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RADIOSYNTHESIS AND USE OF [^{18}F]F_2 DERIVATIVES [^{18}F]SELECTFLUOR *BIS*(TRIFLATE) AND [^{18}F]ClF

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"The Art of Adventure

Be unfraid of new ideas,

new theories and new philosophies

have the curiosity to experiment …

to test and try new ways

of living and thinking.

(Wilfred AS. Peterson)

To my boys

ABSTRACT

Anna Kirjavainen

RADIOSYNTHESIS AND USE OF [^{18}F]F_2 DERIVATIVES [^{18}F]SELECTFLUOR *BIS*(TRIFLATE) AND [^{18}F]ClF

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Positron emission tomography (PET) is a non-invasive functional and metabolic imaging technique that allows the quantification of specific biological and pharmacological processes in humans and animals *in vivo*. PET uses molecules labeled with short-lived positron (β^+) emitters, such as ^{18}F.

Although fluorine is rare in natural compounds, medicinal fluorine chemistry is based on the fluorination of natural compounds or their close derivatives. The oxidizing strength of fluorine is high and easily leads to exothermic radical chain reactions and the formation of undesirable side products. Because of the vigorous reactivity of elemental fluorine, electrophilic radiofluorination often has low regioselectivity and poor yield. Therefore, one goal in electrophilic radiofluorination is to develop a non-hazardous electrophilic fluorine source with less reactivity and better selectivity.

The [^{18}F]F_2 derivatives [^{18}F]Selectfluor *bis*(triflate) ([^{18}F]SF) and [^{18}F]ClF were synthesized with high specific activity and their use demonstrated in electrophilic synthesis of model molecules. Two precursors of 6-[^{18}F]FDOPA, stannylated and boronic ester compounds, were labeled using [^{18}F]SF. [^{18}F]NS12137, a norepinephrine transporter (NET)-selective tracer for PET imaging, was fluorinated with two electrophilic labeling agents, [^{18}F]SF and [^{18}F]F_2, using a nucleophilic approach. A method to produce [^{18}F]ClF via [^{18}F]F_2 was developed and its use demonstrated with the electrophilic addition of [^{18}F]ClF to a C-C double bond.

Keywords: Fluorine-18, electrophilic substitution, electrophilic addition, [^{18}F]Selectfluor *bis*(triflate), [^{18}F]monochloro fluoride.

TIIVISTELMÄ

Anna Kirjavainen

[^{18}F]F_2-JOHDANNAISTEN [^{18}F]SELECTFLUOR *BIS*(TRIFLAATIN) JA [^{18}F]ClF:N SYNTEESI JA KÄYTTÖ

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Positroniemissiotomografia (PET) on kajoamaton kuvantamismenetelmä, jolla voidaan tutkia biologisia ja farmakologisia prosesseja elävissä ihmisissä ja eläimissä. PET käyttää yhdisteitä, joihin on liitetty lyhytikäinen positroni (β^+) säteilijä, kuten ^{18}F.

Lääketieteellinen fluorikemia perustuu luonnonyhdisteiden tai niiden johdosten fluoraukseen, vaikka luonnonyhdisteissä fluori onkin harvinainen. Fluorin hapetuskyky on korkea, mikä johtaa helposti lämpöä vapauttaviin radikaaliketjureaktioihin ja epätoivottujen sivutuotteiden muodostumiseen. Alkuaine fluorin voimakkaasta reaktiivisuudesta johtuen elektrofiilisessa radiofluorauksessa saavutetaan usein huono paikkaselektiivisyys ja matala saanto. Tästä johtuen elektrofiilisessa radiofluorauksessa on tavoitteena kehittää helpommin käsiteltäviä ja vähemmän reaktiivisia elektrofiilisen fluorin lähteitä, joilla saavutetaan myös parempi paikkaselektiivisyys radiofluorauksessa.

[^{18}F]F_2:n johdokset, [^{18}F]Selectfluor *bis*(triflate) ([^{18}F]SF) ja [^{18}F]ClF, tehtiin korkealla ominaisaktiivisuudella ja niitä käytettiin malliyhdisteiden elektrofiilisessa synteesissä. Kaksi 6-[^{18}F]FDOPA:n lähtöainetta, tina- ja booriesteriyhdiste, leimattiin käyttäen [^{18}F]SF:a. [^{18}F]NS12137, norepinefriinin kuljettajaproteiini (NET) -selektiivinen PET-merkkiaine, fluorattiin käyttäen kahta elektrofiilista fluorauslähtöainetta, [^{18}F]SF ja [^{18}F]F_2, sekä nukleofiilista synteesimenetelmää. [^{18}F]ClF:lle kehitettiin tuotantomenetelmä käyttäen [^{18}F]F_2:a, ja [^{18}F]ClF:n käyttöä tutkittiin hiili-hiili kaksoissidoksen elektrofiilisessa reaktiossa.

Avainsanat: Fluori-18, elektrofiilinen substituutio, elektrofiilinen additio, [^{18}F]Selectfluor *bis*(triflatti), [^{18}F]monokloorifluoridi.

CONTENTS

ABBREVIATIONS

amu	Atom mass unit
BDE	Bond dissociation energy [kJ/mol]
Boc	*tert*-Butyloxycarbonyl
CD_2Cl_2	Deuterated dichloromethane
CNS	Central Nervous System
CP	Chemical purity
DBH	1,3-Dibromo-5,5-dimethylhydantoin
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
EF1,2A	2-(2-Nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide
[^{18}F]EF5	2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-[^{18}F]pentafluoropropyl)acetamide
[^{18}F]EF4Cl$_a$	2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-(2,3,3,3-[^{18}F]tetrafluoro-2-monochloropropyl)acetamide
[^{18}F]EF4C$_b$	2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3-[^{18}F]tetrafluoro-3-monochloropropyl)acetamide
EF_3Cl_2	2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-(2,3,3-trifluoro-2,3-dichloropropyl)acetamide
EWG	Electron-withdrawing group
Elec	Electrophile
EOB	End of bombardment
EOS	End of synthesis
[^{18}F]F$^-$	[^{18}F]Fluoride ion
[^{18}F]FDG	2-[^{18}F]Fluoro-2-deoxy-D-glucose
[^{18}F]FDM	2-[^{18}F]Fluoro-2-deoxy-D-mannose
6-[^{18}F]FDOPA	4,5-Dihydroxy-2-[^{18}F]fluoro-*L*-phenylalanine
F-TEDA-X	*N*-fluoro-1,4-diazabicyclo[2.2.2]octane
GC	Gas chromatography
HPLC	High performance liquid chromatography
H_3PO_4	Trihydrogen phosphate
K2.2.2	1,10-Diaza-4,7,13,16,21,24-hexaoxabicyclo[8,8,8]-hexacosane; Kryptofix 2.2.2®
LG	Leaving group
LC-MS/MS	Liquid chromatography mass spectrometry
MeBr	Methyl bromide
MeCN	Acetonitrile
MeI	Methyl iodide
Me-OTs	Methyl tosylate
Me-OTf	Methyl triflate
Mesylate	Methanesulfonic acid

m/z	Mass per charge
MW	Molecular weight
NaI	Sodium iodide
n.c.a.	No-carrier-added
NFSi	N-fluorobenzene sulfonamide
Nosylate	3-Nitrobenzenesulfonic acid
[^{18}F]NS12137	(3-[(6-Fluoro-2-pyridyl)oxy]-8-azabicyclo[3.2.1]octane)
Nuc	Nucleophile
OTf^-	Triflate
OTs^-	Tosylate
PET	Positron emission tomography
QMA	Anion exchange cartridge
RCP	Radiochemical purity
RCY	Radiochemical yield
RP	Reversed phase
RP-HPLC	Reversed-phase high performance liquid chromatography
RT	Room temperature
R_xN-F	Alkylaminofluoride compound
$R_v(N)$	Normalized retention volume
SA	Specific activity
S_EAr	Electrophilic aromatic substitution
SET	Single electron transfer
SF	Selectfluor bis(triflate), 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]-octane *bis*(triflate)
SFP	(3-[(6-Trimethylstannyl-2-pyridyl)oxy]-8-azabicyclo-[3.2.1]octane-8-carboxylate triflate
S_N2	Bimolecular nucleophilic substitution
S_NAr	Nucleophilic aromatic substitution
SPE	Solid phase extraction
SS	Stainless steel
TFA	Trifluoroacetic acid
THF	Tetrahydrofurane
TLC	Thin layer chromatography
TMATf	2-Acetyl-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate
TMS	Trimethyl silane

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I. Stenhagen I, Kirjavainen AK, Forsback S, Jørgensen C, Robins EG, Luthra S, Solin O, Gouverneur V. [^{18}F]Fluorination of Arylboronic Ester using [^{18}F]Selectfluor bis(triflate): Application to 6-[^{18}F]Fluoro-*L*-DOPA. Chem Comm. 2013; 49:1386-1388.

II. Kirjavainen AK, Forsback S, López-Picón FR, Marjamäki P, Takkinen J, Haaparanta-Solin M, Peters D and Solin O. Electrophilic and nucleophilic fluorination of a ^{18}F-labeled norepinephrine transporter tracer for PET-imaging. 2014; Manuscript.

III. Kirjavainen A, Forsback S, Grönroos TJ, Haavisto L, Haaparanta M, Solin O, Electrophilic addition of chlorine monofluoride for PET tracers. Mol Imaging Biol, 2013; 15:131-135.

In addition some previously unpublished data are presented.

1. INTRODUCTION

In 1903, Henri Becquerel and Pierre and Marie Curie were awarded the Nobel Prize for their research on spontaneous radioactivity, and in 1935 Irène and Frédérick Joliot-Curie were awarded the Nobel Prize for their discovery of artificial radioactivity. Ernst Lawrence was awarded the Nobel Prize for his findings related to the acceleration of particles using a cyclotron in 1939, and only four years later George de Hevesy was awarded the Prize for discovering the usefulness of radiotracers in chemistry. In 1937, the artificial fluorine radioisotope fluorine-18 was first produced by Arthur H. Snell (Snell 1937). Fluorine-18 has suitable chemical and nuclear properties for use in radiotracer chemistry; its relatively long half-life (109.8 min), decay route (97%, β^+ emission), weak positron energy (635 keV), and low average range in tissue make fluorine-18 a suitable radioisotope for positron emission tomography (PET)-tracer chemistry. Fluorine also has several other radioactive isotopes with shorter half-lives (National Nuclear Data Center, Brookhaven National Laboratory, Upton, NY 11973-5000, De Kleijn 1977).

Elemental fluorine was isolated for the first time by Henri Moissan in 1886 (Moissan 1886). Moissan was awarded the Nobel Prize in chemistry in 1906. However, the honor of discovering fluorine belongs to Carl W. Scheele, who discovered a fluorine-containing mineral, fluorspar (CaF_2), in 1771. Fluorine is the 13th most abundant element in the Earth's crust, but it is almost unknown in natural compounds. Since the 1930s, fluorine-containing compounds have had an important role in the development of technology, such as freons and fluoropolymers, as well as in the pharmaceutical and agrochemical fields (Dolbier 2005, O'Hagan 2003).

PET is a non-invasive functional and metabolic imaging technique that allows the quantification of specific biological and pharmacological processes in humans and animals *in vivo*. PET uses molecules labeled with short-lived positron (β^+) emitters. The most commonly used PET radioisotopes are ^{11}C ($t_{½}$=20.4 min), ^{13}N ($t_{½}$=10.0 min), ^{15}O ($t_{½}$=2.0 min), and ^{18}F ($t_{½}$=109.8 min) (Crane and Lauritsen 1934, Crane et al. 1934, Barkas 1939, Joliot and Curie 1934, Livingston and McMillan 1934, Snell 1937).

Fluorine-18 is the most commonly used radioisotope in the field of PET-radiochemistry due to its near optimal decay characteristics and relative ease of production. Because of the relatively short half-life of ^{18}F-fluorine, rapid synthesis and purification processes are needed. After six half-lives only 1.6% of the initial activity remains. Nucleophilic fluorination is the

preferred approach for radiolabeling and produces tracers with high specific activity (SA). The greatest limitation of electrophilic ^{18}F-radiolabeling is low SA, which is caused by the inherent dilution with carrier-F_2. The synthesis with highly reactive [^{18}F]F_2 can lead to complex and time-consuming deprotection and purification processes. Therefore, one goal in electrophilic radiofluorination is to develop tamed high SA electrophilic fluorinating agents - in other words, non-hazardous electrophilic fluorine sources with less reactivity and better selectivity, such as alkylaminofluoride (R_xN-F) reagents (Teare et al. 2008, Teare et al. 2010, Furuya et al. 2008a, Furuya et al 2009a, Lee et al. 2011, Tredwell et al. 2012 Liang et al. 2013, Brandt et al. 2014, Campbell et al. 2014).

In the field of fluorine radiochemistry, new fluorinating agents and precursors containing different leaving groups (LG) need to be developed, as well as new labeling methods, so that a broader variety of organic compounds can be radiofluorinated. In this thesis I will present the syntheses and evaluation of two novel electrophilic radiofluorinating agents: [^{18}F]Selectfluor *bis*(triflate) ([^{18}F]SF) and [^{18}F]monochloro fluoride ([^{18}F]ClF).

2. REVIEW OF THE LITERATURE

2.1. Fluorine in chemistry

Interest in fluorine has increased steadily since World War II, and our everyday lives are filled with fluorinated compounds. The research into fluorine chemistry (i.e., reaction methods and number of fluorinated compounds) has developed at an accelerated pace. During World War II the Manhattan Project involved research into fluorine-containing compounds compatible with F_2 and UF_6. Elemental fluorine (F_2) is the most reactive halogen and the most reactive pure element. The fluorine atom is the most electronegative element of the periodic table and has the smallest van der Waals radius if hydrogen and its isotopes are not considered (Dolbier 2005). The electron configuration of fluorine is $1s^2 2s^2 2p^5$; because the valence electrons of fluorine are tightly held close to the nucleus by the nuclear charge, removing an electron from a fluorine atom to produce F^+ is particularly difficult and energy consuming (endothermic; -1678.6 kJ/mol). In contrast, it is relatively easy (exothermic, 327.8 kJ/mol) for the fluorine atom to accept an electron (F^-), filling the 2p-orbital and stabilizing the electropositive nucleus (O'Hagan 2008). Due to the tightly bonded electrons, the polarizability of the atom is very low. Elemental fluorine reacts aggressively with all other elements except the light noble gases He and Ne. The reactions are highly exothermic (Smart 2001, Kirk 2006, Dolbier 2005, O'Hagan 2008).

The bond between fluorine and carbon is a strong covalent bond, but the bond is highly polarized by the large difference in the electronegativity of fluorine and carbon (4.0 vs. 2.5 on Pauling's scale). The C-F bond is intermediate in length (Table 1). Due to the high dipole moment the C-F bond generates, the electron density is concentrated around the fluorine atom and introduces a partial charge on the C-F bond ($C^{\delta+}$-$F^{\delta-}$). The high dipole moment of the C-F bond enables dipole-dipole interactions (Smart 2001, O'Hagan 2008). The partial charge also gives the bond its unusual strength.

Unlike the effect of other atoms, when the number of fluorine atoms increases, the strength of the C-F bond increases. The bond dissociation energies (BDE) of some simple organofluorine compounds (C-F bond) are: CH_3-F, 453 kJ/mol; CH_2F-F, 500 kJ/mol; CHF_2-F, 534 kJ/mol; and CF_3-F, 546 kJ/mol. The high reactivity of fluorine can be explained by the weak F-F bond (BDE 159 kJ/mol), whereas the presence of fluorine in an organic molecule strengthens the bonds between other nearby atoms in the compound. The BDEs of C-C bonds are: CH_3-CH_3, 372 kJ/mol; CH_3-CF_3, 372 kJ/mol; and CF_3-CF_3, 413 kJ/mol (Dolbier 2005).

