced relative to CFCl\textsubscript{3}. High-resolution mass spectra were obtained from the Princeton University mass spectrometer facility by electrospray ionization (ESI). High-performance liquid chromatography (HPLC) was performed on an Agilent 1100 series instrument with a binary pump and a diode array detector.

Radiochemistry experiments were performed at the Merck facility in West Point, PA in a shielded lead hot-cell using a Gilson automated liquid handler. RadioHPLC was performed on either an Agilent 1100 or Agilent 1200/1290 DAD system. Shielded QMA resins charged with \([^{18}\text{F}]\)fluoride were delivered by Siemens Molecular Imaging, Inc., North Wales, Pennsylvania 19454.
2.4.2 Preparation of simple substrate precursors and the $^{19}$F authentic standards

2,4-Dichlorophenoxyacetic acid (precursor of 1) was purchased from Aldrich and used as received. The precursor of 2 was prepared according to the following procedure:

![Scheme 2.11 Synthesis of precursor of 2.](image)

**Step 1:** Methyl 4-methyl-4-piperidinecarboxylate hydrochloride (968 mg, 5 mmol) was dissolved in 20 mL DCM and cooled in an ice bath. Triethylamine (3 ml, 20 mmol) and DMAP (54 mg, 0.4 mmol) were added, followed by addition of 2-chloro-4-fluorobenzoyl chloride (1158 mg, 6 mmol). The reaction mixture was warmed to room temperature and stirred overnight. After the completion of the reaction, the crude reaction mixture was purified by flash chromatography on silica (EtOAc/hexanes) to obtain methyl 1-(3-chloro-4-fluorobenzoyl)-4-methylpiperidine-4-carboxylate (1.4 g, 89%).

**Step 2:** Methyl 1-(3-chloro-4-fluorobenzoyl)-4-methylpiperidine-4-carboxylate (1.4 g, 4.45 mmol) and LiOH·H$_2$O (257 mg, 10 mmol) were dissolved in a mixture of water and THF (1:1 v/v, 20 mL). The solution was heated to reflux overnight, cooled to room temperature, acidified to pH 2 (with aqueous 1 M HCl), and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The product was purified by flash chromatography to provide 1-(3-chloro-4-fluorobenzoyl)-4-methylpiperidine-4-carboxylic acid (1.13g, 85%).
$^1$H NMR (500 MHz, CDCl$_3$) δ 7.49 (dd, $J = 6.9, 2.1$ Hz, 1H), 7.33 – 7.26 (m, 1H), 7.19 (t, $J = 8.6$ Hz, 1H), 4.34 (br s, 1H), 3.57 (br s, 1H), 3.26 (br s, 1H), 3.18 (br s, 1H), 2.22 (br s, 1H), 2.12 (br s, 1H), 1.51 (br s, 1H), 1.40 (br s, 1H), 1.31 (s, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 181.37, 168.32, 158.78 (d, $J = 252.6$ Hz), 132.74, 129.76, 127.14 (d, $J = 7.6$ Hz), 121.52 (d, $J = 18.1$ Hz), 116.81 (d, $J = 21.5$ Hz), 45.60, 41.85, 40.13, 26.00 ppm; HR-MS (ESI) m/z cal’d C$_{14}$H$_{16}$ClFNO$_3$ [M+H]$^+$: 300.0803, found 300.0791.

The $^{19}$F authentic standards of 1 and 2 were prepared according to literature methods.$^{11,34}$

![Image of compound 1](image1)

$^{19}$F NMR (282 MHz, CDCl$_3$) -116.42 (dq, $J = 60.8, 19.9$ Hz) ppm. MS (EI) m/z cal’d C$_8$H$_7$Cl$_2$FO [M]$^+$: 208.0, found 208.0.

![Image of compound 2](image2)

Mixture of two isomers (1:1). Isomer 1: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.50 (dd, $J = 7.0, 2.1$ Hz, 1H), 7.35 – 7.26 (m, 1H), 7.19 (t, $J = 8.6$ Hz, 1H), 4.49 (br s, 1H), 3.81 – 2.94 (br m, 3H), 1.90 (br m, 4H), 1.46 (s, 3H); $^{19}$F NMR (282 MHz, CDCl$_3$) -112.65, -154.04 ppm; HR-MS (ESI) m/z cal’d C$_{13}$H$_{15}$ClF$_2$NO [M+H]$^+$: 274.0810, found 274.0831.

Isomer 2: $^1$H NMR (300 MHz, CDCl$_3$) δ 7.50 (dd, $J = 7.0, 2.1$ Hz, 1H), 7.35 – 7.26 (m, 1H), 7.19 (t, $J = 8.6$ Hz, 1H), 4.49 (br s, 1H), 3.81 – 2.94 (br m, 3H), 1.90 (br m, 4H), 1.39 (s, 3H); $^{19}$F NMR (282 MHz, CDCl$_3$) -112.65, -154.04 ppm.
2.4.3 Procedure of small-scale radiosynthesis

General procedure of a $^{18}$F radiosynthesis using $K^{18}F$ aqueous solution:

A QMA resin containing $[^{18}\text{F}]$fluoride was eluted with a 1.0 mL solution with 5 mg $K_2CO_3$ in Milli-Q water (~100% elution efficiency). 40 µL of the eluted activity was diluted in 1 mL CH$_3$CN. 200 µL of the diluted $^{18}$F acetonitrile solution (~ 400 µCi) was injected into a 1.5 mL reaction vial with a screw cap, containing 20 mg substrate, 10 mg PhIO in 0.4 mL CH$_3$CN and a stir bar (2 × 5 mm). 1 mg of Mn catalyst was added and the resulting solution was stirred at 50 ºC for 10 minutes. The crude reaction mixture was diluted with acetonitrile and injected into radioHPLC. The product identity was confirmed by subjecting both the unlabeled authentic product and crude reaction mixture to radioHPLC.

General procedure of a dry-down free $^{18}$F radiosynthesis:

A QMA resin containing $[^{18}\text{F}]$fluoride was eluted with a 1 mL solution of 15 mg Mn catalyst in acetonitrile. 100 µL of the eluted Mn(TMP)$^{18}F$ solution (~ 5 mCi) was injected into a 1.5 mL reaction vial with a screw cap, containing 20 mg substrate, 10 mg PhIO in 0.3 mL CH$_3$CN and a stir bar (2 × 5 mm). 1 mg of Mn catalyst was added and the resulting solution was stirred at 50 ºC for 10 minutes. The crude reaction mixture was diluted with acetonitrile and injected into radioHPLC. The product identity was confirmed by subjecting both the unlabeled authentic product and crude reaction mixture to radioHPLC.
Figure 2.6 Analytic HPLC trace of the crude reaction mixture of 1 spiked with the authentic standard.

Figure 2.7 Analytic HPLC trace of the crude reaction mixture of 2 spiked with the authentic standard.

2.4.4 General protocol of large-scale radiosynthesis of $^{18}$F labeled molecules

A QMA resin containing $[^{18}\text{F}]$fluoride (200 mCi - 1 Ci) was slowly eluted (1 mL/min) with a 0.5 mL solution with 8 mg Mn(TMP)OTf in acetone (~90% elution efficiency). 10 mg precursor and 5 mg PhIO are added into a Wheaton 1 mL conical glass
vial with a septum cap. The reaction vial is clamped in a microwave cavity and fitted with an 18 gauge outlet needle. Gilson automated liquid handler was used for following steps:

1. 200 µL of the eluted Mn(TMP)\(^{18}\)F solution was added into the reaction vial.
2. 150 µL of acetonitrile was added into the reaction vial.
3. 200 µL of the eluted activity was transferred to the reaction vial.
4. 200 µL of the solution containing 1 mg Mn(TMP)OTf in acetonitrile was added into the reaction vial.
5. The reaction vial was heated in a microwave cavity (80 \(^{\circ}\)C, 50W) for 600 s.
6. 800 µL of water was added into the reaction vial.
7. The solution in the reaction vial was mixed completely using an automated liquid handler (take out 500 µL of the bottom layer of the solution and then add it back on the top of the solution).
8. 1500 µL of the solution in the reaction vial was injected into the preparative radioHPLC for purification.

The product identity was confirmed by subjecting both the authentic standard and the purified labeled product to analytical radioHPLC.

Preparative radioHPLC method to isolate \([^{18}\text{F}]2\):

Column: Gemini C18, 5 µm, 10×150 mm

Conditions: 25%-95% CH\(_3\)CN in H\(_2\)O (0.1% TFA), 0-15 min; 95% CH\(_3\)CN in H\(_2\)O (0.1% TFA), 15-25 min.
**Figure 2.8** Preparative HPLC traces of the authentic standard and the crude reaction mixture of 2.

**Figure 2.9** Analytical HPLC traces of purified $[^{18}\text{F}]2$ spiked with the authentic standard.
2.4.5 Preparation and characterization of $^{19}$F authentic samples of compound 3-7

The authentic samples of $[^{19}\text{F}]3$-7 were prepared from their corresponding carboxylic acid precursors using literature methods.$^{35}$

\[
\text{F} \quad \text{[}^{19}\text{F]}3
\]

Purified by flash chromatography (hexanes). $^{19}$F NMR (376 MHz, CDCl$_3$) -137.41 (m, $J = 22.0$ Hz) ppm; MS (EI) m/z cal’d C$_9$H$_{11}$F $[M]^{+}$: 138.1, found 138.1.

\[
\begin{array}{c}
\text{F} \\
\text{[}^{19}\text{F]}4
\end{array}
\]

Purified by flash chromatography (ethyl acetate/hexanes). Mixture of two isomers. Major isomer: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.42 – 7.17 (m, 5H), 3.71 – 3.28 (m, 4H), 3.18 – 2.97 (m, 2H), 2.14 – 1.80 (m, 2H), 1.47 (s, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) Isomer 1: $\delta$ 154.40, 135.72, 129.96, 128.40, 127.01, 102.67 (d, $J = 178.9$ Hz), 79.52, 55.42 (d, $J = 24.9$ Hz), 44.57, 42.00 (d, $J = 23.3$ Hz), 35.75 (d, $J = 23.3$ Hz), 28.48; $^{19}$F NMR (376 MHz, CDCl$_3$) -149.08 ppm; HR-MS (ESI) m/z cal’d C$_{16}$H$_{23}$FNO$_2$ $[M+H]^{+}$: 280.1713, found 280.1755.

Minor isomer: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.42 – 7.17 (m, 5H), 3.71 – 3.28 (m, 4H), 3.18 – 2.97 (m, 2H), 2.14 – 1.80 (m, 2H), 1.47 (s, 3H); 154.33, 135.72, 129.96, 128.40, 127.01, 101.97 (d, $J = 178.9$ Hz), 79.52, 55.06 (d, $J = 24.7$ Hz), 44.14, 42.00 (d, $J = 23.2$ Hz), 34.98 (d, $J = 23.6$ Hz), 28.48; $^{19}$F NMR (376 MHz, CDCl$_3$) -149.08 ppm.
Purified by flash chromatography (ethyl acetate/hexanes). Mixture of two isomers (1:1.2). Major isomer: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.52 – 7.06 (m, 5H), 4.02 – 3.43 (m, 4H), 2.46 – 2.12 (m, 2H), 1.40 (s, 9H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 154.43, 138.41 (d, $J = 22.6$ Hz), 128.57, 124.67 (d, $J = 8.2$ Hz), 102.56 (d, $J = 178.3$ Hz), 79.72, 58.03 (d, $J = 25.6$ Hz), 44.56, 37.51 (d, $J = 24.5$ Hz), 28.51; $^{19}$F NMR (376 MHz, CDCl$_3$) -147.8 ppm; HR-MS (ESI) m/z cal’d C$_{15}$H$_{21}$FNO$_2$ [M+H]$^+$: 266.1556, found 266.1531.

Minor isomer: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.52 – 7.06 (m, 5H), 4.02 – 3.43 (m, 4H), 2.46 – 2.12 (m, 2H), 1.40 (s, 9H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 154.30, 138.41 (d, $J = 22.6$ Hz), 128.46, 124.67 (d, $J = 7.9$ Hz), 101.74 (d, $J = 178.3$ Hz), 79.69, 57.30 (d, $J = 25.6$ Hz), 44.97, 38.34 (d, $J = 24.5$ Hz), 28.51; $^{19}$F NMR (376 MHz, CDCl$_3$) -147.1 ppm.

Purified by flash chromatography (ethyl acetate/hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.48 – 7.21 (m, 5H), 4.14 (br s, 2H), 3.20 (br s, 2H), 2.16 – 1.89 (m, 4H), 1.52 (s, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 154.80, 144.04 (d, $J = 21.4$ Hz), 128.45, 127.73, 123.84 (d, $J = 9.2$ Hz), 94.28 (d, $J = 174.5$ Hz), 79.74, 38.83, 36.56 (d, $J = 28.1$ Hz), 28.48;
\(^{19}\text{F} \text{NMR (376 MHz, CDCl}_3\text{)} -162.71 \text{ ppm, HR-MS (ESI) m/z cal’d C}_{16}\text{H}_{23}\text{FNO}_2 [M+H]^+: 280.1713, \text{ found } 280.1691.\)

\[
\text{Purified by flash chromatography (ethyl acetate/hexanes). Mixture of two isomers.}
\]
\(^1\text{H NMR (500 MHz, CDCl}_3\text{)} \delta 7.30 – 7.08 (\text{m, 5H}), 4.05 – 3.57 (\text{m, 2H}), 3.13 – 2.68 (\text{m, 4H}), 1.84 – 1.61 (\text{m, 2H}), 1.44 (\text{s, 2H}), 1.38 (\text{s, 9H}); \text{ }\(^{13}\text{C NMR (126 MHz, CDCl}_3\text{)} \delta 155.10, 135.34, 130.40, 128.22, 126.81, 92.45 (\text{d, } J = 177.9 \text{ Hz}), 79.74, 51.74, 51.54, 43.52 (\text{d, } J = 24.6 \text{ Hz}), 43.09, 33.26 (\text{d, } J = 24.4 \text{ Hz}), 28.37, 21.23; \text{ }\(^{19}\text{F NMR (376 MHz, CDCl}_3\text{)} \text{ major isomer: } -158.60, \text{ minor isomer: } -159.35 \text{ ppm; HR-MS (ESI) m/z cal’d C}_{17}\text{H}_{25}\text{FNO}_2 [M+H]^+: 294.1869, \text{ found } 294.1891.\)

2.4.6 Characterization of \(^{18}\text{F}-\text{labeled compound 3-7 using radio-HPLC}\)

Radio chemical conversions (RCCs) of \(^{18}\text{F} \text{ labeling reactions of compound 3-7 and the identification of }^{18}\text{F} \text{ labeled products were determined by analytical radio-HPLC using following method:}

\text{Column: Gemini 5 µm C18 110 Å, 150×4.6 mm, Eau}

\text{Conditions: water (0.1% TFA, A) and acetonitrile (B), 5%->95% B, 0-5 min; 95% B, 5-10 min, 1.5 mL/min}
Figure 2.10 Analytic HPLC trace of reaction mixture of $[^{18}\text{F}]3$ spiked with the authentic sample.

Figure 2.11 Analytic HPLC trace of reaction mixture of $[^{18}\text{F}]4$ spiked with the authentic sample.
Figure 2.12 Analytic HPLC trace of reaction mixture of $[^{18}\text{F}]5$ spiked with the authentic sample.

