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FLUORINE AND ^{18}F-FLUORINE IN RADIOPHARMACEUTICAL PREPARATION

by

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To my family

ABSTRACT

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Recently the use of fluorine has increased in synthetic pharmaceuticals since its unique physicochemical characteristics can confer better efficiency and potency in a pharmaceutical. The effect of fluorine substitution on the pharmacokinetics of a lead compound can be versatile, i.e. it can lead to modulations in lipophilicity, pKa, metabolic stability and even evoke conformational changes.

The radionuclidic properties of the positron emitter ^{18}F have made it one of the most important radioisotopes in positron emission tomography (PET). Its comparatively long half-life (109.8 min) and the low β^+-energy enable lengthy PET-imaging protocols and can contribute to obtaining high-resolution images. ^{18}F can be produced in large quantities enabling the synthesis of radiopharmaceuticals with high yields and high specific radioactivities (SA).

The incorporation of ^{18}F into organic molecules is usually accomplished either via nucleophilic or electrophilic routes. The electrophilic method is useful in labelling electron-rich structures, such as alkenes and aromatics, but often suffers from low yields and low SA. In this study, [^{18}F]F_2, produced with a "post-target" method, was used as an electrophilic labelling reagent. The aim was to evaluate the efficiency of "post-target" [^{18}F]F_2 chemistry in electrophilic fluorodestannylation and electrophilic addition reactions as ways of producing high quality radiopharmaceuticals with reasonable yields and with elevated SA.

The catecholamine analogues 4-[^{18}F]fluorometaraminol (4-[^{18}F]FMR) and 6-[^{18}F]fluorodopamine (6-[^{18}F]FDA) were produced with reasonable yields and with adequate SA, although the selectivity of ^{18}F-incorporation in 6-[^{18}F]FDA production was not optimal. 3-[[4-(4-[^{18}F]fluorophenyl)piperazin-1-yl]methyl]-1*H*-pyrrolo[2,3-b]pyridine ([^{18}F]F5P) was produced with a low radiochemical yield due to the formation of numerous side-products. In contrast, [^{18}F] 2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide ([^{18}F]EF5) was produced at a sufficient yield despite the formation of several side products.

Key words: fluorine, fluorine-18, electrophilic substitution, electrophilic addition, specific radioactivity

TIIVISTELMÄ

Olli Eskola

FLUORI JA ^{18}F-FLUORI RADIOLÄÄKEVALMISTUKSESSA

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Fluoriatomi esiintyy yhä useammin uusissa synteettisissä lääkkeissä, koska fluorin erikoislaatuisilla fysikokemiallisilla ominaispiirteillä voidaan myötävaikuttaa lääkkeen tehokkuuteen ja vaikutuskykyyn. Fluorisubstituution vaikutukset lääkkeen farmakokinetiikkaan voivat olla moninaiset, mukaan lukien vaikutukset lääkkeen rasvaliukoisuuteen, pK_a-arvoon, metaboliseen pysyvyyteen ja konformaatioon.

Positroniemitteri fluori-18 isotoopin radionuklidiset ominaisuudet ovat myötävaikuttaneet siihen, että se on eräs tärkeimmistä radionuklideista positroniemissiotomografian (PET) alalla. Suhteellisen pitkä puoliintumisaika (109.8 min) sekä matala emittoituvan positronin energia mahdollistavat pitkät PET-kuvausprotokollat sekä PET-kuvantamisen korkealla erotuskyvyllä. Fluori-18 isotooppia voidaan tuottaa suuria määriä, mikä mahdollistaa radiolääkeaineen tuoton korkealla saaliilla ja korkealla ominaisradioaktiivisuudella (OR).

Nukleofiiliset ja elektrofiiliset synteesit ovat tyypillisimmät menetelmät liittää ^{18}F isotooppi orgaanisiin molekyyleihin. Elektrofiilinen menetelmä on käytännöllinen leimattaessa elektronirikkaita rakenteita, kuten alkeeneja ja aromaattisia yhdisteitä, mutta haittapuolena ovat menetelmän matalat radiokemialliset saaliit sekä matala OR. Tässä työssä käytettiin elekrofiilisenä leimausreagenssina $[^{18}F]F_2$ kaasua, joka tuotettiin sähköpurkauksella ("post-target" menetelmä). Tavoitteena oli tutkia sähköpurkauksella tuotetun $[^{18}F]F_2$ kaasun kemian tehokkuutta elektrofiilisissa fluoridestannylaatio- ja additioreaktioissa kun päämääränä on tuottaa hyvälaatuisia radiolääkeaineita kelvollisilla saaliilla ja riittävän korkealla ominaisradioaktiivisuudella.

Katekoliamiinianalogit 4-[^{18}F]FMR ja 6-[^{18}F]FDA syntetisoitiin kohtuullisilla saaliilla ja riittävällä ominaisradioaktiivisuudella, joskin ^{18}F-substituution selektiivisyys 6-[^{18}F]FDA:n synteesissä ei ollut optimaalinen. [^{18}F]F5P:n synteesi tuotti matalan radiokemiallisen saaliin, mikä johtui useista muodostuneista sivutuotteista. [^{18}F]EF5 syntetisoitiin riittävällä saalisprosentilla huolimatta lukuisista muodostuneista sivutuotteista.

Avainsanat: fluori, fluori-18, elektrofiilinen substituutio, elektrofiilinen additio, ominaisradioaktiivisuus.

CONTENTS

ABBREVIATIONS

Ac	Acetyl
AHF	Anhydrous hydrogen fluoride
CFC	Chlorofluorocarbon
[^{18}F]CFT	2β-carbomethoxy-3β-(4-[^{18}F]fluorophenyl)tropane
CT	Computerised tomography
DAST	Diethylamino sulphur trifluoride
Deoxofluor	Bis(2-methoxyethyl)aminosulfur trifluoride
DFI	2,2-difluoro-1,3-dimethylimidazolidine
DFMBA	*N,N*-diethyl-α,α-difluoro(m-methylbenzyl)amine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EC	Electron capture
[^{18}F]EF5	[^{18}F] 2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide
EMIM	1-ethyl-3-methyl imidazolium
EOB	End of bombardment
EOS	End of synthesis
[^{18}F]F5P	3-[[4-(4-[^{18}F]fluorophenyl)piperazin-1-yl]methyl]-1*H*-pyrrolo[2,3-b]pyridine
6-[^{18}F]FDA	6-[^{18}F]fluorodopamine
Fluorspar	Calcium difluoride
4-[^{18}F]FMR	4-[^{18}F]fluorometaraminol
Freon-11	CCl_3F, trichlorofluoromethane
GC	Gas chromatography
GMP	Good manufacturing practice
His	Histidine
HPLC	High performance liquid chromatography
K2.2.2	4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane
LC-MS	Liquid chromatography mass spectrometry
MOST	4-morpholinosulfur trifluoride
MRI	Magnetic resonance imaging
n.c.a.	no carrier added
NFBTSI	*N*-fluorobis[(trifluoromethyl)sulfonyl]imide
NFOBS	*N*-fluoro-*o*-benzenedisulfonimide
NFPCB	*N*-fluoro-2,6-dichloropyridinium tetrafluoroborate
NFPT	*N*-fluoropyridinium triflate
NFQT	*N*-fluoroquinuclidinium triflate
NFSI	*N*-fluorobenzene sulfonimide
Nuc	Nucleophile
PET	Positron emission tomography
Phe	Phenylalanine
PPHF	Polypyridinium hydrogen fluoride
RA	Radioactivity
RCP	Radiochemical purity

RP	Reversed phase
R_t	Retention time
SA	Specific radioactivity
Selectfluor	1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)
Ser	Serine
TBABF	Tetrabutylammonium bifluoride
TBAF	Tetrabutylammonium fluoride
TBAOH	Tetrabutylammonium hydroxide
TFA	Trifluoroactic acid
THF	Tetrahydrofurane
TMAF	Tetramethylammonium fluoride
Tyr	Tyrosine

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. Eskola O, Bergman J, Lehikoinen P, Haaparanta M, Grönroos T, Forsback S, Solin O. Synthesis of 3-[[4-(4-[^{18}F]fluorophenyl)piperazin-1-yl]methyl]-1*H*-pyrrolo[2,3-b]pyridine. J Label Compd Radiopharm. 2002; 45:687-96.

II. Eskola O, Grönroos T, Bergman J, Haaparanta M, Marjamäki P, Lehikoinen P, Forsback S, Langer O, Hinnen F, Dollé F, Halldin C, Solin O. A novel electrophilic synthesis and evaluation of medium specific radioactivity (1R,2S)-4-[^{18}F]fluorometaraminol, a tracer for the assessment of cardiac sympathetic nerve integrity with PET. Nucl Med Biol. 2004; 31:103-10.

III. Eskola O, Grönroos TJ, Naum A, Marjamäki P, Forsback S, Bergman J, Länkimäki S, Kiss J, Savunen T, Knuuti J, Haaparanta M, Solin O. Novel electrophilic synthesis of 6-[^{18}F]fluorodopamine and comprehensive biological evaluation. Eur J Nucl Med Mol Imaging. 2012; 39:800-10.

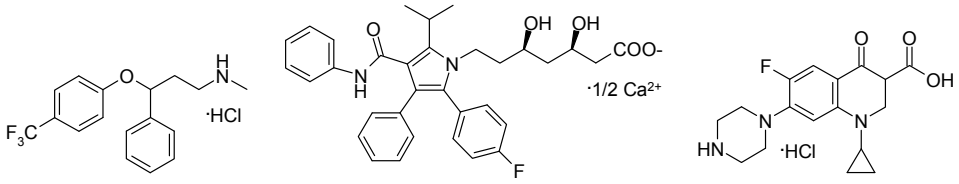
IV. Eskola O, Grönroos TJ, Forsback S, Tuomela J, Komar G, Bergman J, Härkönen P, Haaparanta M, Minn H, Solin O. Tracer level electrophilic synthesis and pharmacokinetics of the hypoxia tracer [^{18}F]EF5. Mol Imaging Biol. 2012; 14:205-12.

1. INTRODUCTION

The 1906 Nobel Prize in chemistry was awarded to Henri Moissan for his groundbreaking work to isolate fluorine, a new element, the work having been done in 1886 with electrochemical methods. In the 16th century, a fluoride containing mineral CaF_2 (also known as fluorite or fluorspar) was described as a substance which facilitated the melting of ores. In the subsequent centuries, studies on the chemical nature and reactions of fluorspar continued, particularly with hydrogen fluoride which was obtained by reacting fluorspar with acids. In the 19th century it was realized that hydrogen fluoride contained a new element, the properties of which resembled chlorine. The new element was found to be extremely reactive and attempts to isolate it proved difficult, laborious and in some unfortunate cases even fatal for the scientists working with it. Finally, Henri Moissan succeeded in isolating elemental fluorine, which he prepared by the electrolysis of a solution containing potassium hydrogen fluoride KHF_2 and liquid hydrogen fluoride (Moissan 1886, Groult 2007, Flahaut 1986, Banks 1986).

The usage of elemental fluorine grew considerably during the mid 20th century, when methods were developed to enrich ^{235}U from natural uranium by using uranium hexafluoride UF_6. Gradually during the 20th century, scientists were able to both control and then exploit the high reactivity of fluorine which had previously limited its use as a versatile fluorinating reagent. The rapid progress of industrial organofluorine chemistry can be considered to stem from the invention of several familiar compounds such as Teflon®, a landmark in fluoropolymer chemistry, and Freons®, which initiated the vast commercial use of chlorofluorocarbons (CFC's) as refrigerants.

Fluorine-containing molecules were rare in agrochemical and pharmaceutical applications before the 1970's. The development of selective, less reactive and safe fluorination reagents (see next paragraphs) turned the tide and allowed scientists to investigate fluorine incorporation reactions for both academic and industrial purposes. At present, hundreds of fluorinated drugs exist; in fact they account for more than 20 % of all pharmaceuticals (Müller 2007).



Prozac **Lipitor** **Ciprobay**

Figure 1. Top-selling fluorinated pharmaceuticals. The antidepressant Prozac®, cholesterol-lowering drug Lipitor® and quinolone antibiotic Ciprobay®.

In addition to the naturally occurring stable ^{19}F-isotope, fluorine has several radioactive isotopes (Lasne 2002). ^{18}F, a new radioisotope of fluorine was first described by Arthur Snell in 1936. This isotope was produced by the bombardment of neon gas with 5 MeV deuterons. The isotope was found to emit "positive electrons", had a half-life of 112 ± 4 minutes and it decayed to ^{18}O. Since it was neither of the then known radioisotopes of fluorine, i.e. ^{17}F or ^{20}F, it was deduced to be ^{18}F. Absorption measurements of the positron indicated that it had a maximum energy of about 500 keV (Snell 1937).

Over the decades, ^{18}F (and to a lesser extent ^{17}F) has become a widely used radionuclide in the field of nuclear medicine, especially with positron emission tomography (PET) (Phelps 2000, Phelps 2004). PET is a nuclear medical imaging modality that uses biologically active molecules labelled with short-lived positron emitters (β^+ emitters) (Welch 2003, Ametamey 2008). Whereas MRI and CT scans provide accurate anatomical information, PET scans offers a non-invasive tool for monitoring the pharmacokinetics (such as biodistribution, metabolism and excretion) of these radiolabelled molecules *in vivo*. The most widely used PET-radionuclides are ^{11}C ($t_{½}$ = 20 min), ^{13}N ($t_{½}$ = 10 min), ^{15}O ($t_{½}$ = 2 min) and ^{18}F ($t_{½}$ = 110 min). These radionuclides are produced with cyclotron bombardment of an appropriate target, and are immediately incorporated into the radiotracer prior to its PET use. Due to the favourable chemical properties of fluorine and the useful radionuclidic properties of ^{18}F-isotope, in many ways ^{18}F has proved to be a near ideal radionuclide for PET.

2. REVIEW OF THE LITERATURE

2.1. General properties of fluorine

Fluorine is the 13th most common element in the earth's crust. Fluorine is a small atom, the smallest of the halogens, with a van der Waals radius of 1.47 Å (Bondi 1964). As such it can be considered the smallest possible substituent in organic chemistry, if one excludes hydrogen and its isotopes. Fluorine is the most electronegative element in the periodic table, with a value of 3.98 on the Pauling electronegativity scale. It has a very low polarizability. Elemental fluorine F_2 is not only the most reactive halogen, but arguably the most reactive pure element in the periodic table. It can react with all other elements, with the exception of the lighter noble gases, He and Ne. The high reactivity of F_2 is a result of the very weak F-F bond (159 kJ/mol) combined with the ability of fluorine to form very strong bonds with other atoms (Dolbier 2005, Groult 2007).

Table 1. Physical properties of most common natural elements and halogens (Begue 2008, Weast 1982).

Element [X]	van der Waals radius [Å]	Electronegativity [Pauling scale]	C-X bond length [Å]	C-X bond strength [kJ/mol]
H	1.20	2.20	1.09	337
C	1.70	2.55	1.70	607
N	1.55	3.04	1.47	770
O	1.52	3.44	1.43	1077
F	1.47	3.98	1.39	536
Cl	1.75	3.16	1.77	397
Br	1.85	2.96	1.94	280
I	1.98	2.66	2.13	209

2.2. Natural occurring fluoro-organic compounds

The presence of fluorine in organic compounds is rare in nature and organofluorides are the least abundant organohalides of the natural compounds (see Figure 2). Most fluorides are found in minerals such as fluorspar, cryolite and fluorapatite. The fluoride ion has a high energy of solvation in water, which debatably has hindered its reactivity and uptake in bio-organisms (Dolbier 2005, Müller 2007). Consequently, the vast majority of organofluorocompounds that we have today are mostly unnatural, essentially man-made synthetic compounds.

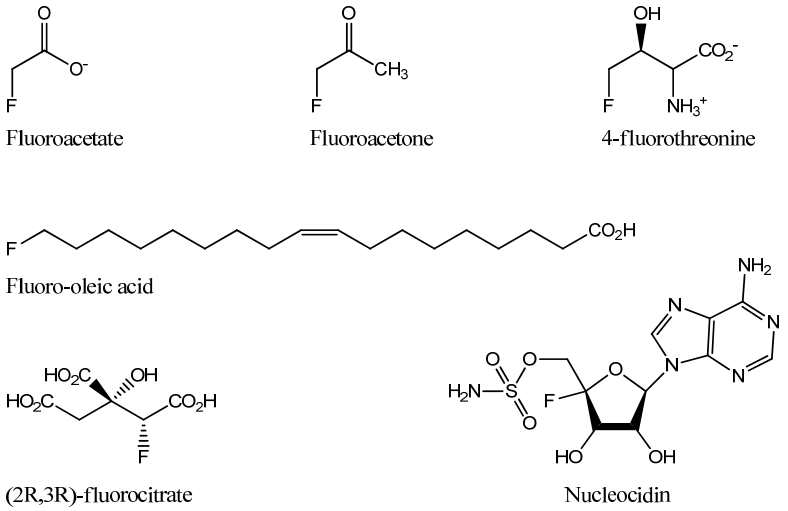


Figure 2. Some fluoro-organic compounds found in nature (Dolbier 2005).

2.3. Fluorine in pharmaceuticals

Over the last 25 years, the number of fluorine containing drugs and biomolecules has increased significantly. This is largely due to the development and commercial availability of selective fluorinating agents (see paragraph 2.5). On the other hand, the ever-growing knowledge of how fluorine substitution can modulate the physicochemical and biochemical properties of lead compounds has been a source of inspiration for scientists to develop novel fluorinated biomolecules and drugs.

The incorporation of fluorine into a drug achieves the simultaneous modulation of electronic, lipophilic and steric parameters, and all of these properties can influence both the pharmacokinetic and pharmacodynamic properties of drugs (Elliot 1995). The size and electronegativity of fluorine as well as the length and the strength of C-F bond are the key factors related to fluorine substitution and its outcome. In this chapter, fluorine substitution and its exploitation in pharmaceutical development are discussed.

