DIRECT ELECTROPHILIC FLUORINATION OF AROMATIC AMINO ACIDS

Dedicated to my loving parents, Hossein and Zarrin Behnam Azad

THE DIRECT ELECTROPHILIC FLUORINATION OF AROMATIC AMINO ACIDS AND THEIR ROLE IN DIAGNOSTIC IMAGING

By

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ABSTRACT

Fluorine-18 labeled 6-fluoro-3, 4-dihydroxy-phenyl-L-alanine (6-FDOPA) has been used in conjunction with Positron Emission Tomography (PET) to study the dopamine metabolism in the living human brain and also to monitor gastrointestinal carcinoid tumors. Elemental fluorination of L-DOPA in anhydrous HF (aHF) or aHF/BF_3 has been shown to be an efficient method for the synthesis of 6-fluoro-L-DOPA. Utilization of aHF, however, is not desirable in a hospital environment owing to its hazardous nature. This work has consequently focused on the development of new methodologies for the direct electrophilic fluorination of aromatic amino acids, which circumvent the use of aHF.

The present work has shown that the reactivity and selectivity of F_2 towards L-DOPA in CF_3SO_3H is comparable to that in aHF. The discovery and versatility of this new synthetic procedure has led to the production of 6-[^{18}F]fluoro-L-DOPA, 6-[^{18}F]fluoro-D-DOPA, 4-[^{18}F]fluoro-L-*m*-tyrosine (4-FMT) and 6-[^{18}F]fluoro-L-*m*-tyrosine (6-FMT) in high radiochemical yields that are not only suitable for small animal imaging, but are also suitable for clinical use in human subjects. Because of the low volatility of CF_3SO_3H, its removal from the reaction mixture was accomplished by use of an anion exchange resin in acetate form. The syntheses of 2-, 4- and 6-FMT were also achieved by the direct fluorination of *m*-tyrosine (MT) in H_2O. The effect of temperature on the fluorination of MT was investigated and it was shown that, unlike CF_3SO_3H, optimal conditions in H_2O were attained at elevated reaction temperatures.

There have been several reports relating to the formation of $[^{18}F]OF_2$ as a major byproduct (up to 20%) in the gas phase nuclear reaction, $^{18}O(p,n)^{18}F$. This reaction is used for the routine production of $[^{18}F]F_2$ which, in turn, is utilized for the syntheses of PET tracers such as radiofluorinated aromatic amino acids. Because the reactivity of OF_2 has been reported to be similar to that of F_2, its selectivity as a fluorinating agent towards aromatic amino acids was investigated. The effect of solvent acidity on the fluorination of MT using OF_2 was studied and it was shown that, in contrast with the reactivity of F_2 in superacids, OF_2 is a more efficient fluorinating agent in less acidic solvent media. The use of H_2O as the solvent medium for fluorination of MT resulted in the formation of ^{19}F-FMT isomers in 4.35 ±0.04% yield. Consequently, the potential use of OF_2 as a fluorinating agent for aromatic amino acids was also investigated for L-phenylalanine, 3-nitro-L-tyrosine, 4-nitro-DL-phenylalanine, L-DOPA, 3-*O*-methyl-L-DOPA, 3,4-dimethoxy-L-phenylalanine, *m*-, *p*- and *o*-tyrosine. In these studies, the only aromatic system fluorinated by OF_2 was MT, indicating that the presence of $[^{18}F]OF_2$ as a byproduct resulting from the nuclear reaction, $^{18}O(p,n)^{18}F$, does not have a significant impact on the syntheses of radiofluorinated aromatic amino acids that have applications in PET imaging.

ACKNOWLEDGMENT

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LIST OF ABBREVIATIONS AND SYMBOLS

General

aHF	anhydrous hydrogen fluoride
i.d.	inner diameter
o.d.	outer diameter
FDG	2-fluoro-2-deoxy-D-glucose
FEP	perfluoroethylene/perfluoropropylene copolymer
PFA	Polyfluoroalkoxy
Kel-F	chlorotrifluoroethylene polymer
HPLC	high performance liquid chromatography
Teflon (PTFE)	tetrafluoroethylene polymer
DOPA	3,4-dihydroxy-phenyl-alanine
FDOPA	fluoro-3,4-dihydroxy-phenyl-alanine
NMR	nuclear magnetic resonance

Nuclear Magnetic Resonance

ppm	parts per million
Hz	Hertz
δ	chemical shift (ppm)
J	nuclear spin-spin coupling constant (Hz)

$\Delta\nu_{1/2}$	line width at half height (Hz)

Radiochemistry

α	alpha particle
β^+	positron
β^-	beta particle
d	deuteron
γ	gamma ray
λ	decay constant
n	neutron
p	proton
t	triton
$t_{1/2}$	half-life
ν	neutrino
A_o	radioactivity at time zero
A_t	radioactivity at time t
Ci	Curie
MRI	magnetic resonance imaging
PET	positron emission tomography
RCY	radiochemical yield

CHAPTER 1

INTRODUCTION

The applications of fluorine-containing compounds have developed tremendously during the twentieth and twenty first centuries and include their use as inert fluorinated oils, greases, polymers,[1,2] and the growing large scale industrial applications of anhydrous HF (aHF). The biological applications of fluorinated compounds have been of particular interest,[3] owing to the fact that a common method of studying the physiological properties of biologically significant molecules is by incorporation of fluorine or fluorinated groups into their structures.

1.1 Medical Applications of Fluorine

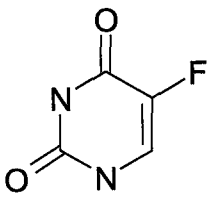
1.1.1 Pharmaceuticals

Fluorinated corticosteroids were the first commercial products where useful modifications of biological activity were achieved through the introduction of a carbon-fluorine bond. Consequently, the interest of the pharmaceutical industry along with the number of known fluorine-containing products grew significantly. [4]

The presence of fluorine or trifluoromethyl substituents in biologically active molecules provides many advantages, which include enhancement of the lipophilicity of the substrate and the subsequent increase in the rate of transport of the drug to the active site (e.g., the anti-inflammatory drug triflamizole).[5] Fluorine substitution can also be used to study the binding properties of pharmaceuticals by varying the sites and extent of fluorination, which alters the dipole moment of the molecule. For example, the anti-

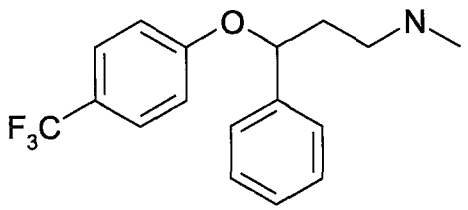
metabolite 5-fluorouracil (5-FU) makes use of covalent bonding to inhibit cell division. As a result, 5-FU is used as an anti-cancer drug. The antibacterial agent Ciprofloxacin® (Bayer) and Prozac® (Eli Lilley) are the top 20 best-selling pharmaceuticals in the world with annual sales of approximately $2 billion US. The structures of these pharmaceuticals are shown in Figure 1.1.[4]

5-fluoro-1-H-pyramidine-2,4-dione

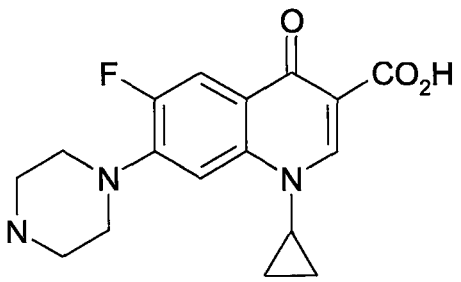


5-Fluorouracil
(anti-cancer)

N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine



Prozac®
(anti-depressant)



Cyprofloxacin®
(anti-bacterial)

1-cylcopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-quinoline-3-carboxylic acid

Figure 1.1 Examples of pharmaceuticals containing fluorine.

1.1.2 Nuclear Medicine

One of the goals of medicine is to identify the abnormal biochemistry associated with a given disease and to observe the abnormality directly and as early as possible in the afflicted organism. This is achieved, to a certain extent, by use of standard techniques in nuclear medicine in which a patient is given a radiotracer and its fate in the body is followed by means of external detectors.[6]

Fluorine-18 is one of the most commonly used isotopes in nuclear medicine. It has a half-life of 109.7 minutes and decays 97% by positron emission and 3% by electron capture. This characteristic enables the monitoring of compounds containing ^{18}F by Positron Emission Tomography (PET), which is a non-invasive imaging technique used to study *in vivo* metabolic processes.[7] The image obtained from PET is a representation of the activity distribution which can be used in quantitative assessment of biological functions. Other common radioisotopes used in PET are ^{11}C ($t_{½}$, 20.3 min), ^{13}N ($t_{½}$, 10.0 min) and ^{15}O ($t_{½}$, 2.1 min) which can be prepared in a proton cyclotron by the $^{14}N(p,\alpha)^{11}C$, $^{16}O(p,\alpha)^{13}N$ and $^{15}N(p,n)^{15}O$ nuclear reactions, respectively. Because carbon, nitrogen and oxygen are constituents of organic molecules, they can be incorporated into the structures of biomolecules without changing their biological properties. The disadvantage of using ^{13}N, ^{15}O, and ^{11}C labels, however, is the need to synthesize, purify and identify the labeled compounds within much shorter time periods. In addition, the labelled compounds may not have sufficient time to accumulate in the targeted areas. The advantage of using fluorine, aside from the sufficiently long half-life, is its substitution for a hydrogen or hydroxyl group without concern for steric alteration. This relates to one

of the main goals of radiochemistry which is to discover, develop and produce radio-labeled compounds for direct use in biological research and clinical diagnostics.[8]

1.2 Isotopes of Fluorine

Fluorine has seven isotopes (Table 1.1) that includes six radioactive isotopes and one naturally occurring isotope, ^{19}F. Fluorine-17 and -18 have a deficit of neutrons and decay by positron emission whereas ^{20}F, ^{21}F, ^{22}F and ^{23}F have a surplus of neutrons and decay by negative beta emission.

Table 1.1 Isotopes of Fluorine

Nuclide	Decay Mode	Product	Half-Life
^{17}F	β^+	^{17}O	64.7 sec
^{18}F	β^+	^{18}O	109.7 min
^{19}F	stable		
^{20}F	β^-	^{20}Ne	11.0 sec
^{21}F	β^-	^{21}Ne	4.2 sec
^{22}F	β^-	^{22}Ne	4.2 sec
^{23}F	β^-	^{23}Ne	2.2 sec

Fluorine-18 is an ideal radioactive isotope having a half-life that is long enough to be of use in radiotracer studies. This isotope decays by emission of a 0.64 MeV positron which annihilates an electron to produce two 0.511 MeV gamma rays in almost opposite directions. The emitted gamma rays are then simultaneously detected by gamma detectors enabling the determination of the line along which positron annihilation occurred. This

makes ^{18}F a useful isotope with which to study the metabolic pathways of biologically active fluoro-organics.

1.3 Production of Fluorine-18

There have been numerous reports related to the production of ^{18}F by various nuclear reactions, including $^{18}O(p,n)^{18}F$, $^{16}O(^{3}He,n)^{18}Ne$:^{18}F, $^{16}O(\alpha,np)^{18}F$ and $^{20}Ne(d,\alpha)^{18}F$. [9-17] The most common production route for ^{18}F, however, is the ^{18}O $(p,n)^{18}F$ reaction, which results in the highest yields relative to the aforementioned reactions.[9] In addition, the availability of low energy proton accelerators has made this the reaction of choice despite the cost of enriched ^{18}O target material. Another commonly used reaction, that is favored for the production of electrophilic fluorine, is $^{20}Ne(d,\alpha)^{18}F$.[18] Wolf and Ruth[9] determined the thick target yields for both reactions at 10 MeV and showed that $^{18}O(p,n)^{18}F$ is three times more efficient than $^{20}Ne(d,\alpha)^{18}F$. The remaining reactions require relatively higher energies (15-40 MeV), which cannot be provided by common commercially available low-medium energy cyclotrons. Reactions carried out in a nuclear reactor, namely, $^{6}Li(n,\alpha)^{3}H$, followed by $^{16}O(^{3}H,n)^{18}F$, are not ideal because of possible tritium contamination in the final product.[8]

Early target body material for the production of $[^{18}F]F_2$ was nickel, which is inert after passivation by fluorine gas. It was later shown that aluminum could also be used as the target materials because it also forms fluoride layers and therefore can be passivated.[19] The use of aluminum is preferred because of its superior activation properties (in terms of long-lived radioisotopes produced during bombardment), resulting

in reduction of the radiation dose received during cleaning and maintenance of the target.[20,21]

Nickels and co-workers[22] developed a "single shoot" and "double shoot" method for the production of $[^{18}F]F_2$ via $^{18}O(p,n)^{18}F$ nuclear reaction. The double shoot method was shown to be more economical because it allowed for quantitative recovery of $[^{18}O]O_2$ from the target.[6,13] The first step in the double shoot method involves the bombardment of isotopically enriched $[^{18}O]O_2$ resulting in the production of ^{18}F, which binds to the target surface and remains behind while the remaining $[^{18}O]O_2$ is retrieved cryogenically. The second step involves the bombardment of an 0.01-0.1% F_2 in Ne, He, Kr, or Ar carrier mixture, which induces fluorine exchange. This procedure recovers useful quantities of $[^{18}F]F_2$ which can then be used in further synthetic studies.[13] The production of nucleophilic $^{18}F^-$ requires an $[^{18}O]H_2O$ liquid target, where the activity is recovered in an aqueous solution as $^{18}F^-$. Fluorine-18 can then be separated from the $[^{18}O]H_2O$ water either by distillation or by the use of an anion exchange resin column.[23-25]

1.4 Methods for Radiofluorination

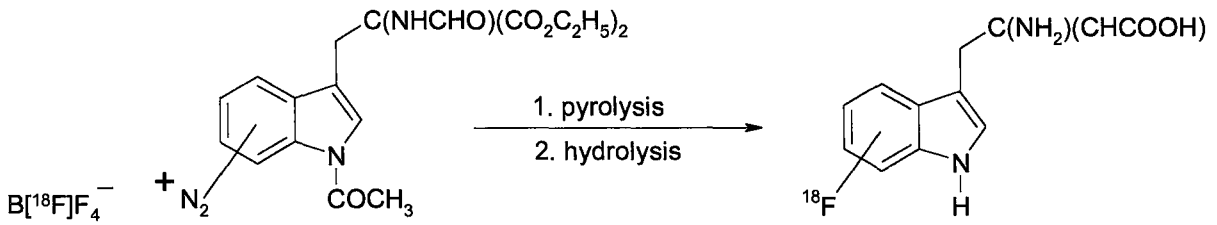
The fluorination of the organic compounds to produce radiotracers can be accomplished by either electrophilic or nucleophilic fluorination reactions and involves a number of considerations including yield, purity, and specific activity. The reaction yield must provide sufficient quantities of the radiotracer for *in vivo* studies. The purity (chemical, radiochemical and radionuclidic) of the product also determines the suitability

of the synthetic procedure for routine production of the radiotracer. With the aid of flash chromatography and high performance liquid chromatography, the task of purification of the products is often feasible. High specific activity levels are also usually required for the successful use of a radiopharmaceutical, the degree of which is dependant on the uptake of the radiotracer under study.[7]

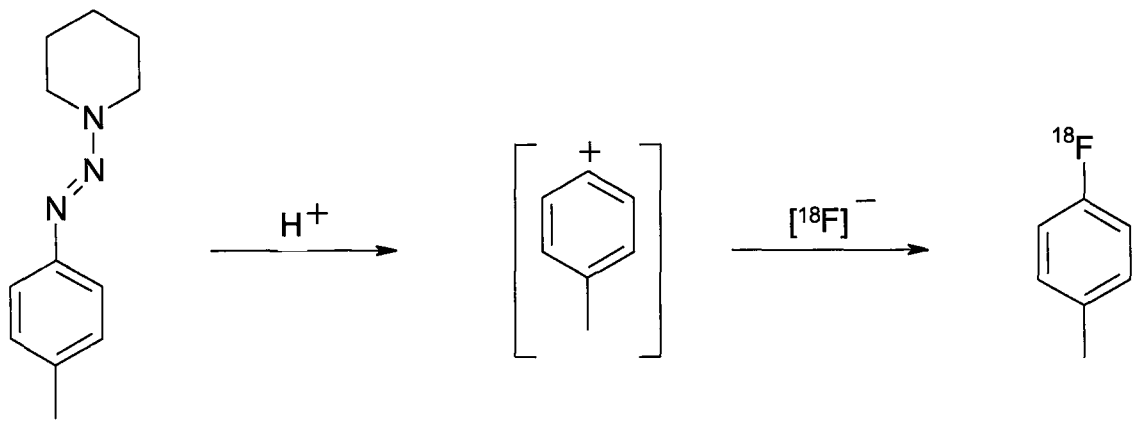
1.4.1 Nucleophilic Fluorination

The use of aromatic nucleophilic substitution was first reported in 1956 by Finger and co-workers,[26] and involves attack by fluoride on an electron rich center with a good leaving group such as a halide (other than fluorine) and a sulfonate ester. The use of ^{18}F-fluoride as fluorinating agent should, in principle, lead to a much smaller number of side products when compared to that of the electrophilic fluorinating agents. The radiochemical yield of the nucleophilic fluorination reaction can be influenced by a number of variables such as the solvent medium, cations and anions present in the solution, catalysts, carrier ^{19}F levels, reaction vessel surfaces, and substrate concentration.