Figure 2.13 Analytic HPLC trace of reaction mixture of $[^{18}\text{F}]6$ spiked with the authentic sample.
2.4.7 Preparation and characterization of the precursor and the $^{19}$F authentic standard of $[^{18}$F]F13640 analogue

The precursor of 8 was obtained according to following procedure:

Scheme 2.12 Synthesis of precursor of 8.
**Step 1**: 1.43 g (5 mmol) of the amine starting material was dissolved in 20 mL DCM. 2.8 mL (1 eq.) N, N-diisopropylethylamine was added followed by 0.7 mL trifluoroacetate (1.2 eq.). The reaction was stirred overnight, and solvent was removed under reduced pressure. The crude reaction mixture was used for the next step directly.

**Step 2**: The crude mixture from step 1 was dissolved in 7 mL dioxane. 5 mL 4M HCl dioxane solution was added and the reaction was stirred for 4 hours. The solvent was then removed and the crude was used directly for next step.

**Step 3**: The crude material from step 2 was dissolved in 20 mL DCM and cooled in an ice bath. Triethylamine (3 ml, 20 mmol) and DMAP (54 mg, 0.44 mmol) were added followed by 3-chloro-4-fluorobenzoyl chloride (1.14 g, 5.94 mmol). The reaction mixture was allowed to warm to room temperature and was stirred overnight. After the completion of the reaction, the crude reaction mixture was purified by flash chromatography (EtOAc/hexanes) to obtain 1.14 g product (2.60 mmol, 52% yield after 3 steps).

**Step 4**: 1.14 g (2.60 mmol) of the product obtained from step 3 was dissolved in 50 mL of absolute ethanol. 700 mg (18.2 mmol, 7 equiv.) of NaBH₄ was added portion-wise to the ethanol solution. The reaction was then stirred for 6 hours. After the reaction was completed as monitored by TLC, ethanol was removed, and the crude reaction mixture was purified by flash chromatography (methanol/DCM) to afford the amine intermediate (782 mg, 2.28 mmol, 88% yield).

**Step 5**: The purified primary amine (782 mg, 2.28 mmol) and 5-methyl-2-pyridinecarboxaldehyde (278 mg, 1 equiv.) was stirred in dichloroethane for 2 hours. The reaction was monitored by TLC. After the consumption of the amine, sodium triacetoxyborohydride (731 mg, 1.5 equiv.) was added and the reaction was stirred at room
temperature for 3 hours. When the imine was fully reduced, water was added, and the reaction mixture was extracted with DCM. The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered, concentrated in vacuo and purified by flash chromatography (methanol/DCM) to afford 815 mg product (1.80 mmol, 79% yield).

**Step 6**: 815 mg (1.80 mmol) of the product in step 5 was then dissolve in DCM. DMAP (5 mg, 0.05 equiv.) was added followed by 590 mg (1.5 equiv.) di-tert-butyl decarbonate. The reaction was stirred at the room temperature and monitored by TLC. After the completion of the reaction, the reaction mixture was purified by flash chromatography (methanol/DCM) to obtain 875 mg (1.60 mmol, 89% yield) product.

**Step 7**: 875 mg (1.60 mmol) of the product in step 6 and LiOH·H$_2$O (202 mg, 4.81 mmol) were dissolved in a mixture of water and THF (1:1 v/v, 20 mL). The solution was heated to reflux for 48 h, cooled to room temperature, acidified to pH 2 (with aqueous 1 M HCl), and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), filtered, concentrated in vacuo and purified by flash chromatography (methanol/DCM) to provide the precursor of 8 (260 mg, 31% yield).

Mixture of two isomers. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.23 (s, 1H), 7.42 (d, $J = 8.0$ Hz, 1H), 7.35 (d, $J = 8.0$ Hz, 1H), 7.17 (br s, 1H), 7.11 – 6.94 (m, 2H), 4.47 (s, 2H), 4.32 (s, 1H), 3.44 (br d, 3H), 3.13 (br s, 1H), 2.85 (br s, 1H), 2.23 (s, 3H), 2.12 (br s, 2H), 1.38 (s, 2H), 1.21 (d, 9H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 167.90, 158.65 (d, $J = 252.3$ Hz), 156.45, 155.05, 149.07, 137.72, 133.18, 131.95, 129.72, 127.14, 127.08, 121.38(d, $J = 17.6$ Hz), 120.52, 116.72 (d, $J = 21.6$ Hz), 80.57, 56.22, 54.00, 47.75, 45.66, 40.02, 29.72, 28.24, 18.11; HR-MS (ESI) m/z cal’d C$_{26}$H$_{32}$ClFN$_3$O$_5$ [M+H]$^+$: 520.2014, found 520.2050.

Compound 8 was obtained according to following procedure:
**Scheme 2.13** Synthesis of compound 8.

**Step 1-3:** These steps were the same as the preparation of precursor of 8.

**Step 4:** 768 mg (2 mmol) of the product obtained in step 3 was dissolved in 10 mL THF. 10 mL of 1 M LiOH aqueous solution was then added. The reaction mixture was stirred overnight. After the completion of the reaction, the THF was removed and the aqueous solution was extracted with ethyl acetate three times. The crude material was purified by flash chromatography (methanol/DCM) to provide 510 mg amine intermediate (1.78 mmol, 89% yield).

**Step 5 and step 6:** These steps were the same as described in the preparation of precursor of 8.

Mixture of two isomers (1:2.5). Major isomer: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.38 (s, 1H), 7.52 – 7.44 (m, 2H), 7.31 (m, 1H), 7.19 (m, 1H), 7.16 – 7.02 (m, 1H), 4.74 – 4.39 (m, 3H), 3.79 – 3.06 (m, 5H), 2.33 (s, 3H), 2.02 – 1.59 (m, 4H), 1.37 (9H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 168.10, 158.78 (d, $J = 252.3$ Hz), 156.38, 155.23, 149.69, 137.05, 132.87 (d, $J = 4.1$ Hz), 131.45, 129.78, 127.12 (d, $J = 7.6$ Hz), 121.54 (d, $J = 18.1$ Hz), 120.29,
116.78 (d, J = 21.4 Hz), 95.72 (d, J = 174.5 Hz), 80.41, 53.90 (d, J = 20.2 Hz), 53.58 (d, J = 3.4 Hz), 43.58, 38.04, 33.11 (d, J = 16.2 Hz), 32.49 (d, J = 12.8 Hz), 28.21, 18.12; 19F NMR (282 MHz, CDCl3) -112.71, -161.52 ppm; HR-MS (ESI) m/z cal’d C25H31ClF2N3O3 [M+H]+: 494.2022, found 494.2036.

Minor isomer: 1H NMR (500 MHz, CDCl3) δ 8.38 (s, 1H), 7.52 – 7.44 (m, 2H), 7.31 (m, 1H), 7.19 (m, 1H), 7.16 – 7.02 (m, 1H), 4.74 – 4.39 (m, 3H), 3.79 – 3.06 (m, 5H), 2.33 (s, 3H), 2.02 – 1.59 (m, 4H), 1.50 (9H); 13C NMR (126 MHz, CDCl3) δ 168.10, 158.78 (d, J = 252.3 Hz), 155.79, 154.81, 149.61, 137.27, 132.87 (d, J = 4.1 Hz), 131.58, 129.69, 127.12 (d, J = 7.6 Hz), 121.54 (d, J = 18.1 Hz), 121.35, 116.89 (d, J = 21.4 Hz), 95.06 (d, J = 174.5 Hz), 80.67, 53.90 (d, J = 20.2 Hz), 52.87, 43.58, 38.04, 33.11 (d, J = 16.2 Hz), 32.49 (d, J = 12.8 Hz), 28.21, 18.12; 19F NMR (282 MHz, CDCl3) -112.42, -163.07 ppm.

2.4.8 Purification and characterization of [18F]F13640 analogue

Preparative radioHPLC method to isolate [18F]8:

Column: Gemini C18, 5 µm, 10×150 mm

Conditions: 5%-90% CH3CN in H2O (0.1% TFA), 0-15 min; 90% CH3CN in H2O (0.1% TFA), 15-20 min.

Figure 2.15 Preparative HPLC traces of authentic sample and crude reaction mixture of 8.
Analytical HPLC method to identify $[^{18}\text{F}]8$:

Column: Gemini 5 µm C18 110 Å, 150×4.6 mm, Ea

Conditions: water (0.1% TFA, A) and acetonitrile (B), 5%->95% B, 0-5 min; 95% B, 5-10 min, 1.5 mL/min

![Radio trace of purified $^{18}\text{F}$ product spiked with authentic sample](image1)

![UV trace of purified $^{18}\text{F}$ product spiked with authentic sample](image2)

**Figure 2.16** Analytical HPLC traces of purified $[^{18}\text{F}]8$ spiked with the authentic sample.

### 2.4.9 Identification of $[^{19}\text{F}]\text{F13640}$ analogue in Mn-catalyzed decarboxylative fluorination

$[^{19}\text{F}]8$ in Mn-catalyzed decarboxylative fluorination reaction mixture was identified by high-resolution mass spectrum and $^{19}\text{F}$ NMR.

![HRMS of Mn-catalyzed decarboxylative $^{19}\text{F}$-fluorination of 8](image3)

**Figure 2.17** HRMS of Mn-catalyzed decarboxylative $^{19}\text{F}$-fluorination of 8.
2.4.10 Preparation and characterization of the precursor and the $^{19}\text{F}$ authentic standard of [${}^{18}\text{F}$]MK-9470 analogue

The starting compound (phenol) for the synthesis of precursor of 9 was prepared by Merck via a previous reported method. The precursor of 9 was obtained according to the following procedure:

Scheme 2.14 Synthesis of precursor of 9.
**Step 1:** A vial charged with the phenol precursor (500 mg, 1.13 mmol), ethyl bromoacetate (0.336 ml, 3 equiv.) and cesium carbonate (982 mg, 3 equiv.). 40 mL anhydrous DMF was added. The reaction mixture was stirred at room temperature for 24 h. The reaction progress was monitored by TLC. After the completion of the reaction, the mixture was filtered, and the solvent was removed under reduced pressure. The crude mixture was purified by flash chromatography (EtOAc/hexanes) to obtain the ester intermediate (490 mg, 1.04 mmol, 92% yield).

**Step 2:** The purified ester (490 mg, 1.04 mmol) was dissolved in 10 mL of THF followed by addition of 10 mL aqueous solution of LiOH (4M). The reaction mixture was stirred overnight and the reaction progress was monitored by TLC. After the completion of the reaction, the THF was removed from the reaction mixture under reduced pressure. The aqueous solution was extracted twice with diethyl ether and then acidified to pH ~ 3.3 (white precipitate formed). The aqueous solution was then extracted three times with ethyl acetate. The combined organic layers were dried over sodium sulfate and the solvent was removed to afford the white solid product (310 mg, 0.789 mmol, 76% yield).

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.07 (br s, 1H), 7.99 – 7.83 (m, 1H), 7.48 (dd, $J$ = 8.4, 2.5 Hz, 1H), 7.43 (dt, $J$ = 7.4, 1.5 Hz, 1H), 7.31 – 7.23 (m, 2H), 7.20 (d, $J$ = 1.8 Hz, 1H), 6.76 – 6.61 (m, 6H), 4.58 (s, 2H), 4.34 (td, $J$ = 8.8, 6.7 Hz, 1H), 3.07 (dd, $J$ = 13.9, 4.2 Hz, 1H), 2.84 (ddd, $J$ = 10.7, 8.4, 4.3 Hz, 1H), 2.71 (dd, $J$ = 13.9, 10.8 Hz, 1H), 2.21 (s, 3H), 1.69 (s, 3H), 1.65 (s, 3H), 0.86 (d, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 174.42, 172.19, 159.48, 155.94, 146.22, 142.82, 140.40, 133.07, 132.43, 132.31, 130.41, 129.85, 129.06, 127.56, 118.82, 114.37, 112.31, 112.17, 81.21, 65.07, 53.53, 48.52, 37.81, 25.62,
25.04, 18.48, 17.41; HR-MS (ESI) m/z cal’d C_{29}H_{32}N_{3}O_{5} [M+H]^+: 520.2342, found 520.2330.

The authentic sample $^{[19}F]9$ was prepared from the precursor of 9 using a literature method.$^{34}$

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.90 (d, $J = 2.3$ Hz, 1H), 7.46 (dt, $J = 7.6$, 1.7 Hz, 2H), 7.35 – 7.25 (m, 2H), 7.22 (d, $J = 1.8$ Hz, 1H), 6.87 – 6.68 (m, 5H), 6.25 (d, $J = 9.1$ Hz, 1H), 5.65 (d, $J = 54.8$ Hz, 2H), 4.43 – 4.29 (m, 1H), 4.21 – 4.05 (m, 1H), 3.18 (dd, $J = 13.0$, 3.3 Hz, 1H), 2.90 – 2.71 (m, 2H), 2.21 (s, 3H), 1.72 (s, 3H), 1.66 (s, 3H), 0.90 (dd, $J = 6.8$, 1.1 Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 174.44, 159.74, 155.08, 146.34, 142.70, 139.99, 134.32, 133.00, 132.30, 130.47, 129.89, 129.08, 127.17, 118.78, 116.39, 112.31, 112.28, 100.83 (d, $J = 218.1$ Hz), 80.86, 53.88, 48.25, 38.01, 25.92, 24.85, 18.46, 17.44; $^{19}$F NMR (282 MHz, CDCl$_3$) -148.21 (t, $J = 54.8$ Hz) ppm; HR-MS (ESI) m/z cal’d C_{28}H_{31}FN_{3}O_{3} [M+H]^+: 476.2350, found 476.2365.

### 2.4.11 Purification and characterization of $^{[18}F]MK-9470$ analogue

Preparative radioHPLC method to isolate $^{[18}F]9$:

Column: Gemini C18, 5 µm, 10 ×150 mm

Conditions: 53% acetonitrile and 47% water (0.1% TFA) in 20 minutes.
Analytical HPLC method to identify $[^{18}\text{F}]9$:

Column: Gemini 5 µm C18 110 Å, 150×4.6 mm, Ea

Conditions: water (0.1% TFA, A) and acetonitrile (B), 5% B, 0-2 min; 5%→95% B, 2-15 min; 95% B, 15-20 min, 1.5 mL/min

Figure 2.19 Preparative HPLC traces of the crude reaction mixture of 9.

Figure 2.20 Analytic HPLC trace of purified $[^{18}\text{F}]9$ spiked with the authentic sample.
2.4.12 Identification of $[^{19}F]$MK-9470 analogue in Mn-catalyzed decarboxylative fluorination

$[^{19}F]9$ product in Mn-catalyzed decarboxylative fluorination reaction mixture was identified by high-resolution mass spectrophotometry and $^{19}F$ NMR spectroscopy.

**Figure 2.21** HRMS of Mn-catalyzed decarboxylative $^{19}F$-fluorination of 9.

**Figure 2.22** $^{19}F$ NMR spectrum (in CDCl$_3$) of Mn-catalyzed decarboxylative $^{19}F$-fluorination of 9.
2.4.13 NMR spectra

precursor of 2
\[ \text{[^{19}F]} \text{1} \]

\[ \text{[^{19}F]} \text{2} \]
2.5 References


Chapter III

Development of C-H Fluorination Mediated by a Non-Heme Manganese Complex

3.1 Introduction

Halogenated organic molecules are widely distributed in nature. Currently, around 5000 halogenated natural products have been reported, in which the carbon-halogen bonds are generated in enzymes.¹ Two types of halogenating enzymes have been found to mediate chlorination, bromination, and iodination.² The first type, which includes heme- and vanadium-dependent enzymes, use hydrogen peroxide as the oxidant, and therefore, are called haloperoxidases. The mechanism of halogenation is thought to proceed via oxidation of the halide ion to a bound hypohalite intermediate (−OX) making an electrophilic halogenating agent (“X⁺” equivalent) (Scheme 3.1, a and b).³⁻⁶ By contrast, the second type of halogenating enzymes, including flavin-dependent and non-heme iron-dependent enzymes, work with dioxygen directly rather than hydrogen peroxide, and so, are termed O₂-dependent halogenases. In the flavin-dependent halogenase, a hypohalite intermediate is produced via the oxidation of halide ion by an active FAD-OOH species (Scheme 3.1, c),⁷ but the non-heme iron(II) halogenases exhibit a different halogenation mechanism. For example, in SyrB2, a chloride ion first binds to the iron(II) center. Then, O₂ activation followed by a decarboxylation of α-ketoglutarate (αKG) results in a highly active oxo-iron(IV) intermediate, which abstracts a hydrogen atom from the substrate leading to a reduction to cis-chlorohydroxyl-iron(III). The radical rebound step involves Cl atom
transfer to the substrate radical providing the observed chlorinated molecule (Scheme 3.1, d).