Table 1. Van der Waals radii, C-X, bond lengths, C-X bond dissociation energies, and electronegativities of some common elements.

X	Van der Waals radius* [Å]	Bond length C-X [Å]	BDE C-X [kJ/mol]	Electronegativity's on Pauling's scale
H	1.2	1.09	413.7	2.1
C	1.7	1.54	347.9	2.5
N	1.55	1.47	291.8	3.0
O	1.52	1.43	351.7	3.5
F	1.47	1.35	441.7	4.0
Cl	1.74	1.77	328.7	3.2

*Bondi 1964

Table 2. Bond lengths and BDEs of some single bonds.

Compound	Bond length [Å]	BDE [kJ/mol])
S-F	156	284
N-F	136	283
O-F	142	190
F-F	135	159
Cl-F	163#	255
Cl-Cl	175#	243

#Van der Waals radius

(Wilson et al. 1989, Luo 2003)

The size of fluorine and hydrogen is often claimed to be nearly equal, and in radiochemistry the fluorinated analogs are designed by the replacement of hydrogen with fluorine-18. The steric size of fluorine attached to carbon is more similar to the oxygen attached to carbon due to the steric repulsion of fluorine and oxygen; correspondingly, the lengths of C-F and C-O bonds are more similar than the length of the C-H bond (135, 143, and 109 pm, respectively). The fluorination of an alkyl group always increases the steric size compared to non-

fluorinated hydrocarbon groups. Over the years there has been much discussion about whether the fluorine atom acts as a hydrogen bond acceptor. The current opinion is that the covalently bonded fluorine hardly ever accepts a hydrogen bond, and conversely for the fluoride ion (Duniz 2004, Duniz and Taylor 1997). The exchange of the hydrogen atom for a fluorine atom affects the lipophilicity more than the oxygen-fluorine exchange (Smart 2001, Lasne et al. 2002, Kirk 2006, Müller et al. 2007, Purser et al. 2008).

2.2. Lipophilicity of fluorine-containing molecules

The affinity of a molecule for a lipophilic environment is called lipophilicity. Lipophilicity is usually measured by a molecule's distribution in a biphasic system, either a liquid-liquid system using the partition coefficient in water/1-octanol or a solid-liquid system using retention on reversed-phase high performance liquid chromatography (RP-HPLC) or thin-layer chromatography (TLC) (IUPAC 1997). The term LogD refers to a compound's apparent partition coefficient in a biphasic water/1-octanol system at physiological pH (7.4). LogD takes into account both ionized and unionized forms of the compound. LogP refers to the actual partition coefficient, which takes into account only the unionized form of the compound and is not determined in physiological pH. LogD better describes the partition of the compound in the biological system.

Lipophilicity is one of the key parameters affecting pharmacokinetic behavior, including uptake into different tissues and passage of the blood brain barrier (Smart 2001, Park et al. 2001, Kirk 2006). High lipophilicity may increase non-specific binding (Dischino et al. 1983). The lipophilicity can be determined at physiological conditions (logD) using shake flask methods (Wilson et al. 2001) or approximated by commercial softwares (computed logP).

In contrast to the fluorination of aromatic rings, which always increases the lipophilicity, the fluorination, perfluorination, or polyfluorination of aliphatic compounds does not always increase their lipophilicity. Fluorination of saturated alkyl compounds decreases their lipophilicity, especially if fluorine is introduced near oxygen or nitrogen atoms. A great decrease in logD usually occurs when fluorine is introduced near basic nitrogen (Smart 2001, Lasne et al. 2002, Kirk 2006, Müller et al. 2007, Purser et al. 2008). Lipophilicity decreases when the terminal carbon of an alkane is mono-, di-, or trifluorinated (Smart 2001).

2.2.1. Solid-phase extraction

Differences in the lipophilicity of molecules are utilized in solid-phase extraction (SPE). SPE is based on the sorption of a compound from a solution onto the solid-phase. The mechanisms

underlying the interaction between solid phase resin and the compound include van der Waals interactions, hydrogen bonding, dipole-dipole forces, size exclusion, and cation anion exchange (Thurman and Mills 1998).

SPE is used for the purification and concentration of various materials and is highly suitable and amenable to PET-radiochemistry, partly due to the very low amounts of chemical entities encountered in PET-radiochemistry. From the radiochemistry point of view, SPE is used for the purification of radiotracers, removing impurities and organic solvents, and for assisting in formulation of the radiotracer for preclinical or human use (Thurman and Mills 1998, Lemaire et al. 1999). In some cases SPE has replaced HPLC purification of radiopharmaceuticals. In addition, the SPE approach is used in tracer hydrolysis, which is carried out while the compound is trapped on the solid phase (SP) material (Mulholland 1995, Lemaire et al. 1997, Mosdzianowski et al. 1999, Mosdzianowski et al. 2002, Lemaire 2002). Recently, Libert et al. reported the nucleophilic, no-carrier-added (n.c.a.), and enantioselective synthesis of 6-[^{18}F]fluoro-L-dopa ([^{18}F]FDOPA) in which the alkylation step is performed using the SPE cartridge (Lemaire et al. 2004, Libert et al. 2013).

In SPE the mechanisms of retention can be divided into normal phase, reversed phase (RP), and ion exchange. The SPE process can be divided into four steps (Figure 1). First, the solid-phase resin is conditioned by passing a solvent through the sorbent to wet the packing material. With all methods the steps are similar, only the solvents that are used vary. For example, using an RP-cartridge, the sorbent is wetted with an organic solvent and rinsed with water or buffer. The aqueous solvent activates the cartridge for aqueous samples. Second, the diluted compound is loaded onto the SPE cartridge by gravity feed, pressure, or vacuum aspiration depending on the SPE system and the sample volume. Next, the SPE cartridge is washed with the appropriate solution to separate the compound from impurities and organic solvents. Finally, the compound is extracted from the SPE resin using a small volume of a suitable eluent for human injection, such as ethanol, and diluted with physiological buffers or sodium chloride solution (typically less than 10% of ethanol) (Lemaire et al. 1999).

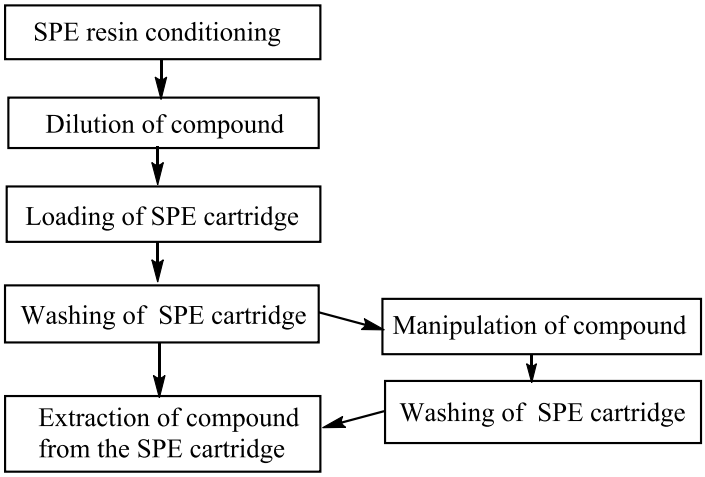


Figure 1. Flow chart of SPE.

2.3. Fluorine in radiochemistry

Although fluorine is rare in natural compounds, medicinal fluorine chemistry is based on the fluorination of natural compounds or their close derivatives (Böhm et al. 2007). Fluorine has only one stable isotope (^{19}F), and several artificial radioisotopes with half-lives from nanoseconds to minutes exist. The properties of fluorine-18 and some other commonly used positron emitters are presented in Table 3.

Table 3. Some properties of commonly used β^+ emitters.

Radionuclide	Maximum β^+ energy [MeV]	Maximum range in water [mm]	Mean range in water [mm]	Decay product
^{11}C	0.96	4.12	1.03	^{11}B
^{13}N	1.19	5.39	1.32	^{13}C
^{15}O	1.72	8.20	2.01	^{15}N
^{18}F	0.635	2.39	0.64	^{18}O
^{64}Cu	0.653	2.9	0.64	^{64}Ni
^{68}Ga	1.89	8.9	2.24	^{68}Zn

(National Nuclear Data Center, Brookhaven National Laboratory, Upton, NY 11973-5000, Welch and Redvanly 2003, Biersack and Freeman 2007, Miller et al. 2008, Cal-Gonzáles et al. 2009)

Fluorine-18 can be produced in a cyclotron via several nuclear reactions; two of the most common nuclear reactions are presented in Table 4. The $^{18}O(n,p)^{18}F$ reaction is preferred due to the high yield and ease of production with modern cyclotrons. Batches of up to several hundreds of GBq of $[^{18}F]F^-$ are routinely produced.

Table 4. The most common nuclear reactions for F-18.

Nuclear reaction	Target material	Product	Typical specific activity [GBq/μmol]
$^{20}Ne(d,\alpha)^{18}F$	Ne/F_2	$[^{18}F]F_2$	0.030-0.37
$^{20}Ne(d,\alpha)^{18}F$	Ne	$[^{18}F]F^-$	100-1000
$^{18}O(p,n)^{18}F$	$H_2{}^{18}O$	$[^{18}F]F^-$	10-7000
$^{18}O(p,n)^{18}F$	$^{18}O_2/F_2$*	$[^{18}F]F_2$	~ 1.85
$^{18}O(p,n)^{18}F$	$H_2{}^{18}O$	post-target produced $[^{18}F]F_2$	55

* Two-shoot method (Nickles et al. 1984)

(Guillaume et al. 1991, Bergman and Solin 1997, Lasne 2002, Barnhart et al. 2003a)

Fluorine-18 has increased in popularity as a radioisotope in the PET field due to its near optimal decay characteristics and relative ease of production also by low energy cyclotrons (Coenen 2007).

2.4. Specific activity (SA)

The SA is the amount of radioactivity per the mass unit of a radiolabeled compound. The SA decreases over time as a radioactive tracer decays and the unlabeled compound remains (Figure 2).

The need for high SA depends on the target of the PET tracer, as many tracers are highly potent or toxic. Additionally, many receptor systems in the CNS exist in nanomolar concentrations, and low SA will lead to saturation of the receptors. As a consequence true tracer conditions (i.e. <5% occupancy by carrier) will not be satisfied at low SA. The injected mass of the tracer should be minimized, but the amount of injected radioactivity must be high enough for a statistically meaningful PET scan. SA is also dependent on the labeling method, i.e., nucleophilic vs. electrophilic. The methods of producing $[^{18}F]F_2$ require the addition of

carrier-F_2, which decreases the SA, in contrast to nucleophilic [^{18}F]F^-, which can be produced as n.c.a and, thus, with high SA, see Table 4.

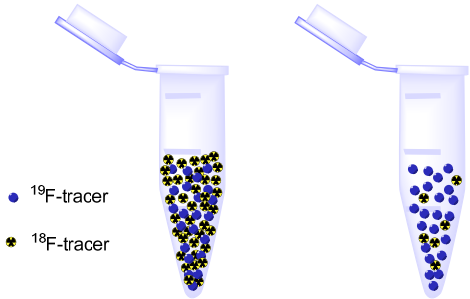


Figure 2. SA decreases over time with radioactive tracer decay, but the amount of unlabeled tracer remains the same.

2.5. Fluorination methods in radiochemistry

The radiofluorination methods can be divided into five groups as follows:

1) Nucleophilic substitution
2) Electrophilic substitution
3) Electrophilic addition
4) Radiofluorination via built-up procedures
5) Radiofluorination via prosthetic groups

Substitutions and additions are direct fluorination methods, and the last two methods are indirect methods. On the contrary to direct methods in built-up procedures and radiofluorination of prosthetic groups the carbon skeleton structure is changed. In built-up syntheses the easily ^{18}F-fluorinated small molecule, such as ^{18}F-fluoroaryls, is used to synthesize more complex molecules, which cannot be radiofluorinated directly due to mechanistic reasons, i.e. their low stability. In radiofluorination reactions via prosthetic groups the primary ^{18}F-labeled functionalized compound is coupled with a second molecule, such as fluoroalkylations and fluoroacylations. Radiofluorination via prostethic group is used in labeling reactions of proteins, peptides and antibodies (Guillaume 1991, Coenen 2007, Banister et al. 2010, Ermert 2014). In this literature review I will concentrate on the direct substitution and addition labeling procedures. In radiosynthesis, the ratio of radioactive labeling agent and precursor is generally different from that of traditional organic chemistry.

The amount of unlabeled precursor is often 10^3 or 10^4-fold of the radiolabeling agent (Ametamey et al. 2008). This is particularly true for high SA nucleophilic fluorination.

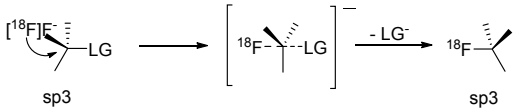
Nucleophilic and electrophilic fluorination methods are complementary processes, with the method of choice being dependent on the reactivity profile of the precursor toward fluorination (Chambers 2004). Nucleophilic fluorination is the preferred method for radiolabeling, because this process produces radiotracers with high SA. However, it is not always possible to use it due to the labeling conditions required or the labeled structure. Though electrophilic ^{18}F-radiolabeling is a useful approach for labeling electron-rich structures, its greatest limitation is the isotopic dilution introduced by the carrier-added methods that produce $[^{18}F]F_2$, leading to radiotracers with low SA (Berridge and Tewson 1986). Selective electrophilic radiofluorination using $[^{18}F]F_2$ is challenging and easily leads to time-consuming deprotection and purification processes. Therefore, the major goals in electrophilic radiofluorination are to develop non-hazardous electrophilic fluorine sources with less reactivity and better selectivity, such as R_xN-^{18}F reagents (Teare et al. 2008, Teare et al. 2010, Furuya et al. 2008a, Tredwell et al. 2012, Liang et al. 2013, Brandt et al. 2014, Campbell et al. 2014), and to develop methods to produce these electrophilic labeling agents with high SA.

2.5.1. Nucleophilic ^{18}F-fluorination

Nucleophilic radiofluorination reactions are mostly aliphatic S_N2 or aromatic S_NAr substitution reactions in which the target molecule has a suitable leaving group that is substituted with the fluoride anion. Fluoride anion is a weak base rather than a nucleophile (Nuc). $[^{18}F]F^-$ is most often produced from highly ^{18}O-enriched water by the nuclear reaction $^{18}O(p,n)^{18}F$. The $[^{18}F]F^-$ is then solvated in target water and a poor nucleophile due to hydrogen bonding between the fluoride anion and water molecules. The content of the water target is passed through the anion exchange cartridge, and $[^{18}F]F^-$ is eluted from an anion exchange resin with an aqueous alkali metal carbonate or alkali metal oxalate solution. Soft metal cations with large atomic radii (Cs^+, Rb^+) have also been used (Welch and Redvanly 2003, Ametamey et al. 2008). A third group of counter ions are tetra-alkylammonium salts (*t*Bu_4N^+, Et_4N^+), which have been used as counter ions without cryptands (Welch and Redvanly 2003, Lasne et al. 2002, Schirrmacher et al. 2007, Cai 2008, Lu and Pike 2008). Because fluoride pairs tightly with alkali metal cations, especially potassium, the crown-ethers (18-crown-6) and cryptands (polyaminoethers; K2.2.2) have been used to chelate alkali metal cations that enable fluoride to be more reactive toward the molecule that is to be labeled

and enable the fluoride ion to be solubilized in apolar aprotic solvents (e.g., MeCN, DMSO, DMF, THF, CD_2Cl_2). The crown ether or cryptand is added to aqueous potassium [^{18}F]fluoride and carbonate solution, and water is removed by azeotropic distillation with MeCN. The azeotropic distillation steps are repeated to ensure a dry anhydrous complex (Lasne et al. 2002, Cai 2008, Lu and Pike 2008, Ametamey et al. 2008, Dollé 2008).

