Radiosynthesis of [^{18}F]fluorophenyl-L-amino acids by isotopic exchange on carbonyl-activated precursors

Inaugural Dissertation

zur
Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Universität zu Köln

vorgelegt von

Johnny Castillo Meleán

aus Caracas, Venezuela

Berichterstatter:	Prof. Dr. H. H. Coenen Prof. Dr. A. Griesbeck
Tag der letzten mündlichen Prüfung:	01.02.2011

Die vorliegende Arbeit wurde in der Zeit von April 2008 bis Dezember 2010 am Institut für Nuklearchemie (INM-5) der Forschungszentrum Julich GmbH unter der Anleitung von Herrn Prof. Dr. H. H. Coenen (Lehrstuhl für Nuklearchemie der Universitat zu Köln) durchgeführt.

KURZZUSAMMENFASSUNG

Aromatische [^{18}F]Fluoraminosäuren sind als vielversprechende Radiodiagnostika für die Positronen-Emissions-Tomographie entwickelt worden. Eine breitere Anwendung dieser radiofluorierten Verbindungen ist jedoch aufgrund der bisher notwendigen aufwändigen Radiochemie eingeschränkt. In dieser Arbeit wurde eine vereinfachte, dreistufige Radiosynthese von 2-[^{18}F]Fluor-L-phenylalanin (2-[^{18}F]Fphe), 2-[^{18}F]Fluor-L-tyrosin (2-[^{18}F]Ftyr), 6-[^{18}F]Fluor-L-*m*-tyrosin (6-[^{18}F]Fmtyr) und 6-[^{18}F]Fluor-L-DOPA (6-[^{18}F]FDOPA) entwickelt. Dazu wurden entsprechende Vorläufer durch einen nukleophilen Isotopenaustausch ^{18}F-fluoriert, die entweder durch Entfernen der aktivierenden Formylgruppe mit $Rh(PPh_3)_3Cl$ oder durch deren Umwandlung mittels Baeyer-Villiger Oxidation und anschließend durch saure Hydrolyse in die entsprechenden aromatischen [^{18}F]Fluoraminosäuren überführt wurden.

Zwei effiziente synthetische Ansätze wurden für die Synthese von hoch funktionalisierten Fluorbenzaldehyden und -ketonen entwickelt, die als Vorläufer benutzt wurden. Die Verbindungen (2*S*,5*S*)-*tert*-Butyl-2-*tert*-butyl-5-(2-fluor-5-formylbenzyl)-3-methyl-4-oxoimidazolidin-1-carboxylat (**1a**), (2*S*, 5*S*)-*tert*-Butyl-5-(5-acetyl-2-fluorbenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidin-1-carboxylat (**1c**), (2*S*,5*S*)-Benzyl-2-*tert*-butyl-5-(2-fluor-5-formylbenzyl)-3-methyl-4-oxoimidazolidin-1-carboxylat (**1d**), 4-Fluor-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)me-thyl)benzaldehyd (**1e**) und 1-(4-Fluor-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)phenyl)-ethanon (**1f**) konnten in sechs Schritten und in einer Gesamtausbeute von 41%, 48%, 37%, 27% und 32% hergestellt werden. (2*S*,5*S*)-*tert*-Butyl-5-(4-(benzyloxy)-2-fluor-5-formylbenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidin-1-carboxylat (**1b**) wurde in zehn Schritten mit einem Gesamtausbeute von 19% synthetisiert, während die Verbindungen (2*S*,5*S*)-*tert*-Butyl-5-(5-(3,5-bis(trifluormethyl)-benzoyl)-2-fluorbenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidin-1-carboxylat (**1g**) und (2*S*,5*S*)-*tert*-butyl-*tert*-butyl-5-(2-fluor-5-(2,2,2-trifluoracetyl)benzyl)-3-methyl-4-oxoimidazolidin-1-carboxylat (**1h**) durch ein neues dreistufiges Verfahren in Ausbeuten von 54 % und 40 % erhalten wurden. Alle Verbindungen wiesen eine hohe Diasteromerenreinheit von > 99% auf.