2.3.1. Typical fluorine substitutions and steric perturbation

Bioisosterism refers to the capacity of atoms and functional groups with similar sizes or shapes to be interchanged without significantly altering the biological behaviour, such as affinity (Patani 1996).

Frequently, fluorine is introduced to replace hydrogen in biomolecules. In terms of size, the Van der Waals radius of fluorine (1.47 Å) is closer to oxygen (1.52 Å) than that of hydrogen (1.20 Å) (Ismail 2002). Despite the slight difference in size, the C-F bond can often replace and mimic the C-H bond with minimal steric consequences (Kirk 2006). Nonetheless, fluorine substitution always increases the steric size of alkyl groups. As an example, the trifluoromethyl group $–CF_3$ is much larger than the methyl group $–CH_3$, with steric volume close to isopropyl (Smart 2001) or ethyl group (Müller 2007), albeit with a very different shape.

Fluorine and oxygen are nearly isosteric from a structural point of view and the bond length of C-F (1.39 Å) is close to the bond length of C-O (1.43 Å) (Müller 2007). Replacement of hydroxyl group –OH with fluorine is therefore possible without adding exessive steric strain. Bioisosterism of C-OMe versus C-F has also been observed (Schweizer 2006).

Some examples of substituting a carbonyl group with fluorinated moieties exist, for instance, the trifluoromethyl fragment $–CF_3$ has also been introduced as a substitute for –C=O (Black 2005). Fluoromethylene C=CHF and difluoromethylene $C=CF_2$ groups have been used as bioisosters of the peptide bond (Zhao 2003) and phosphate esters (Berkowitz 1994).

2.3.2. Fluorine substitution effects on pKa

Due to its strong electron withdrawing nature, fluorine substitution has a profound impact on acidity and basicity of the neighbouring functional groups via inductive effects. Depending on the position of fluorine substitution, pKa shifts of several log units can be observed. Generally, alcohols, carboxylic acids, heterocyclics and phenols become more acidic with adjacent fluorine substitution. Similarly, linear and cyclic amines become much less basic with β-, γ- and in some examples even with δ-fluorine substitution (Hagmann 2008, Böhm 2004).

Often a change in pKa has a major impact on the pharmacokinetics of the molecule and its binding affinity. A nice example of this was reported by van Niel et al. (see figure 3) who developed novel fluorinated indole derivatives **3.1 - 3.3** as selective $5HT_{1D}$ receptor ligands (van Niel 1999). With sequential fluorine incorporation, the pKa values of the compounds were found to decrease. This reduction of basicity, with concomitant

weakening of the affinity to the receptor, had a strong beneficial effect on oral absorption of the drug. However, the difluoro compound **3.3** was no longer sufficiently basic to achieve high binding affinity for the $5HT_{1D}$ receptor (see Figure 3).

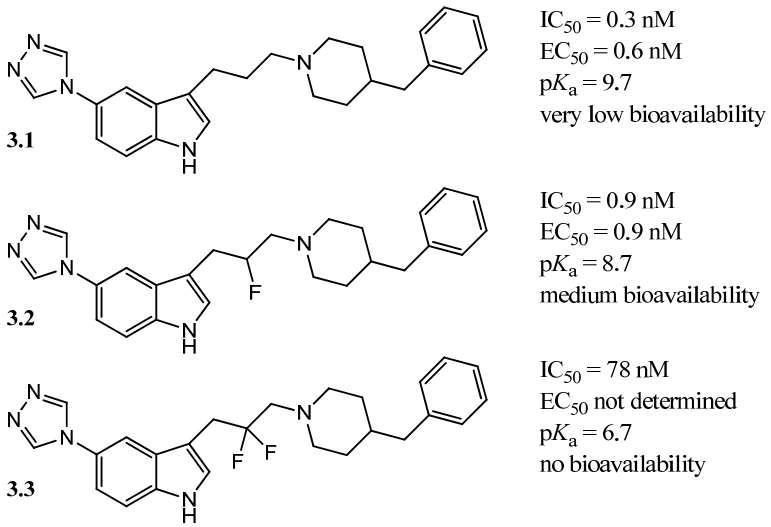


Figure 3. Effect of sequential fluorine substitution on the pKa of a set of 5HT1D agonists (van Niel 1999).

2.3.3. Fluorine substitution effects on lipophilicity

Lipophilicity is an important parameter that influences the in vivo distribution of the drug, for instance, it can enhance the binding affinity to the target protein. No common rule to explain how fluorine substitution affects lipophilicity can be provided. The change in lipophilicity after fluorine substitution is very much affected by the atoms and functional groups in close vicinity to the substitution site. For example, the presence of a fluorine close to an oxygen atom can increase the overall polarity of the molecule and thus enhances its solvation in polar medium. Likewise, fluorine may polarize the neighbouring oxygen atom leading to stronger hydrogen bonding between oxygen and water molecules (Böhm 2004).

Lipophilicity increases with aromatic fluorination, per/polyfluorination and with fluorination adjacent to atoms with π-bonds (with the exception of some α-carbonyl compounds) (Smart 2001).

Terminal mono-, di- and trifluorination and trifluoromethylation of saturated alkyl groups decreases lipophilicity. If heteroatoms are present in the alkyl chain, then the effect is less predictable (Smart 2001).

2.3.4. Hydrogen bonding and intermolecular interactions

Electronegativity considerations would indicate that C-F behaves similarly to C-O and C-N fragments and acts as a good hydrogen bond acceptor, but this does not seem to be the case (Dunitz 1997). Organic fluorine has a very low proton affinity and is weakly polarizable (Müller 2007). Nevertheless, the importance of C-F in hydrogen bonding has been debated intensively within recent years. Some investigators have concluded that organic fluorine is at best a weak hydrogen bond acceptor (Shimoni 1994, Howard 1996). A more accurate interpretation seems to be that organic fluorine hardly ever accepts hydrogen bonds and does so only in the absence of better acceptors (Dunitz 1997, Dunitz 2004). Thus in intermolecular interactions, such as in protein-ligand complexes, the probability that a covalently bound fluorine engages in hydrogen bonding is very small. In most cases, the non-bonding interactions of a C-F unit are better described in terms of weak polar interactions (Böhm 2004).

Interactions of the C-F moiety with strong H-bond donors (such as N-H of protein backbone amide bonds, His side-chains, OH groups of Tyr, Ser and bound water) have been reported in the literature. Possible interactions can also be formed between C-F and lipophilic side chains such as aromatic residues of Phe. Furthermore, an aromatic C-F can influence aromatic-aromatic interactions through alterations of the electronic characteristics of the aromatic ring (Kirk 2006).

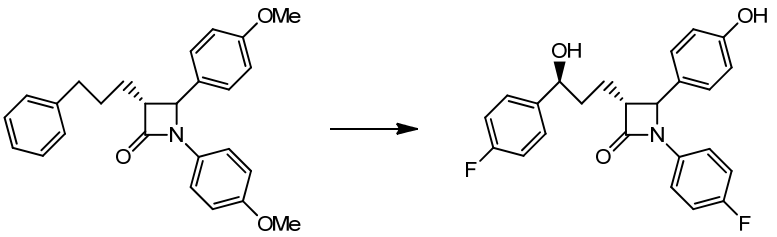
2.3.5. Fluorine substitution effects on metabolism

Lipophilic compounds have a tendency to be oxidized by liver enzymes like cytochrome P450. Hence, the modulation of oxidative metabolism by fluorine substitution has become a noteworthy strategy in drug development. This can be used not only to prolong or modulate the biological half-life of the drug, but also to prevent the formation of potentially toxic products via oxidative metabolism (Kirk 2006).

The ability of fluorine to block oxidative metabolism in saturated aliphatic systems is apparently not merely due to the fact that the C-F bond is stronger than the C-H bond. In fact, the high bond energy and heat of formation of the C-O bond and O-H bond

relative to the F-O bond essentially excludes an oxidative attack on fluorine. Oxidation of the C-H bond adjacent to $–CF_3$ group and perfluoro groups are retarded mainly by field effects as steric and the conformational changes are imposed as compared to the lead structure (Purser 2008).

Fluorine substitution can also block, or at least slow down, oxidation in the aromatic ring. This is typically accomplished by introducing fluorine at the 4-position of the phenyl ring.



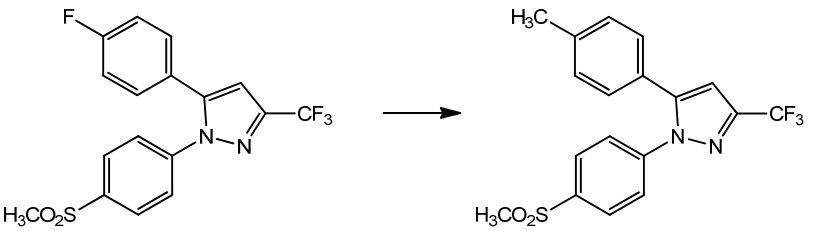
4.1: SCH 48461
ED_{50} (hamster) = 2.2 mg/kg/day

4.2: Ezetimib (SCH 58235, oral cholesterol absorption inhibitor)
ED_{50} (hamster) = 0.04 mg/kg/day

Figure 4. Development of ezetimib by optimization of the lead structure SCH 48461. As part of the optimization, two metabolically labile sites were blocked by fluorine substitution (Rosenblum 1998).

A good example of how fluorine substitution can be utilized to modify drug metabolism, is exemplified in the optimization of the cholesterol uptake inhibitor ezetimib (see Figure 4). The lead compound SCH48461 **4.1** was metabolised extensively and some metabolites were more potent than the drug itself. Fluorine was introduced into the *para*-position of the phenyl ring to prevent oxidation to a phenol. Furthermore, the 4-methoxy group was replaced by fluorine to avoid metabolic demethylation. These fluorinations, along with the addition of some supplemental functional groups, contributed to the "optimized" drug ezetimib **4.2**, which was 400 times more potent than the lead compound (Rosenblum 1998).

Conversely, sometimes it has been advantageous to replace the fluorine atom from lead compounds with metabolically labile groups. For instance, the replacement of fluorine of the cyclo-oxygenase 2 (COX 2) inhibitor **5.1** with methyl group led to celecoxib **5.2** (see Figure 5) and reduced the very long half-life of **5.1** (220 h in rat) to a more acceptable level (3.5 h in rat) (Penning 1997).

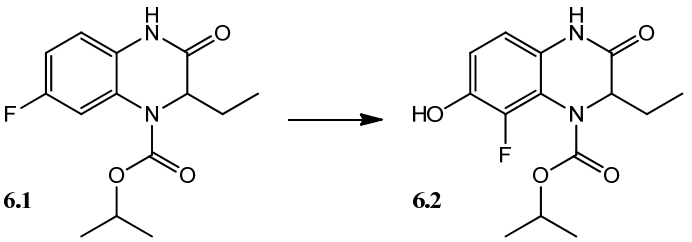


5.1: Early COX II inhibitor
$t_{1/2}$ (rat) up to 220 h

5.2: Celecoxib
$t_{1/2}$ (rat) = 3.5 h

Figure 5. Development of celecoxib. Replacement of fluorine by metabolically labile methyl group reduced the half-life of the lead compound to acceptable level (Penning 1997).

Naturally, there are examples where aromatic fluorine substitution does not prevent oxidative metabolism at the substitution site. This is observed particularly for phenyl rings with nitrogen substituent at the *para* position to the fluorine substituent. During P450-catalyzed oxidation, rearrangement (NIH-shift) takes place in which the fluorine moves to the adjacent carbon and the phenol metabolite is formed *para* to the nitrogen substituent (see Figure 6) (Dear 2000, Park 2001).



6.1 **6.2**

Figure 6. Formation of the NIH-shift metabolite **6.2** of the novel quinoxazoline reverse transcriptase inhibitor GW420867X **6.1** (Dear 2000).

Figure 7 illustrates the *in vivo* epimerisation of thalidomide, a notorious drug that was developed as a sedative hypnotic for the treatment of nausea in pregnancy until it was withdrawn from the market in 1962. The (*R*)-enantiomer is responsible for the clinically effective sedative hypnotic effects while the (*S*)-enantiomer is responsible for the teratogenic side effects. Epimerisation makes the biological evaluation of the individual enantiomers quite difficult. The epimerisation of thalidomide under physiological conditions is due to the presence of an acidic hydrogen atom in the stereogenic centre adjacent to the carbonyl group. The replacement of this hydrogen with fluorine is able to prevent the *in vivo* epimerisation process (Purser 2008).

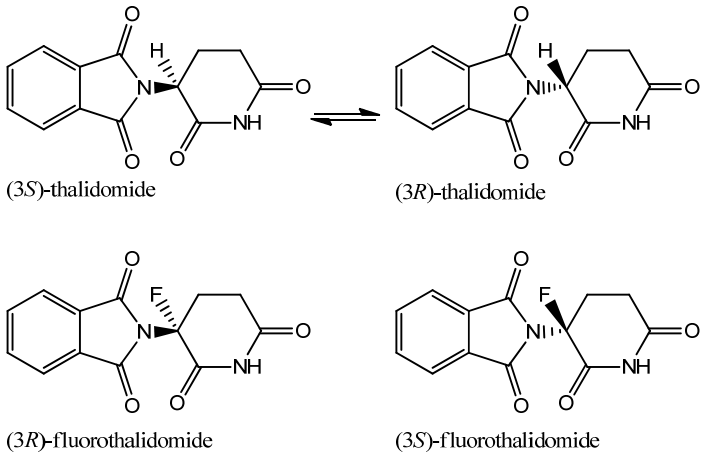


Figure 7. In vivo racemization of thalidomide. (3*R*)- and (3*S*)-fluorothalidomide are not racemized due to the replacement of the acidic hydrogen with fluorine (Purser 2008).

2.3.6. Fluorine substitution effects on molecular conformation

Substitution of H by F can profoundly change the conformational preferences of small molecules and sometimes these changes are quite subtle and difficult to predict beforehand. A tutorial example can be seen with conformations of methoxyphenyl and trifluoromethoxyphenyl groups. The methoxyphenyl group lies in the plane of the phenyl ring whereas the trifluoromethoxy group tends to turn out of plane because of its larger size and stereoelectronic effects (Leroux 2005, Müller 2007).

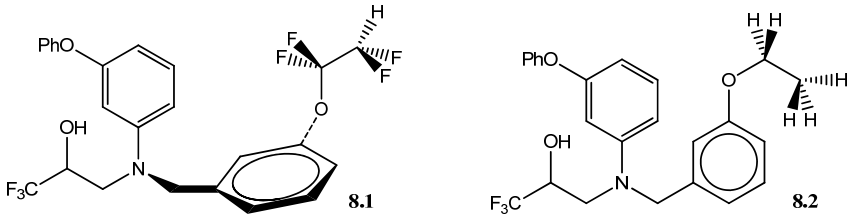


Figure 8. Cholesteryl ester transfer protein inhibitors. Ethoxy substituent in **8.2** favours in-plane orientation. Tetrafluoroethyl side chain in compound **8.1** favours the out-of-plane orientation with enhanced binding affinity (Massa 2001).

The difference in conformational preference induced with fluorine substitution was exploited in the development of superior inhibitors for cholesteryl ester transfer protein. (see Figure 8). When the tetrafluoroethoxy substituent of **8.1** was changed to an ethoxy substituent, an 8-fold loss of potency was observed. Molecular modelling experiments revealed that the tetrafluoroethyl group preferred an out-of-plane orientation with

respect to the phenyl ring, which promoted more efficient binding to the target protein (Massa 2001).

2.4. Fluorine in radiopharmaceuticals

The chemical properties of ^{18}F are the same as those of the stable ^{19}F isotope. Subsequently, the effects of ^{18}F-substitution on biochemical characteristics of pharmaceuticals, such as lipophilicity and pKa, are the same as with ^{19}F-substitution. Furthermore, the ^{18}F-labelled radiotracer has essentially the same properties as the non-radioactive ^{19}F-analogue, the small isotope effect is usually negligible (Matsson 1993). For tracer applications, the ^{18}F-labelling strategy is usually directed toward the position that will have as little effect as possible on the characteristics on the parent molecule. It is common, that ^{18}F is introduced into a radiopharmaceutical to replace either hydrogen or a hydroxyl group of the lead compound. As with stable fluorine, ^{18}F can be used to block the metabolism of the radiotracer, but the ^{18}F-substitution can also be used to detect *in vivo* metabolism as a function of time through analysis of the ^{18}F-labelled metabolites. The ability of fluorine to alter drug lipophilicity can be used in PET-studies, for instance by determining the ability of the ^{18}F-labelled compound to cross the blood brain barrier.

^{18}F is considered an excellent positron emitting radionuclide because of its nuclear and chemical properties. Compared to ^{15}O ($t_{1/2}$ = 2.03 min), ^{13}N ($t_{1/2}$ = 9.97 min) and ^{11}C ($t_{1/2}$ = 20.4 min), the comparatively long half-life of ^{18}F (109.77 min) allows time for complex and multi-step radiolabelling procedures. The appropriate ^{18}F-labelled tracers can be used as tools for following biochemical processes with slow kinetics (for as long as six hours) with a PET-camera. In addition, ^{18}F-labelled tracers can be obtained with high SAs, typically > 400 GBq/μmol at EOS.

^{18}F decays largely by positron emission (β^+: 97 %, EC 3 %) and the positron energy of ^{18}F is the lowest (max 0.635 MeV) of the common positron emitters. As a consequence, the positron has the shortest linear range in tissues which greatly contributes to its ability to provide high resolution images if one uses ^{18}F-labelled tracers (Lasne 2002).

Finally, in many cases ^{18}F-labelled radiopharmaceuticals can be produced in large quantities. This, coupled with the relatively long half-life of ^{18}F, enables shipping of

these radiopharmaceuticals to centres which do not have access to an on-site cyclotron or a radiochemistry laboratory.