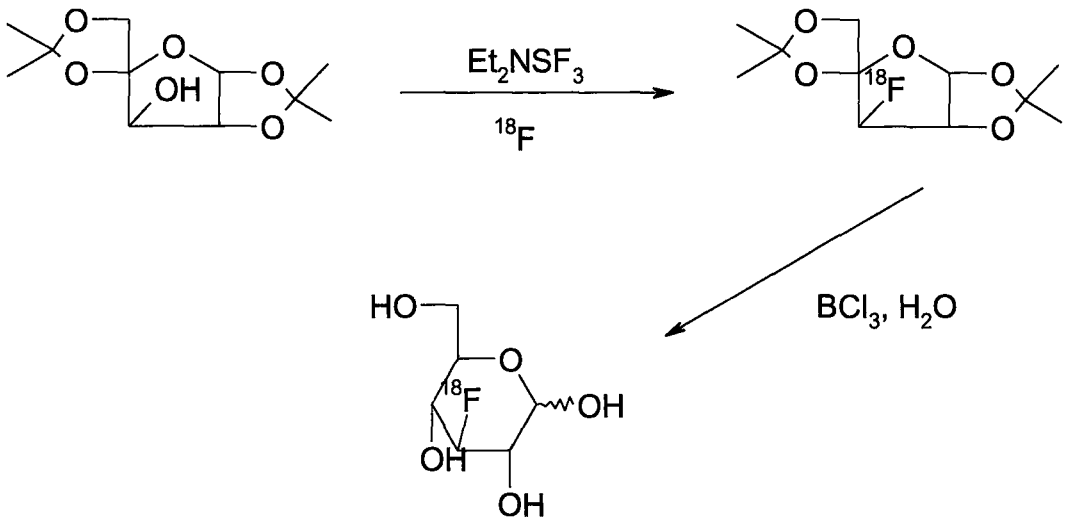
Other nucleophilic fluorination reactions include the Balz-Schiemann (Figure 1.2),[27] Wallach (Figure 1.3),[28] diethylaminosulfur trifluoride (DAST) (Figure 1.4),[29] halofluorination (Figure 1.5),[7] fluorodecarboxylation (Figure 1.6),[30] and fluoride exchange reactions.



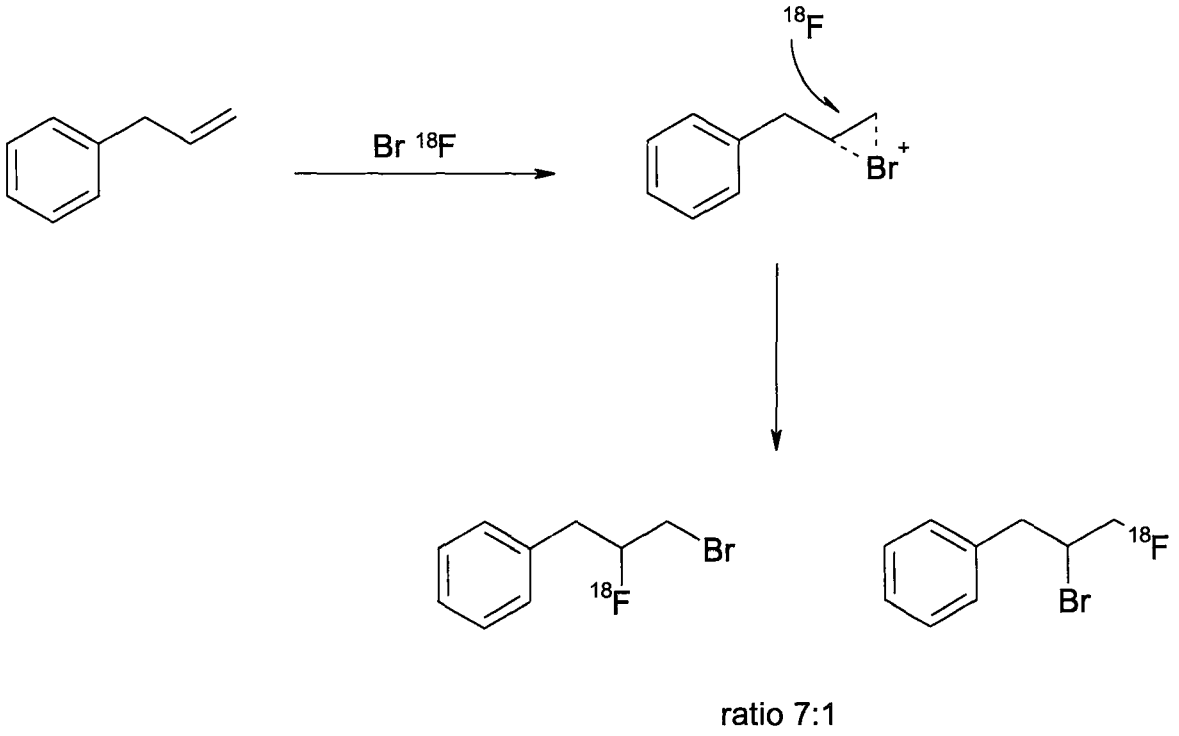
Scheme 1.1 Synthesis of fluorotryptophans by use of the Balz-Schiemann reaction.



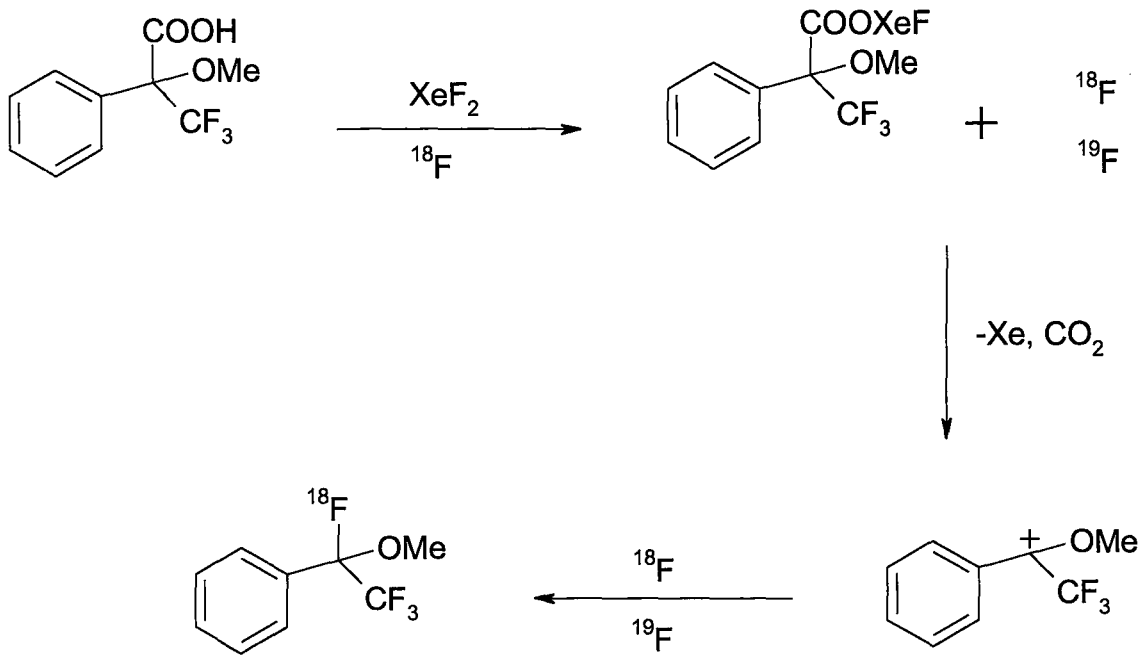
Scheme 1.2 Synthesis of aryl[^{18}F]fluorides using the Wallach reaction.



Scheme 1.3 Synthesis of 3-deoxy-3-[^{18}F]fluoroglucose by reaction of 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose with DAST and $^{18}F^-$.



Scheme 1.4 Reaction scheme for halofluorination.



Scheme 1.5 Reaction scheme for fluorodecarboxylation and formation of alkyl fluorides.

1.4.2 Limitations of Nucleophilic Fluorination

Although nucleophilic fluorination reactions provide high specific activities and regioselectivity, they can be time consuming and costly in terms of precursor syntheses and the number of intermediate steps involved in the overall reaction scheme.[7] High reaction temperatures are also often required which can lead to low product yields, the degree of which varies with the stability of the starting materials at the selected temperatures. In addition, $[^{18}F]F^-$ is often produced from $[^{18}O]H_2O$ water targets, thus obtaining anhydrous $[^{18}F]F^-$ is extremely difficult. The presence of water reduces the nucleophilicity of fluoride ions and, consequently, the product yield. A similar result is

obtained in the presence of metallic ions from the target, the degree of which varies with the nuclear reaction and the target design.[31]

1.4.3 Electrophilic Fluorination

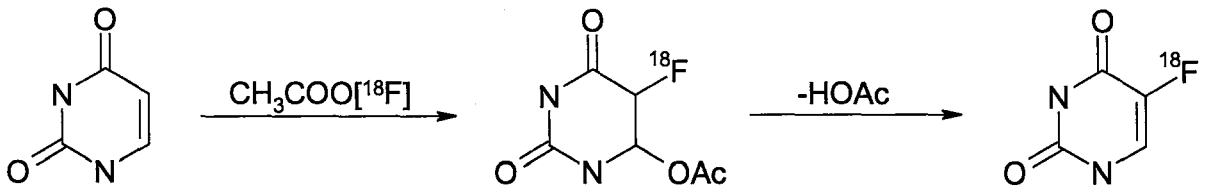
Electrophilic fluorination is the process by which a highly polarized (electropositive) fluorine is delivered to an electron-rich reactant, such as an alkene, aromatic ring, or carbanion, to form a carbon-fluorine covalent bond.[7] The simplest reagent for this purpose is elemental fluorine (F_2) gas, which was thought to be a poor reagent for selective fluorination because of its strong oxidizing power.[4] However, it was later shown that the reactivity of F_2 could be moderated and controlled by dilution with an inert gas, such as He or Ne, and by use of lower reaction temperatures.[32,33] Other labeled fluorinating agents include XeF_2,[34] ClO_3F,[35,36] nitrosyl fluoride,[37] N-fluoro-2-pyridone,[38] N-fluoro-pyridinium triflates,[39] and N-fluoro-N-alkylsulfonamides.[40] The syntheses of these reagents, however, are not trivial and tend to be very time consuming. Because elemental fluorine is one of the starting materials for the aforementioned reagents, it would be much easier if F_2 gas was used as the fluorinating agent, however, the radiochemical yield and choice of solvent medium also need to be considered.

When $[^{18}F]F_2$ is used as the fluorinating agent, the maximum radiochemical yield for the production of a fluorinated radiotracer by electrophilic fluorination is 50%. This also applies to other fluorinating reagents containing two fluorine atoms, e.g., $[^{18}F]XeF_2$. The use of a monofluorinated reagent such as acetyl hypofluorite or perchloryl fluoride would result in a maximum yield of 100% for the fluorination step, however, it should be

noted that the starting material for all of these reagents is $[^{18}F]F_2$ and in such syntheses, the maximum attainable reaction yield is 50%.[7]

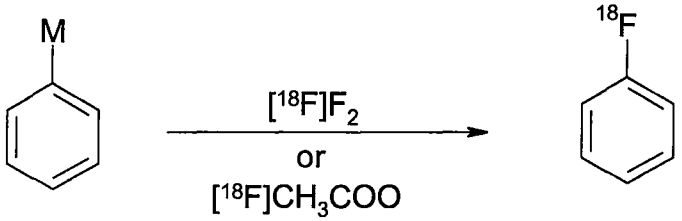
The choice of solvent medium should be an important consideration in the fluorination of aromatic systems because the acidity of the solvent medium dictates the reactivity of fluorine as an electrophile. It has been previously shown that polar protic solvents promote electrophilic fluorinations of aromatic systems.[4] This solvent effect was in particular demonstrated for the aromatic amino acids L-DOPA and *m*-tyrosine and it was shown that aHF and aHF/BF_3, both of which are superacidic protic solvent media, are ideal for syntheses of 6-FDOPA and 6-FMT.[41,42] This is because HF is a very strong polar protic solvent, which becomes more acidic with the addition of a Lewis acid such as BF_3. In addition, HF is resistant to oxidation by F_2, making it an ideal solvent for direct fluorination reactions.[43] Because of the hazardous nature of HF, however, its handling and storage require costly equipment and significant expertise.

Electrophilic fluorination can also be used for fluorination of alkenes (Figure 1.7), where the product is often monofluorinated and arises from a subsequent hydrolysis or elimination step.[7]



Scheme 1.6 Synthesis of 5-$[^{18}F]$fluorouracil by means of electrophilic aromatic fluorination of uracil.

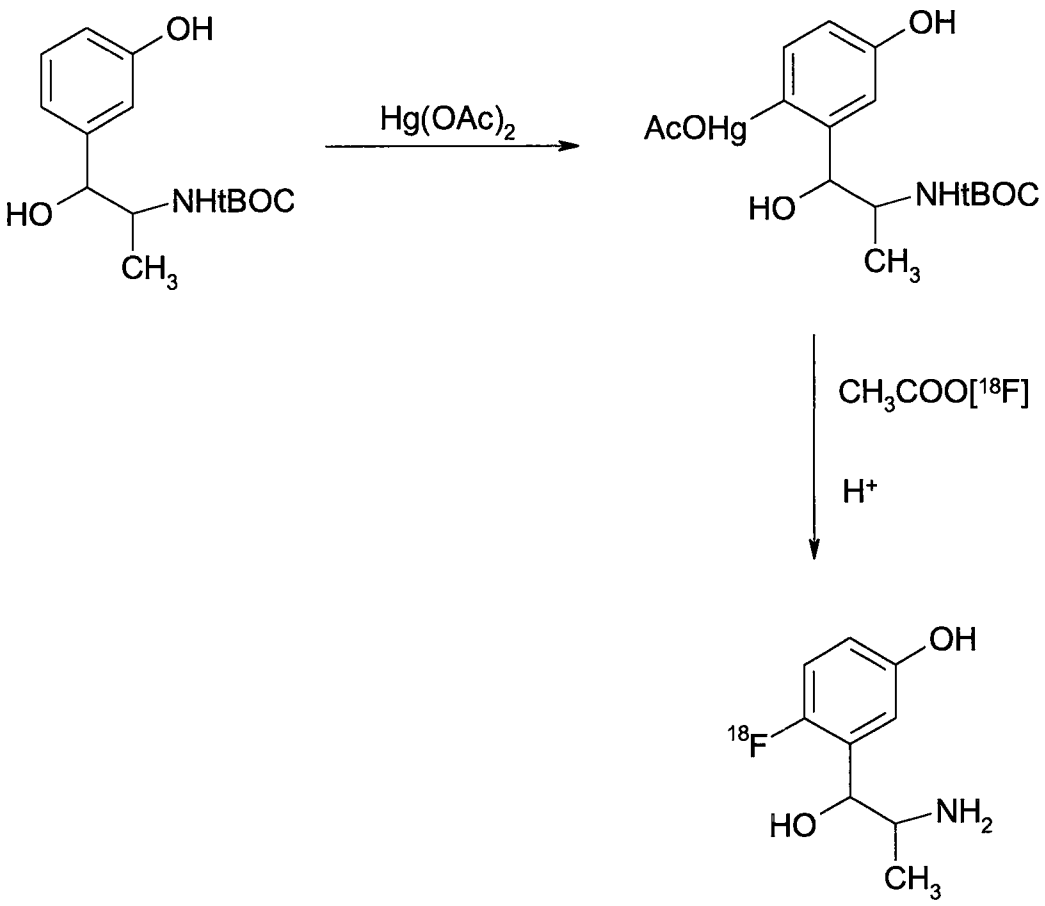
The electrophilic fluorination of aromatic systems can be carried out either by direct fluorination of the aromatic rings (using F_2, acetylhypofluorite, or XeF_2), or by fluorodemetallation reactions (Figure 1.8).[7] In the case of fluorodemetallation, F_2 or AcOF is satisfactory, but with less reactive silicon or germanium derivatives, F_2 is the preferred reagent.[44]



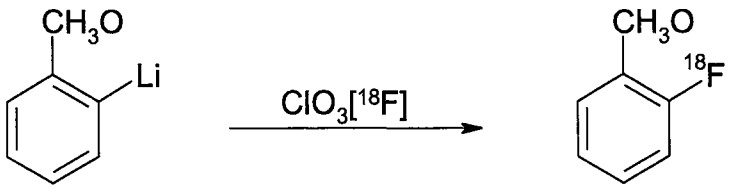
Scheme 1.7 Synthesis of aryl[^{18}F]fluorides by fluorodemetallation; M = Si, Sn, Hg, Ge.

Use of fluorodemercuration reactions (Figure 1.9),[45] in particular, is advantageous because the aryl mercury compounds are easily and directly prepared from aromatics and mercury salts. Furthermore, fluorodemetallation reactions are highly regioselective; thus only one fluorinated isomer is formed as the major product. Preparation of other metallated species (Sn, Ge, Si) involves more synthetic steps. The drawbacks of fluorodemetallation reactions, however, include the protection and deprotection of functional groups in order to promote regioselective fluorination, as well as HPLC purification to separate the fluorinated products from residual mercury precursors.[46]

Another approach to electrophilic fluorination is by reaction of an aryl Grignard reagent with a source of polarized fluorine (Figure 1.10).[47,48]



Scheme 1.8 Synthesis of 6-[^{18}F]fluorometaraminol by means of a fluorodemercuration reaction.