Die Vorläufer **1a**, **1d** und **1e** wurden unter Anwendung einer Decarbonylierungsreaktion für die Synthese von 2-[^{18}F]Fphe eingesetzt, während **1b** für die von 2-[^{18}F]Ftyr verwendet wurde. Die Radiosynthesen konnten entweder unter konventioneller Erhitzung oder über Mikrowellenheizung durchgeführt werden. Konventionelle Heizung erbrachte die gewünschten Produkte 2-[^{18}F]Fphe und 2-[^{18}F]Ftyr in radiochemischen Ausbeuten (RCA) von 43% und in 49%, während RCA von 34% und 43% über Mikrowellenheizung erzielt wurden. Mit der letzten Methode konnten jedoch 38 min der Gesamtsyntheseszeit gespart und somit vergleichbare Produktaktivitäten erhalten werden. Der Enantiomerenüberschuß für 2-[^{18}F]Fphe war 88%, während im Fall von 2-[^{18}F]Ftyr 92% erreicht wurden.

6-[^{18}F]Fmtyr wurde aus dem Seebach-Vorläufer **1c** in 13 % Gesamt-RCA mit hoher Enantiomerenreinheit von > 93% gewonnen. Eine vergleichbare Gesamt-RCA von 11 % an 6-[^{18}F]Fmtyr wurde mit Schöllkopf-Vorläufer **1f** erzielt, während die Enantiomerenreinheit in diesem Fall nur bei 87 % lag. Über den ^{18}F-für-^{19}F Austausch an den Vorläufern **1g** und **1h** konnten relativ hohe RCA erzielt werden, hingegen nur niedrigere bei der Baeyer-Villiger Oxidation. So wurde 6-[^{18}F]Fmtyr ausgehend von **1g** und von **1h** mit einer Gesamt-RCA von nur 6% und 13% gewonnen. Die Enantiomerenreinheit des Endprodukts unter Verwendung beider Vorläufer war jedoch > 98%.

Die auf einer früheren Arbeit basierende nukleophile Radiosynthese von 6-[^{18}F]FDOPA mittels Isotopenaustausch konnte durch Anpassung vieler Parameter weiter optimiert werden, so dass ca. 40% RCA bei einer hohen Enantiomerenreinheit von > 96% erzielt werden konnte. Die spezifische Aktivität der hier unter Entwicklungsbedingungen dargestellten [^{18}F]Fluoraminosäuren war mit der durch elektrophile Methoden erreichten vergleichbar. Außerdem konnte in ersten Untersuchungenden demonstriert werden, dass die Automatisierung der dreistufigen Radiosynthese grundsätzlich durchführbar ist, jedoch weitere technische Entwicklungen erforderlich sind.

ABSTRACT

Aromatic [^{18}F]fluoroamino acids have earlier been developed as promising probes for diagnostics using PET. However, a wider use of these radiofluorinated compounds has been limited due to radiosynthetic constraints. The work here presents an amenable three-step radiosynthesis pathway for the preparation of 2-[^{18}F]fluoro-L-phenylalanine (2-[^{18}F]Fphe), 2-[^{18}F]fluoro-L-tyrosine (2-[^{18}F]Ftyr), 6-[^{18}F]fluoro-L-*m*-tyrosine (6-[^{18}F]Fmtyr) and 6-[^{18}F]fluoro-L-DOPA (6-[^{18}F]FDOPA). For this, corresponding precursors were ^{18}F-fluorinated by nucleophilic isotopic exchange, followed by either removal of an activating formyl group with $Rh(PPh_3)_3Cl$ or its conversion by Baeyer-Villiger oxidation, respectively, and subsequent hydrolysis of protecting groups in acidic medium.