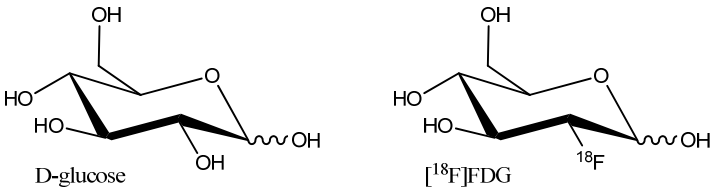


Figure 9. D-glucose and its ^{18}F-labelled analogue [^{18}F]FDG, the most widely used PET-radiopharmaceutical.

The most frequently used radiopharmaceutical for PET is 2-deoxy-2-[^{18}F]fluoro-D-glucose [^{18}F]FDG, originally developed in the late 1970's (Ido 1978), with applications in oncology, neurology and cardiology. [^{18}F]FDG is a glucose analogue and it can be used to assess glucose metabolism *in vivo*. [^{18}F]FDG is a good example on how ^{18}F-fluoride can be introduced as a bioisoster of hydroxyl group while maintaining the desired biochemical characteristics of the parent compound D-glucose. It also illustrates how metabolism of the parent compound can be modulated with fluorine substitution. [^{18}F]FDG is phosphorylated in the same manner as D-glucose, but due to the absence of a hydroxyl group in C2-position, it cannot undergo glycolysis and is therefore trapped inside the cell.

2.5. Formation of C-F bond

The selective introduction of fluorine into biomolecules is of paramount importance if one wishes to exploit the advantages of fluorine substitution discussed in the previous chapter. Nonetheless, the preparation of organofluorine compounds remains a formidable challenge. The traditional techniques of fluorination involve unusual reagents that are often hazardous and corrosive (elemental fluorine, hydrofluoric acid, sulfur tetrafluoride), and the handling of these requires special laboratory equipment. Moreover, they are often poorly selective and incompatible with elaborate and fragile substrates.

However, thanks to the development of selective fluorination agents and building blocks, today there are many ways to introduce fluorine in a regio- and stereoselectively controlled way to organic molecules. There are many excellent books, reviews and monographs describing in detail the broad array of reactions available today for

scientists and fluorine chemists. Fluorination reactions to form organofluorine compounds utilize the nucleophilic, electrophilic and radical forms of fluorine. The goal of this section is to highlight the principle methodologies used to achieve organofluorine substitutions. The emphasis will be placed on aliphatic and aromatic monofluorinations.

2.5.1. Nucleophilic fluorinations

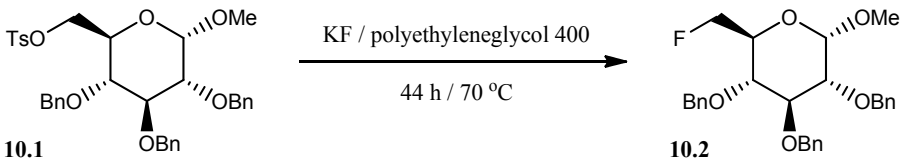
Nucleophilic fluorination implies that the C-F bond is created through the reaction of fluoride anion F^- with a suitable substrate. This is not as straightforward as it appears. The small size of fluorine and its low polarizability encourages F^- to behave as a base rather than a nucleophile (Wilkinson 1992), sometimes F^- has even been successfully used as a mild base in organic synthesis (Clark 1980). Moreover, the fluoride anion is generally strongly solvated in protic solvents (hydration energy 123 kcal/mol) and is prone to form tight ion pairs, which render F^- poorly reactive (Bégué 2008, Kirk 2008).

Traditional fluorinating agents: Nucleophilic substitution of halogens with F- was first achieved in 1863 by Borodine (Borodine 1863). Since then, many reagents have been developed to overcome traditional problems like poor solubility, substitution versus elimination in nucleophilic substitution reactions, high price, high toxicity and low stability of the fluorinating reagents. Some of these first-generation fluorinating reagents are presented in Table 2. Many of these are still in use, in spite of their occasionally non-optimal characteristics such as toxicity and very high reactivity.

Table 2. Traditional first-generation fluorinating reagents (Wilkonson 1992, Dmowski 1986, Rozen 2005)

Reagent	
TASF	Tris(dimethylamino)sulfonium difluorotrimethylsiliconate
AgF	Silver(I) fluoride
CuF_2	Copper(II) fluoride
HgF_2	Mercury(II) fluoride
ZnF_2	Zinc(II) fluoride
SiF_4	Silicon tetrafluoride
BrF_3	Bromine trifluoride
SF_4	Sulfur tetrafluoride
FAR	Fluoroalkylamine reagents; Yarovenko's reagent, Ishikawa's reagent
XeF_2	Xenon difluoride
AHIF	Aromatic hypervalent iodine fluorides

Alkali metal fluorides: "Classical" alkali metal fluorides such as LiF, NaF, KF and CsF have been used to introduce fluorine into a variety of compounds such as alkyl sulfonates, alkyl halides and aromatic halides. The fluorinations are often carried out in high-boiling solvents which improve the solubility of the ionic fluorides or alternatively in anhydrous solvents. Various activation methods, that direct fluoride ion to act as a nucleophile rather than as a mild base, are in most cases required. The reactions can be conducted in the presence of crown ethers, which solvate inorganic fluorides by complexation and enhance their solubility in nonpolar solvents such as benzene. Other cation complexing agents such as glycols and glymes can also be used (See figure 10) (Wilkinson 1992, Halpern 1995, Begue 2008, Kirk 2008, Furuya 2008).



10.1 **10.2**

Figure 10. Replacement of *O*-tosyl group of **10.1** using potassium fluoride as the nucleophilic source of fluorine in a glycol solvent (Wilkinson 1992).

Tetra-alkyl ammonium fluorides: Tetra-alkyl ammonium fluorides were developed to overcome the problems related to alkali metal fluorides. They provide a soluble source of F^-. In addition, by replacing the metal cation with a bulky organic cation, the ion pairing is reduced and the nucleophilicity of F^- is enhanced. The most widely used reagent is the commercially obtainable tetrabutyl ammonium fluoride TBAF, available as a trihydrate. It is a potent source of nucleophilic fluoride, but also a strong base. Furthermore, it is difficult to obtain TBAF in completely anhydrous form, which can lead to variability in some cases, for instance, by hydrolysis of the leaving group or through elimination reactions (see Figure 11) (Cox 1984, Halpern 1995, Furuya 2008, Sun 2005). Elimination side-reactions can be avoided by using tetramethylammonium fluoride TMAF which can be obtained as an anhydrous salt (Furuya 2008). Tetrabutylammonium bifluoride TBABF is a non-corrosive analogue of TBAF with good solubility properties and high thermal stability (Bosch 1987, Kim K-Y 2008).

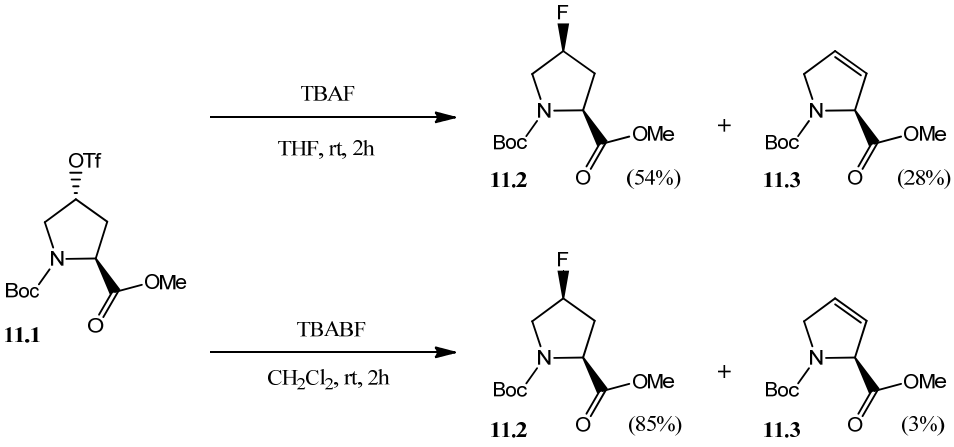


Figure 11. Synthesis of the 4-fluoroproline derivative **11.2** with TBAF and TBABF. With TBABF higher yields are achieved due to the decreased formation of the elimination product **11.3** (Kim K-Y 2008).

HF and its derivatives: Anhydrous hydrogen fluoride (AHF) is one of the most popular fluorination reagents, but due to its corrosive nature and low boiling point (19 ºC), alternatives are required. AHF can be "tamed" with suitable donor solvents such as alkyl amines Et_3N and Et_2NH or with pyridine to form polypyridinium hydrogen fluoride PPHF, commonly known as Olah's reagent. PPHF has mainly been used to fluorinate secondary and tertiary alcohols, alkenes and alkynes and in halogen exchange reactions (Wilkinson 1992).

Alkyl amine hydrogen fluorides such as $Et_3N{\bullet}3HF$ are other useful sources of F^-; they are less corrosive than PPHF. $Et_3N{\bullet}3HF$ has been utilized in bromofluorinations of double bonds and allylic alcohols. $Et_2NH{\bullet}3HF$ has been used in regioselective ring opening of epoxides (see figure 12) (Wilkinson 1992, Muehlbacher 1988).

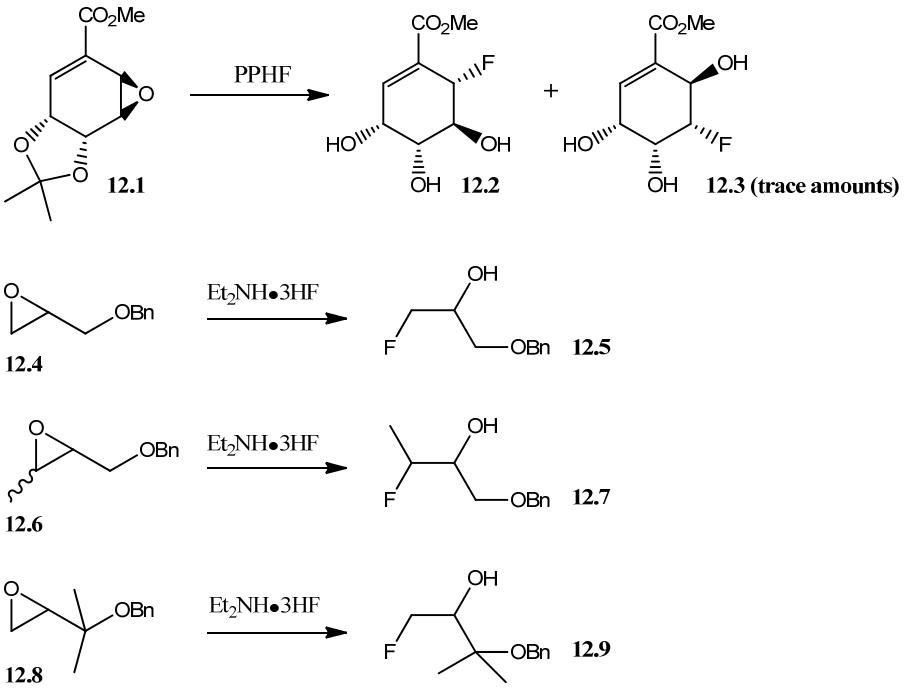


Figure 12. Use of Olah's reagent (PPHF) and $Et_2NH•3HF$ in ring opening of epoxides. The ring strain of the epoxide itself provides the activation for the reaction to proceed. With $Et_2NH•3HF$, the nucleophilic fluoride was generally found to attack the least hindered carbon of the epoxide ring (Muehlbacher 1988, Kirk 2008).

Sulfur fluorides and other novel fluorination reagents: Diethylamino sulfur trifluoride DAST can be considered as the main reagent for nucleophilic fluorination (Hudlicky 1995, Middleton 1975) and its use is quite versatile (Singh 2002). Direct transformation of a C-OH bond to a C-F bond is possible with primary, secondary and tertiary alcohols. These reactions are in most cases stereoselective and inversion of configuration is observed. Ketones and aldehydes can be reacted to form difluoroalkyl compounds. Other, more stable, DAST related reagents such as Deoxofluor™ (Lal 1999) and MOST (Furuya 2008) are also available. DFI (Hayashi 2002) and DFMBA (Kobayashi 2004) also belong to the family of second-generation fluorination reagents.

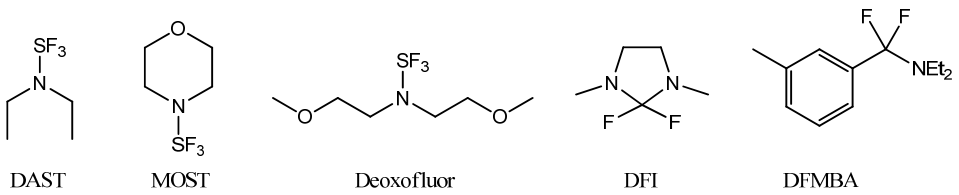


Figure 13. Novel nucleophilic fluorination reagents.

Various types of fluorinations can be accomplished by using sulphur fluorides as nucleophilic fluorination reagents. Some examples are shown in Figure 14.

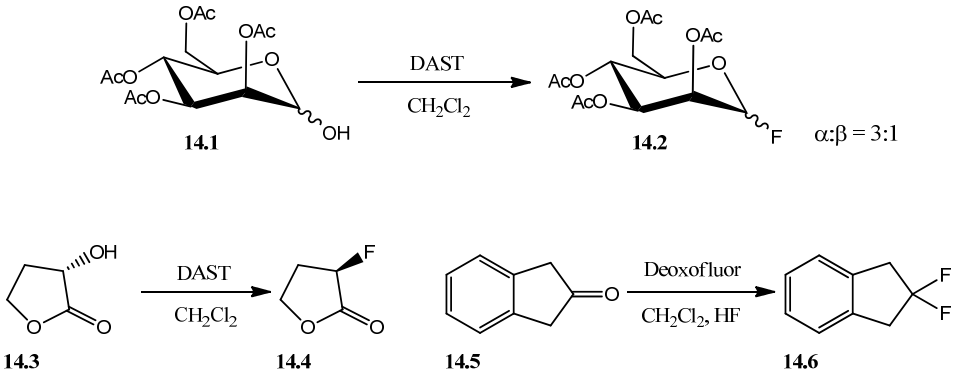


Figure 14. Examples of fluorinations with DAST and Deoxofluor. The C-1 fluorination of 2,3,4,6-tetra-O-acetyl-β-D-mannopyranose **14.1** (Albert 2000). The secondary –OH group replacement of 2*S*-hydroxy-γ-butyrolactone **14.3** with the inversion of the configuration (Shiuey 1988). Cyclic ketone fluorination of **14.5** with deoxofluor to produce the *gem*-difluoride compound (Singh 2002).

2.5.2. Electrophilic fluorinations

Electrophilic fluorination means that the C-F bond is created through the reaction of the fluoride "cation" F^+ with a substrate that has a high electron density. The ability of fluorine to behave as a F^+ electrophile is not easily achieved, since fluorine is the most electronegative element. There are ways to overcome this problem e.g. by either withdrawing the electronic charge from fluorine through inductive effects or by introducing the presence of a good leaving group adjacent to fluorine substitution site or by combination of these effects (Wilkinson 1992).

Initially, molecular fluorine F_2 was the sole source of electrophilic fluorinations. Due to its extreme and uncontrollable reactivity, the development of alternate electrophilic reagents was necessary (Rozen 1980*a*). The "second generation" electrophilic reagents included fluoroxytrifluoromethane CF_3OF, perchlorylfluoride $FClO_3$, xenon difluoride XeF_2, nitrogen oxide fluorides (Barton 1968, Patrick 1995, Nyffeler 2005, Rozen 1975, Schmutzler 1968, Tius 1995) and other hypofluorites, acetohypofluorite in particular (Appelman 1985, Lerman 1981, Lerman 1984, Navarrini 1999, Rozen 1979, Rozen 1980*b*, Rozen 1981*a*). These reagents served as safer alternatives for F_2, but the need

for more stable and less toxic reagents still remained. These reagents will be discussed in the following chapters.

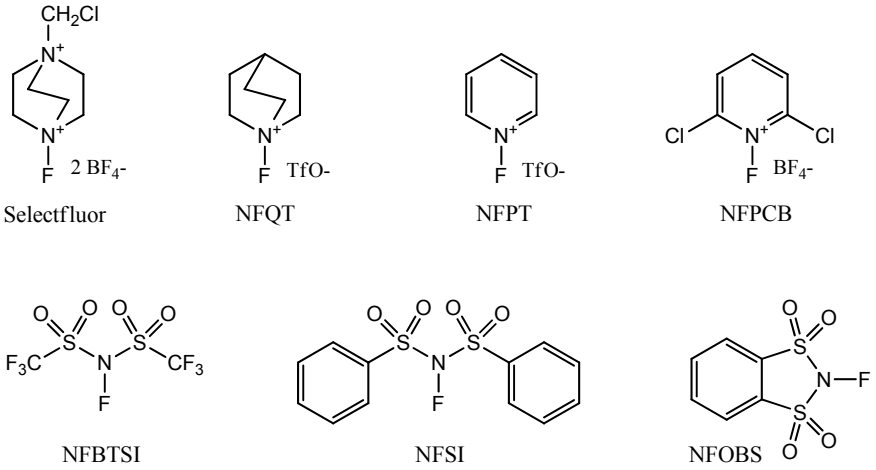


Figure 15. N-F type electrophilic fluorination reagents: 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (Selectfluor®), *N*-fluoroquinuclidinium triflate (NFQT), *N*-fluoropyridinium triflate (NFPT), *N*-fluoro-2,6-dichloropyridinium tetrafluoroborate (NFPCB), *N*-fluorobis[(trifluoromethyl)sulfonyl]imide (NFBTSI), *N*-fluorobenzene sulfonimide (NFSI) and *N*-fluoro-*o*-benzenedisulfonimide (NFOBS).