**Scheme 3.1** Outline of mechanisms for (a) heme-dependent haloperoxidase (CPO), (b) vanadium-dependent haloperoxidase (V-BPO), (c) flavin-dependent halogenase (Bmp5) and (d) nonheme iron-dependent halogenase (SyrB2).

However, within these halogen-containing natural products, only thirty organic molecules are fluorinated, though the relatively high amount of fluoride in nature (the 13th most abundance element in Earth’s crust). Fluorinases are the only known enzymes that are capable of incorporating fluoride ion into organic molecules. These enzymes mediate the carbon-fluorine bond formation of 5-fluorodeoxyadenosine (FDA) via a simple
nucleophilic substitution, in which fluoride attacks the polarized C-S bond of S-adenosylmethionine (SAM) (Scheme 3.2). This process is distinct from the oxidative reactions for other halogenations, where chloride, bromide, and iodide are oxidized so as to react as electrophiles or radical-like species rather than nucleophiles. Because of the extremely high oxidation potential for fluoride ion, fluorination via a biological oxidative pathway has not yet been observed.

Scheme 3.2 Fluorinase-catalyzed $S_N2$ fluorination of SAM and the active site of fluorinase (PDB: 1RQR).

Recently, we reported a cytochrome P450-inspired oxidative C-H fluorination using biomimetic manganese porphyrin and salen catalysts. Key realizations from this work were the high binding affinity of manganese for fluoride ion under moist ion-exchange and catalytic conditions and the facile, low-barrier transfer of fluorine atoms to
carbon radicals from L-Mn(IV)-F. Together, these properties enabled highly efficient $^{19}$F and $^{18}$F labeling of a variety of natural products and drug molecules with fluoride ion.\textsuperscript{15-16}

Given this successful example, we wondered if an oxidative C-H ‘fluorinase’ catalyst could be developed in a non-heme coordination scaffold. We envisaged that an SyrB2-like \textit{cis}-coordination motif could be a useful mediator for C-H fluorination, because the hydrogen abstraction, and subsequent fluorine rebound process, could occur within a solvent cage without the need for freely-diffusing substrate carbon radicals. A non-heme strategy could also take advantage of the variety of ligand systems that have been developed for regio- and stereoselective C-H hydroxylation reactions.\textsuperscript{17-20}

But it is of significance to consider whether the rebound process can be controlled to achieve fluorine transfer in a non-heme coordination motif. Since the oxygen rebound rate (on the order of $10^{10}$ M$^{-1}$ s$^{-1}$) is extremely fast, redirecting the oxygen rebound to other halogen atom transfer is challenging, even for nature. For example, in SyrB2-mediated chlorination, substrate positioning is of primary importance in achieving chlorination selectivity. It was found that SyrB2 binds its substrate, L-threonine, positioning the reactive C-H bond away from the oxo/hydroxo ligand and near to the chloride ligand sacrificing hydrogen abstraction efficiency for the selectivity of chlorine rebound. Upon changing the substrate from L-threonine to L-norvaline, Bollinger and coworkers solely observed the alcohol product.\textsuperscript{21}

Despite the difficulty, there have been several examples implying the feasibility to redirect the oxygen rebound to a halogen atom transfer process (Scheme 3.3). The wildtype SyrB2 was found to direct C-Br or C-N bond formation via the same chemical logic when chloride was replaced by bromide, azide, or nitrite.\textsuperscript{22} Early in 1993, Que and
coworkers synthesized a series of $[\text{Fe}^{\text{III}}X_2(\text{TPA})]\text{ClO}_4$ (TPA = tris(2-pyridylmethyl)amine, and $X = \text{Cl, Br, N}_3$) complexes and treated them with a stoichiometric amount of tert-butylhydroperoxide (TBHP) and excess amount of cyclohexane to afford halocyclohexanes in good yield.\(^{23}\) An active species, $[\text{O} = \text{Fe}^{\text{V}}(\text{TPA})X]^{2+}$, was proposed to homolytically abstract a hydrogen atom from the cycloalkane and then transfer the heteroatom to the resulting carbon radical. Following this promising result, Comba conducted a catalytic chlorination of cyclohexane using an iron(II) complex bearing the tetradeutate bispidine ligand and iodosylbenzene (PhIO) as the oxidant. Though trace oxygenated products were observed, only 3.7% yield of chlorination was achieved based on the oxidant.\(^{24}\) Very recently, Que has synthesized and characterized a high-spin oxo-iron(IV) halide species $[\text{Fe}^{\text{IV}}(\text{O})(\text{TQA})X]^+$ (TQA = tris(quinolyl-2-methyl)amine, and $X = \text{Cl, Br}$) that can preferentially halogenate cyclohexane.\(^{25}\)

**Scheme 3.3** (a) Directing SyrB2-mediated chlorination to bromination, azidation, and nitration. (b) Halogenation reactivity of several nonheme model complexes.
However, no fluorination has been observed in either enzymatic or synthetic models. Notably, all of these examples were iron-mediated. Given the success with manganese porphyrins and salens as fluorination catalysts, in this chapter, we turned our attention to non-heme manganese complexes that have shown accessible Mn(IV) oxidation states and oxo-transfer or hydroxylation activity.

3.2 Results and discussions

3.2.1 Synthesis and characterization of a cis-difluoroMn(III) complex

To demonstrate the possibility of a non-heme manganese complex to mediate C-H fluorination, an important first step was to test the feasibility and efficiency of manganese-to-carbon fluorine atom transfer from a non-heme manganese(III) fluoride. A cis-Difluoromanganese(III) complex, [Mn^{III}(MEP)F_{2}]PF_{6} (MEP = N,N'-dimethyl-N,N'-bis-(2-pyridyl methyl)-ethane-1,2-diamine), was chosen as the model complex for this study as its symmetrical structure would simplify the synthesis and characterization.

[Mn^{III}(MEP)F_{2}]PF_{6} was prepared by reacting MEP with MnF_{3} in methanol/THF (1:1) at room temperature for an hour. Introduction of tetrabutylammonium hexafluorophosphate (NBu_{4}PF_{6}) induces precipitation and aids crystallization (Scheme 3.4).

![Scheme 3.4](image)

Scheme 3.4 The synthesis of [Mn^{III}(MEP)F_{2}]PF_{6}.
The ESI mass spectrum of the complex showed a large peak at m/z 363.1, which is the mass of [Mn$^{III}$\(\text{MEP}\)F$_2$]$^+$, supporting the coordination of MEP ligand and fluorides to the manganese center (see section 3.4.3, Figure 3.11). An X-band electron paramagnetic resonance (EPR) study was performed for this complex, and the spectrum (see section 3.4.3, Figure 3.12) indicated that this complex is EPR-silent, supporting the oxidation state of Mn(III).

The UV-Vis spectrum of the acetonitrile solution of [Mn$^{III}$\(\text{MEP}\)F$_2$]PF$_6$ is shown in Figure 3.1. Bands of low intensity at 448, 454, 520 and 981 nm are detected, which may be assigned to d-d transitions.

![UV-Vis spectrum of \([\text{Mn}^{III}(\text{MEP})\text{F}_2]\text{PF}_6\) in acetonitrile.](image)

Figure 3.1 UV-Vis spectrum of [Mn$^{III}$\(\text{MEP}\)F$_2$]PF$_6$ in acetonitrile.

A splitting energy diagram is drawn in Figure 3.2, showing the splitting of the spectroscopic terms when lowering the symmetry from O$_h$ to C$_2$. The asymmetry from O$_h$ to C$_{2v}$ results from the elongation distortion developing perpendicular to the equatorial plane. In C$_{2v}$ symmetry, only three out of four d-d transitions are allowed. But when lowing
the symmetry from C\textsubscript{2v} to C\textsubscript{2}, a forbidden transition from the $^5\text{B}_1$ ground state to the $^5\text{B}_2$ exited state is allowed. As a result, the 981 nm band is assigned to the $^5\text{B}_1 \rightarrow ^1\text{A}_1$ transition, and bands at 448, 454, and 520 nm are attributed to the transition from the $^5\text{B}_1$ ground state to the three exited states split from the $^5\text{T}_{2g}$ state. These assignments are consistent with those published for other Mn(III)-F complexes.\textsuperscript{26-28}

![Diagram](image)

**Figure 3.2** Splitting of the spectroscopic terms when lowering the symmetry from O\textsubscript{h} to C\textsubscript{2}. The right column shows the corresponding unoccupied d orbitals. The solid and dashed arrows represent the allowed and forbidden d-d transitions, respectively.

The X-ray diffraction crystal structure of [Mn\textsuperscript{III}(MEP)F\textsubscript{2}]PF\textsubscript{6} showed the manganese ion to be coordinated by two fluorides which are cis to one another. The Mn\textsuperscript{III}-F bond lengths of 1.8172(7) Å and 1.8214(7) Å are similar to analogous structurally characterized fluoro-Mn\textsuperscript{III} species (Figure 3.3).\textsuperscript{26,29}
Figure 3.3 X-ray crystal structures of [Mn$^{III}$(MEP)F$_2$]PF$_6$. Thermal ellipsoids represented the 50% probability surfaces (hydrogen atoms are omitted for clarity).

The electrochemistry of [Mn$^{III}$(MEP)F$_2$]PF$_6$ was studied in acetonitrile with 0.1 M NBu$_4$PF$_6$ as the supporting electrolyte. All potentials were referenced in mV versus a Ag/AgCl working electrode. The cyclic voltammogram of [Mn$^{III}$(MEP)F$_2$]PF$_6$ recorded at room temperature is shown in Figure 3.4. A quasi-reversible process was observed at $E_{1/2} = 1383$ mV ($\Delta E_p = 128$ mV), corresponding to the redox couple Mn(III)/Mn(IV).

![Cyclic voltammograms of 1 mM [Mn$^{III}$(MEP)F$_2$]PF$_6$ in acetonitrile at 25 °C with 0.1 M NBu$_4$PF$_6$ as the supporting electrolyte (scan rate = 100 mV·s$^{-1}$).]
3.2.2 Experimental studies of fluorine transfer reactivity of cis-difluoroMn(III) complex to alkyl radicals

With well-characterized [Mn^{III}(MEP)F_{2}]PF_{6} in hand, we then tested its fluorine transfer reactivity to alkyl radicals. Previously, Sammis and coworkers demonstrated the fluorine transfer reactivity of N-fluorobenzenesulfonimide (NFSI) to alkyl radicals using tert-butyl peresters.\textsuperscript{30} Inspired by this work, we chose tert-butyl-2-phenylpropaneperoxoate as the precursor of the alkyl radical.

We found that α-phenethyl radicals generated by thermal decomposition of the precursor in the presence of stoichiometric amounts of [Mn^{III}(MEP)F_{2}](PF_{6}), afforded a 40% yield of 1-fluoroethylbenzene (Scheme 3.5). The color of the reaction mixtures changed from red to yellow, showing the conversion of [Mn^{III}(MEP)F_{2}]PF_{6} to Mn(II) species. The yield was determined by $^{19}$F NMR based on the added tert-butyl-2-phenylpropaneperoxoate with 4-nitrofluorobenzene as an internal standard (see section 3.4.4, Figure 3.13).

\[
\begin{array}{c}
\text{Scheme 3.5} \text{ Fluorine transfer activity of [Mn}^{\text{III}(\text{m})F_{2}]}(\text{PF}_{6}) \text{ to the alkyl radical.}
\end{array}
\]

3.2.3 Synthesis and characterization of non-heme oxo-Mn(IV) complexes

A second key criterion for a functional manganese fluorinase model is the accessibility of oxoMn^{IV} intermediates. Treating Mn^{II}(MCP)(OTf)\textsubscript{2} with m-CPBA in acetonitrile at -30 °C produced a new species, indicated by the appearance of a broad
absorption from 600 nm to 1100 nm in the UV-vis spectra in Figure 3.5, left (see section 3.4.5 for experimental details). This spectroscopic characteristic was consistent with other known non-heme oxo-Mn complexes. The resulting solution was then titrated with 1 equiv. of Bu₄NF under -30 °C which generated a new species indicated by the decreased extinction coefficient (see Figure 3.5, right, section 3.4.5 for experimental details).

**Figure 3.5** UV/Vis spectral changes for the formation of [Mn⁴⁺(O)(MCP)(NCCH₃)]²⁺ in the reaction of Mn¹⁻MCP(OTf)₂ and m-CPBA in acetonitrile at -30 °C. The inset shows the time course of the formation of [Mn⁴⁺(O)(MCP)(NCCH₃)]²⁺ monitored at 650 nm (left). UV/Vis spectra for the titration process from [Mn⁴⁺(O)(MCP)(NCCH₃)]²⁺ to [Mn⁴⁺(O)(MCP)(F)]⁺ (right).

In addition to UV-vis spectroscopy, X-band electron paramagnetic resonance (EPR) spectroscopy was also employed to characterize these two complexes (Figure 3.6, section 3.4.5 for experimental details). All EPR spectra were taken at 5 K with samples frozen in acetonitrile. The EPR spectrum of the starting material, Mn¹⁻(MCP)(OTf)₂, exhibits signals that are characteristic $S = 5/2$ Mn(II) complex ($g = 2.01, 1.42$ for $m_s = ±1/2$ transition, $g = 3.05, 5.53$ for $m_s = ±3/2$ transition with $E/D = 0.2$). After the addition of $m$-
CPBA, a new EPR spectrum was observed with features at $g = 1.85, 2.80$ and 4.80. These $g$ values are in accordance with a $S = 3/2$ state with rhombic symmetry, suggesting the generation of a high-spin oxo-Mn(IV) species.\textsuperscript{33} In addition to the characteristic peaks for Mn(IV) complex, there is some multiline located around the $g = 2$ region, which can be assigned as a 16-line hyperfine splitting for a small amount of Mn(III, IV) dimer in the sample.\textsuperscript{34} Adding 1 equiv. of Bu$_4$NF shifted the transition to another high-spin Mn(IV) species, observed by the change of signals in the EPR spectra with $g$ values at 1.85, 2.97, and 5.17. We interpret this result as indicating the formation of a fluoro-oxo-Mn$^{IV}$ complex.

![EPR spectra](image)

**Figure 3.6** EPR spectra of Mn$^{II}$(MCP)(OTf)$_2$ (blue line), [Mn$^{IV}$(O)(MCP)(NCCH$_3$)]$^2+$ (red line) and [Mn$^{IV}$(O)(MCP)(F)]$^+$ (green line).

### 3.2.4 Computational studies of the hydrogen abstraction process of non-heme high-valent oxo-Mn complexes

To determine the active species mediating the hydrogen abstraction step, DFT calculations were employed to explore the potential energy landscape and electronic structure of intermediates and transition states in an acetone solvent continuum.