In aliphatic nucleophilic substitution (S_N2) reactions the [^{18}F]F^- will attack the sp3 hybridized center at the opposite side relative to the LG, resulting in substitution with inversion of the configuration of the carbon center. In the S_N2 type substitution, the LG is a weak base that can stabilize the resulting negative charge originating from the [^{18}F]F^- substitution (Scheme 1).



Scheme 1. S_N2 type substitution.

Different sulfonate esters are commonly used as a LG in nucleophilic fluorination (Figure 2) and halides are also used. Of the sulfonate esters, the most reactive is triflate; of the halides, the most reactive is iodide. Fluoride is not generally used as a LG in nucleophilic labeling due to the diluting effect of stable fluorine, which decreases the SA. Using nucleophilic radiofluorination, high radiochemical yield and SA is often achieved. On the other hand, the harsh reaction conditions in nucleophilic fluorination may not be suitable for the precursor and can lead to chemical decomposition of the reactant and product.

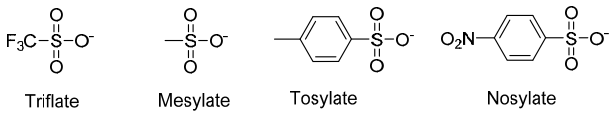
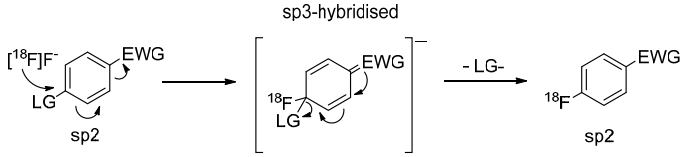


Figure 3. Some sulfonyl-containing LGs used in nucleophilic labeling reactions.

Nucleophilic aromatic substitution (S_NAr) can be divided into homoaromatic and heteroaromatic reactions depending on the aromatic ring structure, but the principles of the reactions are the same. The S_NAr reactions require at least one strong electron withdrawing group (EWG) and a good LG in an *ortho*- or *para*-position in the ring structure of the aromatic ring (Scheme 2).



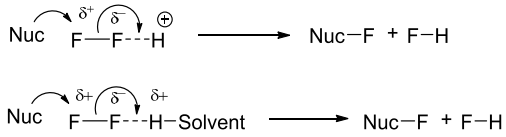
Scheme 2. The S_NAR reaction mechanism.

Typical LGs are the NO_2 group, tetra-alkyl groups, and halides. Good EWGs in the *para*- or *ortho*-position are NO_2-, CF_3-, CN-, and CHO-. The series NO_2 > CF_3 > CN > CHO > COR > COOR > COOH > Br, I, F, Me > NMe_2 > OH, NH_2 represents EWGs in descending order of promotion of the reactivity of the LG in nucleophilic fluorination (Lasne et al. 2002, Cai, Lu and Pike 2008, Dollé 2008).

2.5.2. Electrophilic ^{18}F-fluorination

In electrophilic fluorination, fluorine behaves as a cation (F^+) and reacts with electron-rich structures such as alkenes, aromatic rings, and carbanions. The main reaction mechanisms of electrophilic ^{18}F-fluorination are aromatic substitution (S_EAr) reactions and addition reactions to alkenes (Berridge and Tewson 1986). The oxidizing strength of fluorine is high and easily leads to exothermic radical chain reactions and the formation of undesirable side products. Because of the vigorous reactivity of elemental fluorine, electrophilic radiofluorination often has low regioselectivity and poor yield. These undesirable properties can be affected by 1) the dilution of fluorine with inert gases (0.1-0.5% fluorine in nitrogen or noble gas), 2) using strong acids (e.g., TFA) as a reaction medium, or 3) using less reactive electrophilic labeling agents than $[^{18}F]F_2$ (Lerman et al. 1981, Lerman et al 1984, Taylor 1999, Dollé 2008), 4) specifically-tailored leaving groups. In electrophilic substitution of aromatic rings, an EWG on the ring decreases the electron density of the reaction center and makes it less favorable for electrophilic attack (Namavari et al. 1995).

Rozen and co-workers have studied the effects of temperature and solvent on the electrophilic reaction mechanism. They found that, in electrophilic radiofluorination, decreased reaction temperature and a polar solvent, in particular, promote electrophilic reactions and decrease radical attack. Polar solvents both encourage the polarization of the fluorine molecule and behave as acceptors for the fluorine atom (negatively charged atom of $[^{18}F]F_2$) in the transition state (Scheme 3) (Rozen and Gal 1987a, Rozen and Gal 1987b, Sanford 2007).

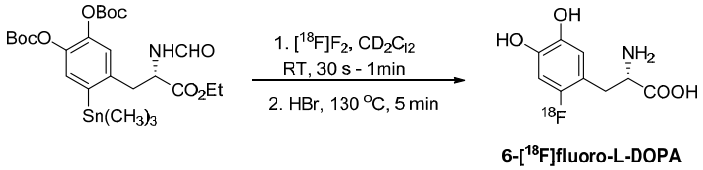


Scheme 3. Polarization of F_2 by protonic acid (upper) and dielectric aprotic solvent (H-solvent) (lower), which makes the F-F bond more prone to nucleophilic attack. Nuc can be a tertiary hydrogen, C-C double bond, or aromatic ring (Sanford 2007).

The development of electrophilic radiofluorination of PET tracers is driven by the desire for a broader and more useful range of electrophilic labeling agents with higher SA than currently achieved. Electrophilic ^{18}F-fluorination, when [^{18}F]F_2 is produced "in-target", suffers from well-recognized drawbacks; the carrier-added method of [^{18}F]F_2 production produces labeled products with low SA. Pleasingly, post-target production of [^{18}F]F_2 has much increased SA (Bergman and Solin 1997). Another drawback is that, the maximum achievable RCY in electrophilic substitution is limited to 50% as only one of the two atoms of [^{18}F]F_2 ends up in the target molecule. On the other hand in electrophilic addition the theoretical RCY is 100 % and subsequently the achievable SA is double that of electrophilic substitution.

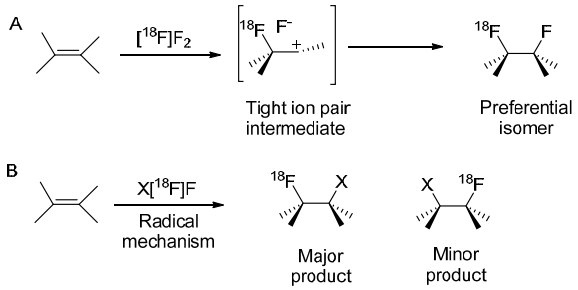
In perfluorination, several hydrogens on the molecule are replaced by fluorine. Perfluorination is usually carried out under conditions that favor the free radical mechanism (Hung et al. 1993, Sanford 2007). In contrast to perfluorination, selective direct monofluorination is promoted by decreasing the amount of carrier-fluorine using polar solvents and good LGs. The reaction conditions attempt to tame free radical processes (Rozen 1988, Navarrini et al. 1999, Sandford 2007). Rozen and co-workers have shown that fluorination of the tertiary hydrogen is less probable when the EWG is located near the reaction center and the electron density of the reaction center is decreased (Gal et al. 1980, Gal and Rozen 1982, Rozen and Gal 1987a, Rozen and Gal 1987b). The reactivity of aliphatic hydrogen has been observed to decrease in the series primary > secondary > tertiary (Coenen 2007).

Selective S_EAr requires an activated aromatic ring with a suitable, easily displaced LG, such as an organometallic group (Scheme 4). Most commonly used LGs are alkylated tin, germanium, and mercury groups (Berridge and Tewson 1986, Namavari et al. 1995, Coenen 2007, Lasne et al. 2002, Forsback et al. 2008, Eskola et al. 2012a). In electrophilic synthesis of 6-[^{18}F]fluoro-L-DOPA, the trialkyltin group has been shown to be very efficient (Namavari 1992, Forsback et al. 2008). Due to the toxicity of these organometallic groups, boronic acids have also recently been used as the LG (Furuya et al. 2008a, Stenhagen et al. 2013).



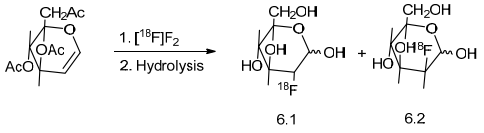
Scheme 4. Selective electrophilic substitution of an aromatic ring (S_EAr).

The addition of $[^{18}F]F_2$ to alkenes follows the bimolecular electrophilic addition route of other halogens. Other halogens mostly form a *trans*-addition product due to the bridged halonium ion transition step. In the addition reaction with fluorine, the main route is *cis*-addition. This is due to the instability of the bridged fluoronium intermediate that would lead to the *trans*-addition product. Subsequently, nucleophilic attack of the double bond towards the fluorine molecule is followed by the formation of an unstable and tightly ion-paired α-fluorocation, which collapses before the rotation of the carbon-carbon bond (Scheme 5A) (Rozen and Brand 1986, Berridge and Tewson 1986, Dollé et al. 2008). With other electrophilic fluorinating agents containing the O-F bond, such as various $[^{18}F]$hypofluorites, the addition reaction primarily leads to *cis*-addition through a radical mechanism (Scheme 5B) (Rozen and Brand 1986).



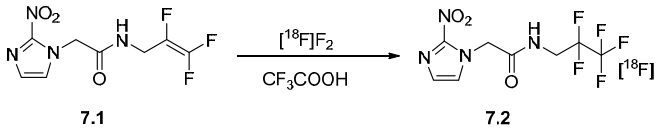
Scheme 5. A: *Cis*-addition of $[^{18}F]F_2$ to a double bond. B: *Cis*-addition of $X[^{18}F]F$, $X=CH_3COO^-$ (Rozen and Brand 1986).

$[^{18}F]$FDG was first synthesized via an electrophilic addition pathway before the nucleophilic synthesis method was developed. Ido et al. (1978) reported the addition reaction between $[^{18}F]F_2$ and triacetoxy glucal resulting in a mixture of $[^{18}F]$FDG (**6.1**) and $[^{18}F]$FDM (**6.2**) (Scheme 6).



Scheme 6. Electrophilic synthesis of $[^{18}F]$FDG (**6.1**) using $[^{18}F]F_2$, $[^{18}F]$FDM (**6.2**) is formed as a byproduct (Ido et al. 1978).

In the synthesis of the hypoxia marker [^{18}F]EF5, nucleophilic substitution of bromine by ^{18}F-fluoride has been unsuccessful (Dolbier et al. 2001). Subsequently, electrophilic labeling of allyl precursor **7.1** with [^{18}F]F_2 was studied (Dolbier et al. 2001) (Scheme 7). In 2012 Eskola et al. published the synthesis of [^{18}F]EF5 (**7.2**) via electrophilic addition using high SA [^{18}F]F_2 (Eskola et al. 2012b).



7.1 7.2

Scheme 7. Synthesis of [^{18}F]EF5 (**7.2**) using [^{18}F]F_2 (Dolbier et al. 2001).

2.6. [^{18}F]MeF

[^{18}F]MeF is the simplest possible ^{18}F-labeled organic molecule. In addition to being used as a cerebral blood flow tracer in PET studies, [^{18}F]MeF is the source of ^{18}F atoms in the isotopic exchange reaction for the production of post-target [^{18}F]F_2 with high SA at Turku PET Centre (Bergman and Solin 1997). The success of [^{18}F]MeF synthesis is crucial for the production of high SA [^{18}F]F_2.

[^{18}F]MeF can be prepared by a variety of methods, including silver oxide-assisted production from MeI and [^{18}F]F^- in MeCN (Gatley et al. 1981, Gatley 1982, Gatley et al. 1991), the use of non-volatile TMATf in DMSO, microwave-assisted synthesis of [^{18}F]MeF from quaternary anilinium salts in DMSO (Banks et al. 1994), and an exchange reaction between [^{17}F]F_2 ($t_{½}$(^{17}F) = 65 s) and methane gas (Stone-Elander 1986, Barnhart et al. 2003b).

The trapping methods of [^{18}F]MeF and [^{11}C]MeF, as well as other gaseous hydrofluorocarbons in SepPak cartridges, have been studied by Gatley and colleagues (Stone-Elander et al. 1986, Gatley et al. 1991, Gatley et al. 1993). In 1991 they presented a method to recover [^{18}F]MeF in SepPak cooled with ethanol/dry ice temperature (Gatley et al. 1991), and in 1993 [^{18}F]MeF was formed trapped on an alumina cartridge (Gatley et al. 1993). Bergman and Solin have published a robust method to trap [^{18}F]MeF in a stainless steel (SS) loop at the temperature of liquid nitrogen after preparative chromatographic purification (Bergman and Solin 1997).

The radiochemical yield (RCY) of [^{18}F]MeF is important for the production of post-target [^{18}F]F_2 (Bergman and Solin 1997), as the subsequent yield of [^{18}F]F_2 depends on this. The chemical purification of [^{18}F]MeF after synthesis has been given little attention. A high

chemical purity (CP) of [^{18}F]MeF is needed to achieve the high SA of post-target [^{18}F]F_2. In the production of high SA [^{18}F]F_2, the amounts of [^{18}F]MeF and carrier-F_2 are low, so even minute impurities can decrease the yield and SA of [^{18}F]F_2. In the synthesis of post-target [^{18}F]F_2, [^{18}F]MeF was purified by gas chromatography and the purified fraction collected in the SS loop at -190°C (liquid nitrogen).

2.7. [^{18}F]F_2

[^{18}F]F_2 gas can be produced using several methods: 1) irradiating neon gas with deuterium to produce ^{20}Ne(d,α)^{18}F (Bida et al. 1980, Casella et al. 1980, Barnhart et al. 2003a) in the presence of carrier-F_2, 2) with the two shoot method in which $^{18}O_2$ gas is bombarded with protons to produce ^{18}O(p,n)^{18}F (Nickles et al. 1984, Chiracal et al. 1995, Roberts et al. 1995, Bishop et al. 1996), and then bombarded again after adding carrier-fluorine diluted with a noble gas, and 3) a post-target method in which ^{18}O-enriched water is bombarded with protons, and the formed [^{18}F]F^- is utilized in the synthesis of post-target [^{18}F]F_2. With "in-target" methods the SAs are significantly lower than with post-target methods. The SA of labeled products directly affects the SA of other electrophilic labeling agents produced from [^{18}F]F_2; therefore, it is important to develop methods to produce [^{18}F]F_2 and tamed [^{18}F]F_2 derivatives with the highest achievable SA.

2.8. Other sources of electrophilic [^{18}F]fluorine

Attempts have been made to tame the reactivity of [^{18}F]F_2 by producing ^{18}F-labeled derivatives of fluorine gas, such as O-^{18}F class ([^{18}F]hypofluorites and perchloryl [^{18}F]fluoride), [^{18}F]XeF_2, or R_xN-^{18}F class reagents. Common to all ^{18}F-labeled derivatives produced from in-target [^{18}F]F_2 is a poor SA, which restricts the use of these derivatives. The scope and limitations of these derivatives should be judged based on various characteristics. Typically, these characteristics are the reactivity profile, availability of suitable methods for production, chemical impurities formed in production, stability, and obviously RCYs and SAs. At present availability of such data is limited for most of the electrophilic ^{18}F-sources.