N-F reagents: A new class of agents with the general structure R_2N-F or R_3N^+-F has revolutionized the field of electrophilic fluorination. In comparison to the earlier reagents, these compounds are milder, safer, more stable and less expensive to produce. Some of these agents possess as high reactivity as the previous reagents but they are also capable of achieving selective fluorination which was not previously possible (Davis 1995, Lal 1996, Banks 1998, Rostami 2007, Furuya 2008, Kirk 2008). The most widely used N-F type electrophilic fluorination reagents are presented in Figure 15. Of these 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate), also known as F-TEDA-BF_4 or Selectfluor®, has proved the most versatile reagent for fluorinating many types of organic compounds and thus it has become a commercially available hazard-free source of fluorine. It is also remarkably stable, non-toxic and does not require harsh reaction conditions (Taylor 1999, Singh 2004, Nyffeler 2005, Begue 2008). Many types of fluorinations have been accomplished with Selectfluor, some examples are shown in figure 16.

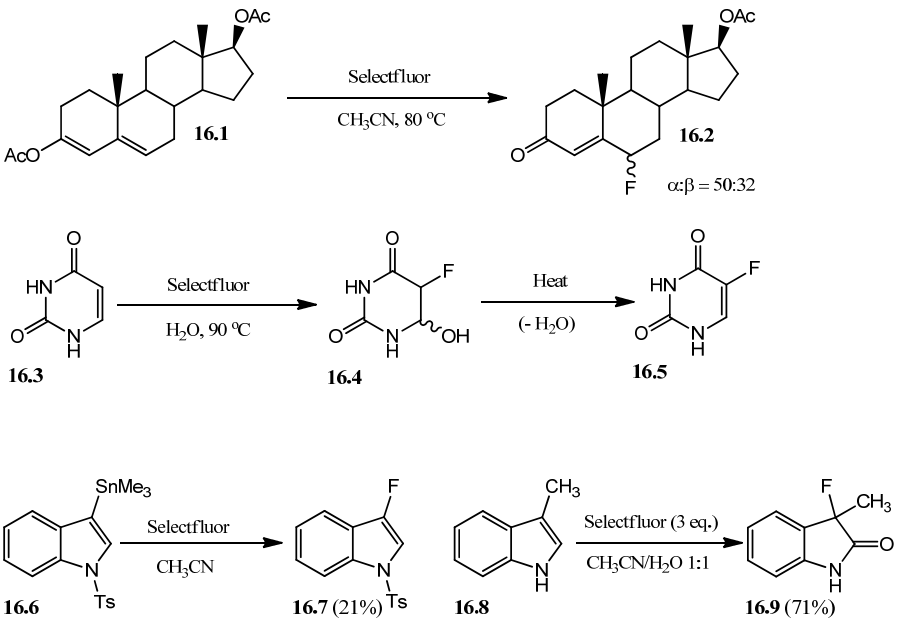


Figure 16. Examples of electrophilic fluorinations reactions with the N-F reagent Selectfluor. 6-fluorination of a testosterone enol acetate **16.1** (Reydellet-Casey 1997). Preparation of 5-fluorouracil **16.5** (Banks 1998). Fluorinations of 3-trimethylstannyl-1-tosylindole **16.6** (Hodson 1994) and 3-methylindole **16.8** (Takeuchi 1999).

Enantioselective fluorination has also progressed significantly within the last 10 years, largely because of the availability of asymmetric electrophilic N-F reagents. Some of these are described in figure 17. The most promising of these is compound **17.4**, an *N*-fluoroderivative of a naturally occurring cinchona alkaloid. Examples of the extensive use of these asymmetric electrophilic N-F reagents can be found in the literature (Muniz 2001, Shibata 2007, Ma 2008, Cahard 2010).

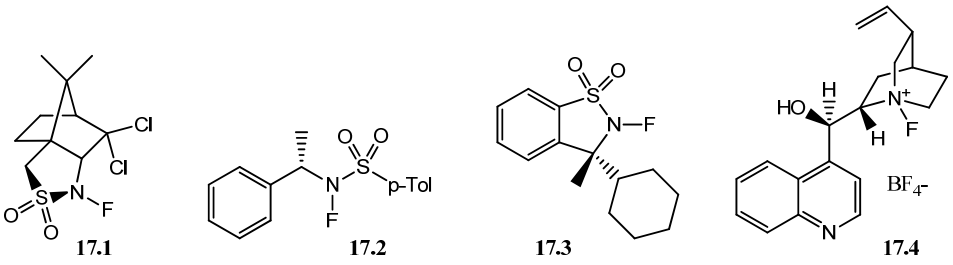


Figure 17. Asymmetric electrophilic fluorination reagents. *N*-fluorocamphorsultam **17.1**. *N*-fluoro-*N*-tosyl-1-phenetyl-1-amine **17.2**. *N*-fluorosulfonamide **17.3**. N-fluorocinchonidium tetrafluoroborate **17.4**.

Elemental fluorine: Elemental fluorine (F_2) is the classical electrophilic fluorination reagent. Unfortunately, due to its chemical character, it is also the most challenging reagent with which to work. The ease of radical F• formation coupled with its high toxicity, strong oxidizing potential with little or no selectivity and potential free radical reactions have limited its use in selective fluorinations (Nyffeler 2005).

(1) $-CH_3 + F_2 \rightarrow -CH_2-F + HF, \ \Delta H = -149$ kcal/mol

Although several selective electrophilic fluorination reagents have been developed, the interest in utilizing F_2 in selective direct fluorinations has not disappeared. Gradually, scientists have been able to control the vigorously exothermic reaction of F_2 with the C-H bond (see Equation 1). In order to assist in the removal of the heat of reaction, the reactions are often performed using fluorine diluted to 5-10 % with nitrogen. Lighter noble gases may also be used. In most cases, cooling of the reaction mixture is advantageous (Moilliet 2001).

The choice of the right solvent is crucial. Previously the solvents tended to be chosen primarily not only for their inertness but also for their ability to dissolve both the substrate and fluorine. Most successful selective fluorination reactions are carried out under conditions which limit any free radical processes and enhance the nucleophilic attack of the substrate to fluorine (Moilliet 2001, Sandford 2007, Hutchinson 1997). Consequently, either high dielectric aprotic solvents such CH_3CN or strong protonic acids such as formic acid or sulfuric acid can be used to make fluorine more susceptible to nucleophilic attack (see figure 18). For instance, in acids, the fluorine molecule is polarized and while the electronegative end of the molecule is protonated by the acid the electropositive end is free to react.

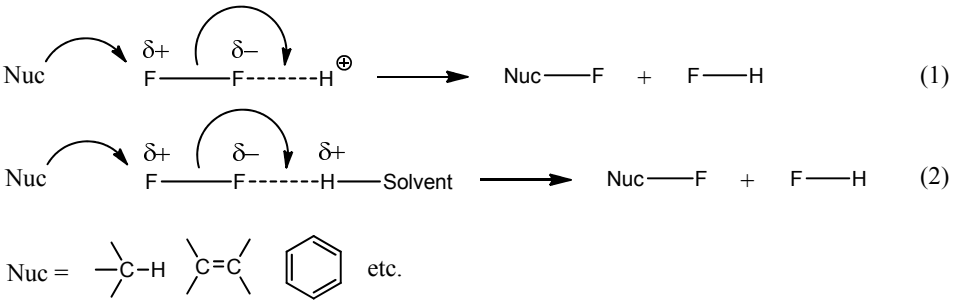


Figure 18. Effects of protonic acid (equation 1) and high dielectric aprotic solvent (such as CH_3CN, equation 2) to F-F bond polarization, which makes the F-F bond more prone to nucleophilic attack.

A polar solvent (Solv-H) not only encourages polarizarion of fluorine molecule and makes it more susceptible to nucleophilic attack, but more importantly, acts as an acceptor for the counter ion (fluoride ion) in the transition state (see figure 19).

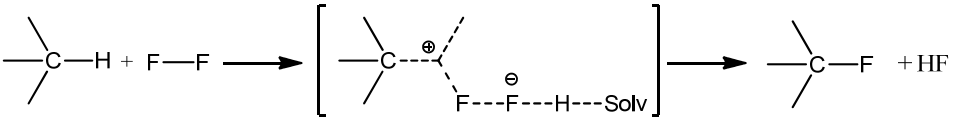


Figure 19. Polarization of F-F bond induced by a polar solvent, which also acts as an acceptor of the fluoride ion in the transition state.

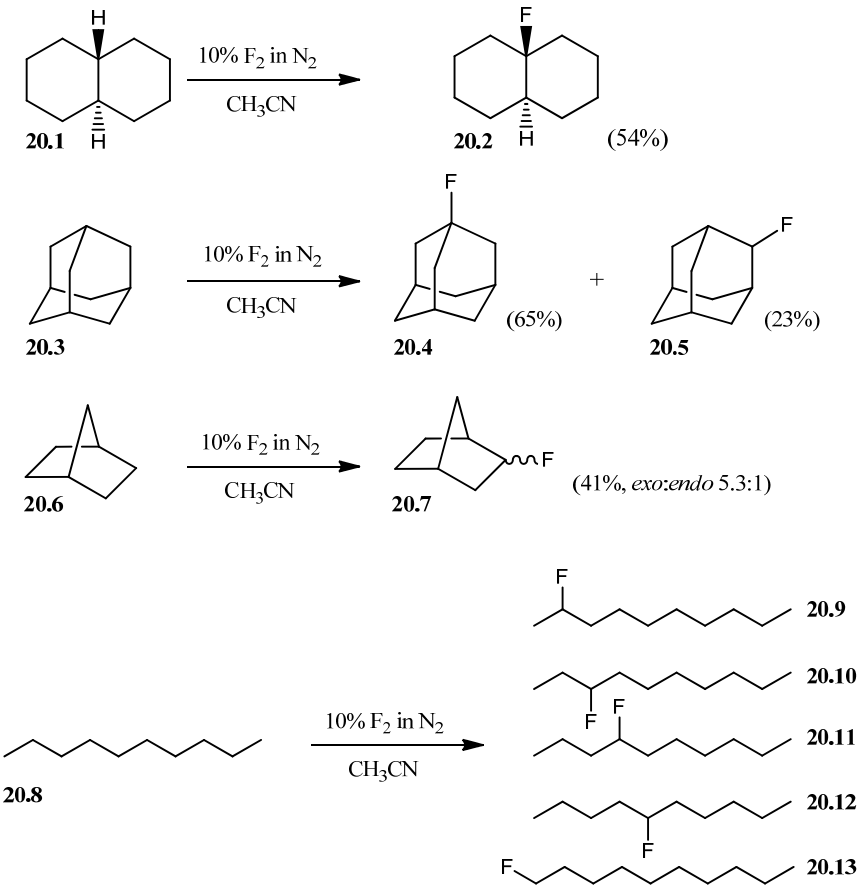


Figure 20. Selectivity of fluorinations of cyclic and aliphatic compounds with dilute F_2 (Chambers 2002).

With aliphatic substrates, hydrogen atoms attached to tertiary sp^3 carbon are selectively replaced with the retention of configuration by fluorine over secondary or primary sites. Examples of this are the fluorination of *trans*-decalin **20.1** and fluorination of adamantine **20.3** (see Figure 20). Secondary sites can also be replaced by fluorine if no tertiary sites exist or if the tertiary C-H bond has a lower p orbital contribution and is therefore less nucleophilic than the available secondary site; fluorination of norbornane **20.6** highlights this case, where the hydrogen attached to the tertiary C-1 carbon is not fluorinated due to the strain induced in the bridged C-1 carbon. Mixtures of several mono-fluorinated products are often obtained with aliphatic non-cyclic substrates, such as in fluorination of *n*-decane **20.8** with F_2 (Chambers 2002, Gal 1980, Gal 1982, Rozen 1981*b,* Rozen 1987*a*, Rozen 1987*b*, Rozen 1988, Sandford 2007).

Selective fluorination of aromatic systems is also possible with elemental fluorine. The products are consistent with electrophilic aromatic substitution processes, where the introduction of fluorine into a certain position of the aromatic ring can be influenced by the presence of electron withdrawing (NO_2, CN) and electron releasing (OH, OMe, NHAc, Me) substituents. Protonic acids (formic, sulfuric, triflic acid and HF) are effective media for promoting selective fluorination of aromatic systems. Fluorine is considered to be made more susceptible towards nucleophilic attack after polarization in the acid (see Figure 21), whilst competing unselective free radical processes are minimized. Even compounds which are very unsusceptible towards electrophilic attack, such as 2,4-dinitro-1-chlorobenzene **21.1**, have been fluorinated in a protonic acid with high yields using dilute F_2. It is, however, important to carefully control the amount of F_2; extensive di-fluorination may also occur if excess of F_2 is used, as seen with the fluorination of 7-methoxycoumarin **21.3** (see Figure 21). Mixtures of organic solvents and acids can also be used, as these also may improve the solubility of the substrate to be fluorinated (Sandford 1997, Moilliet 2001, Sandford 2007).

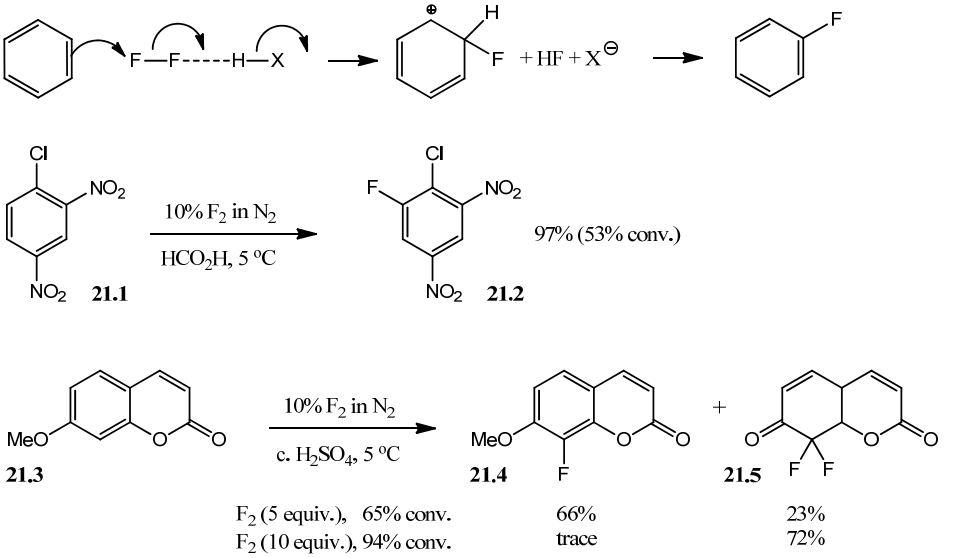


Figure 21. Fluorinations of aromatic compounds with dilute F_2 (Sandford 2007).

2.5.3. Electrochemical fluorination

Electrochemical methods are frequently employed to perform fluorination reactions involving a conversion of C-H bond into its C-F counterpart. Fluorinations are conducted in nickel or steel cells equipped with nickel, steel or platinum anodes and cathodes. Organic substrates are dissolved in mixture of a suitable solvent, often acetonitrile, and a supporting electrolyte medium, which usually serves also as the source of the fluoride ion. Electricity is then conducted through the mixture (Adcock 1995).

Traditionally, electrochemical fluorinations were performed in liquid HF solutions with nickel anodes or KF•2HF melt on carbon anodes. Both these methods mainly produce perfluorinated organic compounds since they convert all of the C-H bonds into C-F bonds (Noel 1997). Selective electrochemical fluorination remained an academic pursuit for a very long time. This is mainly due to the competitive polymerization processes at the high anodic potential generally required to achieve the fluorination process. The breakthrough in selective electrochemical fluorination occurred when triethylamine-HF dissolved in acetonitrile was employed as the electrolyte medium. Even better results were obtained by using Et_3N•*n*HF and Et_4NF•*n*HF, which meant that even aromatic

compounds containing electron withdrawing substituents could be fluorinated selectively (Noel 1997).

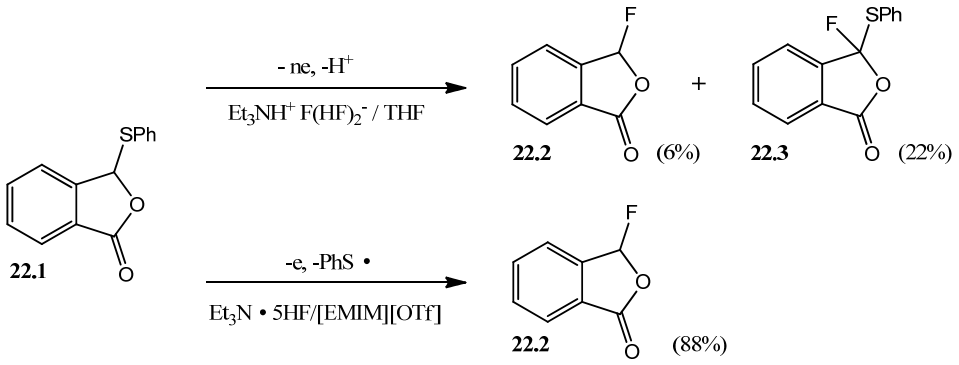


Figure 22. Effect of solvent on the outcome of electrochemical fluorination of 3-phenylthiophthalide **22.1**. Low yields and mixture of products **22.2** and **22.3** are obtained with THF as solvent (upper reaction scheme). **22.2** is obtained exclusively with a high yield using ionic liquid [EMIM][OTf] as solvent (Fuchigami 2007).

Unfortunately the use of organic solvents in electrochemical fluorination has its drawbacks e.g. anodic passivation which results in low efficiency for anodic fluorination. A rather novel method has been described which involves molten salts i.e. ionic liquids (see Figure 22) at room temperature as the sole reaction medium without any organic solvents (Fuchigami 2005, Fuchigami 2007).

2.6. ^{18}F-labelling chemistry

2.6.1. General

In recent decades, PET has advanced to become an important clinical diagnostic and research modality and it is also a valuable tool in drug discovery and development. The number of new targets for nuclear molecular imaging is constantly increasing. Hence, there is an increasing demand for radiolabelled tracers, and concurrently the methodologies to synthesise the compounds.

^{18}F can be used for labelling of simple molecules, such as amino acids, or complex molecules of biological interest including peptides, proteins and oligonucleotides, when the range of the biological process is compatible with the half-life of ^{18}F-fluorine. The labelling chemistry with ^{18}F-ion is however by no means straightforward and the

versatility of possible labelling strategies is somewhat restricted, especially when compared to carbon-11.