Both the oxo-Mn(IV) complex, [Mn$^{IV}$(MCP)(O)(F)]$^+$, and the oxo-Mn(V) complex, [Mn$^{V}$(MCP)(O)(F)]$^{2+}$, have two possible spin states, quartet and doublet for the Mn(IV)
complex, and triplet and singlet for the Mn(V) complex. Our calculation results (Scheme 3.6) indicated the ground state for both complexes are high-spin, which has lower energy than the low-spin state by 15.6 and 18.4 kcal/mol, respectively.

Scheme 3.6 Calculated Gibbs free energy differences between quartet and doublet states for [Mn$^{IV}$($\text{MCP}$)(O)(F)]$^+$ (left), and between triplet and singlet states for [Mn$^{V}$($\text{MCP}$)(O)(F)]$^{2+}$ (right) in an acetone continuum.

The energy diagram of the molecular orbitals and the orbital contour plots of quartet state of [Mn$^{IV}$($\text{MCP}$)(O)(F)]$^+$ and triplet state of [Mn$^{V}$($\text{MCP}$)(O)(F)]$^{2+}$ are shown in Figure 3.7. In [Mn$^{IV}$($\text{MCP}$)(O)(F)]$^+$, $\sigma$-bonding interactions exist between the $d_{x^2-y^2}$ orbital of manganese and the $p_z$ orbital of oxygen while the $d_{xy}$ and $d_{yz}$ orbitals of manganese have $\pi$-bonding interactions with the $p_x$ and $p_y$ orbitals of oxygen. The three d electrons occupy the non-bonding orbital $d_{xz}$ and the $\pi^*(d_{xy}, yz - px, y)$ orbitals. In [Mn$^{V}$($\text{MCP}$)(O)(F)]$^{2+}$, the two d electrons occupy the non-bonding $d_{xz}$ orbital and $\pi^*(d_{yz} - p)$ orbital.
Figure 3.7 Energy diagrams obtained from the calculated energy (eV) of the quasi-restricted orbitals for [Mn$^{IV}$(MCP)(O)(F)]$^+$ (left) and [Mn$^{V}$(MCP)(O)(F)]$^{2+}$ (right).

To simplify the calculation, we chose toluene, which has the BDE of the benzylic C-H bond at 89.8 kcal/mol, as the model substrate when we studied the hydrogen abstraction of [Mn$^{IV}$(MCP)(O)(F)]$^+$ and [Mn$^{V}$(MCP)(O)(F)]$^{2+}$. As is shown in Figure 3.8, hydrogen abstraction by [Mn$^{IV}$(MCP)(O)(F)]$^+$ has a lower energy barrier on the quartet surface, 22.3 kcal/mol, indicating this process can occur at room temperature. [Mn$^{V}$(MCP)(O)(F)]$^{2+}$ shows much higher activity in the hydrogen abstraction from
toluene, with only 6.2 kcal/mol barrier on the triplet surface. In both transition states, the π*(d_zy - p) orbitals of oxo-Mn complexes are engaged and the σ (C-H) bond of toluene is positioned in a side-attack orientation with a bent Mn-O-H angle in order to maximize orbital overlap.

Figure 3.8 Energy landscape of hydrogen abstract of [Mn^{IV}(MCP)(O)(F)]^+ (top) [Mn^{V}(MCP)(O)(F)]^{2+} (bottom) from methylbenzene radical in acetone continuum. The energies are enthalpies in kcal/mol.
3.2.5 Development of a catalytic non-heme manganese C-H fluorination reaction

We found that fluorinated products from simple alkanes such as cylooctane and adamantane were observed in the presence of catalytic amounts of MnII(MCP)(OTf)2. This fluorination is driven by m-CPBA as the oxidant, using silver fluoride/triethylamine trihydrofluoride (TREAT·HF) as the fluoride source. The initial exploratory reaction of cyclooctane afforded the monofluorinated product in 15.1% yield with 15.3% epoxidation and 23.5% ketone (Scheme 3.7). We found that slowly adding the oxidant and the catalyst separately over an extended period of time using a syringe pump could increase the overall conversion of the substrate by disfavoring decomposition of the catalyst and improving overall catalyst productivity.\textsuperscript{35-36} Furthermore, adding trifluoroacetic acid and mixing the catalyst and TREAT·HF before the slow addition was found to improve the selectivity of the fluorination reaction.

\textbf{Scheme 3.7} Initial catalytic C-H fluorination of cyclooctane.

The deuterium kinetic isotope effect (KIE) was also studied. Reaction of a 1:1 mixture of cyclooctane and cyclooctane-\textit{d}_{16} produced intermolecular competitive KIEs of 3.5 and 3.4, respectively, for the reaction with and without fluoride sources (see section 3.4.6 for experimental details). These numbers are similar to the KIE observed for hydrogen abstraction by other reported non-heme mononuclear oxo-Mn\textsuperscript{IV} species.\textsuperscript{32-33, 37}
3.2.6 Computational studies of the fluorine/oxygen rebound step using non-heme Mn(III) complex

To better understand the selectivity of the fluorine/oxygen rebound step of our non-heme manganese complex, DFT calculations were employed to explore the potential energy landscape and electronic structure of intermediates and transition states in an acetone solvent continuum.

Mn(III) complex [Mn$^{III}$MCP(OH)(F)]$^+$ has three possible spin states, quintet ($S = 2$), triplet ($S = 1$), and singlet ($S = 0$). Our calculation results (Scheme 3.8) determined the quintet to be the ground state for this complex which is stabilized by 14.8 and 44.7 kcal/mol versus the triplet and singlet states, respectively, indicating its preference for the $d^4$ high-spin electronic configuration.

Scheme 3.8 Calculated Gibbs free energy difference among quintet, triplet, and singlet states for [Mn$^{III}$MCP(OH)(F)]$^+$ in an acetone continuum.

The energy diagram of manganese $d$ orbitals and the orbital contour plots of the quintet state of [Mn$^{III}$MCP(OH)(F)]$^+$ are shown in Figure 3.9. As expected based on ligand field theory, the energies of $d$ orbitals increase in the order of $d_{xz} < d_{xy} < d_{yz} < d^*_{x^2-y^2} < d^*_{z^2}$ and four $d$ single electrons of manganese sit on the four $d$ orbitals with the lowest energies. Notably, the $d_{xz}$, $d_{xy}$, and $d_{yz}$ orbitals of manganese have $\pi$-antibonding
interactions with $p$ orbitals in fluorine or oxygen while $\sigma$-antibonding interactions exist between the $d_{x^2-y^2}$ orbital and $p$ orbitals in fluorine or oxygen atom.

Figure 3.9 Energy diagrams obtained from the calculated energy (eV) of the quasi-restricted orbitals for [Mn$^{III}$(MCP)(OH)(F)]$^+$. 

We only considered the quintet state of this Mn(III) complex in the computational study of the fluorine/oxygen transfer step, since triplet and singlet states have much higher energies. To simplify the calculation, we chose isopropyl radical as the model radical. Optimized structure of a loosely bound pre-coordination complex containing a Mn(III) complex and an isopropyl radical was used as the starting point in this process assuming the rebound step occurs subsequently after the hydrogen abstraction process within a solvent cage without the need for radical diffusion. There are four possible pathways
through which the isopropyl radical can interact with the Mn(III) complex: rebounding either the fluorine or oxygen atoms of the Mn(III) species on either sextet or quartet energy surfaces. As it is shown in Figure 3.10, both the fluorine transfer and the oxygen transfer have much lower energy barriers on the sextet surfaces. Fluorine atom transfer from [MnIII(MCP)(OH)(F)]+ to the isopropyl radical (F sextet pathway) was predicted to occur with a low activation barrier of only 3.3 kcal/mol. A higher energy calculated transition state was obtained for OH rebound (O sextet pathway, 8.9 kcal/mol), implicating a strong preference for fluorine rebound from a kinetic perspective. However, oxygen rebound is more thermodynamically favored versus fluorine transfer by 19.3 kcal/mol.

In the process of C-F formation (F sextet pathway), the d* x^2-y^2 orbital of the Mn(III) complex, the p_z orbital of fluorine, and the SOMO of the isopropyl radical are involved. The population analysis showed that both the spin population of fluorine and the orbital spin population of the p orbitals of fluorine decrease in the transition state compared with those in the Mn(III) complex. Simultaneously, both the spin population of manganese and the orbital spin population of the d orbitals of manganese increase in the transition state. This result indicated that it is more likely to be a fluoride than a fluorine radical involved during this process.
Figure 3.10 Energy landscape of fluorine/oxygen transfer from \([\text{Mn}^{\text{III}}(\text{MCP})(\text{OH})(\text{F})]^+\) to isopropyl radical in an acetone continuum. The energies are enthalpies in kcal/mol.

3.3 Conclusions

A *cis*-difluoromanganese(III) complex \([\text{Mn}^{\text{III}}(\text{MEP})_2\text{PF}_6]\) has been successfully synthesized and well-characterized. This complex has a capacity to trap alkyl radicals generated in situ and transfer fluorine with a yield of 40% based on the radical precursor. Additionally, non-heme oxo-Mn(IV) complexes \([\text{Mn}^{\text{IV}}(\text{O})(\text{MCP})(\text{NCCH}_3)]^2\) and
[Mn^{IV}(O)(MCP)(F)]^+ have been observed in the oxidation of the Mn(II) complex by m-CPBA.

By contrast, the experimental data for cyclooctane fluorination/oxygenation suggests that fluorine transfer, oxygenation, and desaturation all occur with similar frequency, but we cannot be sure if all catalyst molecules bear fluoride ion during turnover. To probe this mechanistic issue and to explore the applicability of this manganese catalyst platform to $[^{18}\text{F}]$ radiolabeling and PET imaging, we examined the reaction further using $^{18}\text{F}$-fluoride in the next chapter.

3.4 Materials and Methods

3.4.1 General procedures

Unless noted, all reagents and starting materials were purchased from commercial sources with analytical grade and used without purification. Non-heme ligands MEP and MCP were prepared and metallated as previously reported. \textsuperscript{35, 38-39} Solvents were purified by a solvent purification system (Innovative Technology Inc.) and were stored in a N\textsubscript{2}-filled glovebox over molecular sieves. GC/MS analyses were performed on an Agilent 7890A gas chromatograph equipped with an Agilent 5975 mass selective detector. \textsuperscript{19}F NMR spectra (282 MHz) were obtained on a Bruker NB 300 spectrometer and are reported in ppm. High-resolution and low-resolution mass spectra were obtained from the Princeton University mass spectrometer facility by electrospray ionization (ESI). UV-vis spectra were recorded using a Hewlett-Packard 8453 diode array spectrophotometer. Continuous wave (CW) EPR spectra were recorded on an X-band Bruker EMXPlus spectrometer equipped with an EMX standard resonator. The single crystallography was conducted with
a Bruker APEX2 Duo diffractometer equipped with molybdenum and copper X-ray tubes
($\lambda = 0.71073$ and 1.54178 Å respectively).

3.4.2 Preparation of cis-difluoroMn(III) complex [Mn$^{III}$($\text{MEP}$)F$_2$]PF$_6$

[Mn$^{III}$($\text{MEP}$)F$_2$](PF$_6$) was prepared by treating MEP with a stoichiometric amount
of MnF$_3$. Typically, after MEP (270 mg, 1 mmol) was dissolved in 10 mL 1:1 THF/MeOH,
MnF$_3$ (112 mg, 1 mmol) was added into the solution in solid form and the reaction mixture
was stirred at room temperature for an hour. Tetrabutylammonium hexafluorophosphate
(1.55 g, 4 mmol) saturated in THF was added into reaction mixture. The resulting solution
was left without stirring until the crystallization was finished. The product was collected
by filtration (416 mg, 82% yield). Shiny red crystals suitable for X-ray crystal structure
analysis were grown by vapor diffusion (solvent: CH$_3$CN, precipitant: Et$_2$O).

3.4.3 Characterization of [Mn$^{III}$($\text{MEP}$)F$_2$]PF$_6$

A low resolution mass spectrum of [Mn($\text{MEP}$)F$_2$]PF$_6$ is shown in Figure 3.11.

![Figure 3.11 Low resolution mass spectrum of [Mn($\text{MEP}$)F$_2$]PF$_6$ in acetonitrile.](image)
[Mn(MEP)F₂]PF₆ in acetonitrile (10 mM) was frozen by liquid N₂ for measurement of EPR. The EPR was run at 5K (Figure 3.12).

![EPR Spectrum](image)

**Figure 3.12** EPR spectrum of [Mn(MEP)F₂]PF₆ (5 K, in acetonitrile).

### 3.4.4 Radical trapping experiment

The thermal decomposition of t-butyl 2-phenylpropaneperoxoate was conducted at 105 °C in the presence of a stoichiometric amount of [Mn³⁺(MEP)F₂](PF₆). Typically, a 4 mL vial with a screw cap was charged with [Mn³⁺(MEP)F₂](PF₆) (50.8 mg, 0.1 mmol) and t-butyl 2-phenylpropaneperoxoate (22.2 mg, 0.1 mmol) and a stir bar. The vial was purged with N₂ three times. Degassed CH₃CN (1 mL) added into the vial via syringes and the vial was sealed by parafilm. After the solution was heated at 105 °C for 10 minutes, the vial was cooled down to room temperature and the yield of 1-fluoroethyl benzene was determined by ¹⁹F NMR (-166.6 ppm) using 4-nitrofluorobenzene as the internal standard (see in Figure 3.13).
Figure 3.13 $^{19}$F NMR of reaction mixture of fluorine transfer experiment.

3.4.5 Preparation and characterization of non-heme oxo-Mn(IV) complexes

$[\text{Mn(IV)}(\text{O})(\text{MCP})(\text{NCCH}_3)]^+$ was prepared by treating Mn(II)(MCP)(CF$_3$SO$_3$)$_2$ with $m$-CPBA in CH$_3$CN at -30 $^\circ$C. Mn(MCP)(CF$_3$SO$_3$)$_2$ (2 mg, 1 mM) dissolved in 3 mL CH$_3$CN was stirring at -30 $^\circ$C for 5 minutes. $m$-CPBA (2.6 mg, 5 equiv.) in 200 µL CH$_3$CN was added in one portion. Reaction was run under -30 $^\circ$C and UV-vis was used to monitor the reaction progress, showing the appearance of a new absorption from 600 to 1000 nm. $[\text{Mn(IV)}(\text{O})(\text{MCP})(\text{NCCH}_3)]^+$ in CH$_3$CN solution was titrated with 1 equiv. of Bu$_4$NF under -30 $^\circ$C to generate $[\text{Mn(IV)}(\text{O})(\text{MCP})(\text{F})]^+$. After the reaction was finished, the mixture was frozen by liquid N$_2$ for measurement of EPR.
All EPR were run at 5K using CH$_3$CN as solvent. Both EPR of [Mn(IV)(O)(MCP)(NCCH$_3$)]$^+$ and [Mn(IV)(O)(MCP)(F)]$^+$ show characteristic rhombic Mn(IV) signals. In addition to the characteristic Mn(IV) peak, a small amount of multiline was observed at g=2 region, which can be assigned as a small amount of Mn(II) species.