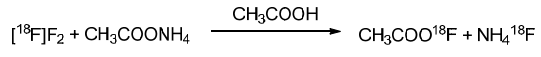
2.8.1. [^{18}F]Hypofluorites – O-^{18}F

The reaction mechanism of hypofluorites with alkenes can follow either a free radical or an electrophilic pathway. In general, polar and steric aspects determine the regio- and stereochemistry of the fluorinated product. Moreover, electron-rich alkenes, polar solvents, low concentration, temperature, and sometimes aerobic conditions encourage an electrophilic reaction and suppress fluorine radical processes (Navarrini et al. 1999). In most cases,

hypochloryl and perchloryl fluorides are labeled using direct electrophilic substitution with $[^{18}F]F_2$. These $[^{18}F]$O-F hypofluorite derivatives of $[^{18}F]F_2$ are less reactive and more selective than $[^{18}F]F_2$. Perchloryl fluoride has been investigated, but not used routinely because of its explosive nature (Fowler et al. 1982, Ehrenkaufer and MacGregor 1982).

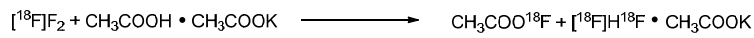
$[^{18}F]$Acetyl hypofluorite

$[^{18}F]$Acetyl hypofluorite ($[^{18}F]CH_3COOF$) has been reported to react with alkenes (double bonds), producing syn-fluorinated products with relatively good regioselectivity. It has also been shown to be milder and more selective than other O-F group labeling agents (CF_3OF, CF_3COOF, CF_3CF_2OF) when using highly activated aromatic rings as a labeling substrate (Rozen and Lerman 1979, Rozen and Menahem 1980, Rozen et al. 1981, Lerman et al. 1981, Lerman et al. 1984). In 1982, Fowler et al. reported the radiosynthesis (Scheme 8) and use of $[^{18}F]CH_3COOF$. Using this liquid phase synthesis method, $[^{18}F]CH_3COOF$ has been produced with high yield and used for labeling reactions without a separate purification process. The highest RCY was achieved using ammonium acetate. Other cations that have been used are K^+, Cs^+, and Na^+. The lowest RCY was obtained with the Na^+ cation (Fowler et al. 1982).



Scheme 8. Synthesis of $[^{18}F]$acetyl hypofluorite (Fowler et al. 1982).

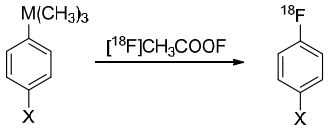
Some years later, the synthesis of $[^{18}F]CH_3COOF$ was developed further by both Jewett et al. (1984) and Chiracal et al. (1988). In this "gas-solid phase method", the content of the target chamber (mostly $[^{18}F]F_2$/neon) was passed through a column containing a complex of alkali metal acetate and acetic acid (Scheme 9). $[^{18}F]CH_3COOF$ was eluted from the column by aqueous solution.



Scheme 9. Synthesis of $[^{18}F]$acetyl hypofluorite (Jewett et al. 1984, Chiracal et al. 1988).

In 1992, Namavari et al. published a paper in which they compared $[^{18}F]F_2$ and $[^{18}F]CH_3COOF$ as labeling agents in the synthesis of 6-$[^{18}F]$FDOPA. The decay corrected RCY with $[^{18}F]CH_3COOF$ was significantly lower than with $[^{18}F]F_2$, 8% and 25% respectively (Namavari et al. 1992). Several papers on fluorodemetallation reactions, i.e. Sn, Ge, Si, and Hg metals, (Adam et al. 1984, Coenen and Moerlein 1987, Namavari et al. 1992,

Adam and Jivan 1988, Chaly et al. 1993,) with [^{18}F]CH_3COOF have been published since the 1980s.



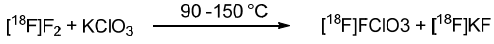
Scheme 10. Radiofluorination of an aromatic ring with [^{18}F]CH_3COOF (M=Si, Ge, or Sn and X= OCH_3, CH_3, H, F, Br, CF_3,or NO_2) (Coenen and Moerlein 1987).

[^{18}F]Trifluoroacetyl hypofluorite

[^{18}F]Trifluoroacetyl hypofluorite ([^{18}F]CF_3COOF) is the oldest of all O-F class reagents (Neirinckx et al. 1978). In 1979 the synthesis and use of CF_3COOF and other non-radioactive fluoroxy compounds was published by Rozen and co-workers (Rozen and Lerman 1979, Rozen and Lerman 1980, Rozen and Menahem 1980).

[^{18}F]Perchloryl fluoride

[^{18}F]Perchloryl fluoride ([^{18}F]$FClO_3$) is a gaseous fluorinating agent produced from [^{18}F]F_2 (Ehrenkaufer and MacGregor 1982) that has been used for the fluorination of unfunctionalized aryllithiums. In 1983, Ehrenkaufer and MacGregor published the results of fluorinating aryl lithiums with pharmacologically interesting functionalized groups using [^{18}F]$FClO_3$ (Ehrenkaufer and MacGregor 1983). In 2008, Hiller et al. published methods for producing n.c.a. [^{18}F]$FClO_3$ from [^{18}F]fluoride (K[^{18}F]F or H[^{18}F]F). The RCYs remained low, only 1-6%, and the reproducibility of n.c.a. [^{18}F]$FClO_3$ has been reported to be poor (Hiller et al. 2008). [^{18}F]$FClO_3$ has to be purified carefully from unreacted [^{18}F]F_2 and the chlorinated oxides formed as side products because these impurities can further decrease the RCY. The formation of [^{18}F]$FClO_3$ is almost quantitative in the reaction shown in Scheme 11, but the highest achieved RCY is 50% due to the formation of [^{18}F]KF (Ehrenkaufer and MacGregor 1983, Hiller et al. 2008).

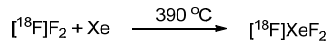


Scheme 11. Synthesis of [^{18}F]perchloryl fluoride (Ehrenkaufer and MacGregor 1983).

2.8.2. [^{18}F]Xenon difluoride

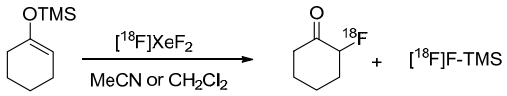
The high reactivity of elemental fluorine indicates that it will react with heavier noble gases, such as xenon (Tius 1995, Lu 2010). [^{18}F]XeF_2 can be produced via several synthesis routes,

both electrophilic and nucleophilic. The thermal reaction between xenon and $[^{18}F]F_2$ is a possible method for producing $[^{18}F]XeF_2$ (Scheme 12) (Chiracal et al. 1984). More recently, Constantinou et al. published a synthesis method in which $[^{18}F]XeF_2$ is produced via an isotopic exchange reaction using Cs^+-Kryptofix222 complex a catalyst for the ionization of XeF_2 (Constantinou et al. 2001).



Scheme 12. Electrophilic synthesis of $[^{18}F]XeF_2$ (Chiracal et al. 1984).

$[^{18}F]XeF_2$ has only a limited number of applications, and the SA of $[^{18}F]XeF_2$ is relatively low regardless of the production method (Chiracal et al. 1984, Constantinou et al. 2001, Lu and Pike 2010). Lu and Pike (2010) achieved a SA of 1.1 GBq/µmol.



Scheme 13. Radiofluorination with $[^{18}F]XeF_2$ (Lu and Pike 2010).

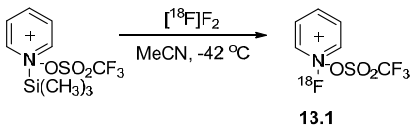
2.8.3. R_xN-^{18}F reagents

R_xN-F reagents can be divided into neutral (R_2NF) or quaternary ammonium salts ($R_3N^+F\ A^-$). Counter ion A^- must be a non-nucleophilic anion, and organonitrogen groups (R_2N and R_3N^+) must be both good LGs and stable enough to survive without fluorine and the counter ion under the reaction conditions.

R_xN-^{18}F reagents are mostly selective, easy to handle, and their reactivity controllable. Though much attention has been paid to the development of electrophilic N-F reagents, only a few have been labeled with ^{18}F-fluorine and used in radiofluorination (Lal et al. 1996, Teare et al. 2007). R_xN-^{18}F reagents can be divided into four classes by their molecular structures: 1) *N*-$[^{18}F]$fluoropyridium reagents (Oberdorfer et al. 1988a), 2) *N*-$[^{18}F]$fluoropyridones (Oberdorfer et al. 1988b), 3) *N*-$[^{18}F]$fluorosulfonamides and *N*-$[^{18}F]$fluorosulfonimides (Satyamurthy et al. 1990, Differding and Hofner 1991, Rostami 2007, Teare et al. 2007), and 4) *N*-$[^{18}F]$fluoro-1,4-diazabicyclo[2.2.2]octane derivates (Banks 1990, Banks 1998, Nyfeller et al. 2005, Teare et al. 2010). N-^{18}F reagents have been used in fluorine/metal exchange reactions of organic compounds.

N-[^{18}F]fluoropyridinium triflate

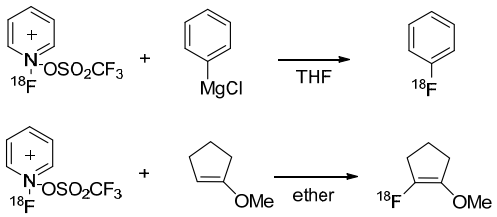
N-fluoropyridinium triflate is stable and nonhygroscopic electrophilical fuorination agent. It has high reactivity compared to other N-fluoropyridinium salts with BF_4, SbF_6 and ClO_4 counter anions. (Umemoto et al. 1986). *N*-[^{18}F]fluoropyridinium triflate (**13.1**) can be prepared from a reaction between [^{18}F]F_2 and *N*-trimethylsilylpyridinium triflate in acetonitrile (Scheme 13). The SA of *N*-[^{18}F]fluoropyridinium triflate has been reported to be ~6 MBq/μmol (Oberdorfer et al. 1988a).



13.1

Scheme 13. Production of *N*-[^{18}F]fluoropyridinium trilate (Obendorfer 1988a).

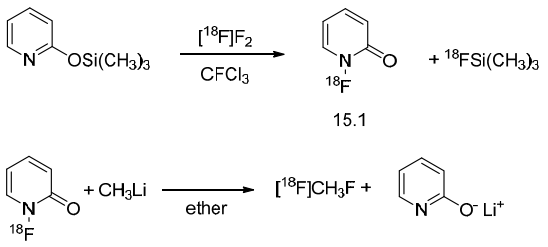
N-[^{18}F]fluoropyridinium triflate has high reactivity with Grignard reagents, carbanions, and enolates (Scheme 14) (Umemoto et al. 1986, Obendorfer et al. 1988a).



Scheme 14. Radiofluorination of Grignards reagent (upper) and enolate (lower) with *N*-[^{18}F]fluoropyridinium trilate (Oberdorfer et al. 1988a).

N-[^{18}F]fluoropyridones

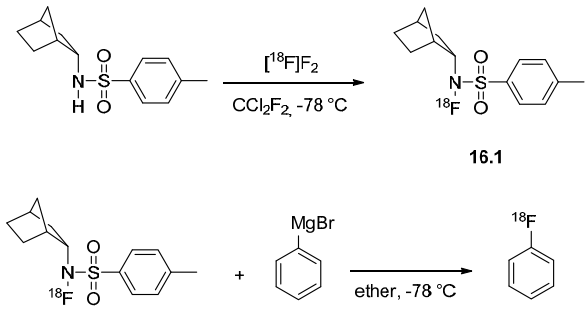
In 1988, Obendorfer et al. published synthesis methods for and the use of 1-[^{18}F]fluoro-2-pyridone (**15.1**), a new electrophilic N-F labeling agent. 1-[^{18}F]fluoro-2-pyridone was produced by passing [^{18}F]F_2 through the substrate solution at -78°C, and the crude product could be used without any purification. Organometallic compounds, such as lithium-containing molecules, can be labeled with 1-[^{18}F]fluoro-2-pyridone (Scheme 15) (Obendorfer 1988b).



Scheme 15. Synthesis and use of 1-[^{18}F]fluoro-2-pyridone (**15.1**) (Obendorfer 1988b).

N-[^{18}F]fluoro-*N*-alkylsulfonamide

Several different *N*-[^{18}F]fluoro-*N*-alkylsulfonamides have been synthesized (Satyamurthy et al. 1990) and tested in fluorination reactions. *N*-[^{18}F]fluoro-*endo*-norbornyl-*p*-tolylsulfonamide (**16.1**) has been shown to be the most reactive of the *N*-[^{18}F]fluoro-*N*-alkylsulfonamides (Scheme 16), which are reactive towards Grignard reagent (R-MgBr) and organilithium (R-Li) reagents. In reactions with bulky *N*-[^{18}F]fluoro-*N*-alkylsulfonamides, the RCY has been relatively low, probably due to steric hindrance between the substrate and the bulky labeling agent (Satyamurthy et al. 1990).

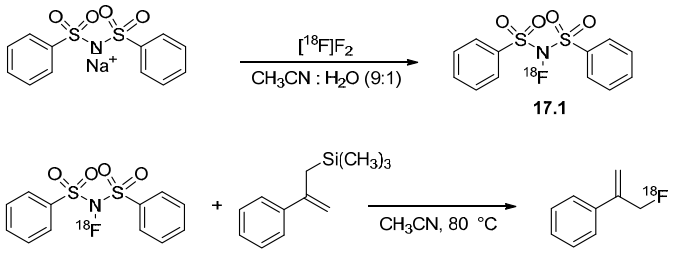


Scheme 16. Synthesis and use of *N*-[^{18}F]fluoro-*endo*-norbornyl-*p*-tolylsulfonamide (**16.1**) (Satuyamurthy et al. 1990).

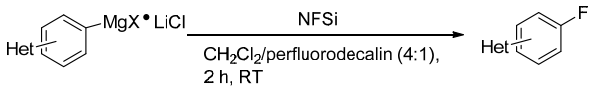
N-[^{18}F]fluorobenzenesulfonimide

N-fluorobenzenesulfonimide (NFSi, **17.1**) is a neutral fluorinating agent that is stable and solid at room temperature (RT). NFSi is soluble in common organic solvents, such as acetone, CH_2Cl_2, MeCN, DMF, and THF, and was found to be suitable for the fluorination of a variety of organometallic compounds, but it also permits the fluorination of slightly activated aromatic compounds (Satyamurthy et al. 1990, Davis et al. 1995, Rostami 2007, Liang et al. 2013). Enantioselective fluorination of β-ketoesters is possible when using NFSi with chiral

palladium complexes (Liang et al. 2013). Organocatalyzed enantioselective fluorinated products can be obtained with [^{18}F]NFSi (Teare et al. 2007).



Scheme 17. Synthesis of [^{18}F]NFSi and its use in the fluorination reaction of allylsilane (Teare et al. 2007).



Scheme 18. Fluorination of Grignard reagent with NFSi (Satyamurthy et al. 1990).

2.8.4. ^{18}F-TEDA-X reagents

F-TEDA-X reagents, *N*-fluoro-1,4-diazabicyclo[2.2.2]octane derivates, form a versatile group of NF class reagents and belong to quaternary ammonium salts ($R_3N^+F\ A^-$). F-TEDA-X reagents include a comprehensive number of derivatives in which the side chain groups and counter ions can be varied. F-TEDA-X reagents have been used in traditional organic chemistry as selective electrophilic fluorinations (Banks 1990, Banks et al. 1996, Banks 1998, Nyfeller et al. 2005, Furuya et al. 2008a, Furuya 2009a, Tang and Ritter 2011, Teare et al. 2010).