^{18}F chemistry is primarily determined by the production method of ^{18}F (see paragraph 3.2). Depending on the nuclear reaction, ^{18}F can be obtained as anionic fluoride $^{18}F^-$, a source for nucleophilic labelling, or as [^{18}F]fluorine gas, used in electrophilic labelling.

The chemical reactions involving positron emitters have to be specially designed to take into account the short half-life of the radionuclide, the limited number of radiolabelled starting materials (or precursors) and the sub-micromolar amounts of these radiolabelled starting materials. Moreover, the reactions must be possible with a minimal addition of the stable isotope, especially with receptor ligands or toxic molecules. Large amounts of reagents are used as compared to the amounts of the radiolabelled precursor, which in many cases, allows for rapid reactions. On the other hand, harsh reaction conditions are often required to achieve fast reactions and unexpected labelled impurities can be formed from side reactions of the reagents present in excess or from reactive impurities in the reaction medium.

Rapidity and robustness are the key words in the production procedure of a radiopharmaceutical. The synthesis route should aim at incorporating the label as late as possible into the sequence. The overall time of production, including labelling chemistry, purification and formulation of the radiopharmaceutical for intravenous injection should be as short as possible, generally not more that 3 hours with ^{18}F-labelled compounds.

Each step of the radiolabelling synthesis requires optimization. Both the reaction conditions (reaction time, temperatures, solvents, reagent concentrations) and purification and formulation procedures entail fine-tuning to achieve a high radiochemical yield and a high radiopharmaceutical quality in the smallest possible time window.

Finally, radiation protection and automation of synthetic procedures have to be considered when planning the synthesis of radiopharmaceuticals. Automation enhances both rapidity and reproducibility of tracer synthesis and perhaps more importantly reduces the radiation burden on the operators by reducing human hand-made manipulations. All the procedures, starting from radionuclide production and ending in

the release of the radiopharmaceutical for injection, have to meet the ever-growing demands of Good Manufacturing Practice (GMP).

Table 3. Selected radionuclides that decay by positron emission and are relevant to PET imaging (Cherry 2004).

Radionuclide	Half-life	β^+ E_{max} [MeV]	β^+ branching ratio
^{82}Rb	1.27 min	2.60, 3.38	0.96
^{15}O	2.03 min	1.73	1.00
^{62}Cu	9.74 min	2.93	0.97
^{13}N	9.97 min	1.20	1.00
^{11}C	20.4 min	0.96	1.00
^{68}Ga	67.6 min	1.89	0.89
^{18}F	109.8 min	0.63	0.97
^{64}Cu	12.7 h	0.65	0.18
^{76}Br	16.2 h	various	0.56
^{124}I	4.17 d	1.53, 2.14	0.23
^{22}Na	2.60 y	0.55	0.90

2.6.2. Properties of ^{18}F

^{18}F is a short-lived ($t_{½}$ = 109.8 min) positron-emitting isotope. It is considered an ideal positron emitter for PET because of its nuclear and physical characteristics. The comparatively long half-life is favourable since it permits longer-lasting radiosyntheses, time-demanding PET-studies and enables long-lasting pharmacokinetic studies such as metabolite analysis. The low positron energy of ^{18}F ensures a short range of positron in tissues leading to acquisition of PET-images of the highest resolution (Jacobson 2010). Some selected physical properties of common positron-emitting PET-radionuclides are presented in table 3.

2.6.3. Production methods of ^{18}F

^{18}F can be produced by several nuclear reactions most of which require the use of an accelerator, typically a cyclotron (Guillaume 1991). The choice of the optimal way to produce ^{18}F is dependent on several factors. Initially, depending on the nuclear reaction needed, different accelerated particles and particle energies are required and their availability is determined by the type of the cyclotron (Le Bars 2006). Secondly, the target-systems available at the cyclotron laboratory have to be considered. Thirdly, the chemical form of fluorine (nucleophilic or electrophilic) and the required amount of the ^{18}F-radioactivity have to be taken into account. Further, the required specific

radioactivity of ^{18}F has to be considered when choosing a suitable ^{18}F-production method. The basic nuclear reactions to produce ^{18}F are summarized in Table 4.

Table 4. Selected nuclear reactions with which to produce ^{18}F-labelled precursors (Ferrieri 2003).

Nuclear reaction	Target	^{18}F-labelled precursor
$^{18}O(p,n)^{18}F$	$[^{18}O]H_2O$	$[^{18}F]F^-$
$^{18}O(p,n)^{18}F$	$[^{18}O]O_2$/ Noble gas + carrier F_2	$[^{18}F]F_2$
$^{20}Ne(d,\alpha)^{18}F$	Ne + carrier F_2	$[^{18}F]F_2$
$^{20}Ne(^3He,\alpha n)^{18}Ne$, $^{18}N \rightarrow ^{18}F$	2% H_2/Ne	$[^{18}F]HF$
$^{16}O(^3He,p)^{18}F$	H_2O	$[^{18}F]F^-$
$^{16}O(\alpha,d)^{18}F$	H_2O	$[^{18}F]F^-$

The most useful and common nuclear reaction to produce ^{18}F is $^{18}O(p,n)^{18}F$, in which ^{18}O-enriched water is irradiated with protons. This nuclear reaction is intrinsically high yielding at low proton energies (< 16 Mev) and produces $[^{18}F]$fluoride with a high specific radioactivity as the $[^{18}F]F^-$ ion in aqueous solution (Ruth 1979, Solin 1988).

Electrophilic fluorine $[^{18}F]F_2$ is produced mainly through two nuclear reactions. The $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction employs neon gas as a target with added F_2 to maintain the produced fluorine as molecular fluorine (Lambrecht 1978, Casella 1980). The $^{18}O(p,n)^{18}F$ nuclear reaction uses $^{18}O_2$ gas as the target material (Nickles 1984). After the irradiation, ^{18}F becomes deposited in the target walls and $^{18}O_2$ is recovered cryogenically. A second irradiation in the presence of noble gas and F_2 is then needed for the isotopic exchange of the adsorbed ^{18}F to obtain $[^{18}F]F_2$. As an alternative, a "post-target" method, developed in Turku PET Centre (Bergman 1997), can be used to obtain $[^{18}F]F_2$ with increased SA. This method will be discussed in more detail in paragraph 2.6.7.

2.6.4. Improving the reactivity of ^{18}F-anion

The first step in radiochemistry with $[^{18}F]$fluoride ion is almost without exception the removal of the bulk $[^{18}O]$water. In the presence of water, the fluoride ion is highly solvated and hydrogen bonded, two properties which decrease the nucleophilicity of $[^{18}F]$fluoride and render it quite unreactive. Some simple, but extremely important, manipulations are therefore required to prepare reactive and nucleophilic $[^{18}F]$fluoride, or "naked" $[^{18}F]$fluoride as some investigators like to call it (Cai 2008, Lasne 2002). This is commonly achieved via two alternative methods (see Figure 23).

(1) [^{18}F]fluoride, dissolved in the target water, is adsorbed onto an ion exchange resin (typically an ion exchange cartridge) from which it is eluted with a small volume of aqueous base, most commonly potassium carbonate. Water is then removed with successive cycles of azeotropic evaporation with acetonitrile in the presence of kryptands, typically aminopolyethers. This method enables the laboratory to recycle the ^{18}O-enriched water for further use.

(2) Another method is to direct the irradiated target water directly to a reaction vessel and then to perform azeotropic evaporation cycles in the presence of base and kryptands or other phase-transfer catalysts.

The [^{18}F]fluoride ion drying procedure in the presence the aminopolyether Kryptofix K2.2.2 and a counter-ion (K^+) leads to a "dry" aminopolyether complex K^+/K2.2.2/[^{18}F]F- (Figure 23). This complex improves the reactivity of [^{18}F]fluoride ion in two ways. First, the aminopolyether serves to capture the counter-ion K^+ and separates it from the [^{18}F]fluoride ion. Second, the complex is readily soluble in organic solvents, where the [^{18}F]fluoride ion is not solvated and remains reactive.

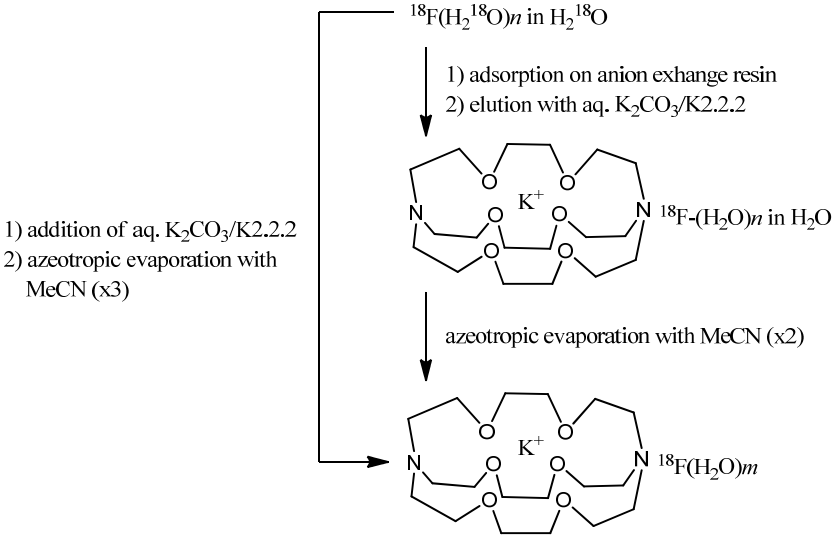


Figure 23. Preparation of reactive ^{18}F-fluorine ion through the formation of [^{18}F]F^-/K2.2.2/K^+-complex ("Kryptofix-complex") with two alternative methods starting from cyclotron-irradiated target water $H_2{}^{18}O$. The amount of residual water is sequentially reduced; the fully hydrated complex is transformed to a "dried" complex containing trace amount of water where $m << n$ (Cai 2008).

There are several variations possible with which to produce the dry and reactive fluoride complex, including the use of different bases (bicarbonate, oxalate), kryptands (18-crown-6) and counterions (Rb^+, Cs^+, Bu_4N^+). A large cation (Cs^+, Et_4N^+, Bu_4N^+) without a kryptand can also serve the same purpose in charge separation. The [^{18}F]fluoride ion is easily rendered non-nucleophilic by protonation, and thus most reactions are conducted in mildly basic conditions with poorly nucleophilic bases such as CO_3^{2-}, HCO_3^- or $C_2O_4^{2-}$. Therefore, the precursor to be labelled should not itself be a source of protons and should not contain base labile structures (Cai 2008).

2.6.5. Specific radioactivity

The specific radioactivity (SA) is defined as the amount of radioactivity per mass unit, the mass usually being expressed as a molar mass. The maximum SA (SA_{max}) of a radionuclide can be calculated using the equation $SA_{max} = N_A * \ln 2/T_{1/2}$, where N_A is Avogadro's number and $T_{1/2}$ is the half-life of the radionuclide. From this one can derive that the theoretical maximum of SA for ^{18}F is 6.34 x 10^4 GBq/µmol. However, this level can never be reached due to the contamination with the stable isotope originating from the radionuclide production, the solvents, chemicals and other non-intentional sources.

SA is a very important topic both in PET radiochemistry and PET imaging. PET is basically a tracer method and the goal of the PET experiment is to probe a physiological process without perturbing that process. In other words, it is necessary to administer low amounts, or "trace" amounts, of the radiolabelled molecule to the study subject. This is particularly important when studying low-density receptor sites, that are readily saturated by the radiotracer, or when the radiotracer itself is potent or toxic. The challenge for the radiochemist is to develop a synthetic strategy in such a way that the highest possible SA can be achieved.

In [^{18}F]fluorine chemistry, the SA depends mainly on the nuclear reaction used to produce ^{18}F. High SA can be obtained by using the ^{18}O(p,n)^{18}F reaction with ^{18}O-enriched water targets, the most common method in use to produce ^{18}F for nucleophilic labelling. The production of the electrophilic labelling reagent [^{18}F]F_2, produced either with in-target or post-target methods, requires the use of carrier-F_2 and so [^{18}F]F_2 cannot be obtained with high SA (Lasne 2002, Satyamurthy 2004).

2.6.6. Nucleophilic fluorinations

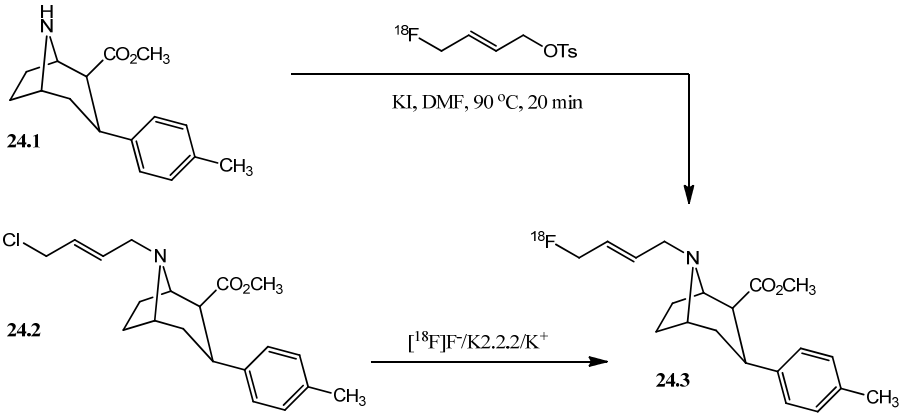


Figure 24. Synthesis of the dopamine transporter ligand [^{18}F]LBT-999 **24.3** via two alternative nucleophilic methods; the indirect labelling of *nor*-fluorobutylene precursor **24.1** with the ^{18}F-labelled prosthetic group; the direct labelling of the chloro-precursor **24.2** with aminopolyether complex (Miller 2008).

Nucleophilic substitutions with [^{18}F]fluoride have been extensively used both in aliphatic and aromatic series. The ^{18}F-fluorinating agent is almost exclusively the dried K^+/K2.2.2/[^{18}F]F^- complex. Usually radiofluorinations do not require any carrier and so the products can be obtained with high SAs. The radiofluorination can be performed either directly on a suitable and complex precursor of the target molecule or indirectly via a simple ^{18}F-fluoroaliphatic derivative i.e. an ^{18}F-labelled prosthetic group (see figure 24). Both methods have their inherent drawbacks. The direct method can result in low radiochemical yields and the indirect method may involve time-consuming and multi-step procedures.

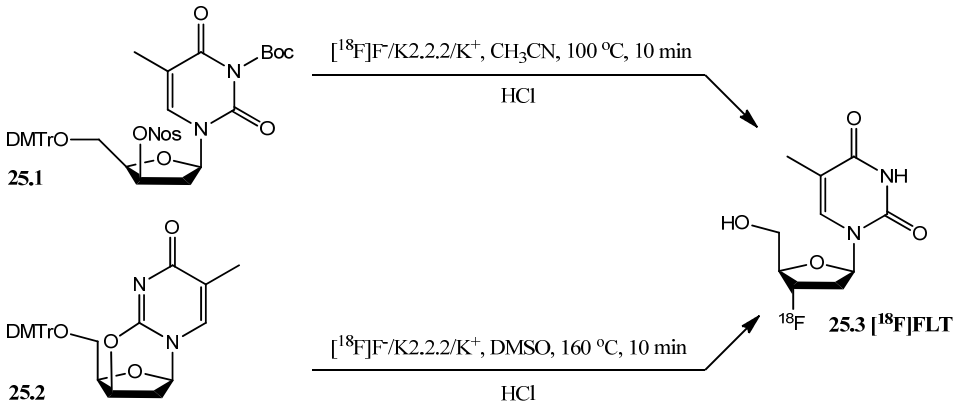


Figure 25. Synthesis of [^{18}F]FLT **25.3** by two alternative aliphatic nucleophilic substitutions; direct and conventional substitution of a sulfonate leaving group of precursor **25.1**; substitution via ring-opening reaction of cyclic precursor **25.2** (Been 2004).

The aliphatic nucleophilic substitution with [^{18}F]fluoride ion is a noteworthy method but the radiochemical yield is very dependent on the chemical structure of the precursor. Precursor reactivity closely follows the pattern of a typical S_N2 type reaction with substitution at the primary carbon favoured for high yield. Substitutions at a secondary carbon may be accompanied by an elimination reaction from the precursor. Usually the leaving groups are sulfonates (triflate, tosylate, mesylate, nosylate) or halides (Cl, Br, I). Certain cyclic systems may also be opened by nucleophilic [^{18}F]fluoride attack (see figure 25) (Lasne 2002, Cai 2008).

Aliphatic nucleophilic substitutions with [^{18}F]fluoride are usually performed in polar aprotic solvents such as DMF, DMSO, THF, CH_2Cl_2 and acetonitrile, which are suitable and effective for many reactions and are also easily removable (Cai 2008). As an alternative to these conventional solvents, the use of polar protic solvents has been explored and successfully applied in many recent studies (see figure 26). Sterically hindered alcohols, such as *tert*-butyl alcohol (*t*-BuOH), have achieved optimal results. This polar medium actually increases the nucleophilicity of the [^{18}F]fluoride ion and thereby increases the rate of nucleophilic fluorination as compared to conventional solvents, especially with aliphatic substrates. The polar medium may also reduce the competing formation of by-products such as alkenes, alcohols or ethers (Kim DW 2008). The reaction mechanism has been proposed to differ from the classical S_N2 reaction. *t*-BuOH, through H-bonding, may act as a Lewis base to weaken the ionic bond between the counter-cation and ^{18}F; also, *t*-BuOH may act as a Lewis acid and

assist the departure of the leaving group from the alkyl chain through H-bonding (Oh 2007, Cai 2008, Schirrmacher 2007).