3.4.6 Screening of reaction conditions of non-heme Mn-catalyzed C-H fluorination

General procedure of non-heme Mn-catalyzed C-H fluorination:

A 4 mL vial with a screw cap and a magnetic stir was charged with AgF (40 mg) and substrate (60 µL, 0.45 mmol). After the vial was capped and purged with nitrogen for 5 minutes, trifluoroacetic acid (0.22 mmol) in acetonitrile (0.2 mL) and CH$_2$Cl$_2$ (0.1 mL) was added by syringe and the vial was placed on a stir plate and heated at 45 °C. A 1 mL solution (solution 1) containing Mn(MCP)(OTf)$_2$ (17.5 mg, 6 mol%) and TREAT·HF (13 µL) in acetonitrile was added dropwise via syringe. After several drops of solution 1, another 1 mL solution (solution 2) with m-CPBA in acetonitrile was added dropwise via syringe. The addition of both solutions was completed over 100-120 minutes. The color of reaction mixture changed from colorless to brown. When the reaction was completed, the solution cooled to room temperature and passed through a short pad of silica gel (washing with CH$_2$Cl$_2$). The filtrate was analyzed by GC/MS (Figure 3.14). The assignment of the products was based on the comparison of GC retention time and mass fragmentation with the authentic standards.
Figure 3.14 GC Trace of non-heme catalyzed C-H fluorination of cyclooctane. GC method: 50 °C, 2 min; 50->100 °C, 5 min; 100->220 °C, 4 min.

Kinetic isotope effect of the fluorination reaction:

Mn(MCP)(CF₃SO₃)₂ (20 mg, 6 mol%), cyclooctane/cyclooctane-₃₁₆ (1:1, 0.5 mmol in total), AgF (45 mg, 1.5 mmol) and Et₃N • 3HF (16 mg 0.1 mmol) were added in a 4 mL vial with a screw cap. No fluoride sources were added in the oxygenation reaction. The vial was purged with N₂ for three cycles. Degassed CH₃CN (0.2 mL) and CH₂Cl₂ (0.1 mL) were added into the vial via syringes. After the reaction mixture stirred at 45 °C for 5 minutes, m-CPBA (43 mg, 0.25 mmol, 0.5 equiv.) in 0.5 mL CH₃CN was injected into the mixture via a syringe and the reaction was further stirred for 30 minutes. Upon reaction completion, the solution cooled to room temperature and was then passed through a short pad of silica gel (washing with CH₂Cl₂). The filtrate was concentrated in vacuum and was analyzed by GC/MS. The kinetic isotope effect was determined by calculating the ratio of peak intensities of corresponding products (Scheme 3.9 for fluorination, Scheme 3.10 for oxygenation).
Scheme 3.9 KIE study of non-heme Mn-catalyzed C-H fluorination. GC method: 50 °C, 2 min; 50->100 °C, 5 min; 100->220 °C, 4 min. are in the same retention time. The amount of epoxidized cyclooctane and deuterated cyclooctanone were determined by using extracted-ion chromatogram for m/z value in 55.0.

Scheme 3.10 KIE study of non-heme Mn-catalyzed C-H oxygenation.
3.4.7 Details of X-ray crystallography

Table 3.1 Crystal data for [Mn(III)(MEP)F₂](PF₆)

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<td>Wavelength</td>
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<td>Space group</td>
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Table 3.2 Structural refinement details for [Mn^{III}(MEP)F₂](PF₆)

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\[ R(F^2 > 2\sigma(F^2)) = 0.0346 \]
\[ wR(F^2) = 0.0893 \]
\[ S = 1.138 \]
Data completeness  = 1.88/0.99
17938 reflections
274 parameters
1 restraint
Primary atom site location: structure-invariant direct methods
Secondary atom site location: difference Fourier map
Hydrogen site location: inferred from neighbouring sites
H-atom parameters constrained
\[ w = 1/\left[ \sigma^2(F_o^2) + (0.0342P) + 0.6550P \right] \]
\[ \Delta \rho_{\text{max}} = 0.814 \text{ e Å}^{-3} \]
\[ \Delta \rho_{\text{min}} = -0.706 \text{ e Å}^{-3} \]

**Table 3.3** Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å²)

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<th>(z)</th>
<th>(U_{iso}/U_{eq})</th>
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Table 3.4: Atomic displacement parameters (Å²) for [Mn<sup>III</sup>(MEP)F₂](PF₆)
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**Table 3.5** Bond lengths and bond angles for [MnIII(MEP)F2](PF6)
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<td>C13-C14</td>
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### 3.4.8 Computational studies

All calculations were carried out with Gaussian 09, Revision C.01, except for orbital diagrams, which were quasi-restricted orbitals generated by ORCA 3.0. Geometry optimizations were conducted at B3LYP/6-31G(d) (SDD for Mn) level of theory without symmetry constraints. The nature of all stationary points was confirmed by frequency analysis at the same level of theory as in geometry optimization. The zero-point vibrational energies (ZPVE) and the thermal correction to Gibbs free energies (TCGE) obtained from
the frequency analysis were used for further correction of the single-point energies (SPE).

Single-point energies in acetone continuum (SPE(ACE)) were calculated using a larger basis set (SDD for Mn and 6-311++G(d, p) for other atoms) with CPCM solvation model and uaks radii. The final free energies of each species ($G$) were obtained using the following equation, $G(ACE) = SPE(ACE) + TCGE$. The reference state of all calculation is 1 atm and 298 K for gas-phase and 1 mol/L and 298 K for solution-phase.
Charge = 2, spin multiplicity = 3

\[ \text{[Mn(V)(MCP)(O)]}^2\Delta \text{ (singlet)} \]

Charge = 2, spin multiplicity = 1

\[ \text{[Mn(V)(MCP)(O)]}^2\Delta \text{ (triplet)} \]
Charge = complex (quartet)  
Transition state of the H-abstraction by oxo-Mn(IV) complex (quartet)  
Charge = 1, spin multiplicity = 4
Transition state of the H-abstraction by oxo-Mn(IV) complex (doublet)
Charge = 1, spin multiplicity = 2

[C]

Transition state of the H-abstraction by oxo-Mn(V) complex (triplet)
Charge = 2, spin multiplicity = 3

[C]
### Transition State of the H-abstraction by oxo-Mn(V)

**Complex (singlet)**

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<th>Coordinates</th>
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**Tolyl radical**

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<td>H</td>
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**Mn**

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**Charge** = 2, spin multiplicity = 1

**Charge** = 0, spin multiplicity = 2

![Diagram of the complex](image-url)

**Transition state of the H-abstraction by oxo-Mn(V) complex (singlet)**

**Tolyl radical**

**Charge** = 0, spin multiplicity = 2

**Charge** = 0, spin multiplicity = 2

![Diagram of the tolly radical](image-url)
Charge = Transition state of fluorine rebound (quartet)

Charge = Transition state of oxygen rebound (sextet)
Transition state of oxygen rebound (quartet)
Charge = 1, spin multiplicity = 4

C 0.15947000 3.34381600 10.95917000
C -0.62486600 1.83926300 9.24701400
C -1.54571000 0.68221800 8.92147500
C -1.83572000 0.30701700 7.60897000
C -2.62836000 -0.82131200 7.39066000
C -3.11055000 -1.53717100 8.48689900
C -2.78314400 -1.90951500 9.76449900
C -2.27453900 3.31446700 10.36184800
C -2.09829300 4.67377300 9.66243800
C -3.42037400 5.43842800 9.51707200
C -1.26987700 0.79526700 12.01118800
H -0.93615500 3.07494400 12.40609800
H -3.52860000 1.36089900 14.29877700
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H -1.66563500 3.09246100 15.98276000
H -1.23940800 1.69714900 13.92604700
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H -5.00219900 3.72887500 11.01338700
H -4.71853500 4.46091600 12.58709100
H -5.01234400 6.17829300 10.80590600
H -3.40177700 6.29293900 11.50598000
H -4.11861000 4.87839400 8.87584900
H -3.24268100 6.39800700 9.01983000
H -1.39619800 5.29719600 10.21542100
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H -0.51940700 4.02555500 10.71812800
H -0.11717400 3.92968100 11.83722200
H -0.57263100 2.53655700 8.99332200
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H -3.73109400 -2.41810800 8.35808800
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F 0.56913900 0.39938700 11.79134900
O -2.07857400 -0.68673370 12.95263800
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H 0.62970800 -2.51664600 14.40985600
H 1.72530000 -3.03041800 13.14951100
H 1.22288400 -1.33897400 13.17057500
H -1.31968300 -3.61724600 12.80760200
H -2.22759300 -2.99389500 13.04576100
H -2.78741200 -1.82433800 12.52435200
H -1.63119400 -4.25207300 11.96492000
H -1.10679100 -4.23882000 13.67959600

\[
\begin{align*}
\text{Transition state of oxygen rebound (quartet)} \\
\text{Charge = 1, spin multiplicity = 4} \\
\text{C} & \text{-4.70378400 -2.58194900 13.10401200} \\
\text{N} & \text{-4.70228100 -1.36920100 13.96045700} \\
\text{C} & \text{-3.92967900 -1.65505800 15.20451100} \\
\text{N} & \text{-3.15503000 0.62144300 14.99254700} \\
\end{align*}
\]
Propan-2-ol

Charge = 0, spin multiplicity = 1

### 3.5 Reference


Chapter IV

Non-Heme Manganese Catalyzed Late-Stage Aliphatic C-H $^{18}$F Labeling

4.1 Introduction

The application of $^{18}$F-labeled small molecules for PET imaging is one of the most promising approaches to diagnose and manage diseases such as cancers, cardiovascular diseases and neurological disorders in vivo. However, the deficiency of chemical tools that can incorporate fluorine-18 into bioactive molecules limit the potential scope of this imaging technique. In addition to the general difficulties of the C-F bond formation, other challenges related to the translation from fluorine-19 chemistry to a radiofluorination reaction come from the intrinsic features of fluorine-18 such as the short half-life (~110 minutes) and the extremely small available amount of fluorine-18 (nanomole scale) in a reaction. Therefore, a $[^{18}\text{F}]$fluorination reaction would be desirable if it meets the following requirements:

- The reaction time of the radiochemistry is short (less than 30 minutes) due to the short half-life of fluorine-18.
- The reaction is efficient enough to work under very low concentrations of fluorine (nM to µM) so as to make fluorine the limiting reagent.
- $[^{18}\text{F}]$Fluoride, which has higher specific activity, is a preferred fluorine-18 source versus electrophilic $[^{18}\text{F}]$fluorine sources prepared from $[^{18}\text{F}]$fluorine gas.
- Fast purification can be achieved using HPLC and/or other methods.
Radiochemical conversion (RCC) and radiochemical yield (RCY), which are used to evaluate fluorine-18 transfer efficiency, are high enough for PET scanning applications.

The reaction is compatible with the presence of water, because rigorously dry \[^{18}\text{F}]\text{fluoride}\) is not accessible.

With respect to radiation safety concerns, the reaction protocol must be operationally simple and preferably fully automated using specialized equipment in compliance with good manufacturing practices (GMPs).

Previous studies (Chapter 3) indicated that non-heme manganese mediated fluorination may be a promising approach to introduce fluorine-18 into an aliphatic position of small molecules via C-H activation. In \(^{18}\text{F}\) chemistry, the transfer efficiency of a substoichiometric amount of \(^{18}\text{F}\), rather than the conversion of the substrate molecule, is mainly considered. The synthetic difluoro-manganese(III) complex, \([\text{Mn(MEP)F}_2]\text{PF}_6\), could trap alkyl radicals generated in situ and transfer fluorine efficiently. DFT calculations of fluorine transfer from a nonheme Mn(III) complex to an isopropyl radical in acetone showed a low energy barrier of only 3.3 kcal/mol. Such a result implicated a fast fluorine rebound process and more importantly, a high potential for this non-heme manganese complex to transfer \(^{18}\text{F}\) at very low concentrations. Preliminary exploration of catalytic C-H fluorination gave successful cases of introducing fluoride to simple molecules such as cyclooctane and adamantane. The use of fluoride as the fluorine source implies its applicability to \(^{18}\text{F}\) fluorination using \(^{18}\text{F}\)fluoride. In addition, all reagents used for this reaction such as the manganese catalyst and oxidant, \(m\)-CPBA, are easy-to-handle and soluble in various organic solvents, facilitating the translation to radiochemistry using an
automation system. Even though significant amounts of oxygenated products were generated, they can be easily separated from the fluorinated product using HPLC.

In this chapter, novel late-stage aliphatic C-H $^{18}$F labeling chemistry using a non-heme manganese catalyst Mn$^{II}$(MCP)(OTf)$_2$ (MCP = N,N'-dimethyl-N,N'-bis(2-pyridylmethyl)-cyclohexane-1,2-diamine) with no-carrier-added $[^{18}$F]fluoride is presented. This labeling method is operationally simple and broadly applicable, taking advantage of a soluble oxidant, $m$-CPBA, and low substrate loading. A variety of building blocks and bioactive molecules have been successfully labeled, demonstrating its potential for the application of PET imaging.

4.2 Results and discussions

4.2.1 Screening of reaction conditions of non-heme Mn-catalyzed C-H $^{18}$F-fluorination

The $[^{18}$F]fluoride was produced from H$_2^{18}$O by the nuclear reaction $^{18}$O(p,n)$^{18}$F in a cyclotron. To efficiently lower the water content in the reaction mixtures, a small portion (~100 μCi) of the $[^{18}$F]fluoride solution, diluted with organic solvent, was used as the $^{18}$F source for the later screening experiments. The radiochemical conversion (RCC) of the $^{18}$F-labeled product was determined by radio-TLC. The identity of the labeled product was confirmed by comparing the radio-HPLC trace of the crude reaction mixture to the HPLC UV trace of the authentic reference sample.

We first explored Mn$^{II}$MCP as a catalyst for direct substitution of aliphatic hydrogen with $[^{18}$F]fluoride. With celestolide as an initial model substrate, exploratory reaction conditions afforded a promising 45% radiochemical conversion (RCC) to $^{18}$F
labeled product (Table 4.1, Entry 1). Changing the solvent from acetonitrile to acetone resulted in the surprisingly high RCC of 76% (Entry 2). Increasing the temperature to 45 °C still produced ~70% RCC (Entry 5). Besides Mn(MCP)(OTf)₂, Mn(PDP)(OTf)₂ (PDP is 2-((2-[1-(pyridin-2-ylmethyl)pyrrolidin-2-yl]pyrrolidin-1-yl)methyl)pyridine) was also found to be reactive for this C-H ¹¹⁸F labeling protocol, albeit with lower RCC (33%) (Entry 3). No ¹¹⁸F labeling product was detected under these conditions with Fe(MCP)(OTf)₂ as the catalyst (Entry 4).