Scheme 1.9 Synthesis of aryl[^{18}F]fluorides by reaction of aryl lithiums with perchloryl fluoride.

Direct electrophilic fluorination was the method of choice in the current research study because it is a versatile, one step synthetic procedure, which is efficient in terms of time and the amounts of materials used.

1.5 Purpose and Scope of the Current Research

The purpose of this research was to investigate the direct electrophilic fluorination of aromatic amino acids by studying the regioselectivity and F-F bond activation processes in various polar protic solvent media including the superacids HF and CF_3SO_3H. Although the use of aHF has been shown to be most favorable for the production of PET tracers,[41,42] its use is not preferred in hospital environments owing to its hazardous characteristics. Therefore, the primary goal of these studies was to find alternative and more facile approaches for the direct electrophilic fluorination of aromatic amino acids, used in diagnostic imaging, without resorting to the use of aHF as the solvent medium. Selected solvent media, however, should also be highly acidic in order to maximize the regioselectivity of the fluorination reactions. Gillespie and Liang[43] determined the Hammett acidity values (used as a measure for acidity for superacidic

media), for a number of superacidic media such as CF_3SO_3H, HSO_3F, H_2SO_4, and $H_2S_2O_7$ (Table 1.2). Trifluoromethanesulfonic acid was used as the fluorination medium in this research because it is a superacid that is resistant to oxidation by elemental fluorine. The direct electrophilic fluorination of the investigated aromatic amino acids was also carried out in weakly acidic media, such as H_2O, in an attempt to find more easily handled solvents.

Table 1.2 H_0 Values for Some Superacidic Media

Acid	H_0 [a]	Acid-SbF_5	H_0
H_2SO_4	−11.9		
CF_3SO_3H	−13.8	CF_3SO_3H - 2% SbF_5	−16.4
$H_2S_2O_7$	−14.1		
HSO_3F	−15.1	HSO_3F - 20% SbF_5	−20
		HSO_3F - 7% $SbF_5.3SO_3$	−19.4
HF	−15.1	HF - 0.5% SbF_5	−21

[a] The acidity of the solvent media increases with more negative values of H_0.

There have been a number of reports on the presence of OF_2 as a major impurity in the nuclear reaction $^{18}O(p,n)^{18}F$.[49,50] The presence of OF_2 as an impurity can be particularly problematic if it results in the syntheses of undesired fluorinated byproducts which may prevent the isolation of the desired fluorinated aromatics by means of HPLC separation. Therefore, an additional goal of the present research was the investigation of the ability of OF_2 to fluorinate aromatic amino acids used in PET imaging.

CHAPTER 2

EXPERIMENTAL

Caution: It is recommended that proper first-aid treatment procedures[51-53] be known and available to all laboratory personnel prior to repeating portions of this study that deal with the use of aHF and F_2. Skin contact with even small amounts of aHF or F_2 may result in painful burns if immediate and proper treatment is not given. Any incident involving direct contact with liquid aHF, HF vapor, F_2 gas and aqueous solutions of HF must be aggressively treated and brought to the attention of qualified medical personnel for appropriate follow-up treatment.

2.1 Standard Techniques

The handling of radioactive isotopes was carried out in a safe and effective manner in compliance with all requirements of the Canadian Nuclear Safety Commission (CNSC) and Radioisotope Protection Committee (RPC).

2.2 Materials

Enriched $[^{18}O]O_2$ (^{18}O, 99 atom%, Isotec), neon (99.999%, Air Products), 1% F_2 in neon (Canadian Liquid Air), OF_2 (1% in neon, Ozark-Mahoning), BF_3 (Matheson, 99.5%), helium (99.9999%, Matheson), aHF (Air Products, 99.9%), CF_3COOH (Caledon, 99.9%), HCOOH (Riedel-de Haën, 98%), glacial CH_3COOH (BDH, 99.7%), D_2O (Cambridge Isotope Laboratories, Inc., 99.9%), CF_3SO_3H (Fluka, 99.8%), 3,4,6-tri-*O*-acetyl-D-glucal (Aldrich, 98%), L-phenylalanine (Aldrich, 98%), *m*-tyrosine (Aldrich,

98%), DL-tyrosine (Aldrich, 99%), *o*-tyrosine (Aldrich, 96%), L-DOPA (Aldrich, 99%), 3-*O*-methyl-L-DOPA (Aldrich), 3-nitro-L-tyrsoine (Aldrich, 98%), 4-nitro-DL-phenylalanine (Aldrich, 98%), *m*-fluoro-DL-tyrosine (Aldrich), anhydrous KF (Aldrich, 99.99+%), $KF{\cdot}2H_2O$ (Aldrich, 98%) and HPLC grade acetonitrile (Caledon, 99.8%) were used without further purification and/or drying. Sterile, deionized water was used in all aqueous procedures.

2.3 Production of $[^{18}F]F_2$

Fluorine-18 labeled F_2 was produced by the nuclear reaction, $^{18}O(p,n)^{18}F$, using a Siemens RDS 112 proton cyclotron, operating at 11 MeV, and the "double shoot" method[50,54] in the Nuclear Medicine Department at Hamilton Health Sciences. An aluminum target (11 mL) was pressurized to 14-16 atm with 99% enriched $[^{18}O]O_2$ and irradiated for 20 min using a 30 μA proton beam (production shoot). After irradiation, the $[^{18}O]O_2$ was recovered from the target by condensation at –196 °C into a cryo-trap consisting of molecular sieves (Varian VacSorb) contained in a 316 stainless steel Whitey® cylinder (75 mL). The target was pumped to remove trace amounts of $[^{18}O]O_2$ and subsequently filled with 1% F_2 (40-50 μmol) in neon, pressurized to 20 atm with neon, and irradiated for 10 min in a 15 μA proton beam (recovery shoot). Portions of the $[^{18}F]F_2$/Ne mixture were periodically released from the target into a continuous stream of helium until the target pressure dropped to 2 atm. Helium was used as the sweep gas to transfer $[^{18}F]F_2$ from the target into the hot cell.

2.4 Electrophilic Fluorination in H_2O, HCOOH, CH_3COOH, CF_3COOH, CF_3SO_3H, CF_3SO_3H/HCOOH, aHF and BF_3/ CF_3SO_3H Using [^{18}F]F_2 and F_2

The substrate (13 mg), dissolved in 0.5 mL of the respective solvent medium, was placed in a $^5/_{16}$" o.d. × $^5/_{32}$" i.d. FEP reaction vessel connected to an FEP Y-piece (Figure 2.1). A $^1/_{16}$" o.d. × $^1/_{32}$" i.d. FEP tube, connected to the [^{18}F]F_2 target at one end, was fed through the sidearm of the Y-piece into the reaction vessel. The other arm of the Y-piece was connected to a separate $^1/_{16}$" o.d. FEP tube, which was immersed in 1M NaOH. The reaction vessel and contents were allowed to equilibrate at selected temperatures in a liquid nitrogen cooled CH_3OH bath. The BF_3/CF_3SO_3H solvent mixture was prepared by bubbling BF_3 into the CF_3SO_3H/DOPA solution at 4 °C until saturation was achieved, and was followed by equilibration at 4 °C for 15 min prior to fluorination.

Fluorine-18 labeled F_2 gas (typically 40 μmoles) was passed through a solution of DOPA in CF_3SO_3H and the effluent gas was passed through 1M NaOH before it was vented into the hot cell. The amount of [^{18}F]F_2 that had reacted was determined by counting the amount of radioactivity present in the reaction mixture.

Removal of CF_3SO_3H from the reaction mixture was achieved using a 250 x 10 mm anion exchange column (Bio-Rad AG 1–X8 in acetate form). The reaction mixture was loaded onto the column and 20 mL of 0.1 M HCl was used as the eluent. The eluate was then evaporated on a rotary evaporator and was subsequently analyzed using high performance liquid chromatography (HPLC).

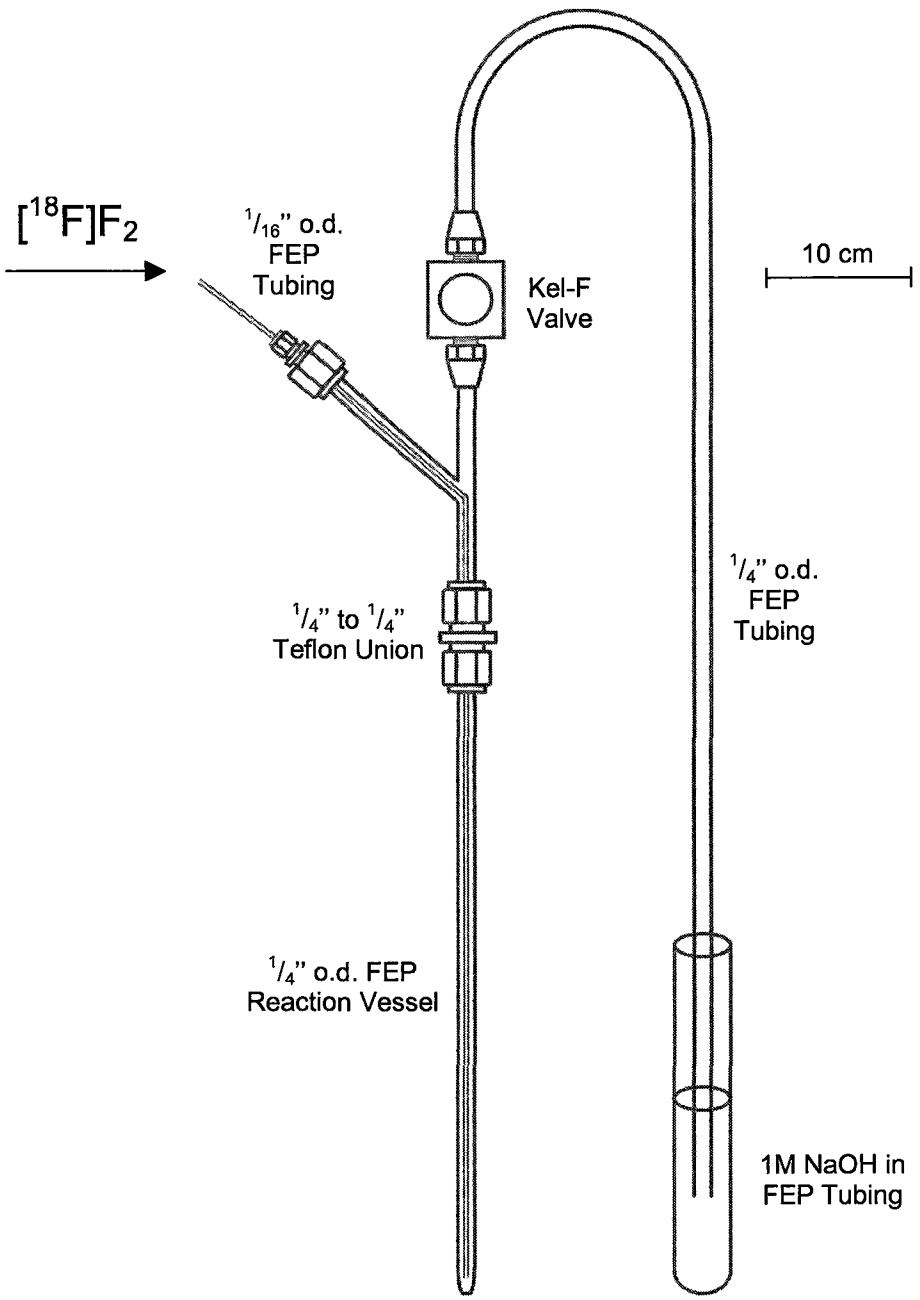


Figure 2.1 A schematic diagram of the experimental apparatus used for direct fluorinations.

In reactions involving aHF as solvent medium, one arm of the Y-piece was connected to the HF line and to a $^1/_{16}$" o.d. × $^1/_{32}$" i.d. Teflon tube was fed through the other arm of the Y-piece into the reaction vessel. Anhydrous HF was condensed into the reaction vessel at -196 °C. The reaction vessel and contents were equilibrated at -65 °C, in a methanol/liquid nitrogen slush bath, and the reaction vessel was disconnected from the HF line. Once the desired amount of $[^{18}F]F_2$ or F_2 gas had passed through the substrate solution, the reaction vessel was disconnected from the target or the F_2 line and the NaOH trap. The hydrogen fluoride solvent was removed under vacuum by pumping through an FEP U-tube cooled to –196 °C.

2.5 Analyses of Reaction Mixtures Using HPLC

The ring-fluorinated aromatic amino acid isomers were isolated using a reverse-phase analytical HPLC column ((Keystone Scientific, Inc., Bellefonte, PA, USA 16823, Fluophase PFP, 5 μm, 150 × 10 mm). A solution of 0.2% CF_3CO_2H in water containing 7% CH_3CN was used as the mobile phase with a flow rate of 2.5 mL min^{-1}. The column eluate was monitored by the use of a Waters 490E Programmable Multi-wavelength Detector set at 280 and 230 nm in conjunction with a Beckman Radioisotope Detector.

The fluorinated isomers were also isolated by the use of a reverse-phase preparative HPLC column (Keystone Scientific, Inc., Bellefonte, PA, USA 16823, Fluophase PFP, 5 μm, 250 × 10 mm). A solution of 17 mg of ascorbic acid in 500 mL of 0.1% CH_3CO_2H was used as the mobile phase with a flow rate of 3.5 mL min^{-1}. The eluate from the column was monitored by the use of a UV detector set at 280 nm and a

Geiger-Müller counter (Bicron SWGM B980C), coupled to a rate meter (Bicron Erick-Tech™).

2.5.1 Analyses of FDOPA Reaction Mixtures by HPLC

A typical UV chromatogram of the FDOPA reaction mixture showed peaks at 11, 12, and 14 min corresponding to DOPA, 2-FDOPA and 6-FDOPA, respectively. The DOPA peak was identified by injection of a standard solution which eluted at 11 min. The peaks appearing at 12 and 14 min corresponded to those appearing in the radiochromatogram at 13 and 15 min, respectively. Each peak was collected and assayed for radiochemical yield. After a 24 h decay period, both samples were combined and analyzed by ^{19}F NMR spectroscopy to obtain the relative molar amounts of products which were shown to be 2- and 6-FDOPA. The relative isomeric ratios of the mono-fluorinated aromatic amino acids were determined by peak integrations of the HPLC radiochromatograms and ^{19}F NMR spectra. The 2- and 6-FDOPA isomers (typically appearing at 12 and 14 min on the radiochromatogram) were also collected using preparative HPLC, assayed and subsequently analyzed using ^{19}F NMR spectroscopy.

2.5.2 Analyses of FMT Reaction Mixtures by HPLC

A typical UV chromatogram of the *m*-tyrosine reaction mixture showed peaks at 10, 11, 12, and 14 min corresponding to MT, 2-FMT, 6-FMT, and 4-FMT, respectively. The MT peak was identified by injection of a standard solution which eluted at 10 min. The peaks appearing at 11, 12 and 14 min corresponded to those appearing in the

radiochromatogram at 11, 13 and 14 min, respectively. Each peak was collected and assayed for radiochemical yield. After a 24 h decay period, both samples were combined and analyzed by ^{19}F NMR spectroscopy to obtain the relative molar amounts of products which were shown to be 2-, 6-, and 4-FMT. The relative isomeric ratios of the mono-fluorinated aromatic amino acids were determined by peak integrations of the HPLC radiochromatograms and ^{19}F NMR spectra. The 2-, 6-, and 4-FMT isomers (typically appearing at 13, 14 and 15 min on the radiochromatogram) were also collected, radio-assayed and subsequently analyzed using ^{19}F NMR spectroscopy.

2.6 Fluorination of Aromatic Amino Acids by OF_2

2.6.1 Attempted Preparation of $[^{18}F]OF_2$

In a typical reaction, 100 mg of $KF{\cdot}2H_2O$ and 300 mg of anhydrous KF were loaded into a $^1/_2$" o.d. FEP U-tube which was attached to two PFA Whitey valves by means of stainless steel Swagelok $^1/_2$" to $^1/_4$" reducing unions and lengths of $^1/_4$" FEP tubing (Figure 2.2). One end of the U-tube was connected to a cylinder of 1% F_2 in Ne (or to the $[^{18}F]F_2$ line from the cyclotron gas target), while the other end was connected to a $^5/_{16}$" o.d. × $^5/_{32}$" i.d. FEP reaction vessel that was, in turn, attached to an FEP Y-piece by means of a $^1/_4$" Teflon Swagelok union. A length of $^1/_{16}$" o.d. x $^1/_{32}$" i.d. FEP tubing, connected to a valve of the U-tube, was fed through the sidearm of the Y-piece into a reaction vessel containing 13 mg of TAG in 0.5 mL of $CFCl_3$. The aforementioned solution was cooled to -65 °C using a methanol/liquid nitrogen slush bath. The other arm of the Y-piece was connected, through a $^1/_4$" to $^1/_{16}$" Teflon reducing union, to a length of

$^1/_{16}$" o.d. FEP tubing that was immersed in 1M NaOH. The U-tube, containing an anhydrous KF/KF·2H_2O mixture, was pressurized with 1% F_2 in neon (typically 60 ±5 μmol of F_2 was used) and was agitated for 110 min at room temperature, after which the gas was passed through the TAG solution. The fluorinated reaction mixture was then analyzed by ^{19}F NMR spectroscopy after hydrolysis using 1.2 M HCl carried out over 17 min at 130 °C.