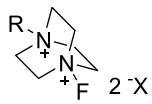
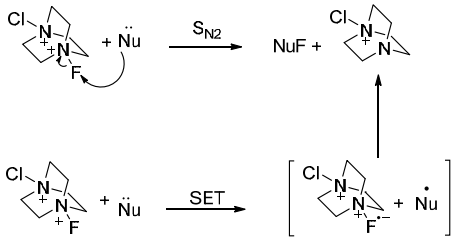


Figure 4. *N*-fluoro-1,4-diazabicyclo[2.2.2]octane derivate (Selectfluor^{TM}), R= Me, CH_2CF_3, or CH_2Cl, and X^- = OTf or $BF4^-$ (Banks 1990, Banks et al. 1992, Banks 1998, Hart and Syvret 1999, Nyfeller et al. 2005, Singh and Shreeve 2005).

With F-TEDA-X derivatives, different organic structures can be effectively fluorinated, from aliphatic chain structures, alkenes, and amines to aromatic rings, glycols, and electron rich organic structures, under regioselective and relatively mild reaction conditions. (Hart and Syvret 1999, Nyfeller et al. 2005, Singh and Shreeve 2004, Furuya et al. 2008a, Furuya et al. 2009a).

In aliphatic fluorinations, F-TEDA-X derivatives have two possible reaction mechanisms: S_N2 or single electron transfer (SET); with aromatic fluorination the mechanisms are S_EAr or SET. The reaction mechanism of fluorination with F-TEDA-X derivatives depends on the nature of the precursor and fluorinating agent. In addition, the reaction solvent impacts the reaction mechanism (S_N2 vs. SET) (Banks et al. 1990, Vincent et al. 1999, Nyfeller et al. 2005, Sorokin et al. 2013).



Scheme 19. S_N2 and single electron transfer (SET) mechanism of fluorination with F-TEDA-X derivatives (Banks et al. 1990).

The reactivity of F-TEDA-X derivatives varies depending on the structure of the side group and the counter ion. Fluorine will have a more electrophilic character if the peripheral alkyl side group is more electron-withdrawing. Of the peripheral side groups, the methyl group affects the reactivity the least. The trifluoroethyl group increases the reactivity compared to the chloromethyl group. Using triflate as a counter ion has been shown to increase the reactivity of F-TEDA-X compared to BF_4 (Banks et al. 1992, Banks et al. 1996, Hart and Syvret 1999, Vincent et al. 1999, Nyfeller et al. 2005). Vincent et al. (1999) observed higher yields and fewer side products when Selectfluor *bis*(triflate) was used instead of Selectfluor tetrafluoroborate (Vincent et al. 1999).

The F-TEDA-X derivatives are soluble in a limited number of polar protic and aprotic solvents: water, nitromethane, MeCN, DMF, DMA, acetone, and CD_2Cl_2 (Nyfeller et al. 2005, Banks 1998, Teare et al. 2010). Currently, ionic liquids, triflate, and tetrafluoroborate are used in the fluorination reactions with F-TEDA-X (Nyfeller et al. 2005).

Selectfluor tetrafluoroborate – F-TEDA-BF_4

N-fluoro-1,4-diazabicyclo[2.2.2]octane with a chloromethyl side chain and tetrafluoroborate counter ions (*N*-fluoro-1,4-diazabicyclo[2.2.2]octane *bis*(tetrafluoroborate), Selectfluor™), F-TEDA-BF_4 (Figure 5), is a quaternary ammonium salt and has the second highest reactivity of the F-TEDA-X derivatives (Banks 1990, Banks 1998, Singh and Shreeve 2004, Nyfeller et al.

2005, Chambers 2010). With F-TEDA-BF_4, a variety of different structures can be fluorinated, from aliphatic chains and alkenes to aromatic rings and amines (Singh and Shreeve 2004).

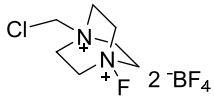
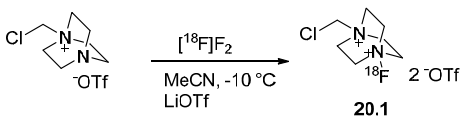


Figure 5. *N*-fluoro-1,4-diazabicyclo[2.2.2]octane *bis*(tetrafluoroborate), commercial Selectfluor.

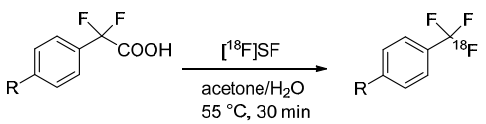
Lately, several applications of the fluorination of aryls with F-TEDA-BF_4 have been published by Ritter and co-workers. They have concentrated on fluorination mediated or catalyzed by transition metals (Furuya et al. 2008a, Furuya and Ritter 2009a, Furuya et al. 2009b, Furuya and Ritter 2010), but also fluorination reactions for aryl boron (Furuya and Ritter 2008b) and aryl silane (Tang and Ritter 2011) using F-TEDA-BF_4. Hodson et al. (1994) and Tageucki et al. (2000) presented an electrophilic fluorination method for fluoroindoles using F-TEDA-BF_4 as a fluorinating agent.

[^{18}F]Selectfluor *bis*(triflate)

The production of radiolabeled *N*-[^{18}F]fluoro-1,4-diazabicyclo[2.2.2]octane *bis*(triflate) ([^{18}F]SF, **20.1**) was published by Teare et al. in 2010 (Scheme 20). They also reported the electrophilic radiofluorination of several model molecules using [^{18}F]SF (Teare et al. 2010). In 2013, Mizuta et al. increased the variety of radiolabeling approaches using [^{18}F]SF by publishing a synthesis method for catalytic decarboxylative fluorination of tri- and difluoromethyl arenes (Scheme 21) (Mizuta et al. 2013).



Scheme 20. Synthesis of [^{18}F]Selectfluor *bis*(triflate) (Teare et al. 2010).

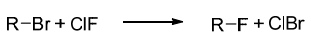


Scheme 21. Synthesis of [^{18}F]trifluoromethyl using [^{18}F]SF (Mizuta et al. 2013).

2.9. ClF and [^{18}F]ClF

In the 1970s and 1980s, Boguslavskaya and co-workers studied the substitution and addition reactions of ClF to halogen-substituted alkanes, vinyl alcohols, and substituted α,β-unsaturated acids. ClF is the lightest interhalogen and has a remarkable dipole moment, 0.89 D, which enables the chlorine atom to act as an electrophile (Boguslavskaya et al. 1980, Boguslavskaya et al. 1982, Boguslavskaya 1984).

Using a mild reaction environment without any catalyst, it is possible to fluorinate bromine LG containing alkanes and esters via a substitution reaction (Scheme 22). The bromine to fluorine substitution is possible in inert, low polarity solvents such as CCl_3, $CHCl_2$, or CF_2ClCCl_2F. The substitution reaction is not temperature sensitive, as substitution occurs from -78°C to +50°C. The alkane substituents cannot contain any reactive double bonds, such as hydroxyl-, carboxylic acid, aryl-, cyano-, or trialkylamine groups, which can react with ClF under these reaction conditions. During the bromine substitution reaction, a carbocation is formed. Accordingly, the reactivity of bromine substitution decreases in the following order due to the stability of the carbocation intermediate: tertiary carbon > secondary carbon > primary carbon (Boguslavskaya et al. 1982).



Scheme 22. Bromine substitution using ClF (Boguslavskaya et al. 1982).

Boguslavskaya et al. have also shown that ClF addition reactions are more regioselective in anhydrous and strongly polar HF than in inert low polarity solvent, as mentioned above, but CH_2Cl_2 can also be used. The addition reaction of ClF to allyl alcohols is trans-stereospecific in both types of reaction media. In an inert solvent, several EWGs on both sides of the double bond can significantly hinder the addition of ClF (Boguslavskaya et al. 1980). Boguslavskaya et al. also observed that the addition of ClF favors the electrophilic mechanism over the radical mechanism. The choice for inert reaction solvent or reaction temperature (-30ºC to +30ºC) did not have a remarkable effect on the yield of the addition reaction (Boguslavskaya et al. 1980).

In addition reactions of ClF to allyl esters in an inert non-polar solvent, the direction of addition does not predominantly depend on the type of double bond substituent and both Markovnikov (60%) and anti-Markovnikov (40%) products were observed (Boguslavskaya 1984).

In 1978, Lambrecht et al. described a production method for [^{18}F]ClF in which neon gas containing chlorine gas was bombarded with deuterons. The recovery yield was reported to be ~5.7%, but the produced [^{18}F]ClF was not used in further reactions (Lambrecht et al. 1978). In 2011, Engle et al. published a method to produce ClF in which the chlorine atom is radioactive: [^{34m,38}Cl]ClF. After production of the radioactive isotope of chlorine-38, they used a method similar to the synthesis of post-target [^{18}F]F_2 (Engle et al. 2012). Paper III reported a method for producing [^{18}F]ClF from post-target [^{18}F]F_2. We have also demonstrated the use of [^{18}F]ClF in an addition reaction of the C-C double bond, which, to the best of my knowledge, is the first demonstration of the ^{18}F-fluorination of an organic molecule with [^{18}F]ClF.

3. AIMS OF THE STUDY

The aims of this study were to develop the new radiolabeling agents [^{18}F]SF and [^{18}F]ClF and to demonstrate their usefulness in labeling reactions of radiotracers with practical utility.

The following objectives were set:

1. To synthesize [^{18}F]Selectfluor *bis*(triflate) ([^{18}F]SF) with high SA and to demonstrate the electrophilic synthesis of model molecules, such as 6-[^{18}F]FDOPA, using [^{18}F]SF.

2. To compare the two electrophilic labeling agents [^{18}F]SF and [^{18}F]F_2 and a nucleophilic approach in the synthesis of [^{18}F]NS12137, a norepinephrine transporter (NET) selective tracer for PET imaging.

3. To develop a method to produce [^{18}F]ClF via [^{18}F]F_2 and demonstrate the electrophilic addition reaction of [^{18}F]ClF to a C-C double bond-containing structure.

4. MATERIALS AND METHODS

4.1. General

All of the fluorinated radiolabeling agents and radiotracers described in this section were synthesized using synthesis devices built at the Radiopharmaceutical Chemistry Laboratory of Turku PET Centre. In the semi-preparative purifications, a Jasco PU-2089 Plus HPLC pump (JASCO Europe s.r.l., Cremella, Italy) with UV and radioactivity detectors was used. A VDC-405 ionization chamber (Veenstra Instruments, Joure, the Netherlands) was used for radioactivity measurements. Helium (99.995%) used in atzeotropic distillation, and 1% chlorine in neon used in [^{18}F]ClF synthesis, were supplied by AGA (Turku, Finland). Neon (99,995%) used as a sweep gas, and the carrier-fluorine, 0.5% F_2 in neon, were supplied by Linde AG - Geschäftsbereich Linde Gas, Unterschleicssheim, Germany. Homemade preparative GC columns were used for the purification of [^{18}F]MeF. A stainless steel HPLC column (7.8 x 300 mm) was emptied of its original filling and filled with gas chromatography (GC) material (Hayesep Q 80-100 mesh, Grace Davison Discovery, Deerfield, IL, USA). More detailed information about the materials and instruments used in radiosynthesis can be found in the original publications (Papers I, II and III).

Analytical radio-HPLC

All of the radiopharmaceuticals described in this section were analyzed using a VWR-Hitachi L-2130 HPLC pump (VWR Hitachi, VWR International GmbH, Darmstadt, Germany) combined with a VWR-Hitachi L-2400 UV-absorption detector and a 2 x 2 inch NaI-crystal for the detection of radioactivity.

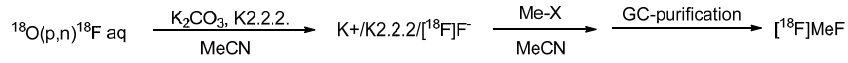
Radio-LC-MS/MS

Liquid chromatography mass spectrometry with radioactivity detection was used for identification of the radiopharmaceuticals in papers II and III. The LC-MS/MS system used a linear ion trap quadrupole mass spectrometer (QTRAP, Applied Biosystems SCIEX, Toronto, Canada) equipped with a turbo ion spray source and an Agilent 1100 series pump (Agilent Technologies, CA, USA). The homemade radioactivity detector placed between the LC outlet and MS inlet consisted of a teflon loop embedded in a plastic scintillator (Meltilex®, Wallac, Perkin Elmer, Turku, Finland). Light from β^+-particles interacting with the scintillator was detected by a double cathode PM-tube and the signal converted to a millivolt signal proportional to the radioactivity concentration eluting from the LC column.

4.2. Synthesis of labeling reagents

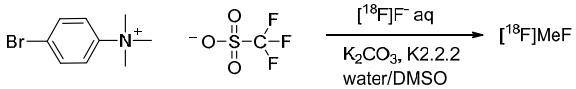
[^{18}F]Fluoride was produced with a CC-18/9 cyclotron (Efremov Scientific Research Institute of Electrophysical Apparatus, St Petersburg, Russia) by irradiating 2.1 ml ^{18}O-enriched water (^{18}O, >98 atom%, Rotem Industries, Israel) in a niobium target with an 18 MeV proton beam. The produced [^{18}F]F$^-$ was isolated from the target liquid on an anion exchange cartridge (QMA, Waters Corporation, Milford. MA, USA). The trapped [^{18}F]F$^-$ was then released by an eluent consisting of potassium carbonate, MeCN, and water and transferred into a vessel containing Kryptofix K2.2.2 in MeCN.

An anhydrous cryptand complex of [^{18}F]F$^-$/Kryptofix K2.2.2 was formed by atzeotropic distillation with MeCN. During evaporation, the reaction vessel was heated to 100°C. The methylation precursor (MeI, MeBr, Me-OTs, or Me-OTf; 90 µl/ml) was dissolved in MeCN and added to the dried residue. [^{18}F]MeF was synthesized through nucleophilic displacement of iodide, bromide, tosylate, or triflate from a methyl precursor (Scheme 23). The formation reaction of [^{18}F]MeF was allowed to occur for 90 s at 100°C. With the methylation precursor MeI, sonication during the atzeotropic distillation and reaction was tested.



Scheme 23. Formation of [^{18}F]MeF using methyl halogens, methyl tosylate, or methyl triflate. X = Br, I, tosylate, or triflate.

With the 4-*N,N,N*-trimethylanilinium triflate precursor (Blecha et al. 2008, Dannoon et al. 2010), the synthesis of [^{18}F]MeF was somewhat different than with other methyl precursors. First, 1.0 ml water containing 7 - 9 mg K_2CO_3 was pushed through a QMA cartridge containing [^{18}F]F$^-$ and into the reaction vial. Next, the methylation precursor 4-*N,N,N*-trimethylanilinium triflate (23-27 mg) was dissolved in a K2.2.2/DMSO solution (21-30 mg in 2.0-3.0 ml) and a QMA cartridge flushed with this solution. Atzeotropic distillation was not necessary. The reaction solution was heated at 190°C for 10 min (Scheme 24) and then cooled for 1 min.

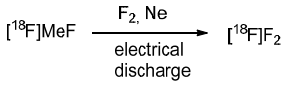


Scheme 24. Formation of [^{18}F]MeF using [^{18}F]F$^-_{aq}$ and 4-*N,N,N*-trimethylanilinium triflate (Blecha et al. 2008, Dannoon et al. 2010).

With all methyl precursors, the formed [^{18}F]MeF was flushed with neon into the self-made semi-preparative GC column (7.8 x 300 mm). In the semi-preparative GC column, [^{18}F]MeF was eluted with neon gas. [^{18}F]MeF gas was separated from gaseous impurities and the purified [^{18}F]MeF fraction trapped in a stainless steel loop at -196°C using liquid nitrogen.