Table 4.1 Reaction screening of C-H ¹¹⁸F-labeling using celestolide as the model substrate.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>T</th>
<th>RCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mn(MCP)(OTf)₂</td>
<td>CH₃CN</td>
<td>r.t.</td>
<td>45%</td>
</tr>
<tr>
<td>2</td>
<td>Mn(MCP)(OTf)₂</td>
<td>Acetone</td>
<td>r.t.</td>
<td>76%</td>
</tr>
<tr>
<td>3</td>
<td>Mn(PDP)(OTf)₂</td>
<td>Acetone</td>
<td>r.t.</td>
<td>33%</td>
</tr>
<tr>
<td>4</td>
<td>Fe(MCP)(OTf)₂</td>
<td>Acetone</td>
<td>r.t.</td>
<td>n.p.</td>
</tr>
<tr>
<td>5</td>
<td>Mn(MCP)(OTf)₂</td>
<td>Acetone</td>
<td>45</td>
<td>71%</td>
</tr>
</tbody>
</table>

4.2.2 Evaluating C-H ¹¹⁸F labeling method with a wide scope of substrates

We next investigated the scope and generality of this ¹¹⁸F-Mn(MCP) labeling protocol. The procedure was found to radiolabel a variety of substrates in 31% - 54% RCC with 10-20 mol% catalyst loading (Scheme 4.1). Functionalities including ester, ether, ketone, nitrile, imide, aryl, and alkyl halide were well tolerated. The reaction efficiency was affected by the electronic properties of the substrate. Labeling usually occurred at the
most electron-rich and the least sterically-hindered C-H bonds. For instance, mono-
substituted cycloalkanes were labeled predominantly at the most remote methylene
position from the hindered and electron-withdrawing substituents (19 and 20). Moreover,
the $^{18}$F-Mn(mcp) method enabled the incorporation of $^{18}$F into common scaffolds in
bioactive molecules, such as indan, dibenzocycloheptene, and tetrahydronaphthalene.

\[
\begin{array}{cccc}
\text{Mn(MCP)(OTf)$_2$ (10 - 20 mol%)} & \text{m-CPBA (1 eq.), } [^{18}\text{F}]\text{F, K$_2$CO$_3$} & \text{acetone, under air, r.t., 10 min} & \text{R-^{18}F} \\
R-H & & & R-^{18}F \\
\end{array}
\]

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction Yield (%)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>41% ± 5%</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>56% ± 3%</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>51% ± 8%</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>53% ± 6%</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>52% ± 5%</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>50% ± 3%</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>51% ± 6%</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>54% ± 4%</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>42% ± 7%</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>46% ± 6%</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>55% ± 3%</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>36% ± 6%</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>40% ± 3%</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>42% ± 10%</td>
<td>5</td>
</tr>
<tr>
<td>17</td>
<td>32% ± 6%</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>43% ± 8%</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>41% ± 8%</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>41% ± 8%</td>
<td>3</td>
</tr>
</tbody>
</table>

Scheme 4.1 Substrate scope of Mn(MCP)(OTf)$_2$ mediated C-H $[^{18}$F$]$fluorination.
This non-heme manganese system for $^{18}$F labeling has several advantages over our previously developed Mn(salen) catalyzed C-H $^{18}$F method (Scheme 4.2). First, a soluble oxidant, $m$-CPBA, could be used instead of solid PhIO in this $^{18}$F labeling reaction. This change facilitates the implementation of automated procedures using laboratory robotics in compliance with GMPs. Low substrate loading is highly desirable in this procedure. Indeed, we found that 10 mg (0.05 mmol) of celestolide afforded $^{18}$F labeled product with $>70\%$ radiochemical conversion (RCC), whereas the Mn(salen) system required more than five times substrate loading to be effective. Such a low substrate loading particularly facilitates high throughput screening assays of potential radiotracers. Moreover, in addition to benzylic substrates, which can be labeled using Mn(salen), this new Mn(mcp) $^{18}$F labeling strategy can also be applied to aliphatic substrates with stronger C-H bonds.

**Scheme 4.2** Comparative C-H $[^{18}\text{F}]$fluorination chemistry catalyzed by Mn(salen)(OTs) and Mn(MCP)(OTf)$_2$. 

Previous work: 

$$
C\equiv H \xrightarrow{\text{Mn(salen)OTs, PhIO, $[^{18}\text{F}]F^-$, 50 °C}} C\equiv ^{18}\text{F}
$$

This work: 

$$
C\equiv H \xrightarrow{m\text{-CPBA, $[^{18}\text{F}]F^-$, r.t.}} C\equiv ^{18}\text{F}
$$
4.2.3 Late-stage $^{18}$F labeling of bioactive molecules

To demonstrate the benefit of a direct C-H [$^{18}$F]fluorination method in late-stage radiolabeling, we applied this system to a panel of complex bioactive molecules (Scheme 4.3). As examples, celestolide, a perfume component and protected dopamine, a biomessenger in the nervous system, could be readily labeled at the benzylic position. The application of PET in pharmacokinetics is a non-invasive approach to investigate molecule biodistribution, target interactions, and study pharmacodynamics.¹ A method for $^{18}$F labeling is particularly useful in medicinal chemistry if it can efficiently radiolabel existing drug molecules. Several widely prescribed drugs, including the Parkinson’s disease drug rasagiline, the anti-inflammatory drug ibuprofen, the immuno-modulating drug fingolimod, and memantine for the treatment of Alzheimer’s disease have been subjected to this method and were successfully radiolabeled within 10 minutes at room temperature. Notably, $^{18}$F-Mn(MCP) produced a different $^{18}$F-fingolimod isomer than was obtained with $^{18}$F-Mn(salen).

Scheme 4.3 Direct C-H $^{18}$F labeling of bioactive molecules.
4.2.4 Proposed mechanism of non-heme Mn-catalyzed C-H $^{18}$F-fluorination

A proposed catalytic cycle for this non-heme manganese catalyzed C-H fluorination is shown in Figure 4.1. Due to the extremely limiting amounts of $[^{18}\text{F}]$fluoride under C-H labeling catalytic conditions, the resting state of the catalyst is likely to be a cis-$^{18}$F-Mn$^{\text{II}}$-OH species, not a difluoride. Further, a large excess of catalyst would necessarily have no fluoride ligand. The cycle proceeds by $m$-CPBA oxidation of the resting Mn$^{\text{II}}$ catalyst to afford a reactive oxoMn(IV)-F intermediate, which subsequently abstracts a hydrogen atom from the substrate, producing a carbon-centered radical and a cis-$^{18}$F-Mn$^{\text{III}}$-OH rebound species. The $^{18}$F-labeled product is formed via $[^{18}\text{F}]$fluorine transfer from the cis-$^{18}$F-Mn$^{\text{IV}}$-OH complex to the substrate radical, a heteroatom rebound reminiscent of SyrB2-mediated chlorination, resulting in the regeneration of the resting Mn$^{\text{II}}$ catalyst.

![Proposed mechanism of C-H $^{18}$F labeling catalyzed by Mn(MCP)(OTf)$_2$.](image-url)
Using radionuclide detection, 66\% (non-decay corrected yield) of [\textsuperscript{18}F]fluoride loaded on an anion exchange cartridge could be extracted using the Mn\textsuperscript{II}(MCP) catalyst in acetone solution, suggesting a highly efficient ligand exchange proceeded during the elution even on the moist column. It also indicated the potential compatibility of our method to a dry-down free protocol, which facilitates the development of automated synthesis and large-scale radiolabeling. The high RCC values observed indicates a high preference for fluorine atom transfer in the portion of the catalyst inventory that does bear a fluoride ligand. It is expected that oxygenated products are also formed under \textsuperscript{18}F-labeling conditions, but these products are inconsequential and easily separated from the fluorinated products.

**Figure 4.2** The elution of \textsuperscript{18}Ffluoride using Mn\textsuperscript{II}(MCP) catalyst.

### 4.3 Conclusions

In summary, we have described a model, non-heme manganese fluorinase capable of cleaving C-H bonds and fluorinating sp\textsuperscript{3} sites in a variety of substrates. Applied to \textsuperscript{18}F
labeling, this Mn-F catalysis enables a facile, no-carrier-added method for straightforward late-stage labeling of a series of small molecules and complex bioactive molecules. Operational simplicity of the labeling, most notably the ability to use a soluble oxidant and low substrate loading, will entrench its application in automated radio-syntheses and high throughput screening of radiotracer candidates. Going forward, we will evaluate the possibility of adapting this transformation to PET imaging applications. The results also suggest that an enzyme using such a strategy is mechanistically feasible.

4.4 Materials and methods

4.4.1 General procedures

Substrates of product 3, 12, 14, 19, 20, 22, 23, 24, and 25 were purchased from commercial sources and were protected according to literature procedures.\textsuperscript{9-12} Mn(MCP)(OTf)\textsubscript{2}, Fe(MCP)(OTf)\textsubscript{2}, and Mn(PDP)(OTf)\textsubscript{2} were synthesized as previously described.\textsuperscript{13-14} 3-Chloroperbenzoic acid (\textit{m}-CPBA) was purified using a literature protocol.\textsuperscript{15} Substrate of product 21 was purchased from Matrix Scientific. Other commercial materials were of the highest purity available from Aldrich and used without further purification. \textsuperscript{1}H NMR spectra were obtained on a Bruker NB 300 spectrometer or a Bruker Avance-III (500 MHz) spectrometer and are reported in ppm using solvent as an internal standard (CDCl\textsubscript{3} at δ 7.26, acetone-\textit{d}\textsubscript{6} at 2.04, or methylene chloride-\textit{d}\textsubscript{2} at 5.32 ppm). Data reported as: chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz); integrated intensity. \textsuperscript{13}C NMR spectra were recorded on a Bruker 500 (125 MHz) spectrometer and are reported in ppm using solvent as an internal standard (CDCl\textsubscript{3} at 77.15 ppm, acetone-\textit{d}\textsubscript{6} at 29.92 ppm, or methylene
chloride-$d_2$ at 54.0). $^{19}$F NMR spectra (282 MHz) were obtained on a Bruker NB 300 spectrometer and were referenced relative to CFCI$_3$. High-resolution mass spectra were obtained from the Princeton University mass spectrometer facility by electrospray ionization (ESI). High-performance liquid chromatography (HPLC) was performed on an Agilent 1100 series instrument with a binary pump and a diode array detector.

4.4.2 Radiosynthesis of $^{18}$F labeled molecules

No-carrier-added $[^{18}$F]fluoride was produced from water 97% enriched in $^{18}$O (ISOFLEX, USA) by the nuclear reaction $^{18}$O(p,n)$^{18}$F using a Siemens Eclipse HP cyclotron and a silver-bodied target at Massachusetts General Hospital Athinoula A. Martinos Center for Biomedical Imaging. The produced $[^{18}$F]fluoride in water was transferred from the cyclotron target by helium push.

A 1.5 mL vial with a screw cap was charged with Mn(MCP)(OTf)$_2$ (6 mg, 0.01 mmol, 20 mol%), substrate (0.05 mmol), and a stir bar (2 × 5 mm). A portion of aqueous $[^{18}$F]fluoride solution (40 – 50 μL, 4 – 5 mCi) obtained from the cyclotron was loaded on to a Chromafix PS-HCO$_3$ IEX cartridge, which had been previously washed with 5.0 mg/mL K$_2$CO$_3$ in Milli-Q water followed by 5 mL of Milli-Q water. Then, the cartridge loaded with $[^{18}$F]fluoride was washed with 2 mL Milli-Q water, and $[^{18}$F]fluoride was released from the cartridge using 1 mL of (80% acetone and 20% 5.0 mg/mL K$_2$CO$_3$ in Milli-Q water) solution. 20 μL of this $[^{18}$F]fluoride acetone solution was added to the vial containing the catalyst and the substrate. After the resulting solution was stirred at room temperature for one minute, $m$-CPBA (9 mg, 0.05 mmol) in 0.1 mL acetone was slowly added into the solution over a few seconds. The vial was capped, and the homogenous
solution was stirred at room temperature in air for 10 minutes. After 10 min, an aliquot of 
the reaction mixture was taken and spotted on a silica gel TLC plate. The plate was 
developed in an appropriate eluent and scanned with a Bioscan AR-2000 Radio TLC 
Imaging Scanner.

4.4.3 Examples of radio-TLC scans

![Figure 4.3 Radio-TLC scan of compound 2](image)

**Figure 4.3** Radio-TLC scan of compound 2
Figure 4.4 Radio-TLC scan of compound 21

Figure 4.5 Radio-TLC scan of compound 22
Figure 4.6 Radio-TLC scan of compound 23

Figure 4.7 Radio-TLC scan of compound 24
4.4.4 Preparation and characterization of $^{19}$F authentic samples

Compounds 19 and 24 were prepared from the corresponding alcohols with DAST. Compound 25 was synthesized via a reported photo-induced method.\textsuperscript{16} Compound 20 was prepared using a manganese-porphyrin catalyzed C-H fluorination protocol.\textsuperscript{17} Other compounds were prepared according to the previously reported manganese salen-catalyzed C-H fluorination procedure.\textsuperscript{18}

\begin{center}
\textbf{Compound 2.} Purification by column chromatography (hexanes to 4\% EtOAc/hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.34 (s, 3H), 1.37 (s, 12H), 2.40 – 2.05 (m, 2H), 2.65 (s, 3H), 6.44 (ddd, $J = 53.9, 5.9, 1.5$ Hz, 1H), 7.43 (t, $J = 1.6$ Hz, 1H), 7.77
(dd, J = 1.8, 0.6 Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 28.77, 93.89 (d, J = 172.2 Hz), 31.50, 31.66, 35.22, 42.84, 48.46 (d, J = 22.7 Hz), 93.89 (d, J = 172.2 Hz), 123.72 (d, J = 2.4 Hz), 125.74 (d, J = 2.6 Hz), 134.90, 135.16 (d, J = 16.3 Hz), 154.26 (d, J = 3.7 Hz), 155.95 (d, J = 4.7 Hz), 199.83; $^{19}$F NMR -158.6 ppm; MS (EI) m/z cal’d C$_{17}$H$_{23}$FO [M]$^+$: 262.2, found 262.2.

**Compound 3.** Purification by column chromatography (10% EtOAc/hexanes). $^1$H NMR (500 MHz, CDCl$_3$) δ 2.70 – 2.17 (m, 2H), 3.98 – 7.81 (m, 2H), 5.54 (ddd, J = 47.8, 8.7, 4.1 Hz, 1H), 7.46 – 7.26 (m, 5H), 7.70 (dd, J = 5.5, 3.0 Hz, 2H), 7.83 (dd, J = 5.4, 3.0 Hz, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 34.66 (d, J = 23.8 Hz), 92.58 (d, J = 171.6 Hz), 123.35, 125.64 (d, J = 6.7 Hz), 128.58 (d, J = 2.0 Hz), 128.63, 132.15, 134.07, 139.34 (d, J = 19.5 Hz), 168.32; $^{19}$F NMR -175.7 ppm; MS (EI) m/z cal’d C$_{17}$H$_{23}$FNO$_2$ [M]$^+$: 283.1, found 283.1.

**Compound 4.** Purification by flash chromatography (hexanes). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.65 – 6.98 (m, 10H), 5.64 (ddd, J = 47.3, 8.2, 4.8 Hz, 1H), 3.58 – 2.74 (m, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 139.89 (d, J = 19.9 Hz), 136.79 (d, J = 4.0 Hz), 129.63, 128.50, 128.48, 128.46, 126.80, 125.77 (d, J = 6.7 Hz), 94.99 (d, J = 174.1 Hz), 44.06 (d, J = 24.5 Hz); $^{19}$F NMR -173.10 ppm; MS (EI) m/z cal’d C$_{14}$H$_{13}$F [M]$^+$: 200.1, found 200.1.

**Compound 5.** Purification by flash chromatography (hexanes) $^1$H NMR (500 MHz, CDCl$_3$) δ 7.43 – 7.36 (m, 2H), 7.36 – 7.31 (m, 2H), 5.48 (ddd, J = 48.1, 8.0,
3.9 Hz, 1H), 3.59 – 3.31 (m, 2H), 2.33 – 1.87 (m, 4H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 139.84 (d, $J = 19.8$ Hz), 128.44, 128.42, 125.41 (d, $J = 6.9$ Hz), 93.76 (d, $J = 171.5$ Hz), 35.74 (d, $J = 23.8$ Hz), 33.36, 28.32 (d, $J = 3.8$ Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) δ -176.05 ppm; MS (EI) m/z cal’d C$_{10}$H$_{12}$BrF [M]$^+$: 230.0, found 230.0.