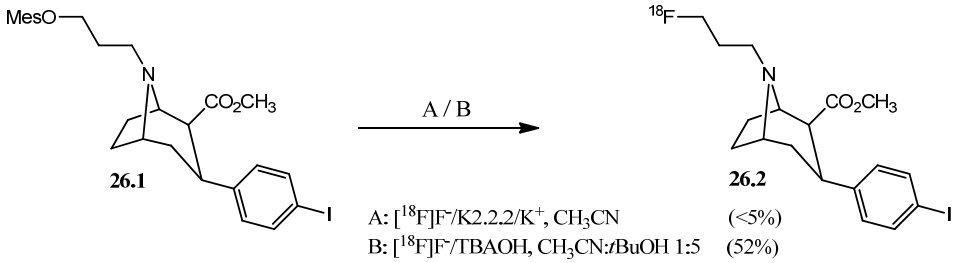


Figure 26. Radiosynthesis of the dopamine transporter ligand [^{18}F]FP-CIT **26.2** in polar aprotic solvent (A) and in polar protic solvent (B). A much higher radiochemical yield is obtained with a polar protic solvent (CH_3CN:*t*-BuOH 1:5) (Chaly 1996, Lee 2007).

Aromatic nucleophilic substitution is an efficient method to introduce fluorine into homo- or heteroaromatic structures. This reaction requires that the aryl ring has a good leaving group, usually at *ortho*- or *para*-position to at least one electron-withdrawing substituent. Normally, quite harsh reaction conditions (120 ºC – 180 ºC on DMSO in the presence of kryptand and K_2CO_3) are mandatory to achieve a sufficient fluoride incorporation yield. Typical leaving groups and their approximate order of increasing reactivity are I < Br < Cl < F < NO_2 ≈ N^+Me_3. Typical electron-withdrawing groups in their order of increasing ability are 3-NO_2 < 4-Ac < 4-CHO < 4-CN ≈ 4-CF_3 < 4-NO_2 (Cai 2008). Synthesis of [^{18}F]-*N*-methylspiperone **27.2** (Figure 27) is a typical aromatic nucleophilic substitution, where *p*-nitro group is substituted with ^{18}F with moderate fluoride incorporation. Only a few examples have been reported for efficient ^{18}F-fluorination reactions with an electron-withdrawing group in the *m*-position. The synthesis of mGluR5 radioligand [^{18}F]FMTEB **27.4** is an example; ^{18}F-fluoride incorporation is enhanced with the use of microwaves but nonetheless a low radiochemical yield has been reported.

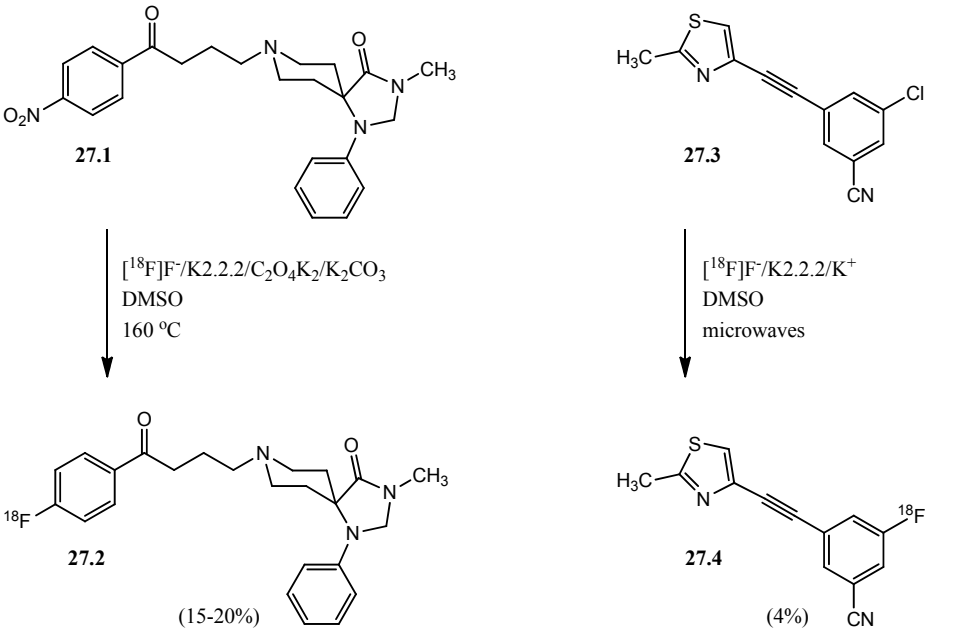


Figure 27. Synthesis of [^{18}F]-*N*-methylspiperone **27.2** and [^{18}F]FMTEB **27.4** with direct nucleophilic aromatic substitution (Hamacher 1995, Guo 2007).

Me_3N+ is generally a good leaving group (with chloride, perchlorate or triflate as a counter-ion) and it permits also a straightforward separation of the precursor and the fluoro-product. Even though the nucleophilic displacement of nitro-group is feasible, the separation of the unreacted nitro-precursor from the fluoro-product can sometimes be very difficult as a result from the co-elution in the HPLC (Cai 2008, Lasne 2002).

The use of heteroaromatic nucleophilic substitutions with [^{18}F]fluorine has lately expanded especially with pyridine structures (Dolle 2005). As in the aliphatic series, only a good leaving group is generally necessary. Figure 28 shows the syntheses of nAChR ligand 2-[^{18}F]fluoro-A-85380 (**28.4** and **28.6**) with two alternative methods using *ortho*-fluorination; higher yields are obtained by using precursor **28.5** with a trimethylammonium leaving group in the labelling synthesis. The presence of a highly electron-withdrawing substituent to activate the heterocycle is recommended to fluorinate the *meta*-position; only a few examples of *meta*-[^{18}F]fluoropyridine derivatives are known to date, one example being *N*-(2-aminoethyl)-5-[^{18}F]fluoropyridine-2-carboxamide **28.2** (see figure 28).

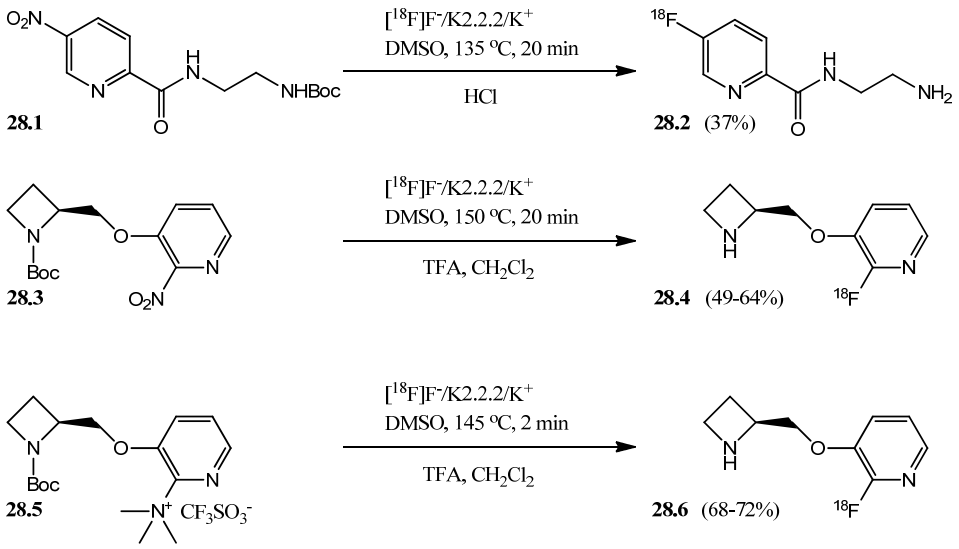


Figure 28. Examples of heteroaromatic nucleophilic substitution reactions to the *meta*-position (**28.2**) and the *ortho*-position (**28.4**, **28.6**). *Meta*-fluorination is generally difficult to achieve (Beer 1995, Dolle 1998, Dolle 1999).

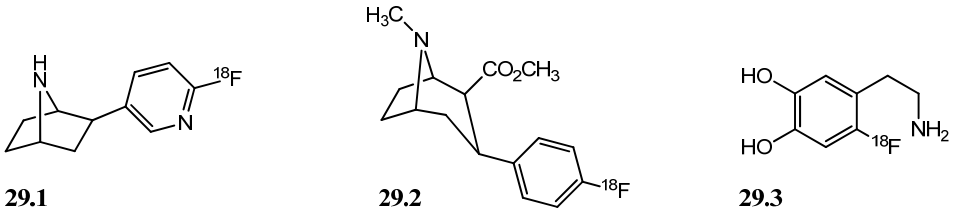
2.6.7. Electrophilic fluorinations

Electrophilic reagents generate a chemical environment in which the fluorine atom is highly polarized with a positive charge. This is not easily achieved since fluorine is the most electronegative atom in the periodic table of elements. With electrophilic fluorination, it is possible to fluorinate a large range of electron-rich substrates such as alkenes, aromatic compounds and carbanions, the labelling of which is not always achievable with nucleophilic n.c.a. ^{18}F-labelling methods (Ferrieri 2003, Coenen 2007).

In brief, electrophilic ^{18}F-fluorinations can be divided into two subgroups; aromatic electrophilic fluorinations (including hydrogen substitutions and demetallation reactions) and aliphatic electrophilic fluorinations. The radiofluorination reactions are typically conducted either in strong protonic acids (acetic acid, trifluoroacetic acid, liquid HF) or in very inert solvents such as acetonitrile or halomethanes. Naturally the reaction solvent and also the protecting groups of the precursor themselves should not be substrates for electrophilic attack.

However, there are several challenges facing the radiochemist when working with ^{18}F-labelled electrophilic reagents; these include low SA, low yields and poor regioselectivity of the ^{18}F-fluoride incorporation.

The classic and most common reagent for electrophilic fluorination is radiolabelled elemental fluorine gas $[^{18}F]F_2$. It can be produced by "in-target" methods using $^{20}Ne(d,\alpha)^{18}F$ or $^{18}O(p,n)^{18}F$ nuclear reactions. In both of these nuclear reactions, the usage of carrier fluorine is mandatory. As a consequence, $[^{18}F]F_2$ cannot be produced with very high SA. This, in turn, has severely limited the use of $[^{18}F]F_2$ gas in radiopharmaceutical preparations, particularly when producing toxic molecules or radiopharmaceuticals for low-density receptors.



29.1 **29.2** **29.3**

Figure 29. Potent radiotracers that require high SA in human studies and are difficult to produce with electrophilic fluorination that results in low SA. *nor*-chloro-[^{18}F]fluoroepibatidine **29.1**, [^{18}F]CFT **29.2** and 6-[^{18}F]fluorodopamine **29.3**.

A "post-target" method (see Figure 30) to produce $[^{18}F]F_2$ with a SA of up to 55 GBq/µmol (decay corrected to EOB) has been developed in the Turku PET Centre (Bergman 1997). This method utilises high-SA ^{18}F-labelled fluoromethane produced from aqueous $[^{18}F]F^-$, which is mixed with low amounts (300–1200 nmol) of carrier F_2 in an inert neon matrix. The constituents are atomised with an electrical discharge; afterwards, rearrangement and ^{18}F for ^{19}F exchange occurs, and high SA $[^{18}F]F_2$ is available for use as a labelling precursor in various types of electrophilic fluorinations.

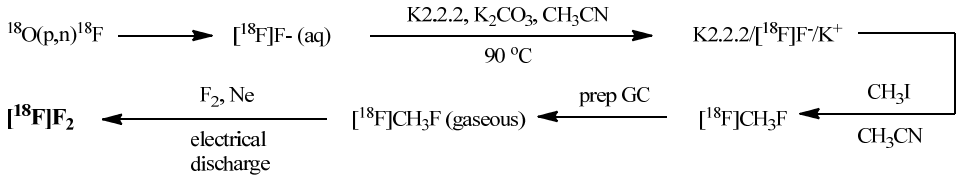


Figure 30. Synthesis of high SA $[^{18}F]F_2$ with a "post-target" method developed at Turku PET Centre (Bergman 1997).

When $[^{18}F]F_2$ is used in electrophilic substitution reactions, only one of the two fluorine atoms is incorporated into the substrate; the maximum achievable radiochemical yield is

therefore only 50%. However, this is hardly ever achieved because of the numerous side reactions due to the high reactivity of $[^{18}F]F_2$. The reactivity of fluorine can be reduced mainly with two methods. Firstly, fluoride can be diluted with an inert gas (typically Ne) resulting in a more controllable gas mixture (Chen 2010). A second option is to convert $[^{18}F]F_2$ into less reactive secondary electrophilic reagents. The most commonly used example of these is ^{18}F-labelled acetyl hypofluorite $[^{18}F]CH_3CO_2F$ (see figure 31) (Fowler 1982, Berridge 1986, Ogawa 2003). Other secondary reagents, derived from $[^{18}F]F_2$, include $[^{18}F]$trifluoromethyl hypofluorite, $[^{18}F]$perchloryl fluoride, $[^{18}F]$xenon difluoride, 1-[^{18}F]fluoro-2-pyridone, *N*-[^{18}F]fluoropyridinium triflate, various *N*-[^{18}F]fluoro-*N*-alkylsulsulfonamides, various *N*-[^{18}F]-sulfonimides and $[^{18}F]$Selectfluor *bis*(triflate) (Ferrieri 2003, Hiller 2008, Constantinou 2001, Oberdorfer 1988, Satyamurthy 1990, Teare 2007, Teare 2010). Although these reagents have been used in various experiments to study the electrophilic ^{18}F-incorporation into small molecules, none of these has yet surpassed the use of $[^{18}F]F_2$ in radiopharmaceutical syntheses.

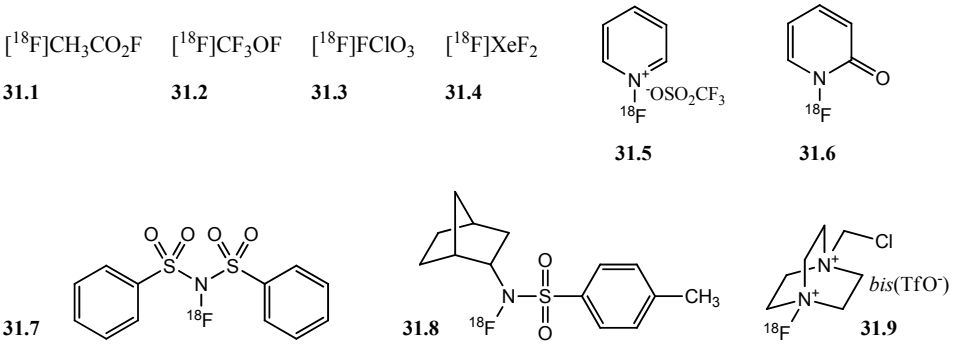


Figure 31. ^{18}F-labelled electrophilic reagents derived from $[^{18}F]F_2$. $[^{18}F]$Acetyl hypofluorite **31.1**, $[^{18}F]$trifluoromethyl hypofluorite **31.2**, $[^{18}F]$perchloryl fluoride **31.3**, $[^{18}F]$xenon difluoride **31.4**, *N*-[^{18}F]fluoropyridinium triflate **31.5**, 1-[^{18}F]fluoro-2-pyridone **31.6**, *N*-[^{18}F]fluorobenzenesulfonimide **31.7**, *N*-[^{18}F]fluoro-*endo*-norbornyl-p-tolylsulfonamide **31.8**, $[^{18}F]$Selectfluor bis(triflate) **31.9**.

Aromatic electrophilic hydrogen substitution reactions with electrophilic $[^{18}F]F_2$ are generally unspecific and can result in the formation of mixtures of ^{18}F-labelled regioisomers (Miller 2008). Thus, aromatic systems are usually fluorinated via demetallation reactions with mercury or tin containing precursors which, through increasing the carbanionic character of the metal bearing carbon, make the labelling much more regioselective (Coenen 2007) (see figure 32).

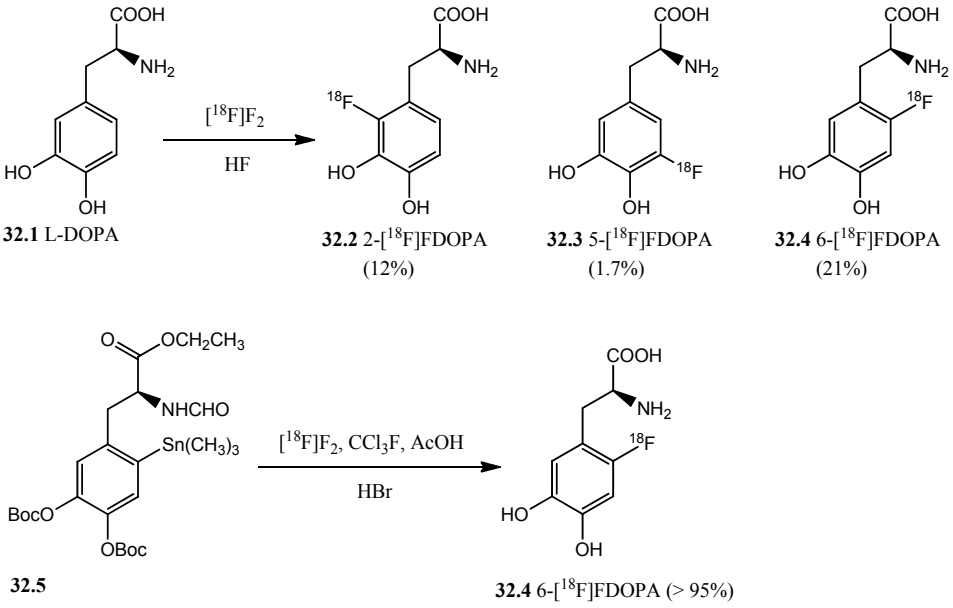


Figure 32. Direct labelling of L-DOPA **32.1** with $[^{18}F]F_2$ is unselective and results in the formation of three regioisomers. Selectivity is improved by using a demetallation reaction with a stannylated precursor **32.5** (Firnau 1984, Forsback 2008).

Aliphatic electrophilic fluorinations are rare as compared to aromatic electrophilic substitutions. The most common reaction is the addition of $[^{18}F]F_2$ to a double bond. This method was used in the original synthesis of [^{18}F]FDG (figure 33) before being replaced with the far more efficient nucleophilic fluorination route. Another example is the synthesis of the hypoxia tracer [^{18}F]EF5 (Dolbier 2001, Eskola 2012*a*) that will be discussed in detail in further chapters of this thesis.