2.6.2 Determination of Reaction Yields for FMT Isomers After the Fluorination of MT by OF_2

A 10.3 mg sample of *m*-fluoro-DL-tyrosine was dissolved in 5.00 mL of 0.100 M HCl for use as an internal standard. Two 0.100 mL aliquots of 0.010 M *m*-fluoro-DL-tyrosine were then added stepwise to each reaction mixture, which was analyzed by ^{19}F NMR spectroscopy after each addition. Each aliquot contained 1.03 ±0.04 μmol of *m*-fluoro-DL-tyrosine, so that ^{19}F NMR peak integrations could be used to calculate the FMT isomer yields.

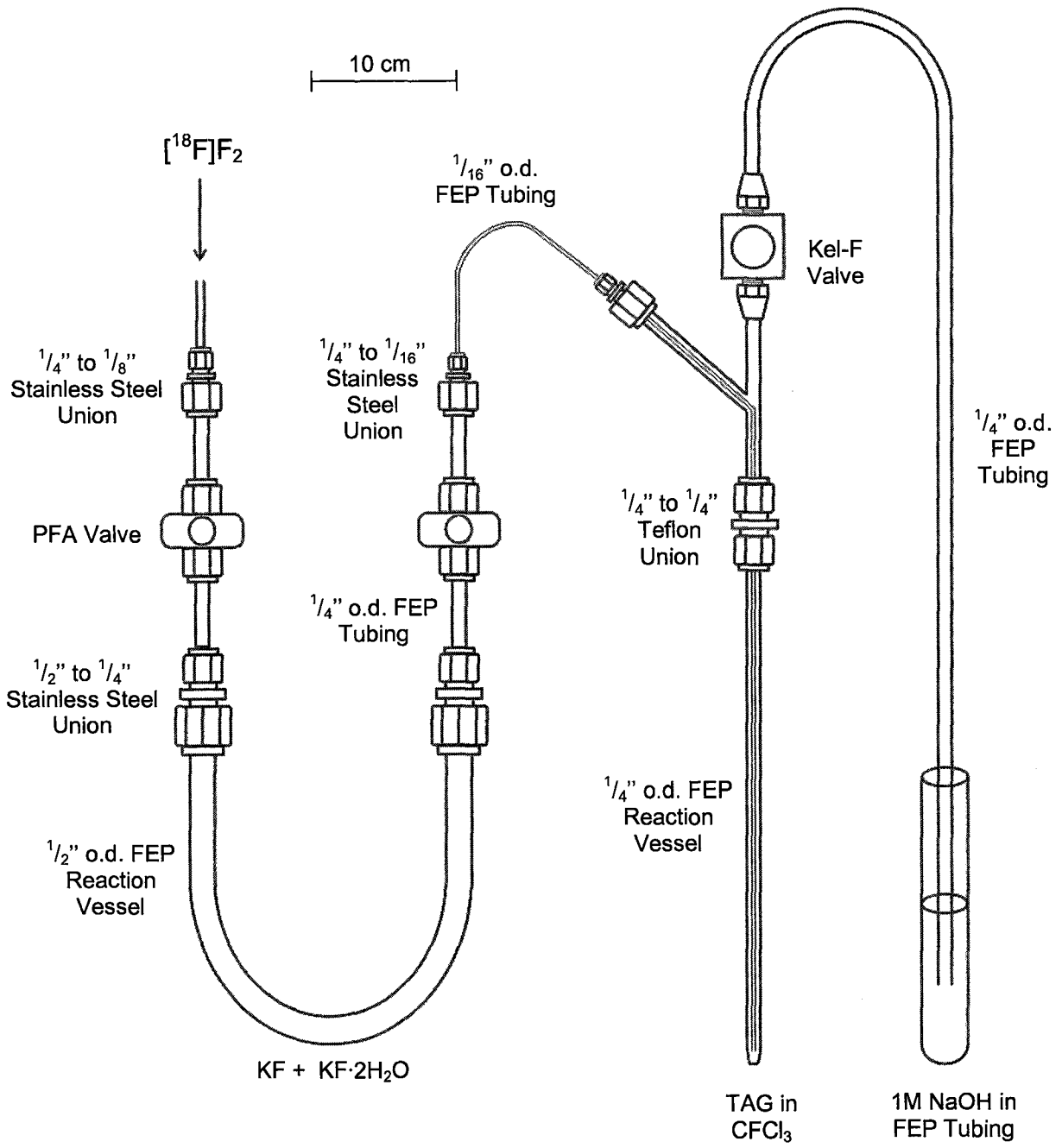


Figure 2.2 A schematic diagram of the experimental apparatus used for the attempted synthesis of $[^{18}F]OF_2$.

2.7 Nuclear Magnetic Resonance Spectroscopy

The ^{19}F NMR spectra were recorded on a Bruker Avance 200 (4.6976 T) or DRX-500 (11.7440 T) spectrometer using pulse widths of 1 μs corresponding to a bulk magnetization tip angle of ~90°. Fluorine-19 NMR spectra were obtained at 11.7440 T and were typically accumulated over a spectral width of 14 kHz (acquisition time, 1.16 s), using 300 scans and 32 K memories, yielding data point resolutions of 0.35 Hz/point. Fluorine-19 NMR spectra obtained at 4.6976 T were accumulated over spectral widths of 17 kHz (acquisition time, 0.94 s), using 200 scans and 32 K memories, yielding data point resolutions of 0.53 Hz/point. Spectra were referenced at 25 °C to external $CFCl_3$. The chemical shift convention used is that positive and negative signs indicate chemical shifts to high and low frequencies relative to that of the reference compound.

2.8 Measurement of Radioactivity

Radioactivity was measured using a radioisotope calibrator (Capintec CRC-12) manufactured by Capintec, Inc. It consisted of a 6 cm i.d. and 25 cm deep measuring well surrounded by an ionization chamber filled with argon gas. The chamber walls were made of aluminum and the outside wall was shielded with $^1/_8$" thick lead. The current produced in the ionization chamber, due to the interaction of photons with the gas molecules, was read using a digital readout. The Capintec could measure radioactivity in the range 10 μCi up to 2 Ci.

In the present work, the measured values for [^{18}F] were decay corrected back to the end of the fluorination step of the experiment. The radioactivity present in the $^1/_{16}$"

o.d. × $^{5}/_{32}$" i.d. FEP reaction vessel at the end of fluorination was taken to be the measured radioactivity (A_i) at zero time (t = 0). Successive measurements of sample radioactivities (A_t) were decay corrected to the theoretical radioactivity at the end of fluorination (A_o) using the equation:

$$A_o = A_t e^{\lambda t} \quad (1)$$

$$\lambda = (\ln 2)/t_{½} \quad (2)$$

where $t_{½}$ is the half-life of ^{18}F (109.7 min) and t is the time (min) from the end of fluorination up to the time when radioactivity is measured. Radiochemical yields (RCY) of the final product were reported by expressing A_o as a percentage of the measured initial activity, A_i.

CHAPTER 3

TRIFLUOROMETHANESULFONIC ACID, AN ALTERNATIVE SOLVENT MEDIUM FOR THE DIRECT FLUORINATION OF DOPA: NEW SYNTHESES OF 6-[^{18}F]FLUORO-L-DOPA and 6-[^{18}F]FLUORO-D-DOPA

3.1 Introduction

Positron emission tomography (PET) is a non-invasive imaging technique, which has been commonly used for the *in vivo* visualization of brain functions such as blood flow, metabolism, enzyme activity, neuroreceptors and neurotransporters.[55] Fluorine-18 labeled 6-fluoro-L-DOPA was initially developed as a routine PET tracer to assess presynaptic dopaminergic function in the human brain.[56] Dopamine is synthesized *in vivo* by the hydroxylation and the subsequent decarboxylation of the amino acid L-tyrosine, stored intraneuronally in vesicles from which it is ejected into the synapse during neurotransmission. The monitoring of intracerebral dopamine by PET requires the presence of dopamine in the brain that is labeled with a positron emitter. Dopamine, however, will not cross the blood-brain barrier upon injection into the blood stream, and is unable to reach the dopaminergic cells of the brain. The immediate dopamine precursor, L-DOPA, can, however, cross the blood-brain barrier and can therefore be employed to monitor *in vivo* intracerebral dopamine metabolism when labeled with ^{18}F.[57] Garnett et al. pioneered the use of 6-fluoro-L-DOPA, in conjunction with PET, to visualize regional distributions of intracerebral dopamine in the human brain.[56] Fluorine-18 labeled 6-fluoro-L-DOPA can be produced by the direct electrophilic fluorination of

L-DOPA in anhydrous HF (aHF), but is formed in admixture with the 2- and 5-fluoro-L-DOPA isomers, which cannot be utilized to study the dopaminergic pathways of the human brain.[58]

Although ^{18}F-FDG has been the benchmark for brain tumor detection, recent studies have shown its use for the detection of low-grade tumors and, in some cases, recurrent tumors, is problematic owing to its low specific to non-specific uptake ratio.[59] Amino acids, on the other hand, usually exhibit higher specific to non-specific uptake ratios and are consequently making major contributions to tumor detection by PET.[60] As a result, there has been a growing interest in the use of ^{18}F-labeled aromatic amino acids and PET for tumor detection. In particular, [^{18}F]6-fluoro-L-DOPA appears to have the potential to improve detection of neuroendocrine tumors and their metastases.[60] Fluorine-18 labeled 6-fluoro-L-DOPA has also been shown to be an excellent candidate for visualization of high-grade and low-grade tumors, and for the analyses of recurrent low-grade gliomas, which are difficult to examine by magnetic resonance imaging and are usually not detected by ^{18}FDG PET.[59]

Although the applications of 6-fluoro-L-DOPA have been well studied and are extensive, drawbacks still remain because 3-*O*-methyl-[^{18}F]6-FDOPA is formed in the blood and brain by the action of catechol-*O*-methyl transferase (COMT). It has been shown that the presence of 3-*O*-methyl-[^{18}F]6-FDOPA causes nonspecific accumulation of radioactivity in the brain and blood, resulting in lower signal-to-noise ratios in PET images.[61] Because aromatic amino acid decarboxylase (AADC) and COMT are specific to L-DOPA, it is speculated that [^{18}F]6-fluoro-D-DOPA may not be as extensively

metabolized in the brain, leading to higher signal-to-noise ratios in tumor images when compared with 6-[^{18}F]fluoro-L-DOPA. In recent *in vivo* and *in vitro* studies, Bauwens et al. showed that the tumor-uptake to background ratio of $^{123/125}$I labeled 2-iodo-D-tyrosine is similar to that of its L-analogue in tumor cells (*in vitro* in LAT1-expressing R1M rat rhabdomyosarcoma cells and *in vivo* in R1M tumor-bearing Wag/Rij rats).[62] These authors also reported similar results for 2-[^{123}I]phenyl-D-alanine and its L-analogue.[63] Their findings suggest that both the D- and L-enantiomers of 6-[^{18}F]FDOPA may also display comparable uptakes in tumors.

Among the various methods that have been used for the synthesis of 6-[^{18}F]fluoro-DOPA,[64,65] the most common synthetic route is regioselective fluorodestannylation[66] although, in the past, fluorodemercuration[67,68] reactions have also been extensively used. Although both methods provide high radiochemical yields, they require costly functionalized precursors, prepared and purified through tedious multi-step procedures, and considerable expertise. Moreover, metal contamination of the product may occur, requiring further purification prior to clinical use.[69] Fluorodestannylation is preferred over fluorodemercuration because of the toxicities associated with the mercury derivatives employed in this procedure. Syntheses using fluorodestannylation have the added drawback of producing insoluble $(CH_3)_3SnF$ which can obstruct tubing and valves used in automated synthetic procedures.[69] Consequently, a simplified and more routine method for the syntheses of D- and L- enantiomers of 6-FDOPA is highly desirable to facilitate further investigation of their biochemical properties in small animal studies.

The use of elemental fluorine has been perceived as a non-selective electrophilic fluorination method for aromatic compounds because the strong oxidant nature of F_2 results in exothermic radical chain reactions that lead to the formation of side products that include tars. Prior work from this laboratory has shown that direct electrophilic fluorination of L-DOPA using F_2 in aHF produced 2-, 5- and 6-fluoro-L-DOPA having a total radiochemical yield of 30% (decay corrected with respect to $[^{18}F]F_2$),[70] which is excellent for electrophilic radiofluorinations because the theoretical maximum radiochemical yield is 50% with respect to $[^{18}F]F_2$. The highly efficient fluorination of L-DOPA and other aromatic compounds in aHF is partly the result of the low reaction temperatures employed and the high solvent polarity, which favor electrophilic substitution reactions. In addition, aromatic substrates in aHF are less susceptible to oxidation by F_2 because the catechol oxygens are protonated.[6] Chambers et al.[4] have shown that protic solvents promote electrophilic fluorination of aromatic compounds and that the reactivity of F_2, as an electrophile, varies significantly with solvent acidity. In prior work from this laboratory, the regioselectivities of aromatic electrophilic fluorinations were shown to be dependent upon the acidity of the reaction medium. For example, although useful quantities of ^{18}F-labeled 2- and 5-fluoro-L-DOPA have been prepared by the direct fluorination of L-DOPA in various weak protic acid solvents such as HCOOH, CH_3COOH and CF_3COOH, 6-fluoro-L-DOPA could only be produced when the superacid, aHF, was used as the solvent.[71] The direct electrophilic fluorination of L-DOPA in BF_3/aHF, in particular, was shown to produce 2-, 5- and 6-fluoro-L-DOPA in a total radiochemical yield of 40%.[70]

Anhydrous HF does not readily lend itself to use in most hospital environments because of its hazardous nature and the specialized fluoroplastic equipment and expertise required for its handling. An alternative route to the production of 6-[^{18}F]fluoro-L-DOPA and 6-[^{18}F]fluoro-D-DOPA by the direct radiofluorination of their respective precursors in the superacidic medium, trifluoromethanesulfonic acid (triflic acid, CF_3SO_3H), that largely circumvents these difficulties is highly desirable and is reported in this Article.

3.2 Results and Discussion

In choosing an alternative solvent medium for electrophilic fluorination of L-DOPA, a high solvent acidity is required to facilitate and enhance the regioselectivity of the reaction. Triflic acid was chosen because of its high Hammett acidity (H_o = –13.8), which places it in the superacidic category along with aHF (H_o = –15.1),[43] and its resistance to oxidation by F_2. Moreover, CF_3SO_3H has a favorable liquid range (f.p. –40 to –45 °C and b.p. 162 °C),[72] both it and its conjugate base are not sources of fluoride ions (even in the presence of very strong Lewis acid fluoride ion acceptors) and the solubilities of amino acids are high in both CF_3SO_3H and aHF, which is expected to promote greater product yields. There have been numerous applications of CF_3SO_3H in organic syntheses such as salt formation, polymerization, ester formation and Friedel-Crafts reactions.[73] Recently, Coe et al.[74] used 10% CF_3SO_3H in $CFCl_3$ solvent to demonstrate the role of CF_3SO_3H in promoting electrophilic fluorinations of aromatic compounds containing the $(CH_3)_3Si$ group. The use of CF_3SO_3H minimizes side reactions encountered in direct electrophilic fluorinations that utilize F_2 and reduces the difficulties

and hazards associated with the use of aHF.[72] Prior studies from this laboratory have shown increased regioselective fluorination of the C6 position in electrophilic fluorinations of L-DOPA, with increasing solvent acidity. The regioselective fluorination of the C6 position of the aromatic amino acid was shown to increase with increasing solvent acidity. Consequently, in the present study, the direct fluorination of L-DOPA was carried out in 99% CF_3SO_3H to maximize the yield of 6-fluor-L-DOPA.[71]

3.2.1. CF_3SO_3H as a Direct Fluorination Medium

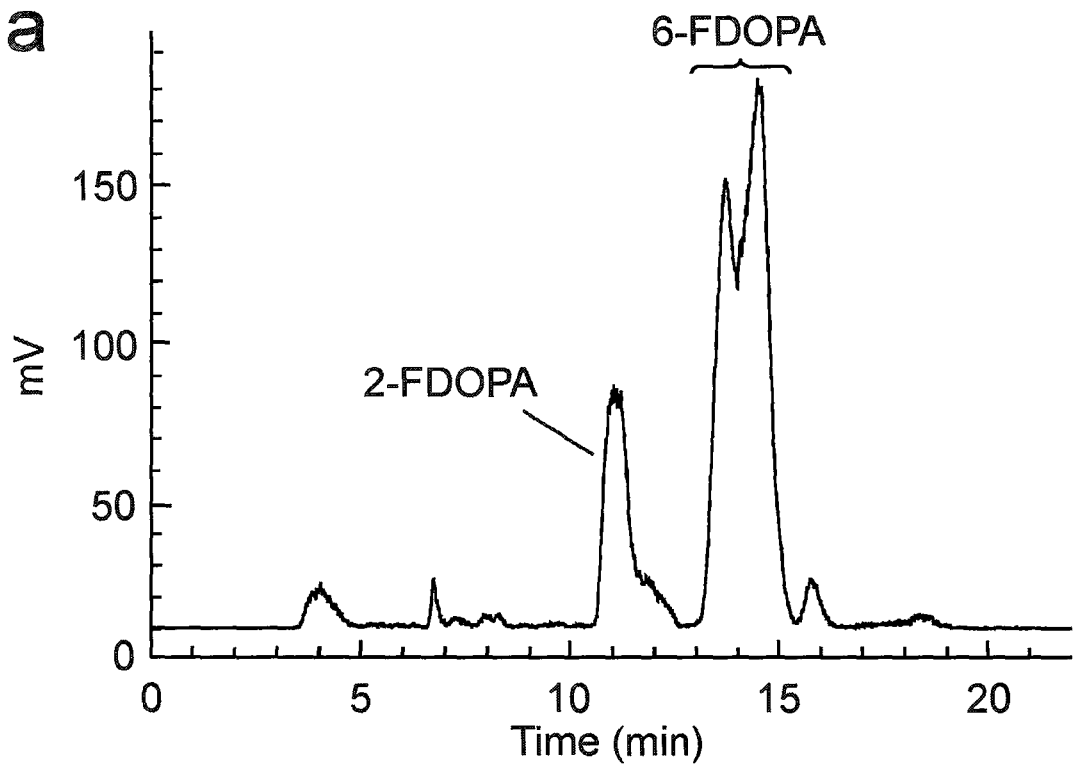
The direct regioselective fluorination of DOPA in 99% CF_3SO_3H (Table 3.1) produced clinically useful quantities of ^{18}F-labeled 2- and 6-FDOPA. Fewer side products and higher relative isomeric ratios of 6-FDOPA (Table 3.1) were observed by preparative HPLC (Figure 3.1) at lower reaction temperatures, suggesting that 6-FDOPA is a kinetically favored product.