**Compound 6.** Purification by column chromatography (4% EtOAc/hexanes). $^1$H NMR (500 MHz, CDCl$_3$) δ 3.86 – 3.35 (m, 2H), 5.84 (ddd, $J = 47.6, 9.9, 2.1$ Hz, 1H), 7.70 – 7.28 (m, 6H), 8.10 – 7.94 (m, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 194.20, 139.16 (d, $J = 19.1$ Hz), 138.64, 136.14 (d, $J = 4.7$ Hz), 134.15 (d, $J = 11.2$ Hz), 132.79, 132.67, 130.63, 130.52, 130.20, 128.82, 127.46, 126.36 (d, $J = 11.4$ Hz), 90.49 (d, $J = 176.3$ Hz), 41.06 (d, $J = 24.0$ Hz); $^{19}$F NMR -168.7 ppm; MS (EI) m/z cal’d C$_{15}$H$_{11}$FO [M]$^+$: 226.1, found 226.1.

**Compound 7.** Purification by column chromatography (hexanes). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.55 – 7.49 (m, 4H), 7.39 – 7.33 (m, 4H), 7.30 – 7.25 (m, 1H), 5.60 (dq, $J = 47.6, 6.4$ Hz, 1H), 1.61 (dd, $J = 23.9, 6.4$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 141.22 (d, $J = 2.1$ Hz), 140.70, 140.41 (d, $J = 19.6$ Hz), 128.82, 127.45, 127.28, 127.16, 125.75 (d, $J = 6.6$ Hz), 90.83 (d, $J = 167.3$ Hz), 22.92 (d, $J = 25.3$ Hz); $^{19}$F NMR -166.6 ppm; MS (EI) m/z cal’d C$_{14}$H$_{13}$F [M]$^+$: 200.1, found 200.1.

**Compound 8.** Purification by column chromatography (1%-20% EtOAc/hexanes). $^1$H NMR (500 MHz, CDCl$_3$) δ 2.53 (m, 3H), 3.00 – 2.85 (m, 1H), 3.94 (s, 3H), 3.98 (s, 3H), 5.68 (dt, $J$=51.0, 4.5 Hz, 1H), 6.97 (s, 1H), 7.51 (s, 1H); $^{13}$C NMR
(126 MHz, CDCl$_3$) $\delta$ 195.60, 153.90 (d, $J$ = 1.5 Hz), 149.91 (d, $J$ = 2.6 Hz), 134.92 (d, $J$ = 17.8 Hz), 125.06, 109.40 (d, $J$ = 5.8 Hz), 108.31, 87.85 (d, $J$ = 171.8 Hz), 56.31, 56.19, 33.63 (d, $J$ = 6.9 Hz), 29.66 (d, $J$ = 21.4 Hz); $^{19}$F NMR -169.6 ppm; MS (EI) m/z cal’d C$_{12}$H$_{13}$FO$_3$ [M]$^+$: 224.1, found 224.1.

**Compound 9.** Purification by column chromatography (hexanes). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.84 (dd, $J$ = 23.8, 6.4 Hz, 3H), 6.36 (dq, $J$ = 46.7, 6.5 Hz, 1H), 7.46 – 7.61 (m, 3H), 7.61 – 7.68 (m, 1H), 7.82 – 7.97 (m, 2H), 8.00 – 8.09 (m, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 22.43 (d, $J$ = 25.1 Hz), 88.87 (d, $J$ = 167.6 Hz), 122.50 (d, $J$ = 10.0 Hz), 123.13 (d, $J$ = 1.3 Hz), 125.33, 125.71, 126.32, 128.77 (d, $J$ = 1.9 Hz), 128.90, 129.96 (d, $J$ = 3.5 Hz), 133.70, 136.98 (d, $J$ = 18.0 Hz); $^{19}$F NMR -169.8 ppm; MS (EI) m/z cal’d C$_{12}$H$_{11}$F [M]$^+$: 174.1, found 174.1.

**Compound 10.** Purification by column chromatography (10% EtOAc/hexanes). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.64 (dd, $J$ = 23.9, 6.5 Hz, 3H), 2.30 (s, 3H), 5.62 (dd, $J$ = 47.5, 6.4 Hz, 1H), 7.03 – 7.18 (m, 2H), 7.30 – 7.46 (m, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 169.55, 150.43 (d, $J$ = 2.3 Hz), 139.04 (d, $J$ = 19.9 Hz), 126.48 (d, $J$ = 6.6 Hz), 121.67, 90.49 (d, $J$ = 167.8 Hz), 22.96 (d, $J$ = 25.2 Hz), 21.18; $^{19}$F NMR -166.4 ppm; MS (EI) m/z cal’d C$_{10}$H$_{11}$FO$_2$ [M]$^+$: 182.1, found 182.1.

**Compound 11.** Purification by column chromatography (hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.82 (ddd, $J$ = 23.8, 6.5 Hz, 3H), 5.87 (dq, $J$ = 47.6, 6.5 Hz, 1H), 7.63 – 7.49 (m, 3H), 8.10 – 7.77 (m, 4H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 138.97 (d, $J$ = 19.4
Hz), 133.31 (d, J = 1.3 Hz), 133.23, 128.51, 128.23, 127.86, 126.45, 126.33, 124.31 (d, J = 8.0 Hz), 123.26 (d, J = 5.7 Hz), 91.23 (d, J = 167.8 Hz), 23.08 (d, J = 25.2 Hz); $^{19}$F NMR -166.7 ppm; MS (EI) m/z cal’d C$_{12}$H$_{11}$F [M]$: 174.1, found 174.1.

**Compound 12.** Purification by column chromatography (5% EtOAc/hexanes). Isolated as a single diastereomer. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.21 (s, 9H), 2.41–2.34 (m, 1H), 2.92–2.67 (m, 1H), 6.15 (ddd, J = 57.4, 6.4, 2.4 Hz, 1H), 6.40 (ddd, J = 7.1, 4.6, 2.3 Hz, 1H), 7.48–7.37 (m, 3H), 7.53 (dd, J = 5.4, 1.8 Hz, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 178.48, 142.71 (d, J = 4.1 Hz), 140.34 (d, J = 16.6 Hz), 130.46 (d, J = 3.7 Hz), 129.45 (d, J = 3.1 Hz), 125.65 (d, J = 1.9 Hz), 125.37 (d, J = 2.2 Hz), 94.50 (d, J = 172.5 Hz), 75.69 (d, J = 1.5 Hz), 40.62 (d, J = 23.6 Hz), 38.72, 27.10; $^{19}$F NMR -164.1 ppm; MS (EI) m/z cal’d C$_{14}$H$_{17}$F [M]$: 236.1, found 236.1.

**Compound 13.** Purification by column chromatography (hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.66 (dd, J = 24.0, 6.4 Hz, 3H), 5.62 (dq, J = 47.5, 6.4 Hz, 1H), 7.16 – 7.42 (m, 2H), 7.49 (d, J = 7.0, 1H), 7.56 (q, J = 1.5 Hz, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 143.84 (d, J = 19.9 Hz), 131.24, 130.18, 128.31 (d, J = 7.4 Hz), 123.79 (d, J = 6.8 Hz), 122.63, 90.05 (d, J = 169.7 Hz), 22.97 (d, J = 24.9 Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) -168.86 ppm; MS (EI) m/z cal’d C$_8$H$_8$BrF [M]$: 202.0, found 202.0.

**Compound 14.** Purification by column chromatography (hexane to 10% EtOAc/hexanes). Two diastereomers 14a and 14b were separated as shown below.
**Diastereomers 14a.** $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 2.56–2.00 (m, 4H), 5.66 (dt, $J$ = 51.2, 3.6 Hz, 1H), 6.31 (t, $J$ = 4.0 Hz, 1H), 7.66–7.35 (m, 7H), 8.01 (d, $J$ = 7.7 Hz, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 166.01, 134.80 (d, $J$ = 3.7 Hz), 134.28 (d, $J$ = 17.1 Hz), 133.06, 130.34, 129.99, 129.94 (d, $J$ = 1.9 Hz), 129.69, 129.66, 129.05 (d, $J$ = 2.8 Hz), 128.37, 87.66 (d, $J$ = 167.2 Hz), 69.35, 25.42 (d, $J$ = 21.5 Hz), 24.19; $^{19}$F NMR (282 MHz, CDCl$_3$) -160.42 ppm; MS (EI) m/z cal’d C$_{17}$H$_{15}$FO$_2$ [M]$^+$: 270.1, found 270.1.

**Diastereomers 14b.** $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 2.06–2.70 (m, 4H), 5.60 (ddd, $J$ = 51.7, 6.3, 4.3 Hz, 1H), 6.21 (dd, $J$ = 7.1, 4.7 Hz, 1H), 7.68–7.30 (m, 7H), 8.09 (dd, $J$ = 8.2, 1.4 Hz, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 166.32, 135.63 (d, $J$ = 3.9 Hz), 134.78 (d, $J$ = 18.0 Hz), 133.17, 130.18, 129.80, 129.81 (d, $J$ = 3.4 Hz), 129.09 (d, $J$ = 4.9 Hz), 128.58 (d, $J$ = 2.4 Hz), 128.44, 128.10 (d, $J$ = 1.4 Hz), 88.05 (d, $J$ = 169.4 Hz), 70.26, 26.62 (d, $J$ = 21.0 Hz), 24.92 (d, $J$ = 4.8 Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) -161.24 ppm; MS (EI) m/z cal’d C$_{17}$H$_{15}$FO$_2$ [M]$^+$: 270.1, found 270.1.

**Compound 15.** Purification by chromatography (hexanes to 20% EtOAc/hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.48 – 7.30 (m, 5H), 5.58 (ddd, $J$ = 47.6, 8.7, 3.9 Hz, 1H), 2.63 – 2.05 (m, 4H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 138.21 (d, $J$ = 19.6 Hz), 128.97, 128.81, 125.30 (d, $J$ = 7.0 Hz), 118.83, 92.12 (d, $J$ = 173.6 Hz), 32.92 (d, $J$ = 24.7 Hz), 13.36 (d, $J$ = 4.8 Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) -179.5 ppm; MS (EI) m/z cal’d C$_{10}$H$_{10}$FN [M]$^+$: 163.1, found 163.1.
**Compound 16.** Purification by column chromatography (hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.63 (dd, $J$=23.8, 6.4 Hz, 3H), 5.61 (dq, $J$=47.5, 6.4 Hz, 1H), 7.06 (ddd, $J$ = 8.7, 7.7, 0.9 Hz, 2H), 7.34 (ddd, $J$ = 8.7, 5.3, 1.4 Hz, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 162.59 (d, $J$ = 246.5 Hz), 137.20 (d, $J$ = 23.0 Hz), 127.14 (dd, $J$ = 8.2, 7.6 Hz), 115.38 (d, $J$ = 21.6 Hz), 90.38 (d, $J$ = 167.4 Hz), 22.90 (d, $J$ = 25.4 Hz); $^{19}$F NMR (282 MHz, CDCl$_3$) -117.2, -166.0 ppm; MS (EI) m/z cal’d C$_8$H$_8$F$_2$ [M]$^+$: 142.1, found 142.1.

**Compound 17.** Purification by column chromatography (hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.65 (dd, $J$=24.1, 6.4 Hz, 3H), 5.94 (dq, $J$=46.6, 6.4 Hz, 1H), 7.19 (td, $J$ = 7.8, 1.7 Hz, 1H), 7.38 (td, $J$ = 7.5, 1.2 Hz, 1H), 7.54 (dq, $J$ = 8.1, 1.5 Hz, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 141.15 (d, $J$ = 21.2 Hz), 132.65, 129.42 (d, $J$ = 1.5 Hz), 127.86, 126.30 (d, $J$ = 10.1 Hz), 120.47 (d, $J$ = 5.9 Hz), 90.09 (d, $J$ = 169.8 Hz), 22.16 (d, $J$ = 25.4 Hz); $^{19}$F NMR -173.71 ppm; MS (EI) m/z cal’d C$_8$H$_8$BrF [M]$^+$: 202.0, found 202.0.

**Compound 18.** Purification by column chromatography (hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.63 (dd, $J$=23.9, 6.5 Hz, 3H), 5.59 (dq, $J$=47.5, 6.4 Hz, 1H), 7.10 - 7.19 (m, 2H), 7.70 - 7.81 (m, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 141.25 (d, $J$ = 19.9 Hz), 137.65, 127.23 (d, $J$ = 6.8 Hz), 93.97 (d, $J$ = 2.5 Hz), 90.33 (d, $J$ = 168.8 Hz), 23.02 (d, $J$ = 25.0 Hz); $^{19}$F NMR -168.7 ppm; MS (EI) m/z cal’d C$_8$H$_8$FI [M]$^+$: 250.0, found 250.0.
Compound 19. Purification by column chromatography (5% EtOAc/hexanes). $^1$H NMR (300 MHz, CDCl$_3$) δ 1.48 – 1.80 (m, 4H), 2.00 – 2.31 (m, 2H), 2.64 (qd, $J = 13.5$, 4.3 Hz, 2H), 4.10 – 4.27 (m, 1H), 4.86 (d, $J = 48.1$ Hz, 1H), 7.76 (ddd, $J = 37.6$, 5.5, 3.1 Hz, 4H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 168.26, 133.87, 131.98, 123.13, 86.86 (d, $J = 169.3$ Hz), 49.65, 30.44 (d, $J = 21.5$ Hz), 23.89. $^{19}$F NMR (282 MHz, CDCl$_3$) -185.90 ppm; MS (EI) m/z cal’d C$_{14}$H$_{14}$FNO$_2$ [M]$^+$: 247.1, found 247.1.

Compound 20. The regiochemical assignment was made on the basis of three-bond F-C2 coupling, 26.48 ppm (d, $J = 8.1$ Hz). $^1$H NMR (500 MHz, CDCl$_3$) δ 1.80 – 2.25 (m, 10H), 4.81 (dtdd, $J = 47.3$, 6.9, 5.0, 3.8 Hz, 1H), 5.19 (dtt, $J = 12.7$, 8.3, 4.6 Hz, 1H), 7.42–7.47 (2H), 7.54 – 7.63 (m, 1H), 8.02 – 8.11 (m, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 17.66 (d, $J = 8.0$ Hz), 26.48 (d, $J = 8.2$ Hz), 28.56 (d, $J = 22.5$ Hz), 34.04, 34.78 (d, $J = 22.0$ Hz), 74.20, 92.87 (d, $J = 167.1$ Hz), 128.34, 129.55, 130.60, 132.66, 165.86; $^{19}$F NMR (282 MHz, CDCl$_3$) -166.58 ppm; MS (EI) m/z cal’d C$_{14}$H$_{17}$FO$_2$ [M]$^+$: 236.1, found 236.1.

Compound 21. $^1$H NMR (500 MHz, Methylene Chloride-$d_2$) δ 0.91 (t, $J = 6.9$ Hz, 3H), 1.22 – 1.54 (m, 10H), 1.73 – 1.90 (m, 1H), 1.95 (s, 3H), 2.08 (s, 6H), 2.12 – 2.25 (m, 3H), 2.57 – 2.72 (m, 2H), 4.33 (s, 4H), 5.44 (ddd, $J = 50$, 8.1, 5.1 Hz, 1H), 5.68 (s, 1H), 7.22 – 7.30 (m, 4H); $^{13}$C NMR (126 MHz, Methylene Chloride-$d_2$) δ 13.85, 20.62, 22.63, 23.89 25.15 (d, $J = 4.5$ Hz), 29.15, 29.26, 29.30, 31.76, 33.62, 37.01 (d, $J =
23.6 Hz), 57.96, 64.36, 94.67 (d, \( J = 168.8 \) Hz), 125.84 (d, \( J = 6.4 \) Hz), 128.35, 138.37 (d, \( J = 19.8 \) Hz), 141.84 (d, \( J = 2.2 \) Hz), 169.89, 170.62; \(^{19}\)F NMR (282 MHz, CDCl\(_3\)) \(-167.49\) (ddd, \( J = 48.0, 27.6, 16.2 \) Hz) ppm; HR-MS (ESI) m/z cal’d C\(_{25}\)H\(_{38}\)FNO\(_5\) [M+H]\(^+\): 451.2734, found 451.2722.