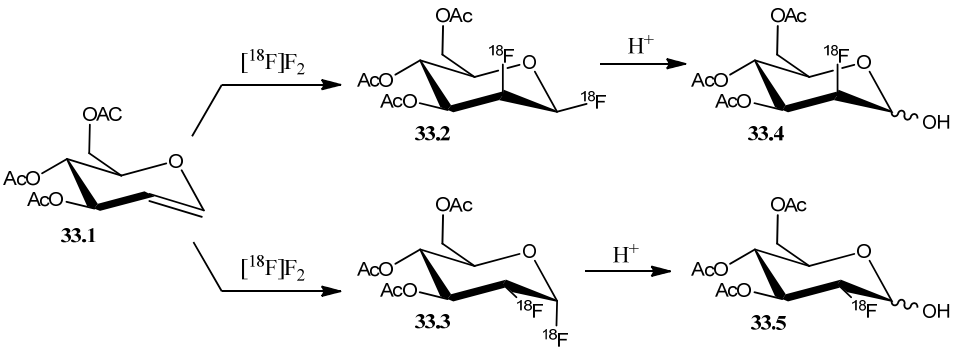


Figure 33. Synthesis of [^{18}F]FDG **33.5** via electrophilic addition of $[^{18}F]F_2$ to the 3,4,6-tri-O-acetyl-D-glucal precursor **33.1**. [^{18}F]-difluoroisomers **33.2** and **33.3** were produced with 1:3 ratio. Subsequent hydrolysis of these compounds led to [^{18}]fluorodeoxymannose **33.4** and [^{18}F]FDG **33.5**. The radiochemical yield of [^{18}F]FDG was 8% (Ido 1978).

2.6.8. Other fluorination methods

In addition to the conventional nucleophilic and electrophilic fluorination methods, a few useful techniques have been devised to incorporate fluorine-18 into radiopharmaceuticals; isotopic exchange reactions can be useful when one does not need to obtain high SA (Langer 2003, Blom 2009); enzymatic reactions offer chemo-selective ways for ^{18}F-fluoride incorporation since these types of reactions are bio-catalytically controlled (Martarello 2003, Deng 2006); various ^{18}F-labelled prosthetic groups, usually synthesised with standard nucleophilic methods, have been widely used to label macromolecules, such as peptides and oligonucleotides (Ametamey 2008, Miller 2008). In particular, recently prosthetic labelling through click chemistry (1,3-dipolar Huisgen cycloaddition reaction) has become rather popular. This offers a fast and selective radiolabelling method for biomolecules with mild reaction conditions (Li 2007, Sirion 2007). The techniques mentioned in this paragraph will not be discussed in a more detailed manner in this thesis.

3. AIMS OF THE STUDY

All the syntheses included in this study were done with [^{18}F]F_2 that was produced with a "post-target" method (Bergman 1997). The aim was to demonstrate the suitability and efficiency of "post-target" produced [^{18}F]F_2 as an electrophilic labelling reagent with which to synthesise high-quality radiopharmaceuticals. This "post-target" technique is advantageous in many ways as compared to the conventional "in-target" method; (1) an elevated SA is obtained, (2) the over-all production time is short and (3) reduced amounts of non-radioactive starting materials can be used, which enables more straightforward purification of the radiopharmaceutical. All these aspects were evaluated in this study while at the same time trying to maintain a sufficient radiochemical yield. The chemical structures of the radiopharmaceuticals chosen for this work were such, that the ^{18}F-fluoride incorporation into these structures could, in theory, be accomplished efficiently via electrophilic fluorination. Efficiency was generally assessed in terms of achieving three properties; high radiochemical yield, high selectivity for the introduction of the ^{18}F-label and high SA.

The following objectives were set:

1. To study the efficiency of aromatic electrophilic fluorodestannylation; introduction of ^{18}F-fluoride into aromatic rings with a carbanionic character induced by a trimethylstannyl leaving group.

2. To study the efficiency of fluorodestannylation with a multiaromatic precursor containing many electron-rich centers; synthesis of [^{18}F]F5P.

3. To produce potent catecholamine analogues through electrophilic aromatic substitution with a high radiochemical yield and an elevated SA; synthesis of 4-[^{18}F]FMR and 6-[^{18}F]FDA.

4. To study the electrophilic addition reaction of [^{18}F]F_2 to a double-bond containing precursor; synthesis of [^{18}F]EF5.

4. MATERIALS AND METHODS

4.1. Production of radiopharmaceuticals

4.1.1. General

All the radiopharmaceuticals described in this section were synthesised using custom-made synthesis units built at Turku PET Centre. A Merck-Hitachi L-7100 HPLC pump (Merck AG, Darmstadt, Germany) and a Merck-Hitachi L-7400 UV-absorption detector (Merck AG, Darmstadt, Germany) were used in the semi-preparative HPLC separations. A 2"x2'' NaI crystal was used for radioactivity detection on the HPLC-column outflow. Radioactivity was measured with VDC-405 ionisation chamber (Veenstra Instruments, Joure, The Netherlands).

The precursor for [^{18}F]F5P (**34.1**) was synthesised in the Turku PET Centre. The precursor for 6-[^{18}F]FDA (**36.1**) was obtained commercially (ABX, Radeberg, Germany). The precursors for 4-[^{18}F]FMR (**35.1**) and [^{18}F]EF5 (**37.1**) were supplied by scientific collaborators. All the other reagents were obtained from commercial suppliers. More detailed information about the materials and instrumentation related to the radiopharmaceutical productions can be found in the following scientific articles (Eskola 2002, Eskola 2004, Eskola 2012*a*, Eskola 2012*b*).

4.1.2. Production of [^{18}F]F$^-$

[^{18}F]F$^-$ was produced using the ^{18}O(p,n)^{18}F nuclear reaction by irradiating 700 µl ^{18}O-enriched water with 17 MeV proton beam produced with an MGC-20 cyclotron (Efremov Institute of Electrophysical Apparatuses, St. Petersburg, Russia).

4.1.3. Production of high SA [^{18}F]F$_2$

[^{18}F]F$_2$ was synthesised in an electrical discharge chamber by the ^{18}F/^{19}F-exchange reaction. The ^{18}F-source was high SA n.c.a. [^{18}F]fluoromethane, which was mixed with a low amount (250-1200 nmol) of carrier fluorine (F_2) inside a discharge chamber. [^{18}F]fluoromethane was produced from iodomethane by a nucleophilic substitution reaction with [^{18}F]F$^-$. The aminopolyether Kryptofix K2.2.2 in dry acetonitrile was used to enhance the nucleophilicity of the [^{18}F]fluoride to improve the S_N2 reaction with iodomethane at an elevated temperature (85-90 °C). A detailed description of this "post-target" [^{18}F]F$_2$ synthesis set-up can be found in the literature (Bergman 1997).

4.1.4. Synthesis of [^{18}F]F5P (I)

3-[[4-(4-[^{18}F]fluorophenyl)piperazin-1-yl]methyl]-1*H*-pyrrolo[2,3-b]pyridine ([^{18}F]F5P) **34.2** was synthesized from precursor **34.1** through electrophilic destannylation with [^{18}F]F_2 gas. 300 µg (0.66 µmol) of **34.1** was dissolved in a solution containing freon-11 (600-700 µl) and dry acetic acid (25-50 µl). [^{18}F]F_2 was bubbled through this reaction mixture at room temperature. Freon-11 was evaporated and the residue was dissolved in 0.1 M HCO_2NH_4-solution, which was injected on the semi-preparative HPLC-column (Waters µBondapak C18, 7.8 x 300 mm, 10 µm). For the first two minutes, the column was eluted isocratically with 0.1 M ammonium formate solution and after that isocratically with a mixture of 0.1 M ammonium formate/MeOH (45:55) with a flow rate of 4 ml/min. Separation of products was monitored with a radioactivity detector and a UV-detector (λ=280 nm). The fraction containing compound **34.2**, eluting at approximately 18 minutes, was collected and the radioactivity was measured. This fraction was then evaporated to dryness and the dry residue was dissolved in 0.9 % NaCl-solution (pH 4.7).

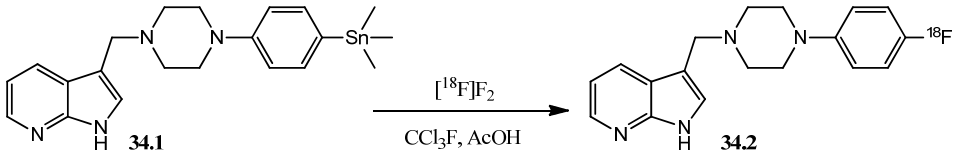


Figure 34. Synthesis of [^{18}F]F5P **34.2** with electrophilic aromatic substitution using [^{18}F]F_2.

4.1.5. Synthesis of 4-[^{18}F]FMR (II)

The synthesis of (1R,2S)-2-amino-1-(4-[^{18}F]fluoro-3-hydroxyphenyl)-1-propanol (4-[^{18}F]FMR **35.3**) is outlined in figure 35. The stannylated precursor **35.1** (1.26 - 1.72 mg, 2.4 - 3.2 µmol) was dissolved in a mixture of freon-11 (500 - 600 µl) and dry acetic acid (50 µl). [^{18}F]F_2 was bubbled through this mixture at room temperature. Freon-11 was evaporated and 300 µl of 47 % HBr was added to the residue. The hydrolysis of the di-Boc-compound **35.2** was carried out at 90 °C for five minutes. The reaction mixture was partially neutralised by addition of 170 µl 10.8 M NaOH and 300 µl HPLC eluent. 4-[^{18}F]FMR **35.3** was purified by semi-preparative HPLC. The HPLC-column (Waters

μBondapak C18, 7.8 x 300 mm, 10 μm) was eluted with 0.9% NaCl-solution containing 2% ethanol and 0.02% AcOH (flow rate 3 ml/min). Separation of products was monitored with a radioactivity detector and a UV-detector (λ=280 nm). The fraction of **35.3** (R_t = ~11.5 min) was collected and measured for radioactivity. This ethanolic saline solution, which was suitable for intravenous injection, was used in preclinical experiments.

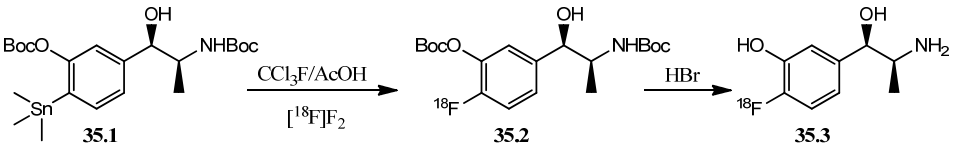


Figure 35. Synthesis of 4-[^{18}F]FMR **35.3** with electrophilic aromatic substitution using [^{18}F]F_2.

4.1.6. Synthesis of 6-[^{18}F]FDA (III)

The synthesis of 4-(2-aminoethyl)-5-[^{18}F]fluorobenzene-1,2-diol (6-[^{18}F]FDA **36.3**) is outlined in figure 36. The stannyl precursor **36.1** (1.26–1.72 mg, 2.4–3.2 μmol) was dissolved in a mixture of freon-11 (500–600 μl) and dry acetic acid (20 μl). [^{18}F]F_2 was bubbled through this mixture at room temperature with neon as the sweep gas. Freon-11 was evaporated and 300 μl of 57% HI was added to the acetic acid residue, after which the hydrolysis was carried out at 125 °C for 10 min. The reaction mixture was then partially neutralised with the addition of 170 μl of 10.8 M NaOH solution diluted with the HPLC eluent. 6-[^{18}F]FDA **36.3** was purified by semi-preparative HPLC. A Waters μBondapak C18 column (7.8 x 300 mm, 10 μm) was eluted with 0.9% NaCl-solution containing 2% ethanol and 0.02% AcOH (flow rate 3 ml/min). Elution of products was monitored with a radioactivity detector and a UV-detector (λ=280 nm). The 6-[^{18}F]FDA fraction (R_t = 12–13 min) was collected and measured for radioactivity. This ethanolic saline solution, applicable for intravenous injection, was used in preclinical experiments.

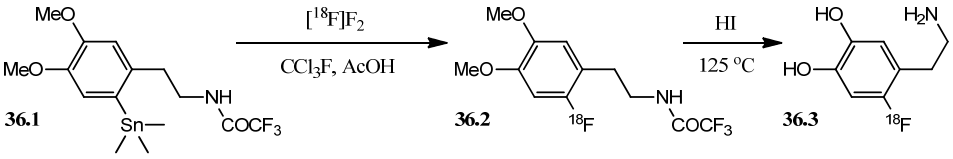


Figure 36. Synthesis of 6-[^{18}F]FDA **36.3** with electrophilic aromatic substitution using [^{18}F]F_2.

4.1.7. Synthesis of [^{18}F]EF5 (IV)

The synthesis of the regioisomers of [^{18}F]EF5 is shown in figure 37. The trifluoroallyl acetamide precursor **37.1** (1.04 - 1.20 mg: 3.9 - 4.5 μmol) was dissolved in TFA (600 - 700 μl). [^{18}F]F_2 was bubbled through this mixture at room temperature with neon as the sweep gas. TFA was evaporated by bubbling neon gas through the reaction vessel heated at 60 °C. The dry residue was dissolved in a solution of 0.1 M ammonium formate (pH adjusted to 4.6) and CH_3CN (75/25 v/v). [^{18}F]EF5 was purified by gradient RP-HPLC using Waters μBondapak C18 column (7.8 x 300 mm, 10 μm). Semi-preparative HPLC separation was achieved using a gradient method with 0.1 M ammonium formate (pH adjusted to 4.6) (Eluent A) and CH_3CN (Eluent B) as mobile phases (A/B 74/26 → 50/50, 15 min linear gradient continued with isocratic conditions with (A/B 50/50) until 20 minutes). The flow rate was 3 ml/min. Separation of products was monitored with radioactivity detector and UV-detector (λ=325 nm). The fraction containing the [^{18}F]EF5 isomers **37.2** and **37.3** was collected, measured for radioactivity and evaporated to dryness with a rotary evaporator. The residue was dissolved in physiological saline and filtered through a 0.22 μm sterile filter into a sterile vial.

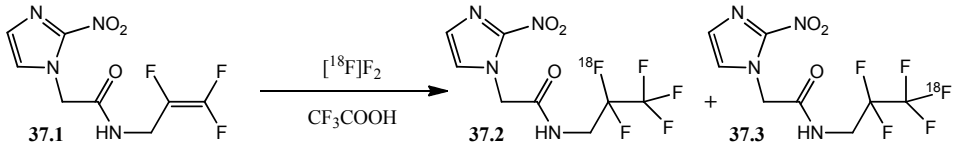


Figure 37. Synthesis of the two regioisomers of [^{18}F]EF5 **37.2** and **37.3** with aliphatic electrophilic addition reaction using [^{18}F]F_2.

4.2. Quality of radiopharmaceuticals

Radiopharmaceutical quality of the end products was determined by analytical HPLC using a Merck-Hitachi L-7100 HPLC pump (Merck AG, Darmstadt, Germany), a Merck-Hitachi L-7400 UV-absorption detector (Merck AG, Darmstadt, Germany) and a 2”x2’’ NaI-crystal for radioactivity detection. Determinations of product identity, chemical purity, radiochemical purity (RCP) and SA were carried out by comparing retention times and peak intensities to reference compounds of known concentrations. Radiochemical yields were calculated from the initial amount of [^{18}F]F^- and were decay-corrected to the end of bombardment (EOB). The SAs of the radiopharmaceuticals were decay-corrected to the end of synthesis (EOS). More detailed information about the

materials and instrumentation related to the quality analyses of the individual radiopharmaceuticals can be found in the following scientific articles (Eskola 2002, Eskola 2004, Eskola 2012*a*, Eskola 2012*b*).

5. RESULTS

5.1. Production of radiopharmaceuticals

5.1.1. Synthesis of [^{18}F]F5P (I)

[^{18}F]F5P **34.2** (see Figure 34) was synthesised from precursor **34.1** via fluorodestannylation with [^{18}F]F_2. The average synthesis time was 50 minutes. The radiochemical yield was low, on average 0.7 ± 0.1 % (decay corrected to EOB) as calculated from the amount of [^{18}F]F^- produced. This was due to the formation of several radiofluorinated side products. The absolute amount of radioactivity in the form of **34.2** was on average 183 ± 32 MBq at EOS. The major non-radioactive side-product that was formed using the strategy was found to be the *des*-fluorophenylpiperazine analogue of **34.2**, where fluorine has been replaced with hydrogen. The SA of **34.2** (at EOS) was in average 14.6 ± 1.8 GBq/μmol.

HPLC analysis of the end product revealed the presence of an unidentified ^{18}F-labelled contaminant. This contaminant, eluting as a bulky broad peak from the semi-preparative HPLC column before compound **34.2**, decreased the radiochemical purity of **34.2**, which was on average 90.3 ± 1.7 %. The chemical purity exceeded 95 %.

5.1.2. Synthesis of 4-[^{18}F]FMR (II)

4-[^{18}F]FMR **35.3** (see figure 35) was synthesised from precursor **35.1** by a fluorodestannylation reaction with [^{18}F]F_2. The synthesis time was 60 minutes. Based on seven production runs, the radiochemical yield of **35.3** was 2.8 ± 1.1 % (decay corrected to EOB). Radioactivity of **35.3** varied from 337 MBq to 1010 MBq at EOS. The SA of **35.3** was 11.8 ± 3.3 GBq/μmol and ranged from 7.7 to 16.8 GBq/μmol at EOS. The radiochemical purity, as analysed with analytical HPLC, exceeded 99 % in every case, and was found to be unchanged for at least three hours after the end of synthesis.