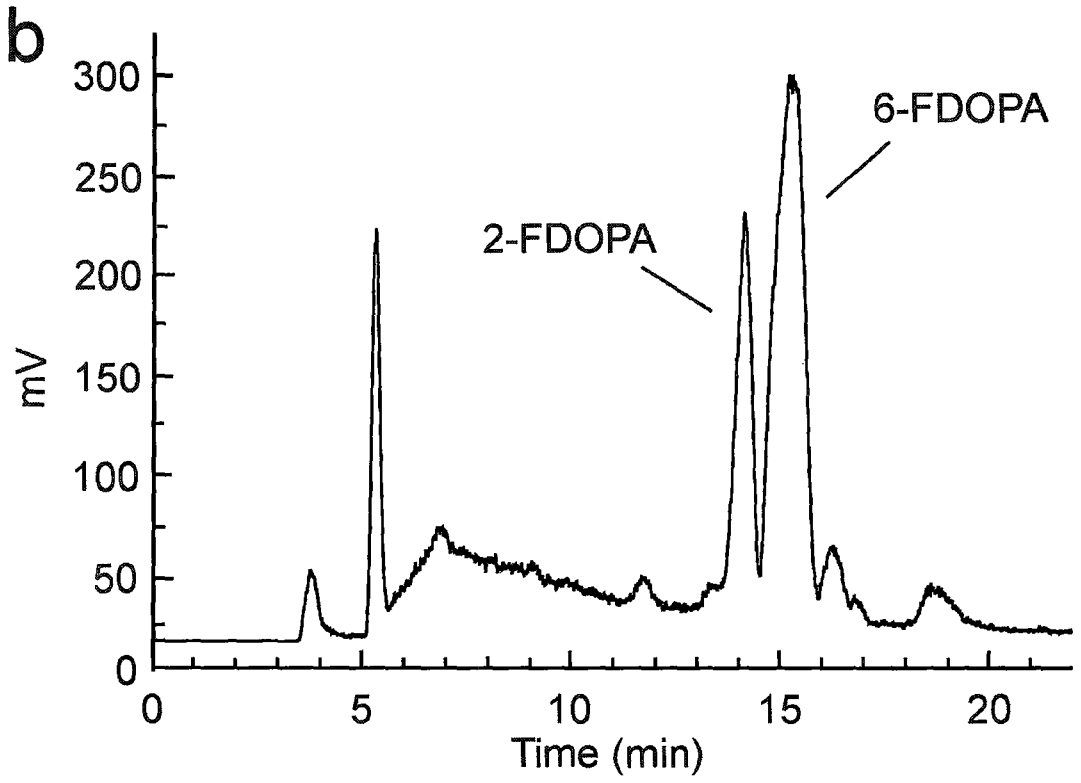
Table 3.1 Radiochemical Yields (RCY) of FDOPA Resulting from Direct Fluorination of DOPA in CF_3SO_3H [a]

Temp (°C)	RCY (%) [b]	Number of Trials	Relative Ratio [c] (2-, 5- and 6-FDOPA)
RT	19.3 ± 2 %	6	38 :15 : 47
4	15.6 ± 3 %	6	36 : 7 : 57
–30	11.4 ± 2 %	2	36 : 5 : 59
–40	14.5 ± 2 %	2	33 : 0 : 67

[a] Reactions were carried out in 99% CF_3SO_3H. [b] Typical synthesis times were 55 min. Radiochemical yields have been decay corrected with respect to $[^{18}F]F_2$. A 2:1 molar ration of DOPA:F_2 was used. [c] Relative intensities were determined from integrated ^{19}F NMR spectra.

Figure 3.1 Typical preparative HPLC radiochromatograms for reaction mixtures resulting from the direct fluorination of L-DOPA in 99% CF_3SO_3H at (a) – 45 °C and (b) 4 °C. Small differences in retention times result from minor variations in mobile phase concentrations or flow rates.


a
6-FDOPA
2-FDOPA
mV
150
100
50
0
0
5
10
15
20
Time (min)

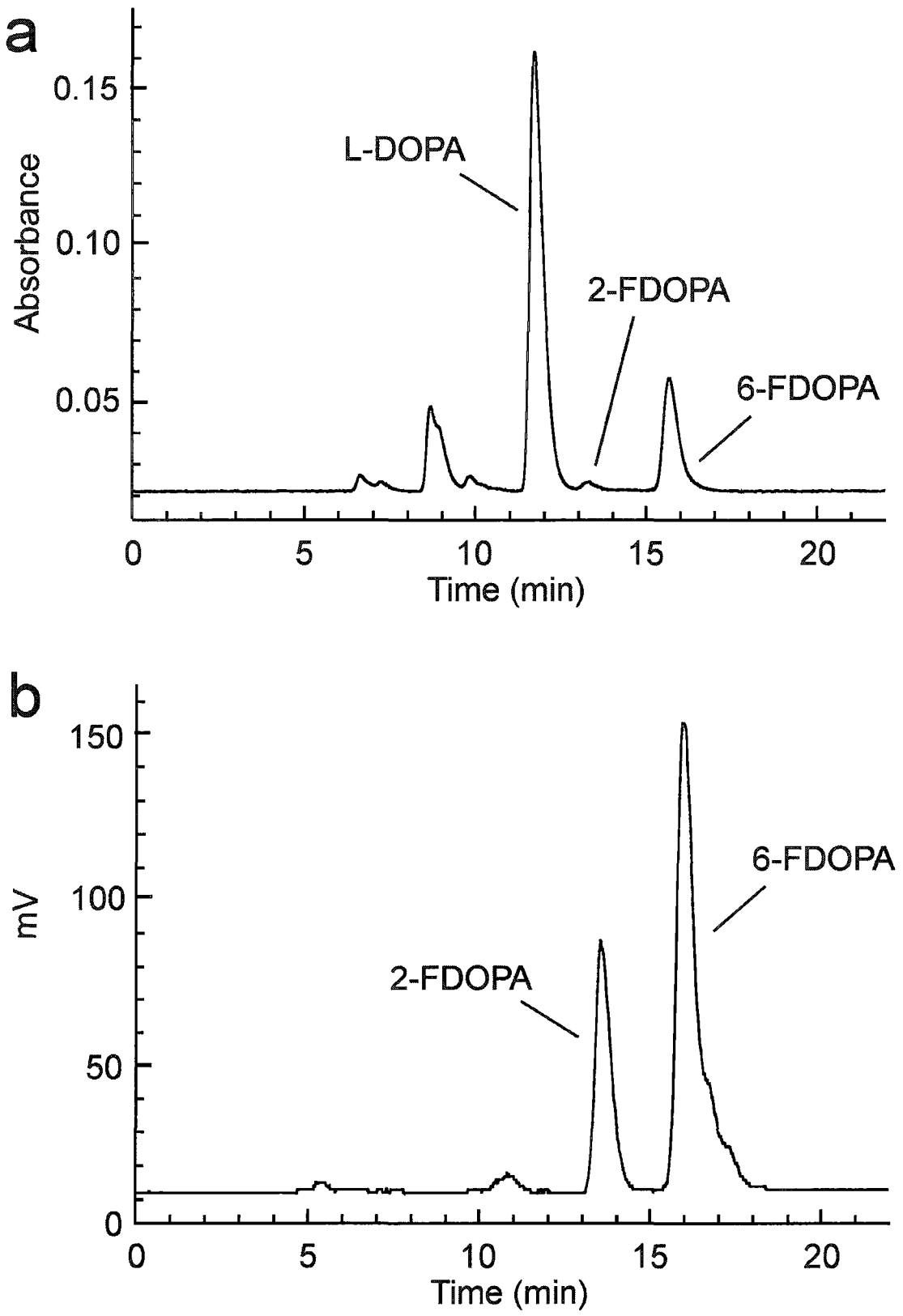

b
6-FDOPA
2-FDOPA
mV
300
250
200
150
100
50
0
0
5
10
15
20
Time (min)

It is noteworthy that the highest radiochemical yield was obtained when a 3:1 molar ratio of DOPA:F_2 was used. A typical fluorination of DOPA in CF_3SO_3H at 4 °C resulted in a radiochemical yield of 19.7% compared to 15.6% when a 1.6:1 molar ratio of DOPA:F_2 was used. The preparative HPLC findings are in agreement with the analytical HPLC UV and radiochromatograms obtained for the products of the direct fluorination of L-DOPA in CF_3SO_3H at –40 °C (Figure 3.2).

Reduction of the number of side products is a major advantage of direct radiofluorination of L-DOPA in CF_3SO_3H (Figures 3.3a and 3.4) over radiofluorinations carried out in BF_3/aHF (Figure 3.3b) or aHF[70] and serves to facilitate the isolation of 6-FDOPA from the final reaction mixture. The use of CF_3SO_3H as the solvent medium resulted in a decay-corrected total radiochemical yield of 19.7% for the FDOPA isomers, which is sufficient for clinical use.

The ^{19}F NMR spectra of 2-FDOPA (–139.6 ppm, broad singlet, $\Delta\nu_{1/2}$ = 16 Hz), 5-FDOPA (–135.6 ppm, doublet, $^3J(F–H_6)$ = 11.3 Hz) and 6-FDOPA (–126.4 ppm, doublet of doublets, $^3J(F–H_5) \approx {}^4J(F–H_2)$ = 9.0 Hz) agreed with those reported in the literature.[75] The peak integrations corresponding to the FDOPA isomers in the ^{19}F NMR spectra matched those of the ^{18}F-labeled FDOPA isomers in the HPLC radiochromatograms. Figure 3.4 shows a typical ^{19}F NMR spectrum after isolation of 6-FDOPA from the final product mixture and Table 3.2 lists the relative intensities of fluorinated species in the aforementioned sample. Higher purity 6-FDOPA samples can be obtained when the corresponding peak in the radiochromatogram is collected at longer HPLC retention times; this, however, results in lower activities.

Figure 3.2 Typical analytical HPLC UV (a) and radiochromatogram (b), for a reaction mixture resulting from the direct fluorination of L-DOPA in 99% CF_3SO_3H at –40 °C.


a
Absorbance
0.15
0.10
0.05
L-DOPA
2-FDOPA
6-FDOPA
0
5
10
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Time (min)
b
mV
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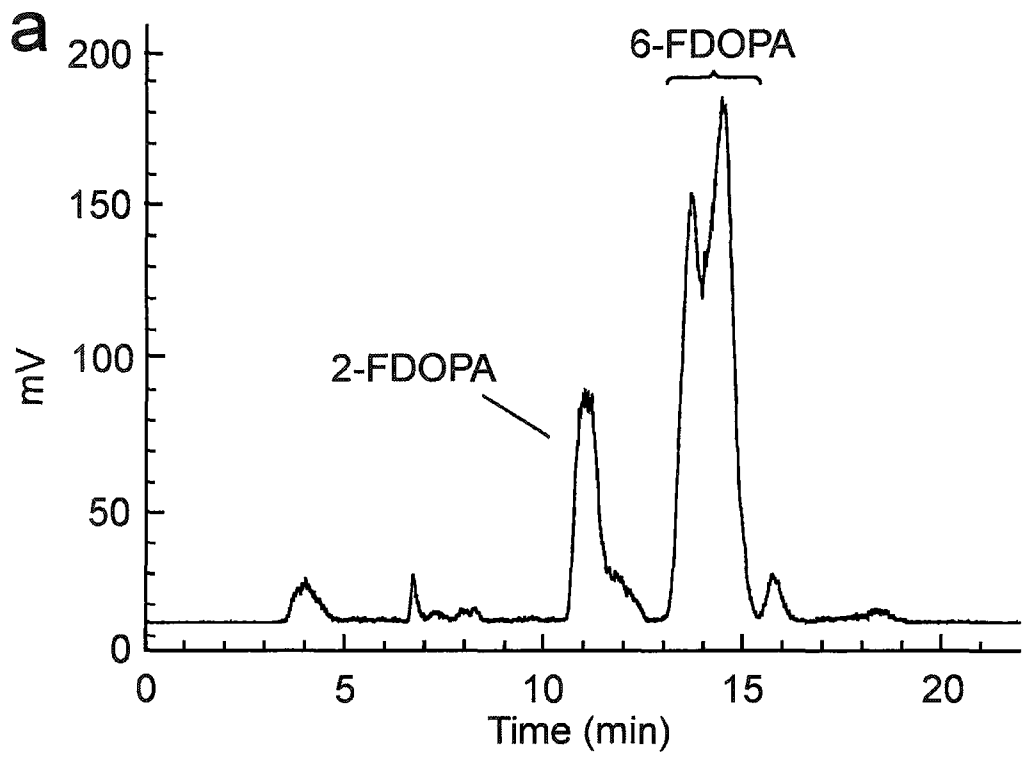
6-FDOPA
2-FDOPA
0
5
10
15
20
Time (min)

Figure 3.3 Typical preparative HPLC radiochromatograms (Fluophase; flow rate, 3.5 mL min^{-1}; mobile phase, 17 mg ascorbic acid in 500 mL of 0.1% acetic acid) for reaction mixtures resulting from the direct fluorination of L-DOPA in (a) CF_3SO_3H at –25 °C and (b) BF_3/HF at –65 °C. Small differences in retention times result from minor variations in mobile phase concentrations or flow rates.


a
6-FDOPA
2-FDOPA
mV
200
150
100
50
0
0
5
10
15
20
Time (min)

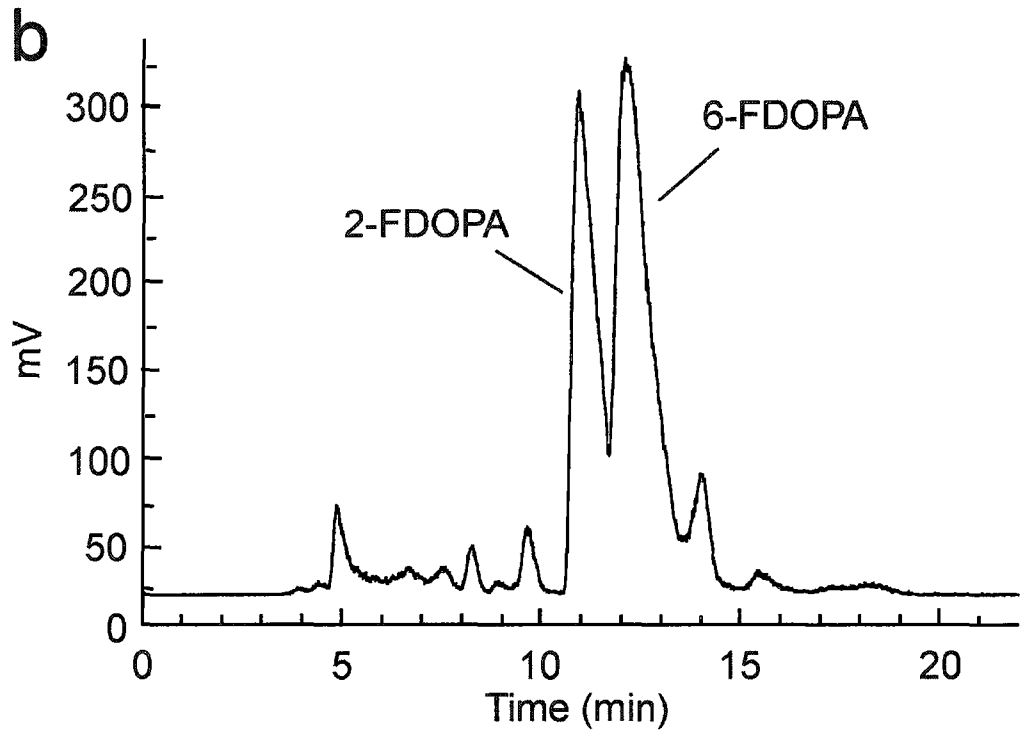

b
6-FDOPA
2-FDOPA
mV
300
250
200
150
100
50
0
0
5
10
15
20
Time (min)

Figure 3.4 Fluorine-19 NMR spectrum of 6-FDOPA isolated from the product mixture using preparative HPLC. Peaks B, C and E correspond to 6-, 5- and 2-FDOPA, respectively. Small amounts of unidentified fluorinated side products were also observed (A and D). The ^{19}F NMR spectrum also showed two singlets at −75.7 (unidentified) and −79.9 ppm (residual CF_3SO_3H) which are not shown.