**Compound 22.** Purification by column chromatography (hexanes to 5% EtOAc/hexanes). Two diastereomers 22a and 22b were separated as shown below.

22a (containing two geometrical isomers in 2.8:1 ratio due to the amide moiety).

Major isomer: \(^1\)H NMR (500 MHz, methylene chloride-\( d_2 \)) \( \delta 7.68 – 7.58 \) (m, 1H), 7.59 – 7.44 (m, 2H), 7.44 – 7.30 (m, 1H), 5.54 (td, \( J = 7.7, 5.1 \) Hz, 1H), 5.36 (t, \( J = 1.1 \) Hz, 1H), 3.89 (ddd, \( J = 140.8, 17.2, 2.5 \) Hz, 2H), 3.18 – 2.99 (m, 1H), 2.66 – 2.47 (m, 1H), 2.23 (t, \( J = 2.5 \) Hz, 1H); \(^{13}\)C NMR (126 MHz, methylene chloride-\( d_2 \)) \( \delta 156.19, 140.58 \) (d, \( J = 18.7 \) Hz), 138.68 (d, \( J = 3.8 \) Hz), 130.75 (d, \( J = 3.4 \) Hz), 130.00 (d, \( J = 2.8 \) Hz), 125.98 (d, \( J = 1.2 \) Hz), 125.10 (d, \( J = 1.2 \) Hz), 116.57 (q, \( J = 287.6 \) Hz), 92.89 (d, \( J = 176.5 \) Hz), 78.42, 71.49, 59.37 (d, \( J = 3.8 \) Hz), 37.56 (d, \( J = 20.8 \) Hz), 32.95; \(^{19}\)F NMR (282 MHz, CDCl\(_3\)) -68.00, -156.46 ppm; HRMS (ESI) m/z cal’d C\(_{14}\)H\(_{11}\)F\(_4\)NNaO [M+Na]\(^+\): 308.0674, found 308.0670.

Minor isomer: \(^1\)H NMR (500 MHz, methylene chloride-\( d_2 \)) \( \delta 7.68 – 7.58 \) (m, 1H), 7.59 – 7.44 (m, 2H), 7.44 – 7.30 (m, 1H), 6.08 – 6.00 (m, 1H), 5.94 (td, \( J = 7.3, 3.4 \) Hz, 1H), 4.07 (ddd, \( J = 110.0, 19.1, 2.5 \) Hz, 2H), 3.18 – 2.99 (m, 1H), 2.66 – 2.47 (m, 1H), 2.32 (t, \( J = 2.8 \) Hz, 1H).
2.5 Hz, 1H); $^{13}$C NMR (126 MHz, methylene chloride-$d_2$) $\delta$ 156.48, 141.02 (d, $J = 18.4$ Hz), 139.63 (d, $J = 5.0$ Hz), 130.52 (d, $J = 3.4$ Hz), 129.56 (d, $J = 2.8$ Hz), 125.81 (d, $J = 1.1$ Hz), 125.35 (d, $J = 2.0$ Hz), 116.24 (q, $J = 287.4$ Hz), 93.51 (d, $J = 176.4$ Hz) 78.73, 72.67, 58.23, 36.51 (d, $J = 20.8$ Hz), 33.54; $^{19}$F NMR (282 MHz, CDCl$_3$) -69.16, -155.56 ppm.

![Diagram](attachment:image.png) 22b (containing two geometrical isomers in 2.4:1 ratio due to the amide moiety).

Major isomer: $^1$H NMR (500 MHz, Methylene Chloride-$d_2$) $\delta$ 2.31 (t, $J = 2.5$ Hz, 1H), 2.69 – 2.92 (m, 2H), 3.45 (dd, $J = 17.4$, 2.5 Hz, 1H), 4.12 (dd, $J = 17.4$, 2.5 Hz, 1H), 5.96 (td, $J = 7.1$, 3.5 Hz, 1H), 6.17 (dddd, $J = 55.0$, 4.9, 2.4 Hz, 1H), 7.35 (d, $J = 7.4$ Hz, 1H), 7.44 – 7.58 (m, 2H), 7.59 – 7.65 (m, 1H); $^{13}$C NMR (126 MHz, Methylene Chloride-$d_2$) $\delta$ 32.65, 38.17 (d, $J = 25.1$ Hz), 61.33 (d, $J = 3.9$ Hz), 71.84, 78.34, 93.76 (d, $J = 171.6$ Hz), 116.50 (q, $J = 287.8$ Hz), 124.54 (d, $J = 2.6$ Hz), 126.54 (d, $J = 2.2$ Hz), 129.81 (d, $J = 3.3$ Hz), 131.14 (d, $J = 4.0$ Hz), 140.11 (d, $J = 16.1$ Hz), 140.45 (d, $J = 4.0$ Hz), 156.81; $^{19}$F NMR (282 MHz, CDCl$_3$) -68.32, -160.81 ppm; HRMS (ESI) m/z cal’d C$_{14}$H$_{12}$F$_4$NO [M+H]$^+$: 286.0855, found 286.0858.

Minor isomer: $^1$H NMR (500 MHz, Methylene Chloride-$d_2$) $\delta$ 2.45 (t, $J = 2.5$ Hz, 1H), 2.69 – 2.92 (m, 2H), 3.91 (dd, $J = 18.8$, 2.4 Hz, 1H), 4.31 (dd, $J = 18.8$, 2.4 Hz, 1H), 6.03 (td, $J = 7.6$, 7.2, 3.2 Hz, 1H), 6.23 (dddd, $J = 55.1$, 6.3, 2.1 Hz, 1H), 7.31 (d, $J = 7.0$ Hz, 1H), 7.58 – 7.44 (m, 2H), 7.59 – 7.65 (m, 1H); $^{13}$C NMR (126 MHz, Methylene Chloride-$d_2$) $\delta$ 35.51, 37.27 (d, $J = 23.9$ Hz), 61.65, 73.24, 78.03, 94.77 (d, $J = 171.4$ Hz), 114.00 (q, $J = 287.8$ Hz), 124.27 (d, $J = 2.4$ Hz), 126.13 (d, $J = 2.1$ Hz), 129.22 (d, $J = 3.2$ Hz), 130.57 (d, $J = 93.76$ (d, $J = 171.6$ Hz), 116.50 (q, $J = 287.8$ Hz), 124.54 (d, $J = 2.6$ Hz), 126.54 (d, $J = 2.2$ Hz), 129.81 (d, $J = 3.3$ Hz), 131.14 (d, $J = 4.0$ Hz), 140.11 (d, $J = 16.1$ Hz), 140.45 (d, $J = 4.0$ Hz), 156.81; $^{19}$F NMR (282 MHz, CDCl$_3$) -68.32, -160.81 ppm; HRMS (ESI) m/z cal’d C$_{14}$H$_{12}$F$_4$NO [M+H]$^+$: 286.0855, found 286.0858.
3.9 Hz), 140.85 (d, J = 16.1 Hz), 140.86 (d, J = 4.0 Hz), 157.10; $^{19}$F NMR (282 MHz, CDCl$_3$) -69.33, -162.23 ppm.

**Compound 23.** Purification by column chromatography (10% EtOAc/hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 0.85 (d, J = 7.0 Hz, 3H), 1.02 (dd, J = 6.8, 1.0 Hz, 3H), 1.50 (d, J = 7.3 Hz, 3H), 2.10 (dq, J = 16.9, 6.7 Hz, 1H), 3.66 (s, 3H), 3.74 (q, J = 7.2 Hz, 1H), 5.08 (dd, J = 47.0, 6.7 Hz, 1H), 7.25 – 7.10 (m, 4H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 174.90, 140.35, 138.28 (d, J = 20.6 Hz), 127.33, 126.47 (d, J = 7.0 Hz), 126.47 (d, J = 7.0 Hz), 52.08, 45.15, 34.27 (d, J = 23.9 Hz), 18.60 (d, J = 1.3 Hz), 18.36 (d, J = 5.7 Hz), 17.57 (d, J = 5.1 Hz); $^{19}$F NMR -179.2 ppm; MS (EI) m/z cal’d C$_{14}$H$_{19}$FO$_2$ [M]$^+$: 238.1, found 238.1.

**Compound 24.** $^1$H NMR (500 MHz, acetone-$d_6$) $\delta$ 7.38 - 7.27 (m, 3H), 6.38 (br, 1H), 5.60 (ddd, J = 47.6 7.6, 4.0 Hz, 1H), 3.58 - 3.44 (m, 2H), 2.29 (s, 3H), 2.28 (s, 3H), 1.43 (s, 9H); $^{13}$C NMR (126 MHz, acetone-$d_6$) $\delta$ 167.74, 167.73, 155.79, 142.65 (d, J = 1.8 Hz), 142.60, 136.61 (d, J = 20.0 Hz), 123.79 (d, J = 6.8 Hz), 123.69, 121.11 (d, J = 7.6 Hz), 92.01 (d, J = 173.2 Hz), 78.22, 45.94 (d, J = 25.6 Hz), 27.67, 19.62 (d, J = 1.4 Hz); $^{19}$F NMR -182.20 ppm; HR-MS (ESI) m/z cal’d C$_{17}$H$_{23}$FNO$_6$ [M+H]$^+$: 356.1509, found 356.1611.

**Compound 25.** Purification by column chromatography (5% EtOAc/hexanes). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.00 (s, 6H), 1.33–1.09 (m, 2H), 1.73 –
1.50 (m, 4H), 2.02–2.20 (m, 4H), 2.58 (d, \( J = 6.2 \text{ Hz}, 2\text{H} \)), 7.78–7.65 (m, 4H).\(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \( \delta \) 169.43, 133.90, 131.72, 122.73, 93.37 (d, \( J = 183.0 \text{ Hz} \)), 62.39 (d, \( J = 13.1 \text{ Hz} \)), 49.07 (d, \( J = 1.6 \text{ Hz} \)), 47.60 (d, \( J = 16.9 \text{ Hz} \)), 44.89 (d, \( J = 1.3 \text{ Hz} \)), 43.83 (d, \( J = 20.6 \text{ Hz} \)), 34.78 (d, \( J = 10.3 \text{ Hz} \)), 29.22 (d, \( J = 1.3 \text{ Hz} \)); \(^{19}\)F NMR (282 MHz, CDCl\(_3\)) -135.68 ppm; HRMS (ESI) m/z cal’d C\(_{20}\)H\(_{23}\)FNO\(_2\) [M+H]+: 328.1713, found 328.1710.

4.4.5 Characterization of the \(^{18}\)F labeled products using radio-HPLC

All labeled molecules were characterized by comparing the radio-HPLC trace of the crude reaction mixture to the HPLC UV trace of the authentic reference sample with methods detailed below. Note: There is a time difference (\( \Delta t \)) between the radio-HPLC trace and the HPLC UV trace due to the delay volume between the diode array detector and the radioactivity detector (for 1.0 ml/min flow rate, \( \Delta t \approx 0.3 \text{ min} \)).

**Method A**

HPLC column: Agilent Eclipse XDB-C18, 5 \( \mu \text{m}, 4.6 \times 250 \text{ mm} \)

Conditions: 3% CH\(_3\)CN/H\(_2\)O → 95% CH\(_3\)CN/H\(_2\)O over 20 min, 1.0 mL/min

**Method B**

HPLC column: Agilent Eclipse XDB-C18, 5 \( \mu \text{m}, 4.6 \times 250 \text{ mm} \)

Conditions: 3% CH\(_3\)CN/H\(_2\)O → 95% CH\(_3\)CN/H\(_2\)O over 25 min, 1.0 mL/min

**Method C**

HPLC column: Agilent Eclipse XDB-C18, 5 \( \mu \text{m}, 4.6 \times 150 \text{ mm} \)

Conditions: H\(_2\)O (0.1% TFA, A) and CH\(_3\)CN (0.1%TFA, B), 3%→5% B, 0-3 min; 5%→50% B, 3-6 min; 50%→95%, 6-9 min; 95% B, 9-19 min, 1.0 mL/min
Figure 4.9 UV and radioHPLC trace of compound 2 (retention time of authentic reference: 18.108 min; labeled product: 18.337 min).

Figure 4.10 UV and radioHPLC trace of compound 3 (retention time of authentic reference: 10.363 min; labeled product: 10.645 min).

Figure 4.11 UV and radioHPLC trace of compound 4 (retention time of authentic reference: 15.820 min; labeled product: 16.100 min).
Figure 4.12 UV and radioHPLC trace of compound 5 (retention time of authentic reference: 10.938 min; labeled product: 11.206 min).

Figure 4.13 UV and radioHPLC trace of compound 6 (retention time of authentic reference: 10.673 min; labeled product: 10.959 min).

Figure 4.14 UV and radioHPLC trace of compound 7 (retention time of authentic reference: 11.479 min; labeled product: 11.698 min).
Figure 4.15 UV and radioHPLC trace of compound 8 (retention time of authentic reference: 8.964 min; labeled product: 9.252 min).

Figure 4.16 UV and radioHPLC trace of compound 9 (retention time of authentic reference: 11.225 min; labeled product: 11.580 min).

Figure 4.17 UV and radioHPLC trace of compound 10 (retention time of authentic reference: 9.849 min; labeled product: 10.144 min).
Figure 4.18 UV and radioHPLC trace of compound 11 (retention time of authentic reference: 11.213 min; labeled product: 11.464 min).

Figure 4.19 UV and radioHPLC trace of compound 12 (retention time of authentic reference: 11.069 min; labeled product: 11.281 min).

Figure 4.20 UV and radioHPLC trace of compound 13 (retention time of authentic reference: 15.481 min; labeled product: 15.791 min).
Figure 4.21 UV and radioHPLC trace of compound 14 (retention time of authentic reference: 11.114 min; labeled product: 11.389 min).

Figure 4.22 UV and radioHPLC trace of compound 15 (retention time of authentic reference: 9.649 min; labeled product: 9.924 min).

Figure 4.23 UV and radioHPLC trace of compound 16 (retention time of authentic reference: 13.908 min; labeled product: 14.116 min).
Figure 4.24 UV and radioHPLC trace of compound 17 (retention time of authentic reference: 16.043 min; labeled product: 16.287 min).

Figure 4.25 UV and radioHPLC trace of compound 18 (retention time of authentic reference: 15.986 min; labeled product: 16.258 min).

Figure 4.26 UV and radioHPLC trace of compound 19 (retention time of authentic reference: 10.454 min; labeled product: 10.619 min).
**Figure 4.27** UV and radioHPLC trace of compound 20 (retention time of authentic reference: 11.267 min; labeled product: 11.606 min).

**Figure 4.28** UV and radioHPLC trace of compound 21 (retention time of authentic reference: 11.900 min; labeled product: 12.125 min);

**Figure 4.29** UV and radioHPLC trace of compound 22 (retention time of authentic reference: 10.195 min; labeled product: 10.459 min).
Figure 4.30 UV and radioHPLC trace of compound 23 (retention time of authentic reference: 10.998 min; labeled product: 11.215 min).

Figure 4.31 UV and radioHPLC trace of compound 24 (retention time of authentic reference: 9.914 min; labeled product: 10.212 min).

Figure 4.32 UV and radioHPLC trace of compound 25 (retention time of authentic reference: 11.626 min; labeled product: 11.877 min).
4.4.6 NMR spectra
4.5 References