4.2.1. [^{18}F]F_2

[^{18}F]MeF was transferred with a low amount of carrier fluorine (~1000 nmol) in neon to a quartz discharge chamber. A high voltage discharge was initiated through this gas mixture (Scheme 25).



Scheme 25. Production of [^{18}F]F_2.

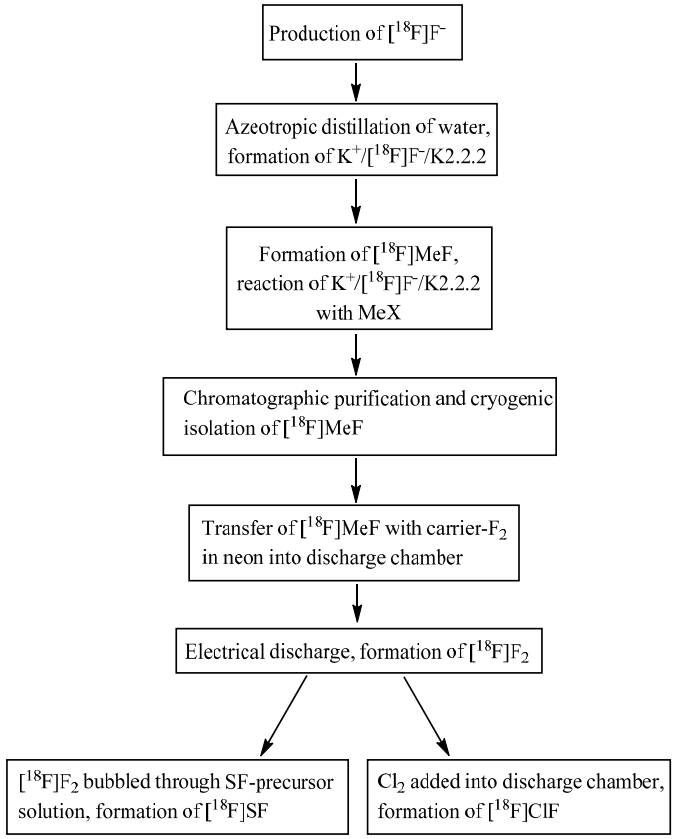
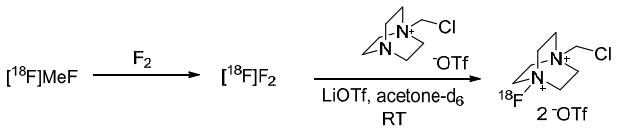


Figure 6. Flow chart of synthesis procedures for [^{18}F]F_2 and its derivatives [^{18}F]SF and [^{18}F]ClF. Me-X, X= I-, Br-, OTf-, or OTs-.

4.2.2. [^{18}F]Selectfluor bis(triflate) (I)

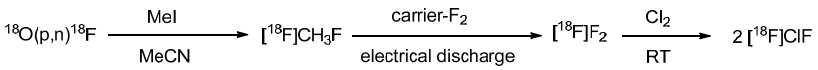
In paper I, the reaction conditions for [^{18}F]SF were further optimized from those in Teare et al. (2010). The influence of the concentration of SF precursor, 2 µmol (2.7 mM) versus 7.5 µmol (10 mM), in labeling reactions was studied. [^{18}F]F_2 was bubbled directly through the reaction solution containing the SF precursor (0.6-2.3 mg, 2.0-5.5 µmol, MW 310 g/mol) and LiOTf (0.3 or 1.2 mg, 2.0 or 7.5 µmol, MW 156.01 g/mol) in acetone-d_6, leading to instantaneous fluorination. The formed crude stock solution of [^{18}F]SF was used without any further purification. These two concentrations of the [^{18}F]SF solution, 2.7 mM and 10 mM, were evaluated in electrophilic labeling of 6-[^{18}F]FDOPA (Scheme 26 and Table 6).



Scheme 26. Synthesis of [^{18}F]SF.

4.2.3. [^{18}F]ClF (III)

For formation of [^{18}F]ClF, chlorine gas (1400 nmol) was added to the quartz vessel the [^{18}F]MeF/F_2 discharge (Scheme 27). The formation of [^{18}F]ClF was not promoted by heating or any other means.



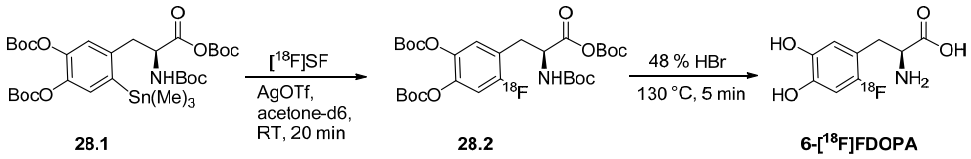
Scheme 27. Formation of [^{18}F]ClF.

4.3. Synthesis of 6-[^{18}F]FDOPA (I)

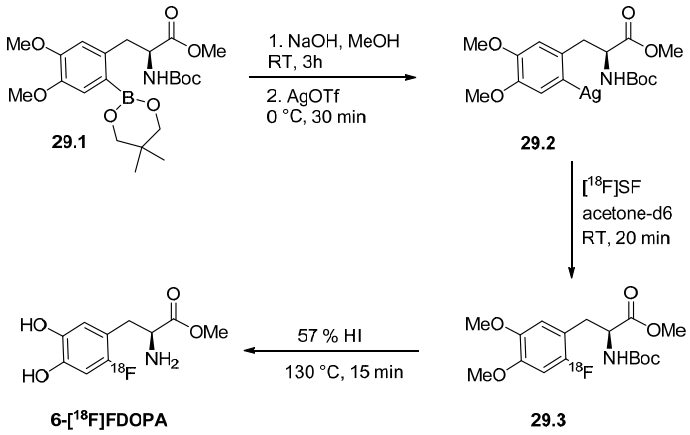
Both tin and silver(I) complex LG-containing precursors were used in the synthesis of 6-[^{18}F]FDOPA (Schemes 28 and 29). The silver(I) complex (**29.2**) was produced from boronic ester (**29.1**) (Scheme 29) in situ. With the stannyl-containing precursor, the synthesis of 6-[^{18}F]FDOPA occurred at both 2.7 mM and 10 mM [^{18}F]SF, but with silver(I) complex only 2.7 mM [^{18}F]SF was used.

[^{18}F]SF in acetone-d_6 (0.2 ml) was added to a reaction vial containing either **28.1** or **29.2** (10 µmol). Silver triflate (20 µmol) was added only for fluorination of **28.2**. The reaction solution was stirred for 20 minutes at RT. Subsequently, acetone was evaporated at RT and the Boc-protecting groups removed with 48% HBr (0.3 ml) at 130°C for 5 minutes (Scheme 28). The

MeO- protecting groups were hydrolyzed with 57% HI (0.3 ml) at 130°C for 15 minutes (Scheme 29).



Scheme 28. Synthesis of 6-[^{18}F]FDOPA using trimethyltin as a leaving group and [^{18}F]SF as a labeling agent.



Scheme 29. Synthesis of 6-[^{18}F]FDOPA using aryl boronic ester (**29.1**) as a leaving group.

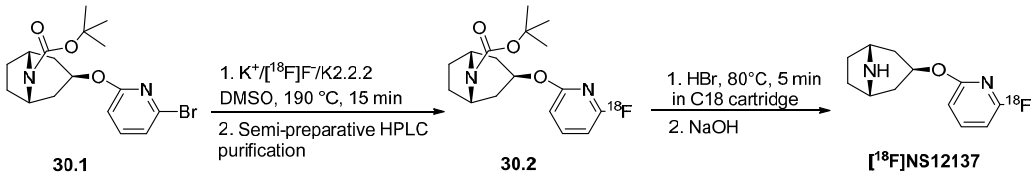
After the radiofluorination samples from the reaction mixtures of both precursors were collected and analyzed with analytical radio-HPLC, the fractions corresponding to the protected intermediates **28.2** and **29.3** were collected from the HPLC outlet. The same analysis was carried out after hydrolysis, when the fraction corresponding to 6-[^{18}F]FDOPA was collected and measured for radioactivity and to calculate the SA.

Statistical analyses were performed using the program Graph Prism, version 5.01 (GraphPad Software, San Diego, CA, USA). RCYs were compared using the unpaired t-test with Welch's correction. Results are expressed as means ± SD for the indicated number of observations (Figure 7). Means were considered significantly different when $p<0.05$.

4.4. Synthesis of [^{18}F]NS12137 (II)

4.4.1. Nucleophilic synthesis

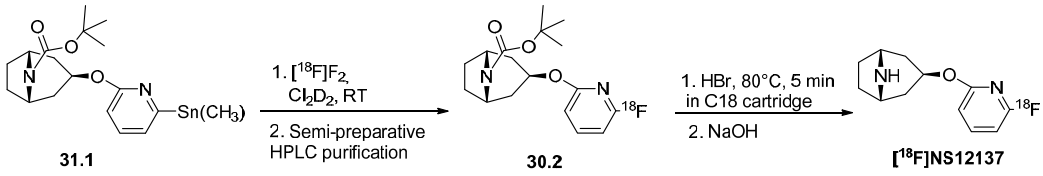
The brominated precursor tert-butyl(1*S*,5*R*)-3-[(6-trimethylstannyl-2-pyridyl)oxy]-8-azabicyclo[3.2.1]-octane-8-carboxylate **31.1** (7.1-10.2 mg; 20.6-26.6 μmol) was dissolved in 0.5-1.0 ml DMSO and added to the dry $^{18}F^-/K_2CO_3$/K222 complex. The reaction solution was heated at 190ºC for 15 minutes (Scheme 30), diluted with 0.5-1.5 ml MeCN, and purified using semi-preparative HPLC.



Scheme 30. Nucleophilic synthesis of [^{18}F]NS12137.

4.4.2. Electrophilic synthesis with [^{18}F]F_2

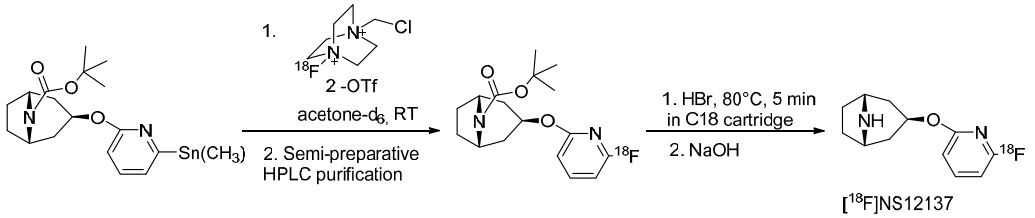
[^{18}F]F_2 was bubbled directly through reaction solution containing the stannylated precursor 3-[(6-trimethylstannyl-2-pyridyl)oxy]-8-azabicyclo-[3.2.1]octane-8-carboxylate **31.1** (1.5-1.7 mg; 3.2-3.6 μmol) in CD_2Cl_2 (750 μl) (Scheme 31). After fluorination, the solvent was evaporated at RT, the residue dissolved into MeCN, and then purified using semi-preparative radio-HPLC.



Scheme 31. Electrophilic synthesis of [^{18}F]NS12137 using [^{18}F]F_2.

4.4.3. Electrophilic synthesis with [^{18}F]SF

[^{18}F]SF stock solution (2.7 mM, 0.2 ml) and silver triflate (5-20 mg, AgOTf) measured to be twice the molar amount of the [^{18}F]SF in stock solution were added to the vial containing stannylated precursor (2.6-5.1 mg; 5.6-17.1 μmol) (Scheme 32). The reaction mixture was stirred for 20 min at RT. After the reaction, the solvent was evaporated at RT. The residue was dissolved in MeCN and purified using semi-preparative radio-HPLC.



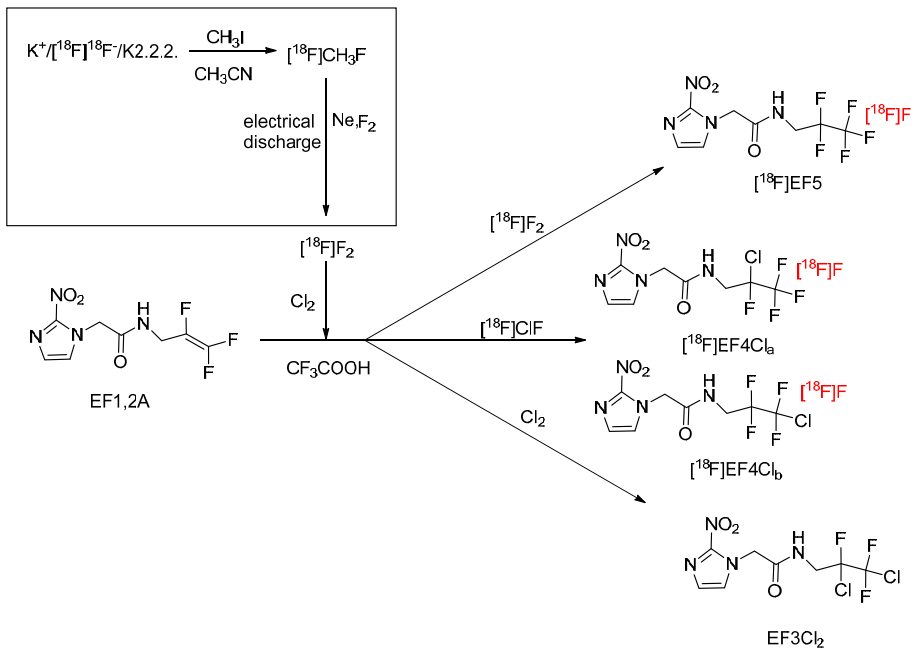
Scheme 33. Electrophilic synthesis of [^{18}F]NS12137 using [^{18}F]SF.

The purified protected intermediate (**30.2**) of the [^{18}F]NS12137 fraction radiofluorinated using either of the above methods was collected, diluted with 30 ml water, and loaded onto a preconditioned C18 SepPak (Waters Corporation, Milford, MA, USA). The SepPak was then washed with 20 ml water. Deprotection was achieved in 4 min on the SepPak using 48% HBr (300 µl). After hydrolysis the SepPak was rinsed with water and [^{18}F]NS12137 eluted using 1 ml EtOH and 4 ml 0.9% NaCl solution. The chemical purity (CP), radiochemical purity (RCP), and SA of the final product were analyzed using analytical radio-HPLC.

RadioLC-MS/MS was utilized for identification of the protected and deprotected [^{18}F]NS12137 based on m/z. Samples were separated on a Waters Atlantis dC18 column (1.0 x 150 mm, 3 µm, Waters Corp., Milford MA, USA) using a flow rate of 50 µl/min. The mobile phase consisted of 80% MeCN with 0.1% formic acid and 20% water (v/v). The turbo ion spray source was operated in positive ion mode.

4.5. Synthesis of [^{18}F]EF4Cl$_{a,b}$ (III)

The gaseous contents of the quartz reaction vessel were bubbled through the precursor solution containing EF1,2A (2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide) in TFA (Scheme 33), and radiolabeled products were isolated and identified by radio-HPLC. The same HPLC system was used for semi-preparative HPLC separation and identification of the radiolabeled products. The HPLC pump was connected to a SunFire C18 column (5 µm, 4.6 x 150 mm, Waters Corp., Milford, MA, USA), and the HPLC column was eluted with 0.1% formic acid in methanol:water (38:62, v/v) at a flow rate of 1.0 ml/min.



Scheme 33. Synthesis of [^{18}F]EF4Cl$_a$ and [^{18}F]EF4Cl$_b$.