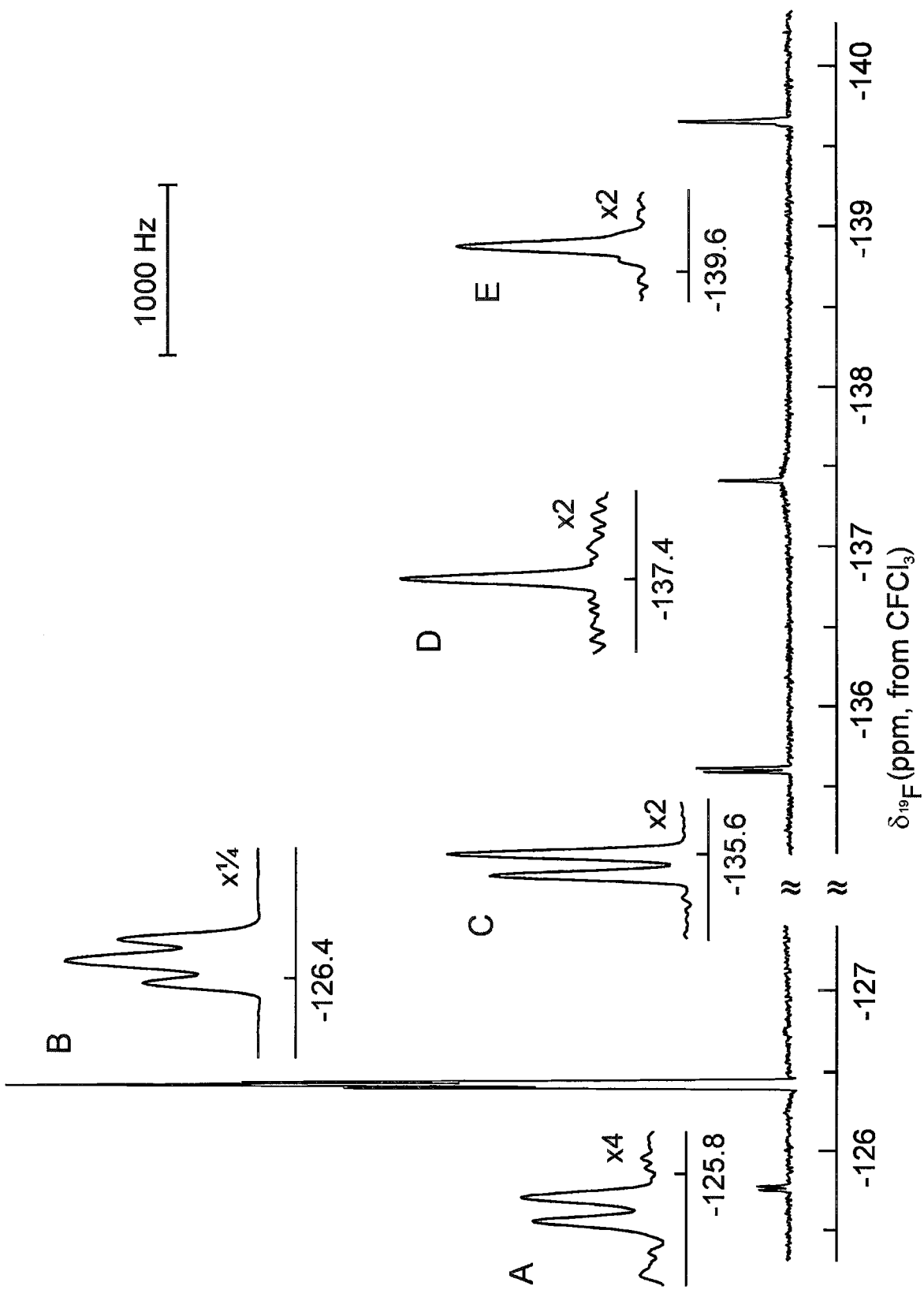

1000 Hz
A
x4
-125.8
B
x¼
-126.4
C
x2
-135.6
D
x2
-137.4
E
x2
-139.6
-126
-127
-136
-137
-138
-139
-140
δ19F(ppm, from CFCl3)

Table 3.2 Relative Intensities of Fluorinated Species in an HPLC-Purified 6-FDOPA Sample Resulting from Direct Fluorination of DOPA in CF_3SO_3H at 4 °C

Fluorinated Product	Relative Ratio [a]
2-FDOPA	7.3
5-FDOPA	6.8
6-FDOPA	79.7
combined unassigned byproducts	6.2

[a] Relative intensities were determined from integrated ^{19}F NMR spectra.

Removal of CF_3SO_3H by evaporation proved to be difficult and time consuming owing to its high boiling point (162 °C) and low vapor pressure (1 Torr at 42 °C).[21] Anion exchange proved to be an efficient method for the removal of CF_3SO_3H over a short time period (< 5 min). Approximately 42 ±2% (decay corrected with respect to $[^{18}F]F_2$) of the theoretical maximum activity was eluted from the column using 30 mL of 0.1 M HCl. The ^{19}F NMR spectrum of the eluent (pH, 4-5) indicated the removal of most of the triflic acid in the reaction mixture. The retention of a small amount of FDOPA remaining on the column (typically < 0.5%) was confirmed by elution of the anion exchange resin with an additional 5 mL of 0.1M HCl followed by HPLC analysis of the eluate. Attempts to recover the remaining FDOPA from the column by using higher HCl concentrations (0.5, 1 and 2M) resulted in the co-elution of CF_3SO_3H with FDOPA.

3.2.2. Other Direct Fluorination Media

3.2.2.1 CF_3SO_3H/HCOOH

Because the direct removal of CF_3SO_3H from the product under dynamic vacuum is problematic owing to its low vapor pressure, solutions of CF_3SO_3H in formic acid were also investigated in an attempt to reduce the amount of CF_3SO_3H. Formic acid has been used as a solvent in the selective fluorination of substrates containing carbon centers of high electron density.[76] In the same study, it was shown that increasing the solvent acidity results in fewer side products and higher yields of 6-FDOPA (Table 3.3). In addition, smaller amounts of 5-FDOPA were produced at higher concentrations of CF_3SO_3H which is significant because 5-FDOPA cannot be fully separated from 6-FDOPA using HPLC, because of their very similar retention times. As a result, CF_3SO_3H is preferred over CF_3SO_3H/HCOOH as the solvent medium for the production of 6-FDOPA.

Table 3.3 Radiochemical Yield (RCY) of FDOPA Resulting from Direct Fluorination of DOPA in CF_3SO_3H/HCOOH and BF_3/CF_3SO_3H

Solvent	Temp (°C)	RCY [a] (%)	Relative Ratio (2-, 5- and 6-FDOPA)
99% CF_3SO_3H	4	19.7	36 : 7 : 57
30% CF_3SO_3H in HCOOH	4	25.8	38 : 26 : 36
20% CF_3SO_3H in HCOOH	-15	8.8	4 : 60 : 36
BF_3/CF_3SO_3H	4	20.5	39 : 4 : 57

[a] Radiochemical yields have been decay corrected with respect to $[^{18}F]F_2$. A 3:1 molar ratio of DOPA:F_2 was used in each reaction.

3.2.2.2 BF_3/CF_3SO_3H

The presence of a Lewis acid, such as BF_3 or AsF_5, in aHF has been shown to increase radiochemical yields of the monofluorinated amino acids in direct fluorinations.[41] In particular, BF_3/HF media have been utilized to significantly improve radiochemical yields of 6-FDOPA.[20] The radiofluorination of L-DOPA in BF_3/CF_3SO_3H at 4 °C did not, however, result in a significantly higher radiochemical yield (Table 3.3). This is in agreement with a previous study by Coenen et al. in which the use of BF_3/CF_3COOH as a solvent medium for the fluorination of phenylalanine did not result in a significant increase in the radiochemical yield when compared with yields obtained in CF_3COOH.[77]

3.3 Conclusions

A fast, efficient and versatile method for the production of 6-fluoro-L-DOPA and 6-fluoro-D-DOPA, in high yields, in CF_3SO_3H has been developed as an alternative to aHF for use in a hospital environment. It has been shown that the direct fluorination of D- and L-DOPA in CF_3SO_3H is a viable method for the production of mono-fluorinated aromatic amino acid isomers in clinically useful quantities. In a typical fluorination reaction, 12±2% mCi of 6-[^{18}F]fluoro-DOPA was produced starting with 200 mCi of [^{18}F]F_2. The radiochemical yield (17 ±2%) obtained from the current method is not only sufficient for investigation of the biochemical properties of 6-[^{18}F]fluoro-D-DOPA using small animal imaging, but is also sufficient for clinical use in human subjects. The

application of 6-fluoro-D-DOPA as a PET tracer for brain tumor imaging is currently under investigation.

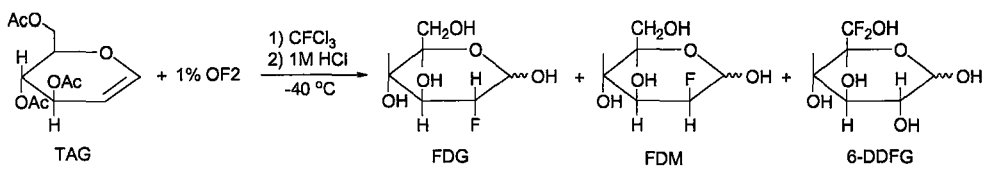
CHAPTER 4

SELECTIVE FLUORINATION OF *m*-TYROSINE BY OF_2

4.1 Introduction

Four fluorides of oxygen have been synthesized and structurally characterized, namely O_4F_2, O_3F_2, O_2F_2, and OF_2, all of which are powerful oxidants and fluorinating agents.[78] It has been shown that the stabilities of the aforementioned compounds decrease with increasing number of oxygen atoms, making OF_2 the most stable oxygen fluoride known.

Since its initial preparation by Lebeau and Damiens in 1927,[79] OF_2 has been utilized as an oxidizing or fluorinating agent in organic and inorganic syntheses. For example, OF_2 has been employed in the oxidation of primary aliphatic amines,[80] fluorination of aromatic systems through fluorodestannylation reactions,[81] and addition reactions, where OF_2 adds across carbon-carbon or sulfur-oxygen double bonds.[82-92] Moreover, the direct fluorination of 3,4,6-tri-*O*-acetyl-D-glucal (TAG) by OF_2 (Eq. 1) has been shown to result in the production of 2-deoxy-2-fluoro-D-glucose (2-FDG). Fluorine-18 labeled 2-FDG is currently the most widely used radiotracer in diagnostic imaging.[91]



(1)

Several synthetic routes have been used to prepare OF_2, including the reaction of fluorine with aqueous alkali solutions,[92] 60% $HClO_4$,[93] H_5IO_6,[94] hydrated alkali fluorides,[95] or HOF.[96] More recently, Satyamurthy et al.[97] reported the formation of $[^{18}F]OF_2$ as a reaction byproduct (up to 5% for double shoot and up to 20% for the single shoot irradiation methods) under the high-pressure and high-energy irradiation conditions of a proton cyclotron gas target using the nuclear reaction, $^{18}O(p,n)^{18}F$ and according to Eq. 2–4. Prior studies from our laboratory have also indicated the presence of $[^{18}F]OF_2$ (up to 5%) in the double shoot irradiation method.[50]

$$O_2 \text{ (stable)} \underset{}{\overset{10 \text{ MeV } H^+}{\rightleftharpoons}} 2O^{\cdot} \quad (2)$$

$$O^{\cdot} + F^{\cdot} \longrightarrow OF^{\cdot} \quad (3)$$

$$OF^{\cdot} + F^{\cdot} \longrightarrow OF_2 \quad (4)$$

The increasing popularity of the "double shoot" method[50,98] for the production of electrophilic agents used for the syntheses of PET imaging agents requires a fuller understanding of the electrophillic fluorination properties of OF_2. Positron Emission Tomography is commonly used for the *in vivo* visualization of brain functions such as blood flow, metabolism, enzyme activity, neuroreceptors and neurotransporters.[55] An important category of PET tracers for brain imaging are ^{18}F-labeled aromatic amino acids such as $[^{18}F]$6-fluoro-3,4,-dihydroxy-L-phenylalanine (6-FDOPA)[56] and $[^{18}F]$6-fluoro-L-*meta*-tyrosine.[99] It is therefore important to investigate the fluorinating ability of OF_2 towards aromatic amino acids and to establish whether or not OF_2 gives rise to other

fluorinated aromatic amino acids occurring in the product mixture that may prove difficult to separate, thus contributing to PET image background noise.

4.2 Results and Discussion

4.2.1 Attempted Synthesis of $[^{18}F]OF_2$

The formation of $[^{18}F]OF_2$ in the gas target of an 11 MeV proton cyclotron in the nuclear reaction, $^{18}O(p,n)^{18}F$, occurs simultaneously with that of $[^{18}F]F_2$, making the separation of the two species very difficult to impossible. Moreover, the isolation of $[^{18}F]OF_2$ has not been reported. In order to quantify the relative amounts of ^{18}F-labeled products that result from the fluorination of aromatic amino acids using OF_2, attempts were made to synthesize and isolate $[^{18}F]OF_2$. The method used in this study involved the direct fluorination of anhydrous $KF/KF{\cdot}2H_2O$ in micromolar quantities and is a modification of the gram-scale synthesis reported by Pullen et al..[95] Parameters reported by these workers could not be replicated in our laboratory owing to the scale and reaction conditions required for the production of radiotracers having applications in diagnostic imaging. Formation of OF_2 can be confirmed by its reaction with TAG in $CFCl_3$ which, unlike the reaction of TAG with F_2, results in the formation of 6-deoxy-6,6-difluoro-D-glucose (6-DDFG).[91] The attempted syntheses did not, however, result in OF_2 formation which was confirmed by the absence of 6-DDFG in the ^{19}F NMR spectra when the effluent gas was passed through $CFCl_3$ solutions of TAG. Consequently, fluorinations of the aromatic systems investigated in this study were carried out using unlabeled OF_2.

4.2.2 Solvent and Isomer effects

Solvent acidity has been shown to play an important role in the direct fluorination reactions of aromatic amino acids.[100] In attempts to find a suitable solvent medium for the fluorination of aromatic amino acids by OF_2, the effect of solvent acidity was also studied using *m*-tyrosine (MT) as the substrate. Direct fluorination of MT using F_2 results in the formation of 2-, 4-, 5- or 6-fluoro-*m*-tyrosine (6-FMT), the degree of which varies with solvent acidity.[42]

Several protic solvent media, namely, aHF, CF_3SO_3H, CF_3COOH, CH_3COOH, HCOOH and H_2O, were utilized for the fluorination of MT (Table 4.1).