5.1.3. Synthesis of 6-[^{18}F]FDA (III)

6-[^{18}F]FDA **36.3** was synthesised by a fluorodestannylation reaction from precursor **36.1** using high SA [^{18}F]F_2 (see figure 36). The synthesis time was typically 60 min. The radiochemical yield of 6-[^{18}F]FDA, decay corrected to EOB, was 2.6 ± 1.1%. The total amount of 6-[^{18}F]FDA after HPLC purification was 663 ± 291 MBq and varied

from 171 MBq to 1006 MBq at EOS. The SA of 6-[^{18}F]FDA, decay corrected to EOS, varied from 10.0 to 18.8 GBq/µmol and was 13.2 ± 2.7 GBq/µmol. As a side reaction, a radiofluorinated compound, tentatively assigned as 2-[^{18}F]FDA, was obtained, the radioactivity of which was 184 ± 92 MBq at EOS. The radiochemical purity of 6-[^{18}F]FDA was determined with analytical HPLC and it exceeded 99.0% in every experiment. The radiochemical purity of the final product remained unchanged over a time period of 3 h after synthesis.

5.1.4. Synthesis of [^{18}F]EF5 (IV)

[^{18}F]EF5 (regioisomers **37.2** and **37.3)** was synthesised by electrophilic addition of high SA [^{18}F]F_2 to the trifluoroallyl precursor **37.1** (see Figure 37). The synthesis time was approximately 65 minutes. The radiochemical yield of [^{18}F]EF5, decay corrected to the EOB, was 2.8±0.6%. The total amount of the HPLC-purified [^{18}F]EF5 was 595±153 MBq, ranging from 406 MBq to 1027 MBq at EOS. The SA, decay corrected to EOS, was 6.6±1.9 GBq/µmol and ranged from 2.3 to 9.8 GBq/µmol. Radiochemical purity was determined by analytical HPLC and exceeded 99.0% in each experiment and was found to be unchanged for at least three hours after the end of synthesis.

5.2. Summary of results

The main results for the radiopharmaceuticals synthesised for this thesis are summarized in table 5.

Table 5. Summary of the main results for the four radiopharmaceuticals produced for this thesis.

Tracer	Synthesis time [min]	RA range at EOS [MBq]	RA at EOS [MBq]	RCY[1)] [%]	SA at EOS[2)] [GBq/µmol]
[^{18}F]F5P	50	132 - 223	183 ± 32	0.7 ± 0.1	14.6 ± 1.8
[^{18}F]FMR	60	337 - 1010	729 ± 281	2.8 ± 1.1	11.8 ± 3.3
[^{18}F]FDA	60	171 - 1006	663 ± 291	2.6 ± 1.1	13.2 ± 2.7
[^{18}F]EF5	65	406 - 1027	595 ± 153	2.8 ± 0.6	6.6 ± 1.9

[1)] Radiochemical yield (RCY) is calculated from the initial [^{18}F]F^- radioactivity at EOB and from the RA of the radiopharmaceutical, decay corrected to EOB.

[2)] SA is decay corrected to EOS. SAs of the different tracers are not completely comparable since different amounts of carrier-F_2 and different amounts of initial [^{18}F]F^- radioactivity have been used with the individual tracers.

6. DISCUSSION

6.1. Synthesis of [^{18}F]F5P (I)

[^{18}F]F5P was synthesised by electrophilic aromatic substitution from a *non*-protected stannyl precursor **34.1** (see Figure 34) using high SA [^{18}F]F_2 as the labelling reagent. Reduced amount of carrier-F_2 was used in order to obtain [^{18}F]F5P with a moderately high SA. A small amount of acetic acid was added to the reaction medium in order to polarize [^{18}F]F_2 and thus to convert it into a better electrophile. The incorporation of radiofluorine into the desired *para*-position of the phenyl ring was not optimal; a low radiochemical yield, on average 0.7 ± 0.1 % (decay corrected to the EOB), was obtained. This was due to the formation of several radiofluorinated side products. A large number of these compounds were more polar than [^{18}F]F5P showing earlier R_t in the RP-HPLC system. These were arguably produced through fragmentation, a common phenomenon with highly reactive and *non*-discriminating [^{18}F]F_2. The unprotected pyrrolo[2,3-b]pyridine moiety of **34.1** has also a high electron density and was, as such, a structure which could attract an electrophilic attack of [^{18}F]F_2. The trimethylstannyl group attached to the *para*-position of phenyl ring thus did not activate this position sufficiently to achieve selective fluorination of this position.

HPLC analysis revealed the presence of an unidentified ^{18}F-labelled contaminant in the end product solution of **34.2**. This contaminant, eluting as a bulky broad peak from the semi-preparative HPLC column before compound **34.2**, reduced the radiochemical purity of **34.2**, which was on average 90.3 ± 1.7 %. The SA (at EOS) was on average 14.6 ± 1.8 GBq/μmol.

6.2. Synthesis of 4-[^{18}F]FMR (II)

The major aim of this work was to obtain 4-[^{18}F]FMR **35.3** with increased SA while at the same time maintain a reasonable radiochemical yield. Increased SA is considered mandatory in 4-[^{18}F]FMR studies, since elevations in blood pressure have been observed in anaesthetized dogs after a 50-125 μg/kg administration of other fluorometaraminol regioisomers (Wieland 1990). The SA we obtained was 7.7 - 16.8 GBq/μmol, which is at least 250-fold higher than the values previously achieved with electrophilic labelling of 6-[^{18}F]FMR (Mislankar 1988). Consequently, the improved SA obtained in our study permits the administration of trace levels of 4-[^{18}F]FMR,

equivalent to a 2.1 – 4.4 μg administration of 4-FMR with the typical 185 MBq tracer injection. Even higher SAs, up to 106 GBq/μmol, have been obtained with nucleophilic methods (Langer 2000, Langer 2001, Ermert 1999). However, the nucleophilic methods used to produce 4-[^{18}F]FMR involve several reaction steps, are quite long-lasting and require the chromatographic separation of stereoisomers, aspects which can be avoided by using the present electrophilic method.

Our initial labelling experiments started with a benzyl protected stannylated precursor **38.1** (see figure 38). However, the use of this precursor in electrophilic synthesis of 4-[^{18}F]FMR was unsuccessful. A series of mass signals, corresponding to mono-, di- and trifluorinated derivatives of precursor **38.1**, were detected with LC-MS. Apparently, [^{18}F]F_2 was unable to displace the trimethylstannyl leaving group of **38.1** and instead it reacted with the electron rich benzyl protecting groups (see figure 38). Thus, very low yields of 4-[^{18}F]FMR, less than 20 MBq, were obtained and the precursor was changed to a Boc-derivative **35.1** which helped to overcome these problems.

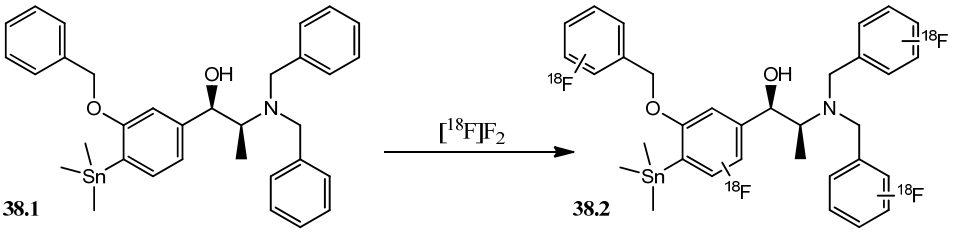


Figure 38. A failed attempt to radiolabel benzyl protected 4-[^{18}F]FMR precursor with electrophilic labelling. [^{18}F]F_2 reacted mainly with benzyl protecting groups and as a rule was unable to displace the stannyl leaving group.

By using the Boc-precursor **35.1**, 4-[^{18}F]FMR was obtained as the major radiofluorinated product. Radiochemical yields were satisfactory and high enough for several injections from a single batch despite the fact that a reduced amount of carrier-F_2 was used to obtain increased SA. Four radiolabelled side-products, eluting within 1-4 minutes after 4-[^{18}F]FMR from the semi-preparative HPLC column, were detected, and these were likely to be fluorinated aromatic regioisomers of 4-[^{18}F]FMR. The major chemical side-product generated in this synthesis was metaraminol, produced through the hydrolysis of the unreacted precursor **35.1**. Finally, the adoption of ethanolic saline

solution as the HPLC eluent enabled the easy formulation of the HPLC-fraction for *in vivo* use through sterile filtration.

6.3. Synthesis of 6-[^{18}F]FDA (III)

Electrophilic aromatic substitution with [^{18}F]F_2 is a noteworthy method to introduce the ^{18}F isotope into electron-rich molecules. Due to its high reactivity, the fluorination chemistry with [^{18}F]F_2 is almost instantaneous and can often be conducted at the last reaction steps. Unfortunately, the high reactivity of [^{18}F]F_2, coupled with its high oxidising strength, also enhances its tendency to create side products, typically through exothermic radical chain reactions (Lasne 2002). Thus, when complex and multifunctional molecules are labelled with [^{18}F]F_2, radiochemical yields tend to be low and a complex mixture of compounds may be obtained. Another challenge is to introduce the ^{18}F label selectively at the desired position by using [^{18}F]F_2 as the labelling reagent. In many cases, the selectivity can be improved by ^{18}F-fluorodemetallation reactions; e.g., by displacement of Hg- or Sn-containing leaving groups with [^{18}F]F_2.

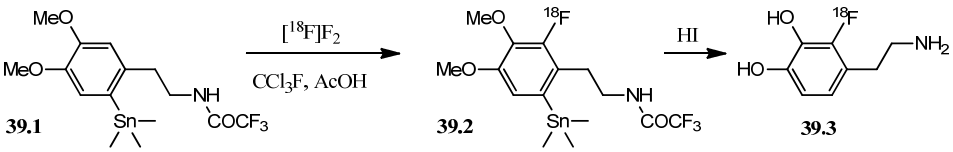


Figure 39. Formation of 2-[^{18}F]FDA as a side-reaction.

The goal of this study was to develop a high-yield electrophilic synthesis 6-[^{18}F]FDA and to obtain a significantly higher SA than that previously achieved with electrophilic productions of 6-[^{18}F]FDA (Chaly 1993, Goldstein 1993, Namavari 1995, Chirakal 1996). Few chemical side products were formed, due to the simplicity of the trimethylstannyl precursor **36.1** (see Figure 36). However, as a result of unselective labelling, a considerable amount of a side-product was formed, the yield of which was on average 29 ± 7% of the amount of 6-[^{18}F]FDA. This side-product was tentatively assigned as 2-[^{18}F]FDA (**39.3**, see figure 39). The presence and formation of 5-[^{18}F]FDA regioisomer, possibly co-eluting with 2-[^{18}F]FDA in our chromatographic system, is also possible and cannot be excluded. However, both the radiochemical side-products and the major nonradioactive chemical side product dopamine were efficiently separated from 6-[^{18}F]FDA using semi-preparative reversed-phase HPLC purification

with ethanolic saline as the mobile phase. Since the mobile phase was suitable for intravenous administration, the HPLC fraction could be sterilised and formulated for intravenous injection via a simple membrane filtration.

The radiochemical yield of 6-[^{18}F]FDA (as calculated from the initial amount of [^{18}F]F$^-$) was low mainly for the following reasons. Firstly, a large amount of [^{18}F]F$^-$ at EOB was required to obtain a sufficient amount of high SA [^{18}F]F_2 (the labelling precursor) and subsequently a reasonable amount of end product. Secondly, in order to obtain 6-[^{18}F]FDA with increased SA, a low amount of carrier-F_2 had to be used, which inevitably decreased the radiochemical yield of the labelling precursor. Thirdly, as a result of unselective labelling, the formation of the side-product, probably 2-[^{18}F]FDA, was the principal factor decreasing the yield. Based on this observation, one would predict that the selectivity of the electrophilic labelling to the 6-position should be increased by using an alternate precursor that contains functional groups which promote the electrophilic attack to 6-position more efficiently.

A nucleophilic method to produce 6-[^{18}F]FDA has been reported by Ding *et al.*; their method afforded 6-[^{18}F]FDA with relatively high SA (up to ~100 GBq/μmol at EOS) and with adequate RCY (20%), albeit several reaction steps were required to create the molecule (Ding 1991). In the previously reported electrophilic syntheses of 6-[^{18}F]FDA, the highest SA achieved has been ~0.4 GBq/μmol at EOS (Chaly 1993, Goldstein 1993, Namavari 1995, Chirakal 1996). By using the "post-target" method for [^{18}F]F_2 production, it was intended to synthesise 6-[^{18}F]FDA with moderately high SA, on the order of 15 GBq/μmol at EOS. The SA range was 10.0–18.8 GBq/μmol, by far the highest value so far reported for 6-[^{18}F]FDA using electrophilic labelling. The amount of cold 6-fluorodopamine, with typical 185 MBq PET-tracer administration, would have been 1.7–3.2 μg, accordingly. A therapeutic dose of dopamine is 2–10 μg/kg/min. The SA obtained in these present experiments can thus be considered as adequate to perform human PET studies at trace levels.

6.4. Synthesis of [^{18}F]EF5 (IV)

[^{18}F]EF5 **37.3** is an example of a molecule which has so far proved impossible to produce via nucleophilic fluorination; neither Br-to-^{18}F exchange nor isotopic exchange of any of the fluorine atoms in authentic EF5 have proved successful. Thus,

electrophilic labelling remained as the only choice, and the electrophilic addition of $[^{18}F]F_2$ gas to the double bond of the trifluoroallyl acetamide precursor **37.1** was demonstrated to be quite suitable (Dolbier 2001, Dolbier 2006). By performing the labelling reaction in a highly acidic medium, the electron density of the nitroimidazole ring was reduced via protonation, and the trifluoroallyl moiety became more susceptible towards electrophilic attack. Dolbier et al. used "in target produced" $[^{18}F]F_2$ gas with a large amount of carrier-F_2 (Dolbier 2001). Due to this large amount of carrier, it is difficult to control the high and unselective reactivity of F_2, the chemical manipulations become more difficult and the specific radioactivity of the end product is inevitably low.

By using the "post-target" method to produce $[^{18}F]F_2$ (Bergman 1997), it was intended to synthesise $[^{18}F]$EF5 with moderately high SA, whilst maintaining a high radiochemical yield. A 200-fold increase in SA, as compared to previous reports, was obtained with the present method making it possible to decrease the injected amount of non-radioactive EF5 significantly. The radiolabelling procedure was simplified from that reported by Dolbier et al. Smaller amounts of reagents were used, in particular the trifluoroallyl precursor **37.1** (1 mg in our study versus 25 mg used by Dolbier) and TFA (0.7 ml versus 5 ml by Dolbier). Bubbling the $[^{18}F]F_2$ gas through the precursor solution was completed within 30 seconds, after which removal of TFA was achieved in approximately 10 minutes. A considerable amount of volatile ^{18}F-labelled compounds was distilled from the reaction vessel during the TFA removal. A rather recent report has described a procedure where the somewhat laborious TFA-removal step could be accomplished with an alternative method (Chitneni 2012); the TFA reaction mixture was at first partially neutralized and then passed through a solid-phase cartridge prior to the HPLC purification; a less complex mixture for semi-preparative HPLC purification was thus obtained. Replacement of the evaporation step with solid-phase extraction also makes the overall synthetic process easier to automate (Chitneni 2012).

The large number of radiofluorinated side-product emphasises the high and uncontrollable reactivity of $[^{18}F]F_2$, even though a fairly simple molecule, such as precursor **37.1**, was radiolabelled. More than ten chemical and radiochemical side-products were generated during the labelling. To obtain sufficient radiopharmaceutical quality, the development of a gradient HPLC purification method was mandatory. The major chemical impurity after the labelling was the unreacted precursor **37.1**. The major

radiolabelled side-products eluted after [^{18}F]EF5, indicating that these products were more lipophilic than [^{18}F]EF5; these products are postulated to be nitroimidazole ring fluorinated products or compounds formed through radical polymerization. The amount of radiolabelled side-products also decreased the radiochemical yield to approximately 3% (decay corrected and calculated from initial ^{18}F-radioactivity). The amount of purified [^{18}F]EF5 produced with the present method was, however, sufficient for at least two consecutive human PET studies from a single batch.

7. CONCLUSIONS

The major conclusions of the work presented in this thesis are:

- Post-target produced [^{18}F]F_2 is a suitable fluorination reagent for achieving electrophilic substitution of a trimethylstannyl group attached to an aromatic ring; three radiopharmaceuticals were produced via aromatic electrophilic fluorodestannylation and the descending order of the ^{18}F-fluorination efficiency was 4-[^{18}F]FMR > 6-[^{18}F]FDA > [^{18}F]F5P. The selectivity of ^{18}F-incorporation was the main reason for lowered efficiency. However, these three radiopharmaceuticals were produced with moderately high SA, a result not achievable with "in-target" produced [^{18}F]F_2.

- Selective ^{18}F-incorporation to the multi-aromatic precursor was poor; many side-products were formed resulting in a low radiochemical yield. Synthesis of [^{18}F]F5P was not efficient.

- The catecholamine analogues 4-[^{18}F]FMR and 6-[^{18}F]FDA were obtained with moderate efficiency. In [^{18}F]fluorometaraminol synthesis, 4-[^{18}F]FMR was the main radiofluorinated product, although some side-products, probably radiofluorinated regioisomers of 4-[^{18}F]FMR, were generated. Similarly in [^{18}F]fluorodopamine synthesis, 6-[^{18}F]FDA was the main radiofluorinated product, but the selectivity was not optimal; 2-[^{18}F]FDA was produced in considerable amounts as a side-product. Nonetheless for both 4-[^{18}F]FMR and 6-[^{18}F]FDA, the SA and the radiochemical yield were high enough to permit preclinical applications.

- Post-target produced [^{18}F]F_2 is a suitable fluorination reagent for use in electrophilic addition reactions. [^{18}F]EF5 was produced through electrophilic addition of [^{18}F]F_2 to a double bond with moderate efficiency. [^{18}F]EF5 was the main fluorinated product but many side-products were formed through competing substitution reactions. The SA and radiochemical yield were high enough for preclinical and clinical applications.

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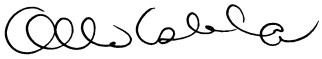

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