RadioLC-MS/MS analysis of [^{18}F]EF4Cl$_{a,b}$

In radioLC-MS/MS analysis, single ion masses were monitored: EF1,2A m/z 263 amu, [^{18}F]EF5 m/z 301 amu, [^{18}F]EF4Cl$_a$ m/z 317 amu, [^{18}F]EF4Cl$_b$ m/z 317 amu, EF3Cl$_2$ m/z 333 amu. The separated products, [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, [^{18}F]EF5, and precursor (EF1,2A), were fragmented using the LC-MS/MS system (Figure 10). Fragmentation conditions varied for different compounds: [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, [^{18}F]EF5, and EF3Cl$_2$ were fragmented using a collision energy of 15 kV. In fragmentation of the precursor, a collision energy of 5 kV was used.

Lipophilicity measurements of [^{18}F]EF4Cl$_{a,b}$

The lipophilicity of [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, and [^{18}F]EF5 was determined under physiological conditions (logD), and ClogP values were calculated using two different commercially available codes: ChemDraw©, version 11 (CambridgeSoft Corporation, Cambridge, MA, USA) and ACD/ChemSketch Freeware, version 12.0 (Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2009).

The apparent partition coefficients (logD) for [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, and [^{18}F]EF5 were determined using the shake flask method (OECD 1995). The radiolabeled compound (3 - 5 MBq) was shaken in a saturated mixture of n-octanol (10 ml) and 0.1 M phosphate buffer (pH

7.4, 10 ml) for one hour at RT. The radioactivity concentration of both phases was measured and logD calculated. The retention volumes ($R_V(N)$) of [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, and [^{18}F]EF5 were calculated from the HPLC chromatograms and normalized to the $R_V(N)$ of EF3Cl$_2$.

5. RESULTS

5.1. Synthesis of labeling reagents

5.1.1. [^{18}F]MeF

The RCYs of [^{18}F]MeF using halogenated precursors are presented in Table 5. The highest RCY of [^{18}F]MeF was achieved using 4-*N,N,N*-trimethylanilinium triflate as a precursor (Entry 6). No significant difference was found in the RCYs of methyl iodide with (Entry 1) or without (Entry 2) sonication. With methyl bromide the formation of [^{18}F]MeF was very low, 0.52 ± 0.33% (Entry 3). Low RCY was observed using methyl triflate as a precursor (Entry 5). The RCY was similar using methyl tosylate (Entry 4) or methyl iodide as a precursor. All of the results are decay corrected to the end of bombardment (EOB) and summarized in Table 5.

Table 5. Radiochemical yields (mean ± SD) of [^{18}F]MeF using different precursors with decay corrected to EOB.

Entry	Precursor	Number of synthesis	Radiochemical yield [% at EOB]
1	MeI[#]	n=5	57.8 ± 2.4
2	MeI[†]	n=4	59.5 ± 4.8
3	MeBr	n=3	0.52 ± 0.33
4	MeOTs	n=3	57.2 ± 5.5
5	MeOTf	n=3	6.4 ± 3.1
6	4-*N,N,N*-trimethylanilinium triflate	n=3	85.6 ± 11.5

[#] with sonication, [†]without sonication

5.1.2. [^{18}F]Selectfluor (I)

[^{18}F]SF was prepared and successfully used for ^{18}F synthesis. [^{18}F]SF stock solutions of 5-10 GBq were used in labeling reactions for up to several hours after preparation. The stock solution of [^{18}F]SF was not homogenous when left without mixing, as layers with different concentrations of radioactivity were observed (Kirjavainen, unpublished data). Remixing the solution before sampling aliquots for reactions homogenized the stock solution.

5.1.3. [^{18}F]ClF (III)

[^{18}F]ClF was not isolated and the RCY was not calculated. Instead, the formation of [^{18}F]ClF was identified from the reaction products produced through electrophilic addition of [^{18}F]ClF.

5.2. Synthesis of 6-[^{18}F]FDOPA (I)

The applicability of [^{18}F]SF was demonstrated with the radiolabeling of stannylated and aryl boronic ester precursors of 6-[^{18}F]FDOPA. The results are presented in Table 6.

Table 6. The measured RCYs (mean ± SD) of 6-[^{18}F]FDOPA with stannylated precursor (n= 6) and boronic ester precursor (n=6). The results are presented as mean +/- SD.

Entry	Precursor of FDOPA	RCY [%]	
		c(SF stock) = 2.7 mM	c(SF stock) = 10 mM
1	arylstannane	12.1 ± 3.7	6.8 ± 1.8
2	boronic ester	19.0 ± 12.2	-

Arylstannane precursor was reacted at RT with [^{18}F]SF (lower or higher molar SF concentration (Table 6 Entry 1), and silver(I) triflate (Table 6 Entry 2) in acetone-d_6 for 20 minutes. The yield of 6-[^{18}F]FDOPA using stannylated precursor did not differ significantly from the yield of 6-[^{18}F]FDOPA using boronic ester precursor. In addition, less SF precursor resulted in significantly higher RCY for 6-[^{18}F]FDOPA (Table 6 Entry 2, Figure 7).

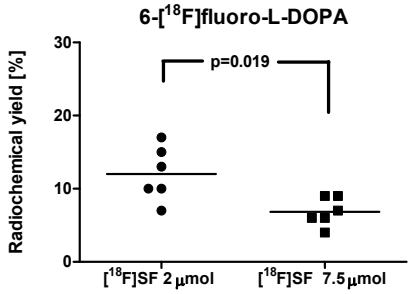


Figure 7. Statistical analysis of the yield of 6-[^{18}F]FDOPA using 2 µmol (n=6) or 7.5 µmol (n=6) stannylated precursor.

5.3. Synthesis of [^{18}F]NS12137 (II)

[^{18}F]NS12137 was synthesized using a nucleophilic and two electrophilic methods. In electrophilic labeling with [^{18}F]F_2, the number of radioactive side products was greater than in electrophilic labeling with [^{18}F]SF or nucleophilic labeling (see Paper III, Suppl. Data).

The RCP of the protected intermediate was equivalent (>95%) in all methods. The RCY, RCP, and SA are presented in Table 7. The deprotection proceeded smoothly at RT, and the RCP of [^{18}F]NS12137 exceeded 96% with all methods. The highest achieved SA using [^{18}F]F_2 as a labeling agent was 29 GBq/µmol. This value was achieved using optimized conditions; very high starting activity and minimized F_2-carrier addition.

Table 7. RCYs, RCPs, and SAs of protected intermediate of [^{18}F]NS12137 (**30.2**) using different labeling approaches. SAs are decay corrected to EOB. Data was achieved using same condition in tracer synthesis. The results are presented as mean +/- SD.

Entry		**RCY* of 30.2 [%]**	**RCP of 30.2 [%]**	**SA* of 30.2 [GBq/µmol]**
1	Electrophilic labeling via [^{18}F]F_2	2.1 ± 0.9	96.2 ± 0.1	1.8 ± 0.5
2	Electrophilic labeling via [^{18}F]Selectfluor	2.3 ± 1.5	> 99.5	1.4 ± 0.3
3	Nucleophilic labeling	48.7 ± 8.2	99.6 ± 0.7	> 500

*Decay corrected to EOB

With LC-MS the SIMs were monitored: [^{18}F]NS12137 m/z 223 amu and Boc-protected [^{18}F]NS12137 m/z 323 amu (Figure 8).

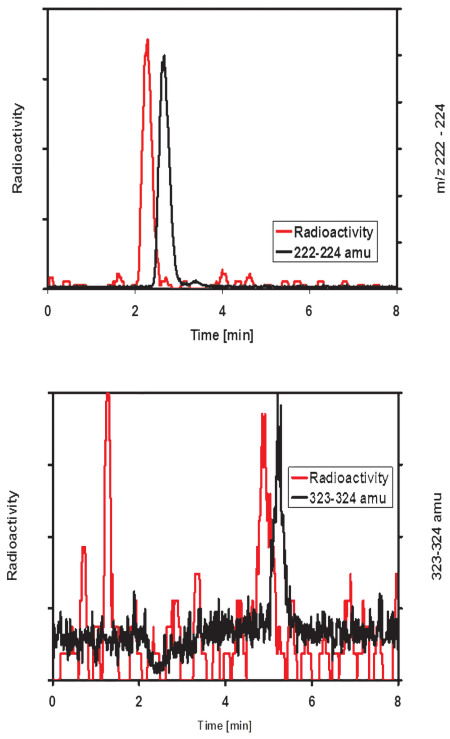


Figure 8. RadioLC-MS chromatograms of [^{18}F]NS12137 (top) and Boc-protected [^{18}F]NS12137 (bottom) analyzed by selected ion masses: m/z 232 for [^{18}F]NS12137 and m/z 323 for Boc-protected [^{18}F]NS12137 (Paper III, Supplementary data).

5.4. Synthesis of [^{18}F]EF4Cl$_{a,b}$ (III)

Radiolabeled products [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, and [^{18}F]EF5 were produced in a single synthesis and separated by HPLC. The RCYs, RCPs, and SAs of all products are presented in Table 8. The SAs of [^{18}F]EF4Cl$_a$ (Entry 1) and [^{18}F]EF4Cl$_b$ (Entry 2) were determined using EF5 as a reference assuming that the UV absorption absorbance of [^{18}F]EF4Cl$_a$ and [^{18}F]EF4Cl$_b$ was equivalent to that of EF5 (Entry 3). The ratio of the SAs of [^{18}F]EF4Cl$_a$ and [^{18}F]EF5 was 0.56 ± 0.03, and the ratio of the SAs of [^{18}F]EF4Cl$_b$ and [^{18}F]EF5 was 0.55 ± 0.05.

Table 8. The RCYs, RCPs, and SAs of [^{18}F]EF4Cl$_a$ (n=5), [^{18}F]EF4Cl$_b$ (n=5), and [^{18}F]EF5 (n=5). The results are presented as mean +/- SD.

Entry	Product	RCY (absolute yields) [%]	RCY (from HPLC chromatogram) [%]	SA* [GBq/µmol]
1	[^{18}F]EF4Cl$_a$	7.7 ± 0.9	8.5 ± 2.0	6.0 ± 1.6
2	[^{18}F]EF4Cl$_b$	4.6 ± 0.5	5.4 ± 1.7	3.3 ± 0.9
3	[^{18}F]EF5	24.6 ± 2.9	29.4 ± 4.2	3.2 ± 0.7

*The SAs are decay corrected to the EOS

Identification of [^{18}F]EF4Cl$_{a,b}$

The turbo ion spray source was operated in negative ion mode. SIMs were monitored: EF1,2A m/z 263 amu, [^{18}F]EF5 m/z 301 amu, [^{18}F]EF4Cl$_a$ m/z 317 amu, [^{18}F]EF4Cl$_b$ m/z 317 amu, and EF3Cl$_2$ m/z 333 amu (Figure 9).

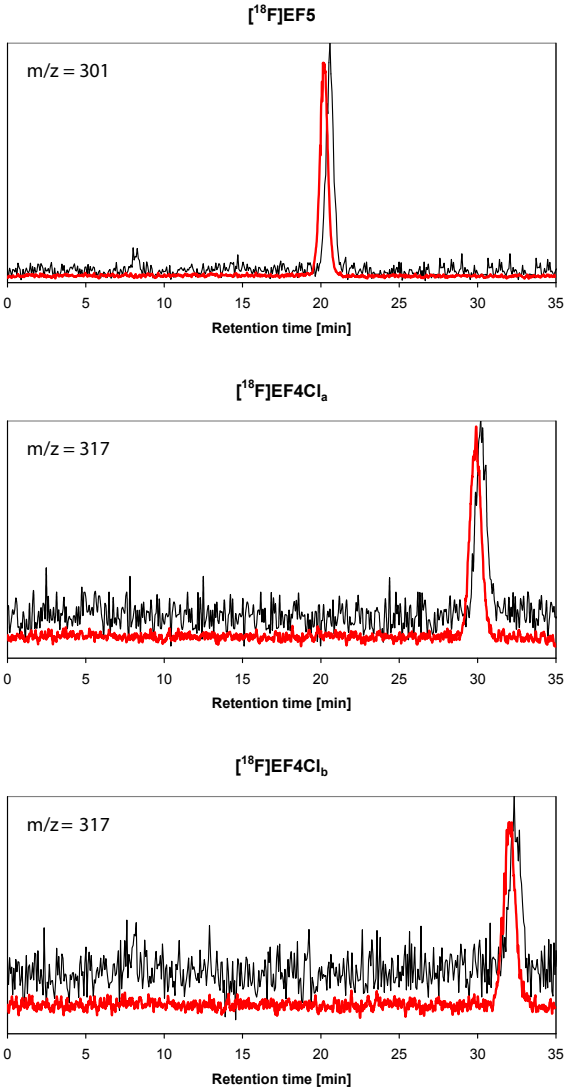
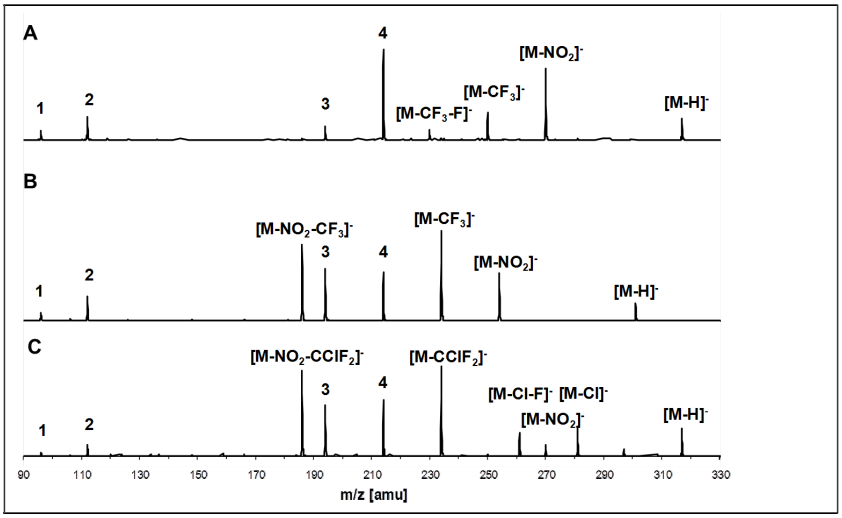
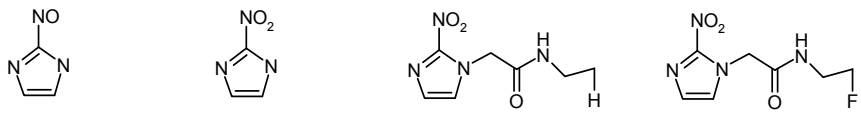


Figure 9. Radio LC-MS/MS (Paper III, Supplementary data).

In the LC-MS/MS spectra of [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, and [^{18}F]EF5, the mass peaks at m/z 96, 112, 196, and 214 amu (1-4, Figure 10) were common to [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, and [^{18}F]EF5. The mass peaks at m/z 270 amu for [^{18}F]EF4Cl$_a$ and [^{18}F]EF4Cl$_b$ were identified as

the parent ion lacking the NO_2 group. The corresponding mass peak for [^{18}F]EF5 was observed at m/z 254 amu. Breakage of the terminal carbon bond of the halogenated tail was seen in the formation of the mass peaks at m/z 250 amu for [^{18}F]EF4Cl$_a$ (parent minus -CF_3), m/z 234 for [^{18}F]EF4Cl$_b$ (parent minus -$CClF_2$), and m/z 230 for [^{18}F]EF5 (parent minus -CF_3). For both [^{18}F]EF5 and [^{18}F]EF4Cl$_b$, an m/z 186 product was identified as 1-(2-((1,1-difluoroethan-1-id-2-yl)amino)-2-oxoethyl)-1H-imidazol-2-ide. In this ion, both the terminal carbohalogen group and the NO_2 group were detached. The corresponding product for [^{18}F]EF4Cl$_a$ at m/z 202 amu was observed in only minute amounts. Furthermore, in the fragmentation pattern of [^{18}F]EF4Cl$_b$, ions were observed at m/z 281 amu (parent minus -Cl) and m/z 261 amu (parent minus -Cl and -F) (Paper III, Supplementary data).