Table 4.1 Effect of Solvent Acidity on the Fluorination of *m*-Tyrosine by OF_2

Solvent	Temp (°C)	Product	Relative Amounts (2:4:6)	Yield ±0.04% [a]	Fluorinated Byproducts [b] (%)
H_2O	23	2-, 4-, and 6-FMT	33 : 20 : 47	4.35	0
HCOOH	23	2-, 4-, and 6-FMT	26 : 8 : 66	2.17	60
CH_3COOH	23	2- and 6-FMT	30 : 0 : 70	2.19	67
CF_3COOH	23	2- and 6-FMT	30 : 0 : 70	2.60	74
CF_3SO_3H	23	2- and 6-FMT	9 : 0 : 91	1.41	85
aHF	–60	FMT not formed	–	–	–

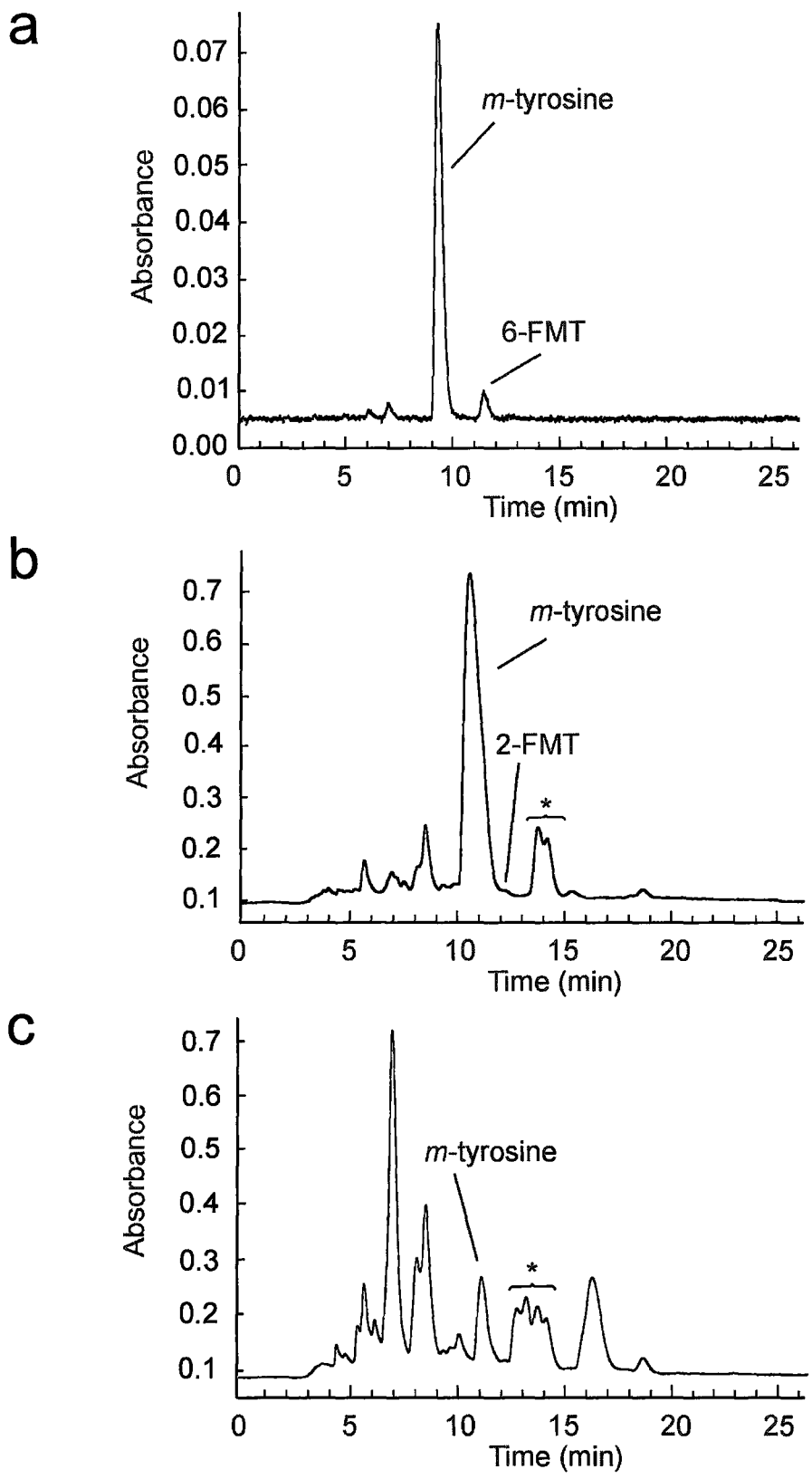
[a] Yields are reported with respect to OF_2. [b] Percentages of fluorinated byproducts were calculated from the total integrated peak intensities in ^{19}F NMR spectra.

It was shown that, unlike F_2, fluorination by OF_2 was less efficient in superacidic media. Fewer byproducts and higher ratios of fluorinated MT isomers were formed in solvent media of lower acidity as determined by analytical HPLC (Figure 4.1). After analysis of

the reaction mixtures by ^{19}F NMR spectroscopy, each sample was spiked with known quantities (1.03 and 2.07 ±0.04 μmol) of the internal standard, *m*-fluoro-DL-tyrosine. Fluorine-19 NMR peak integrations of the spiked reaction mixtures were then used to calculate reaction yields (Table 4.1). It was shown that the highest yield (4.35 ±0.04%) and lowest amounts of fluorinated byproducts were obtained when H_2O was used as the solvent for fluorination of MT with OF_2. The ^{19}F NMR spectrum of 6-FMT (–129.3 ppm, complex multiplet), 4-FMT (–138.4 ppm, doublet of doublet of doublets, $^3J(F–H_5) = 11.3$ Hz, $^4J(F–H_6) = 4.2$ Hz, $^4J(F–H_2) = 7.4$ Hz), and 2-FMT (–142.2 ppm, doublet of doublets, $^4J(F–H_4) \approx {}^4J(F–H_6) = 7.1$ Hz) agree with those reported in the literature.[42]

The effect of the electron donating OH substituent at the *ortho-*, *meta-* and *para-* positions on the fluorination of aromatic amino acids by OF_2 was studied using *o-*, *m-* and *p*-tyrosine as the substrates. Contrary to expectations, MT was the only isomer shown by ^{19}F NMR spectroscopy and HPLC to be fluorinated by OF_2, showing that OF_2 is, in this instance, a highly selective fluorinating agent.

Figure 4.1 UV chromatograms for reaction mixtures resulting from the fluorination of MT at 23 °C in (a) H_2O, (b) CH_3COOH and (c) CF_3COOH. The asterisk (*) denotes peaks corresponding to 4- and 6-FMT isomers which cannot be specifically assigned because of overlap, and more intense byproduct peaks.


a
Absorbance
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
m-tyrosine
6-FMT
0
5
10
15
20
25
Time (min)
b
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
m-tyrosine
2-FMT
*
0
5
10
15
20
25
Time (min)
c
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
m-tyrosine
*
0
5
10
15
20
25
Time (min)

4.2.3 Fluorination by OF_2

In order to investigate the suitability of OF_2 as a fluorinating agent for aromatic amino acids, several systems were studied in which the electron density in the π-system was directed towards different ring sites using electron withdrawing and electron donating ring substituents. The solvent medium used for this study was H_2O because it produced the highest isomeric ratios of 6- and 4-fluoro-*m*-tyrosine and smallest amounts of fluorinated byproducts in the fluorination of MT.

Initially, L-phenylalanine, which contains the *ortho*- and *para*-directing electron donating alanine group, was examined. Although direct fluorination of L-phenylalanine results in the formation of 2-, 3-, and 4-fluoro-L-phenylalanine isomers,[101] analytical HPLC and ^{19}F NMR spectroscopy indicated that fluorination with OF_2 did not result in the formation of fluorinated phenylalanine.

The fluorination of DL-tyrosine, which contains two electron donating (alanine and hydroxyl) groups, was also studied with the aim to further localize the electron density in the π-system in order to promote the electrophilic fluorination by enhancing the nucleophilicity of the phenyl ring at the activated sites. The direct fluorination of DL-tyrosine has been shown to result in the formation of 3-fluoro-DL-tyrosine,[102] however, fluorination using OF_2 did not yield any fluorinated tyrosine isomers.

Compounds with greater electron localization in the π-system were also investigated by the use of additional hydroxyl substituents (L-DOPA) and utilization of the stronger electron donating CH_3O-group (3-*O*-methyl-L-DOPA and dimethoxy-L-DOPA). As previously reported, direct fluorination of L-DOPA in non-superacidic

solvent media results in the formation of 2- and 5-FDOPA,[101] whereas direct fluorination of 3-*O*-methyl-L-DOPA and dimethoxy-L-DOPA results in the formation of 3-*O*-methyl-6-fluoro-L-DOPA and 2-, 5- and 6-fluoro-dimethoxy-L-DOPA isomers.[41] The fluorination of L-DOPA, 3-*O*-methyl-L-DOPA and dimethoxy-L-DOPA by OF_2, however, did not result in any fluorinated aromatic products as indicated by analytical HPLC and ^{19}F NMR spectroscopy. Similar results were obtained when electron withdrawing NO_2 substituted derivatives were used as substrates, namely, 3-nitro-L-tyrosine and 4-nitro-DL-phenylalanine.

4.3 Conclusion

The effect of solvent acidity on the fluorination of MT using OF_2 was studied and it was shown that, in contrast with the reactivity of F_2 in superacids, OF_2 is a more efficient fluorinating agent in less acidic solvent media. The use of H_2O as the solvent medium for fluorination of MT resulted in the formation of FMT isomers in 4.35 ±0.04% yield.

The potential use of OF_2 as a fluorinating agent for aromatic amino acids was also investigated in the cases of L-phenylalanine, 3-nitro-L-tyrosine, 4-nitro-DL-phenylalanine, 3,4-dihydroxyphenyl-L-alanine (L-DOPA), 3-*O*-methyl-L-DOPA, 3,4-dimethoxy-L-phenylalanine, *m*-, *p*- and *o*-tyrosine. In these studies, the only aromatic system fluorinated by OF_2 was MT. These results indicate that the presence of $[^{18}F]OF_2$ (up to 20%) as a major byproduct resulting from the nuclear reaction, $^{18}O(p,n)^{18}F$, does

not have a negative impact on the syntheses of radiofluorinated aromatic amino acids having applications in PET imaging.

CHAPTER 5

SYNTHESES OF 2-, 4- AND 6-FLUORO-*m*-TYROSINE IN H_2O

5.1 Introduction

The growing interest in the utilization of biologically active compounds in oncology and, more specifically, medical imaging, using non-invasive techniques such as PET, has motivated the invention of alternative synthetic routes to imaging agents that involve fewer intermediate steps or less hazardous starting materials. Ever since the pioneering work of Dr. E. S. Garnett who used [^{18}F]6-FDOPA in conjunction with PET to visualize the dopaminergic pathways in the human brain,[56] PET has been commonly used for the *in vivo* visualization of brain functions such as blood flow, metabolism, enzyme activity, neuroreceptors and neurotransporters.[55]

It has been shown that *m*-tyrosine is a good substrate for AADC with a decarboxylation rate similar to that of L-DOPA.[103] Unlike L-DOPA, however, *m*-tyrosine is not a substrate for COMT, making the aforementioned an ideal tracer for investigating the AADC activity in the brain.[104] Both 6- and 4-fluoro-*m*-tyrosine isomers offer a nonpharmacological alternative to the use of COMT inhibitors. After injection, the radiofluorinated *m*-tyrosine analogs accumulate selectively in striatal structures and allow for the detection of additional innervation sites, such as brain stem, which are rich in AADC. In addition, high signal-to-noise ratios are observed on PET scans when using either 4- or 6-FMT, making the aforementioned outstanding candidates for probing the dopaminergic mechanisms in the human brain.[105]

Prior work from this laboratory has shown that direct electrophilic fluorination of *m*-tyrosine in aHF using F_2 produced 2-, 4- and 6-FMT having a total radiochemical yield of 43% (decay corrected with respect to $[^{18}F]F_2$),[42] which is excellent for electrophilic radiofluorinations because the theoretical maximum radiochemical yield is 50% with respect to $[^{18}F]F_2$. The highly efficient fluorination of *m*-tyrosine is partly the result of the low reaction temperatures employed and the solvent polarity, which favor electrophilic substitution reactions. In addition, aromatic substrates in aHF are less susceptible to oxidation by F_2 because the catechol oxygens are protonated.[6] Chambers et al.[4] have shown that protic solvents promote electrophilic fluorination of aromatic compounds and that the reactivity of F_2, as an electrophile, varies significantly with solvent acidity. Prior work from this laboratory, have also shown the regioselectivities of aromatic electrophilic fluorinations to be dependent upon the acidity of the reaction medium.

In Chapter 3, CF_3SO_3H was shown to be a suitable solvent medium for the syntheses of 6-fluoro-D-DOPA and 6-fluoro-L-DOPA by fluorination of D- and L-DOPA. In this work we describe alternative routes to the production of $[^{18}F]$fluoro-*m*-tyrosine isomers by the direct radiofluorination of *m*-tyrosine in CF_3SO_3H and H_2O solvent media.

5.2 Results and Discussion

5.2.1. CF_3SO_3H as a Direct Fluorination Medium

Previous studies from our laboratory showed the improved regioselectivity of direct fluorination of aromatic amino acids with increased solvent acidity, indicating aHF

and aHF/BF_3 to be optimal for such reactions.[42] Due to the hazards associated with handling of aHF, however, an alternative solvent medium, CF_3SO_3H, was found to be suitable for production of clinically useful quantities of PET tracers such as 6-[^{18}F]fluoro-L-DOPA (Chapter 3). The versatility of this synthetic approach was shown in the production of 6-[^{18}F]fluoro-D-DOPA (Chapter 3). Therefore, CF_3SO_3H was the first solvent choice for the direct fluorination of *m*-tyrosine, which resulted in the production of 2-, 4- and 6-[^{18}F]FMT isomers in a 19.9 ±3 radiochemical yield. Because of the decreased solvent acidity, from aHF to CF_3SO_3H, higher isomeric ratios of 4-FMT and lower isomeric ratios of 6-FMT are obtained when CF_3SO_3H was employed. Although the radiochemical yield was lower than that obtained in aHF, it was still sufficient for the production of clinically useful quantities of 4- and 6-FMT tracers.

5.2.2. H_2O as a Direct Fluorination Medium

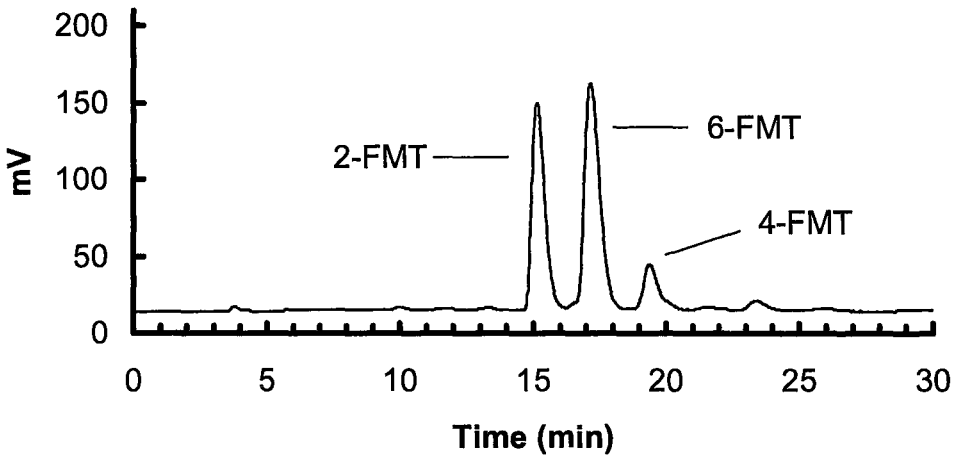
Considering that syntheses of FMT isomers were investigated in superacidic solvent media, it was also necessary and informative to study their syntheses in a very weakly acidic solvent media such as H_2O. Water has been used in a variety of reactions such as nucleophilic additions, Claisen rearrangements, photochemical aromatic nucleophilic substitutions, Barbier-type reactions, transition metal complex catalyzed reactions, and Diels-Alder reactions.[106] There have also been reports on syntheses of fluorinated biomolecules, such as 5-fluorouracil, 2-FDG, and 4-fluoroantipyrine, by direct fluorination of the respective precursors in H_2O.[107,108] If proven successful, utilization of H_2O as solvent medium, for the direct fluorination of *m*-tyrosine and the

subsequent syntheses of 4- and 6-FMT, would be ideal, circumventing the use of "exotic" and potentially hazardous solvent media in hospital environments.

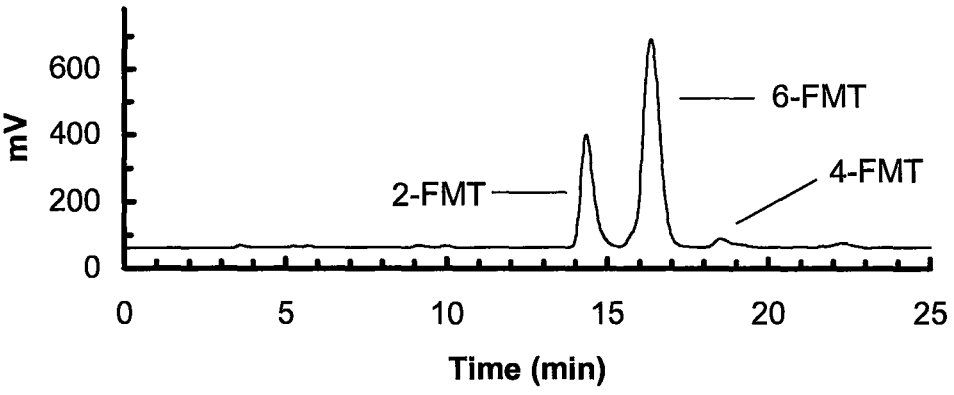
The direct fluorination of *m*-tyrosine, carried out in H_2O at 4 °C, resulted in the production of 2-, 4- and 6-FMT isomers in 22.0 ±8 % yields. Figure 5.1 shows typical analytical HPLC radiochromatograms that result from the direct fluorination of MT in aHF, CF_3SO_3H and H_2O. These radiochromatograms indicate the formation of more fluorinated by-products in H_2O, at lower reaction temperatures, compared to that in CF_3SO_3H and aHF solvent media. The identity of the intermediate fluorinating agent generated in these reactions is unclear. It is well established that the reaction of elemental fluorine with water leads to the formation of HOF,[109] which could act as a fluorinating agent in the present suite of reactions. Further investigations are required in order to clarify the reaction pathway.

Figure 5.1 Typical analytical HPLC radiochromatograms of FMT reaction mixtures when the direct fluorination of MT was carried out in (a) HF at –20 °C, (b) CF_3SO_3H at –18 °C and (c) H_2O at 4 °C. The asterisk (*) denotes unassigned fluorinated species.

a


200
150
100
50
0
mV
2-FMT
6-FMT
4-FMT
0
5
10
15
20
25
30
Time (min)

b


600
400
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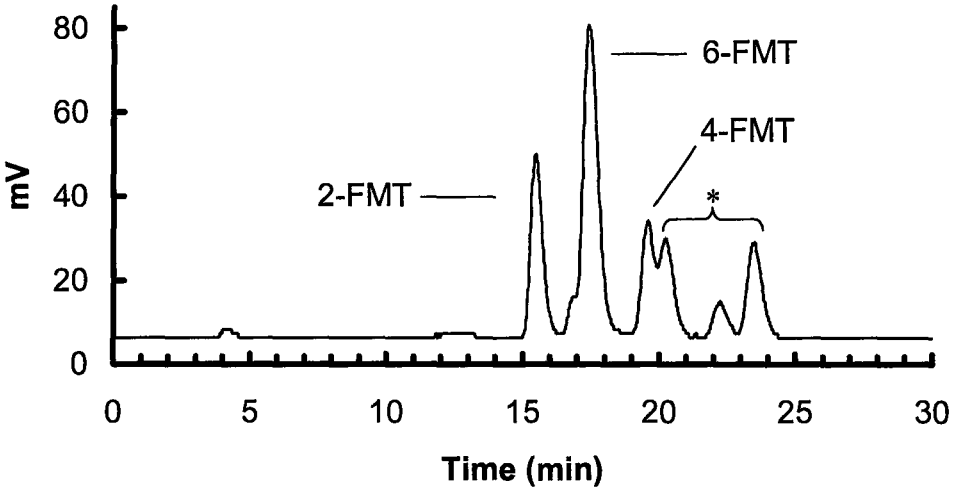
mV
2-FMT
6-FMT
4-FMT
0
5
10
15
20
25
Time (min)

c


80
60
40
20
0
mV
2-FMT
6-FMT
4-FMT
*
0
5
10
15
20
25
30
Time (min)

5.2.3. Effect of Temperature on the Direct Fluorination of MT

In Chapter 3, the direct fluorinations of D- and L-DOPA and the subsequent production of 6-FDOPA were shown to be kinetically favoured as higher isomeric ratios and yields of 6-FDOPA were obtained at lower reaction temperatures. Attempts were therefore made to investigate the presence of similar temperature effects on the direct fluorination of MT in both H_2O and CF_3SO_3H solvents. However, no such trend was observed for the formation of FMT isomers resulting from the direct fluorination of MT in H_2O (Table 5.1). Figure 5.2 shows typical analytical HPLC radiochromatograms of FMT reaction mixtures resulting from the fluorination of MT at varying temperatures.

Unlike the direct fluorination of MT in H_2O, direct fluorination of MT in CF_3SO_3H resulted in the formation of more fluorinated by-products at higher reaction temperatures. Optimal reaction conditions in H_2O were achieved when the direct fluorination of MT was carried out at 50 °C, resulting in the formation of 2-, 4- and 6-FMT isomers in 27.4 ±6 % yields. The direct fluorination of MT in CF_3SO_3H, however, was optimal when the reaction was carried out at –18 °C resulting in the syntheses of 2- and 6-FMT isomers in 19.9 ±3 % radiochemical yields.

Figure 5.3 shows typical ^{19}F NMR spectra of HPLC purified reaction mixtures resulting from the direct fluorination of MT in H_2O at 4 °C and 50 °C. This data further confirmed the formation of fluorinated by-products at lower reaction temperatures. The ^{19}F NMR signal appearing at -137.4 ppm on spectrum (a), may correspond to either difluorinated MT species or the novel 5-FMT isomer, neither of which have been well studied and characterized up to date. If proven successful, this methodology may provide

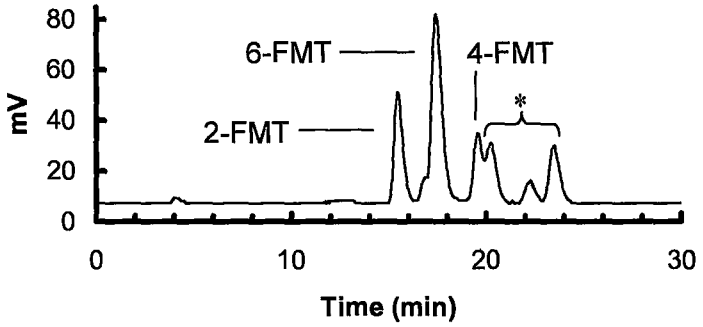
Table 5.1 Radiochemical yields and relative isomeric ratios from variable-temperature fluorinations of *m*-tyrosine

Solvent	Temp (°C)	RCY [a] (%)	Trials	Isomeric FMT Ratio (2:4:6) [b]
CF_3SO_3H	−18	19.9 ±3	2	34 : 0 : 66
	90	28.6 ±3	2	36 : 10 : 54
H_2O	4	22.0 ±8	3	39 : 25 : 36
	23	23.3 ±3	4	41 : 22 : 37
	50	27.4 ±6	4	36 : 29 : 35
	80	19.1 ±3	4	38 : 24 : 38

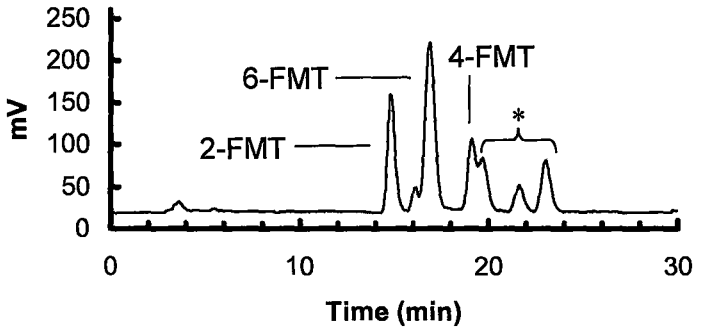
[a] Yields are reported with respect to $[^{18}F]F_2$. [b] Percentages of fluorinated byproducts were calculated from the total integrated peak intensities in ^{19}F NMR spectra.

Figure 5.2 Typical analytical HPLC radiochromatograms of the FMT reaction mixtures when the direct fluorination of MT was carried out in H_2O at (a) 4 °C, (b) 23 °C, (c) 52 °C and (d) 80 °C. The asterisk (*) denotes unassigned fluorinated species.

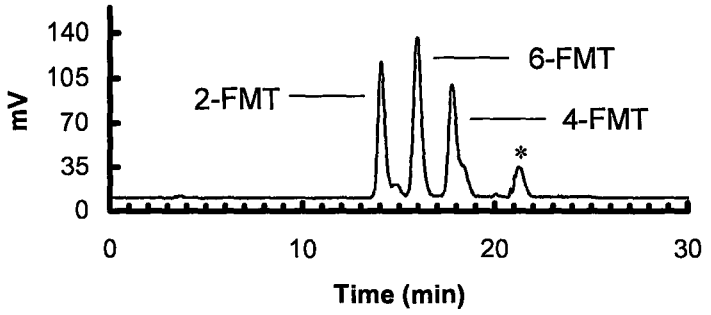
a


80
60
40
20
0
mV
6-FMT
4-FMT
2-FMT
*
0
10
20
30
Time (min)

b


250
200
150
100
50
0
mV
6-FMT
4-FMT
2-FMT
*
0
10
20
30
Time (min)

c


140
105
70
35
0
mV
2-FMT
6-FMT
4-FMT
*
0
10
20
30
Time (min)

d

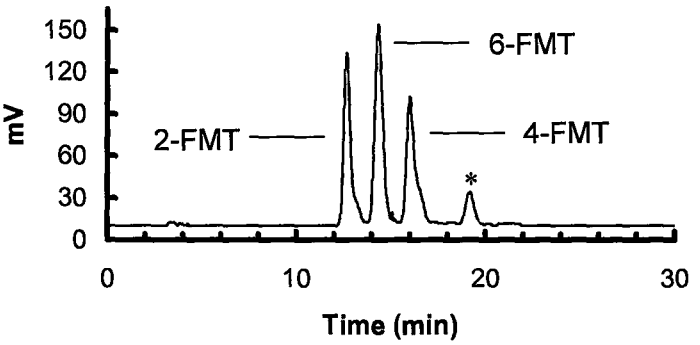
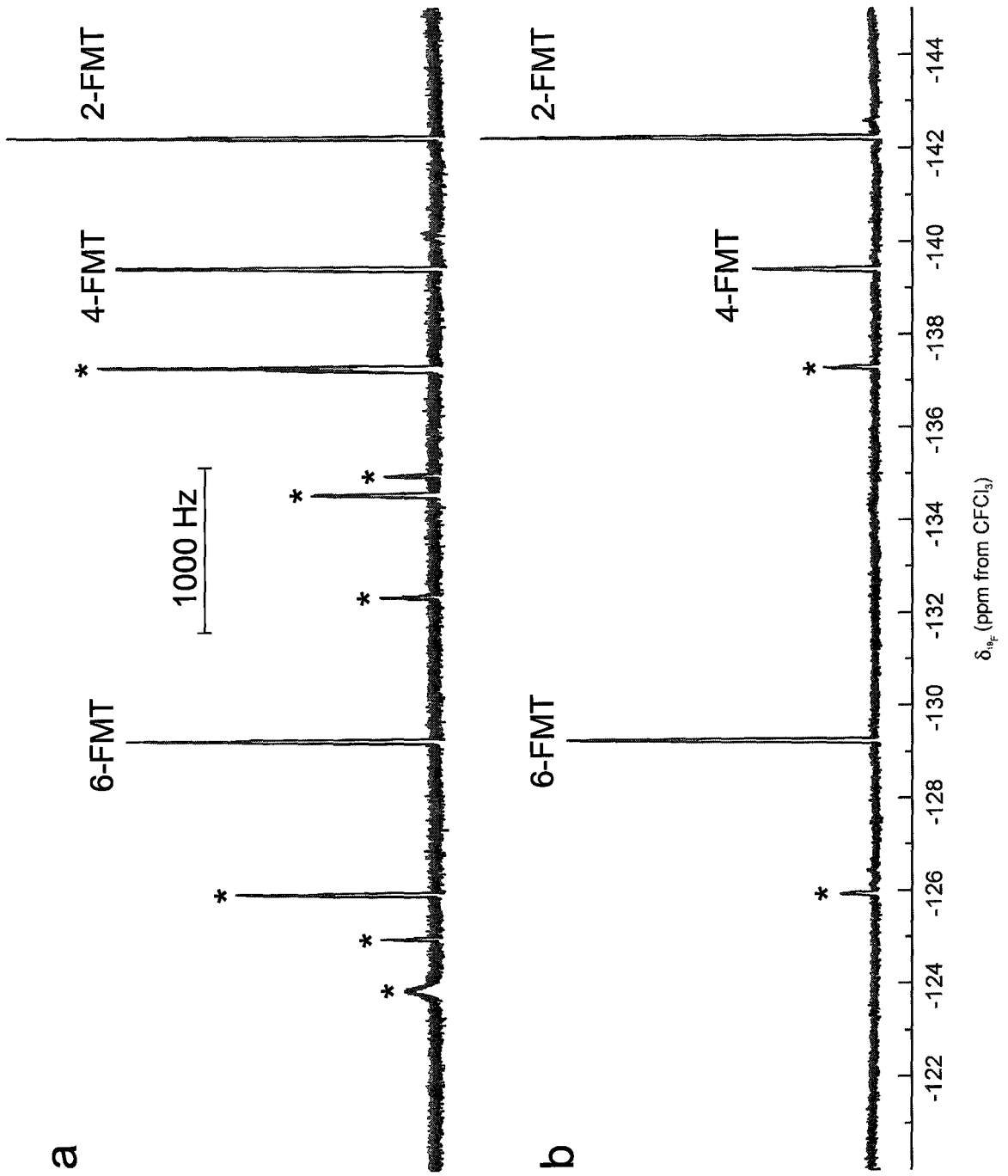

150
120
90
60
30
0
mV
2-FMT
6-FMT
4-FMT
*
0
10
20
30
Time (min)

Figure 5.3 Typical ^{19}F NMR spectra of the FMT reaction mixtures when the direct fluorination of MT was carried out in H_2O at (a) 4 °C and (b) 50 °C. The asterisk (*) denotes unassigned fluorinated species.


a
b
2-FMT
4-FMT
6-FMT
1000 Hz
-122
-124
-126
-128
-130
-132
-134
-136
-138
-140
-142
-144
$\delta_{^{19}F}$ (ppm from $CFCl_3$)

the first synthetic route to clinically useful quantities of 5-FMT, or the syntheses of new difluorinated MT species.

Aside from the production of clinically useful quantities of 4- and 6-FMT tracers, the developed synthetic procedures can also be utilize in studying the biochemistry of 2-, 4- and 6-FMT isomers, both *in vivo* and *in vitro*, as 2-FMT is also produced in useful quantities.

5.3 Conclusions

The aim of this project was to find a more easily handled and/or less hazardous solvent medium, other than HF, that can be used for the fluorination of MT. To that end, it has been shown that the direct fluorination of MT in CF_3SO_3H and H_2O are quick and efficient synthetic procedures for the production of clinically useful quantities of 2-, 4- and 6-FMT. The syntheses of 4- and 6-FMT were optimal in H_2O when the direct fluorination of *m*-tyrosine was carried out at 50 °C giving yields of 27.4 ±6 % for 2-, 4- and 6-FMT. Direct fluorination of MT in CF_3SO_3H, however, was optimal at –18 °C giving yields of 19.9 ±3 % for 2- and 6-FMT.

CHAPTER 6

SUMMARY AND DIRECTIONS FOR FUTURE RESEARCH

6.1 Summary

The present work focused on electrophilic fluorination of aromatic amino acids with applications in diagnostic imaging by means of PET. Utilization of CF_3SO_3H as the solvent medium was shown to be a reliable and efficient method for the direct fluorination of D- and L-DOPA resulting in the syntheses of 6-fluoro-D-DOPA and 6-fluoro-L-DOPA in radiochemical yields of 19.7 ± 2 %. In addition, 6-FDOPA was shown to be a kinetically favored product as higher isomeric ratios and yields were obtained at lower reaction temperatures. A methodology was developed for the removal of CF_3SO_3H from the reaction mixture using acetate based anion exchange columns. The 6-FDOPA isomer was isolated by preparative HPLC and further analyzed using ^{19}F NMR spectroscopy. Radiochemical yields are sufficient for use in human patients and, as well, for small animal imaging studies. This new methodology allows the production of ^{18}F-labelled aromatic amino acids without using aHF which requires costly equipment and significant expertise in its storage and handling.

The ability of OF_2, formed as an impurity in the production of $[^{18}F]F_2$ using the nuclear reaction $^{18}O(p,n)^{18}F$, as a fluorinating agent of aromatic amino acids was investigated. Among the aromatic amino acids (L-phenylalanine, DL-tyrosine, *o*-tyrosine, *m*-tyrosine, L-DOPA, 3-*O*-methyl-L-DOPA, dimethoxy-L-DOPA, 3-nitro-L-tyrosine and 4-nitro-DL-phenylalanine) studied, only *m*-tyrosine was fluorinated by OF_2 resulting in

the formation of 2-, 4- and 6-FMT. The effect of solvent acidity on fluorination by OF_2 was investigated and it was shown that, unlike F_2, fluorinations by OF_2 are more regioselective in less acidic solvent media, such as H_2O. Fluorination of m-tyrosine by OF_2 was optimal in H_2O at 23 °C resulting in the syntheses of FMT isomers in a yield of 4.75 ±0.04%. It was concluded that the presence of OF_2 as a major impurity in the nuclear reaction, $^{18}O(p,n)^{18}F$, has no negative impact on fluorination of aromatic amino acids.

The present work also investigated the direct fluorination of *m*-tyrosine in H_2O and CF_3SO_3H as alternative solvent media to aHF. It was shown that utilization of these solvents resulted in the syntheses of 2-, 4- and 6-FMT in clinically useful quantities. The radiofluorination MT in H_2O at 50 °C resulted in the syntheses of FMT isomers in 27.4 ±6% yields. The use of H_2O as solvent medium for the direct fluorination of *m*-tyrosine is a facile and efficient synthetic method for the syntheses of the PET tracers [^{18}F]6-FMT, [^{18}F]4-FMT and [^{18}F]2-FMT.

6.2 Directions for Future Research

Attempts should be made to identify the enantiomeric purity of 6-fluoro-D-DOPA and 6-fluoro-L-DOPA after direct fluorination of D- and L-DOPA in CF_3SO_3H. The aim of this study will be to investigate the possibility of racemization during the fluorination reaction which may result in lowered signal to noise ratios on PET images.

In order to further study the fluorinating abilities of OF_2, it would be helpful to develop a methodology for ^{18}F-labeling of OF_2 or the isolation of [^{18}F]OF_2 from [^{18}F]F_2

formed simultaneously in the cyclotron gas target. Once [^{18}F]OF_2 has been isolated, it can also be used to label *m*-tyrosine in an attempt to calculate the resulting radiochemical yield. Suggestions for improving the existing apparatus include the use of larger quantities of $KF{\cdot}2H_2O$ and longer reaction periods.

Attempts should also be made to identify the major impurities in the FMT reaction mixture resulting from the direct fluorination of MT in H_2O. These major impurities may be the difluorinated MT compounds, which have not been isolated and well studied up to date. The isolation of the aforementioned will also provide the means to study their ability as PET tracer for tumor imaging and to build a comparison with the reported monofluorinated species.

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