1 (m/z 96 amu) **2** (m/z 112 amu) **3** (m/z 196 amu) **4** (m/z 214 amu)

Figure 11. Consider the m/z spectra of [^{18}F]EF4Cl$_a$ (A), [^{18}F]EF5 (B) and [^{18}F]EF4Cl$_b$ (C) fractions. Parent is assigned the symbol M. Signals marked 1-4 represent common structures for all molecules and are depicted below the figure (Paper III, Supplementary data).

Lipophilicity of [^{18}F]EF4Cl$_{a,b}$

The lipophilicity of [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, and [^{18}F]EF5 was determined under physiological conditions by calculating ClogP using two commercially available software (Table 9).

Table 9. $LogD_{7.4}$ and ClogP values of [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$ and [^{18}F]EF5 (Paper III).

	[^{18}F]EF5	**[^{18}F]EF4Cl$_a$**	**[^{18}F]EF4Cl$_b$**
Shake-flask method ($logD_{7.4}$) (n=3)	0.6 ± 0.04	0.79 ± 0.08	0.78 ± 0.1
ChemSketch (ClogP)	1.35 ± 0.83	1.51 ± 0.79	1.87 ± 0.75
ChemDraw (ClogP)	0.83	1.17	1.43

*logP = 0.6

6. DISCUSSION

In this study, the regioselective radiofluorinating agents [^{18}F]SF and [^{18}F]ClF were developed with high SA. In the synthesis of [^{18}F]SF presented in papers I and II, [^{18}F]F_2 was bubbled through an SF precursor solution at RT. In paper III, the gas mixture of [^{18}F]F_2, [^{18}F]ClF, and Cl_2 was bubbled through an EF5 precursor in order to produce [^{18}F]EF4Cl_a, [^{18}F]EF4Cl_b, and [^{18}F]EF5, which were separated by HPLC.

6.1. Synthesis of labeling reagents

6.1.1. [^{18}F]MeF

High quality [^{18}F]MeF is a prerequisite for the successful production of high SA [^{18}F]F_2. The RCY and SA of [^{18}F]MeF directly affects the SA of [^{18}F]F_2. The RCY was reproducible for the synthesis with methyl iodide, but with methyl triflate the yield varied considerably. The RCY of [^{18}F]MeF did not increase significantly when sonication was used with the MeI precursor. The highest RCY was achieved with 4-*N*,*N*,*N*-trimethylanilinium triflate, but there were some issues achieving high CP for [^{18}F]MeF with the preparative GC system. The approach for synthesizing [^{18}F]MeF with 4-*N*,*N*,*N*-trimethylanilinium triflate does not require atzeotropic distillation, but the reaction time is significantly longer than with other methyl precursors. Furthermore, with other methyl precursors, the only liquids used in the synthesis are water and MeCN, so the synthesis device is not contaminated with other solvents, which can occur during synthesis with 4-*N*,*N*,*N*-trimethylanilinium triflate. This contamination with a high boiling point solvent can also affect the preparative GC purification and function of the GC column. Automation of the synthesis of [^{18}F]MeF using 4-*N*,*N*,*N*-trimethylanilinium triflate as a methyl source is possible and could be achieved easily. On the other hand, the synthesis of [^{18}F]MeF with methyl iodide is already automated in our laboratory and is robust and repeatable. In conclusion, 4-*N*,*N*,*N*-trimethylanilinium triflate appeared to be a promising precursor, but further studies are needed in order to improve the robustness of the synthesis in our hands.

6.1.2. [^{18}F]Selectfluor (I)

[^{18}F]SF has a unique reactivity profile in the sense that it is much milder and selective than elemental fluorine and suitable for the fluorination of electron-rich substrates that cannot be fluorinated using alternative N-F reagents. The advantage of [^{18}F]SF is that the stock solution has high stability and can be divided for several syntheses, or even transported to other laboratories. These characteristics are highly sought after, especially for ^{18}F-labeling precursors that are not suitable for nucleophilic fluorination. Further studies and optimization

using various SF counter-ions will be needed. However, [^{18}F]SF reagent has shown its usefulness and versatility as an electrophilic radiofluorinating agent.

The stock solution of [^{18}F]SF was not homogenous when left without mixing, as uneven activity distribution was observed in the solution. Thus the [^{18}F]SF stock solution has to be stirred before use in radiolabeling. Deuterated acetone has been shown to be a suitable solvent for the production of [^{18}F]SF, due to its superior chemical quality (Kirjavainen, unpublished data). [^{18}F]SF in acetone-d_6 can be used directly in labeling reactions. Acetone can also be evaporated easily due to its low boiling point, thereby allowing wider range of precursors to be solubilized and amenable to [^{18}F]fluorination.

6.1.3. [^{18}F]ClF (III)

Modification of the pentafluoro group of [^{18}F]EF5 by fluorine for chlorine exchange modified the lipophilicity of the chlorinated molecules that were formed. However, preclinical studies showed that the biological behavior of these chlorinated molecules is not altered to a great extent compared to the parent molecule [^{18}F]EF5. This result encourages development of the electrophilic labeling approaches for ^{18}F using [^{18}F]ClF. However, this approach requires a suitable double bond for the addition of [^{18}F]ClF, which can limit the number of candidate tracer molecules. In this study, only one precursor candidate was labeled with [^{18}F]ClF; thus, the broader use of this labeling agent should be studied further.

[^{18}F]ClF could be produced by F_2-carrier-addition via [^{18}F]F_2 and subsequent Cl_2 addition in the quartz chamber at RT, but it was not possible to produce n.c.a [^{18}F]ClF by electrical discharge in the quartz chamber direct from [^{18}F]MeF and Cl_2. In the electrical discharge method, [^{18}F]MeF and F_2, were decomposed to atoms in Ne gas matrix. During the discharge, an F_2 excimer is formed that emits enough highly energetic UV light (157 nm) to cut the C-F bonds of [^{18}F]MeF (dissociation energy of C-F bond is 217 nm). The Cl_2 excimers produced in a similar way by high voltage discharge do not have enough energy (wavelength of emitted UV light is 258 nm). Therefore, research on other methods for excitation of gas molecules in this setting is needed. One worthwhile option for study is a vacuum UV excimer laser. We postulate that with ArF laser photons can be produced with enough energy to cut the C-F bond, with no need for carrier-F_2, and n.c.a. [^{18}F]ClF can be produced. The amount of [^{18}F]ClF formed will be so low that special synthesis and analytical devices will be needed, such as GC-MS, which can analyze gases with low atomic masses. In addition other chamber materials than quartz , i.e. Teflon, will be valuable to study.

6.2. Synthesis of 6-L-[^{18}F]FDOPA (I)

This study showed that [^{18}F]SF is appropriate for the production of high quality 6-[^{18}F]FDOPA. 6-[^{18}F]FDOPA can be prepared in acetone as efficiently as in acetonitrile (Paper II, Teare et al. 2010). A significant difference was found in the RCY of 6-[^{18}F]FDOPA when using different amounts of precursors in the preparation of [^{18}F]SF. Silver triflate-mediated ^{18}F fluorination of electron-rich arylstannane is of particular interest because this transformation, which can now be conducted selectively and rapidly under very mild conditions, indicates that the unprotected alcohol functionality is well tolerated. The boronic ester LG is less toxic than the arylstannane group and may be a better choice when using large amounts of precursor and electrophilic radiofluorinating agents with low SA. On the other hand, with the SA presented in this study, the toxicity of stannylated precursors would not be a problem. Also, from the GMP point of view, the long and demanding synthesis procedures needed with boronic ester precursor are not practical and often not possible.

Elemental fluorine was shown to be so highly reactive that even minute impurities of SF precursor, solvents, or other chemicals can crucially affect the RCY and SA of the formed [^{18}F]SF. Elemental fluorine is the limiting reagent in the reaction and may prefer reacting with these impurities. In contrast to the work of Teare et al. (2010), who used a large excess of SF precursor in the synthesis of [^{18}F]SF from [^{18}F]F_2, we decreased the amount of SF, demonstrating a significant increase in the yield of [^{18}F]SF and subsequent radiofluorinations when using more closely matched stoichiometry for the reagents.

6.3. Synthesis of [^{18}F]NS12137 (II)

In this study, [^{18}F]NS12137 was successfully produced by three different methods. The radiofluorination of [^{18}F]NS12137 with [^{18}F]SF took 20 min at RT due to the lower reactivity of [^{18}F]SF compared to [^{18}F]F_2, but fewer byproducts were observed for the same reason. Regardless of the electrophilic labeling method used, the RCY, RCP, and SA were similar. The electrophilic radiofluorination method for [^{18}F]NS12137 using [^{18}F]F_2 was straightforward and could easily be automated for GMP environments. Thus, both electrophilic labeling methods are suitable for the production of [^{18}F]NS12137.

With the nucleophilic labeling methods the RCY and SA were considerably higher compared to electrophilic methods, and only a few byproducts were observed. However, the reaction conditions were very harsh. With the nucleophilic method, the amount of base, reaction temperature, reaction time, or solvent will need more attention, and the optimization of

reaction conditions is more time-consuming than with electrophilic methods. [^{18}F]NS12137 was evaluated as a NET tracer and high SA was crucial due to the low density of NET in the brain. The SA of the electrophilic labeling agent achieved with the post-target production method can be high, as has been demonstrated earlier in the synthesis of [^{18}F]CFT for clinical use (Laakso et al 1998). On the other hand, in the nucleophilic synthesis the harsh reaction environment could lead to decomposition or racemization of the precursor or desired product; thus, the electrophilic labeling method, even with significantly lower RCY, may be the better choice. However, as a general statement it can be said that, all other things being equal, nucleophilic fluorination is to be preferred over electrophilic fluorination due to the higher SA achievable.

The pharmacokinetics of [^{18}F]NS12137 was evaluated *in vivo* and *ex vivo* in brain and periphery of healthy, adult and juvenile Sprague Dawley rats. The pharmacological specificity in rats was estimated from the decrease in binding after the administration of a dose of nisoxetine, a highly selective NET antagonist. The highest [^{18}F]NS12137 binding was found in the locus coeruleus, which has the highest level of NET expression in rat brain. The highest region-to-cerebellar cortex uptake ratios were obtained in the locus coeruleus and hippocampus. The region-to-cerebellar cortex ratios in thalamus, hypothalamus, and septum did not differ from striatum, which is known of low level of NET expression. In rats that were treated with nisoxetine the binding in locus coeruleus was not detectable.

6.4. Synthesis of [^{18}F]EF4Cl$_{a,b}$ (III)

[^{18}F]EF4Cl$_a$ and [^{18}F]EF4Cl$_b$ were selected as model molecules for [^{18}F]ClF labeling because their precursor contains a suitable double bond for the electrophilic addition reaction. Furthermore, the effects of the added chlorine atom were compared to pentafluorinated [^{18}F]EF5. The biological behavior of [^{18}F]EF5 is well known (Koch et al. 2001, Eskola et al. 2012). [^{18}F]EF4Cl$_a$ and [^{18}F]EF4Cl$_b$ were produced simultaneously. The SAs of [^{18}F]EF4Cl$_a$ and [^{18}F]EF4Cl$_b$ were determined using EF5 as a reference because it was not possible to obtain non-radioactive standards of the monochlorinated products. Because the UV-active structure is the same in all three molecules, the UV absorbance of [^{18}F]EF4Cl$_a$ and [^{18}F]EF4Cl$_b$ was assumed to be equal to the UV absorption of EF5. Furthermore, the SAs of the monochlorinated ^{18}F-labeled products were half the SA values obtained for the [^{18}F]EF5. This outcome was predictable, as the molar amount of [^{18}F]ClF is doubled in the reaction with [^{18}F]F$_2$. [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, and [^{18}F]EF5 have unique MS/MS fragmentation patterns

and were identified by these patterns. LC-MS/MS analysis and fragmentation was a useful method for identifying this kind of regioisomer.

The results of the lipophilicity measurements and calculations varied somewhat due to commercially available codes, but the chromatographic behavior of [^{18}F]EF4Cl$_a$, [^{18}F]EF4Cl$_b$, and [^{18}F]EF5 and the shake flask method confirmed the results. Monochlorinated [^{18}F]EF4Cl$_a$ and [^{18}F]EF4Cl$_b$ were more lipophilic than the pentafluoro group-containing [^{18}F]EF5. Furthermore, the dichlorinated, non-radioactive molecule EF3Cl$_2$ was more lipophilic than the monochlorinated molecules determined by HPLC retention times and ClogP. The position of a single chlorine atom on the two terminal carbon atoms of the chlorinated analog had a slight effect on the lipophilicity according to HPLC analysis and ClogP values. This difference was marginal and the shake flask method is not very sensitive; therefore, the difference was not observed with the shake flask method for logD determinations.

Experimental tumors in mice were achieved by subcutaneous injections of adenocarcinoma cells at three male nude mice and three PET scans were carried out on each tumor-bearing mouse using the Inveon multimodality PET/CT scanner. Compared to [^{18}F]EF5, the chlorine/fluorine exchange of [^{18}F]EF4Cl$_a$ and [^{18}F]EF4Cl$_b$ affected the lipophilicity of these molecules, however it did not significantly affect their biological behavior and the hypoxia avidity. This indicates that [^{18}F]ClF is an useful agent for ^{18}F-labeling of clinical radiotracers.

7. CONCLUSIONS

High SA for the electrophilic labeling agent [^{18}F]SF can be achieved with post-target [^{18}F]F_2. This thesis shows that highly regioselective electrophilic ^{18}F corporation is possible using [^{18}F]SF and suitable good LG-containing precursors. The electrophilic synthesis of 6-[^{18}F]FDOPA using [^{18}F]SF as a labeling agent was presented as an example. The RCY of 6-[^{18}F]FDOPA was higher when using less of the precursor of [^{18}F]SF. The production method for [^{18}F]SF without [^{18}F]F_2, i.e., the nucleophilic synthesis route, could expand the field of electrophilic fluorine-18 chemistry and allow facilities not equipped to handle [^{18}F]F_2 to perform electrophilic [^{18}F]fluorination. However, this will require broad collaboration between "traditional" organic chemists and radiochemists.

The highly NET selective tracer [^{18}F]NS12137 was produced with different electrophilic labeling agents [^{18}F]SF and [^{18}F]F_2 and also with nucleophilic labeling. The number of byproducts was highest with [^{18}F]F_2, and only a few byproducts were observed with [^{18}F]SF. The nucleophilic synthesis was the most effective way to produce [^{18}F]NS12137. [^{18}F]NS12137 has also shown potential as a NET tracer in preclinical studies. Thus, [^{18}F]NS12137 shows characteristics of a PET tracer with potential utility also in clinical imaging. In the future, [^{18}F]NS12137 can easily be put into production for clinical use in GMP environment.

In this thesis, the novel synthesis method for producing high SA [^{18}F]ClF via post-target [^{18}F]F_2 was presented. Its utility was demonstrated by the electrophilic addition of [^{18}F]ClF to a double bond in the synthesis of chlorinated analogs of [^{18}F]EF5: [^{18}F]EF4Cl_a and [^{18}F]EF4Cl_b. These analogs were identified by their splitting patterns using radioLC-MS/MS analysis. Our results indicated that the hypoxia specificity was quite similar for all three tracers, thus [^{18}F]ClF is a suitable labeling agent for radiotracer syntheses. The next step in the synthesis of [^{18}F]ClF will be the development of n.c.a. [^{18}F]ClF.

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