Two efficient synthetic approaches were developed for the preparation of highly functionalized fluoro-benzaldehydes and -ketones which were used as labeling precursors. The compounds (2*S*,5*S*)-*tert*-butyl 2-*tert*-butyl-5-(2-fluoro-5-formylbenzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (**1a**), (2*S*,5*S*)-*tert*-butyl 5-(5-acetyl-2-fluorobenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (**1c**), (2*S*,5*S*)-benzyl 2-*tert*-butyl-5-(2-fluoro-5-formylbenzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (**1d**), 4-fluoro-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)me-thyl)benzaldehyde (**1e**) and 1-(4-fluoro-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)phenyl)ethanone (**1f**), could be prepared in six steps and overall yields of 41%, 48%, 37%, 27%, and 32%, respectively. (2*S*,5*S*)-*tert*-Butyl 5-(4-(benzyloxy)-2-fluoro-5-formylbenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (**1b**) was prepared in ten steps with an overall yield of 19% while compounds (2*S*,5*S*)-*tert*-butyl 5-(5-(3,5-bis(trifluoromethyl)-benzoyl)-2-fluorobenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (**1g**) and (2*S*,5*S*)-*tert*-butyl 2-*tert*-butyl-5-(2-fluoro-5-(2,2,2-trifluoroacetyl)benzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (**1h**) were synthesized by a novel three-step procedure in 54% and 40%, respectively. All compounds were obtained with high diasteromeric purity of > 99%.

Corresponding precursors **1a**, **1d**, and **1e** were used for the radiosynthesis of 2-[^{18}F]Fphe while **1b** was employed for 2-[^{18}F]Ftyr making use of a decarbonylation reaction. The radiosyntheses were performed either under conventional or microwave heating. The conventional heated reactions yielded the desired products 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr in 43% and 49% whereas 34% and 43% RCY, respectively, were obtained when microwave heating was applied. However, 38 min of total preparation time were saved with the latter method, thus providing similar amounts of product activity. The enantiomeric excess achieved for 2-[^{18}F]Fphe was 88% while in the case of 2-[^{18}F]Ftyr 92% was obtained.

6-[^{18}F]Fmtyr was prepared from the Seebach-precursor **1c** in 13% overall RCY with a high enantiomeric purity of > 93%. A comparable overall RCY of 11% of 6-[^{18}F]Fmtyr was achieved with the Schöllkopf-precursor **1f** while the enantiomeric purity in this case was only 87%. Precursors **1g** and **1h** showed a relative high RCY of the ^{18}F-for-^{19}F substitution, but a low one in the Baeyer-Villiger oxidation. Thus from **1g** and **1h**, 6-[^{18}F]Fmtyr was obtained with an overall RCY of only 6% and 13%, respectively. However, the enantiomeric purity of the product using both precursors was > 98%.

Based on earlier attempts the nucleophilic radiosynthesis of 6-[^{18}F]FDOPA by isotopic exchange could also be optimized by changing many parameters from the previous work providing ca. 40% RCY and a high enantiomeric purity of > 96%. The specific activity of the tracers prepared here under developmental conditions was as high as that achieved by electrophilic methods. Furthermore, in preliminary studies it could be demonstrated that the automation of the three-step radiochemical synthesis developed here is principally feasible but requires further technical maturation.

CONTENTS

1 INTRODUCTION

1.1 Basic aspects of positron emission tomography

Positron emission tomography (PET) is a non-invasive molecular imaging technique that has the ability to monitor physiologic processes in living beings. PET relies on the use of exogenous radioactive probes (radiotracers) which emit a detectable photon signal. Imaging techniques such as magnetic resonance imaging (MRI), X-rays, or ultrasound (US) provide valuable information on anatomy (namely, structural imaging), but give limited or no information at all on metabolic or molecular events. Therefore, for the *in vivo* detection of diseases in patients, these imaging methods are restricted to those malfunctions associated with structural abnormalities.

A positron emitter is an unstable atom bearing an excess of protons in its nucleus. The nucleus gets stabilized by the conversion of one proton in a neutron under emission of a positron and a neutrino (Scheme 1). The emitted positron (β^+) is slowed down in the tissue by collisions. After thermalization at the end of the track a positronium, a hydrogen-like atom, is formed by the positron and an electron. The positron and electron are antiparticles, so they annihilate each other. In the annihilation process, two γ photons of 511 keV are generated (annihilation radiation), these photons travel in opposite direction forming 180° angle. Contrary to the β^+-emission, and because of their penetration power, these two γ photons can travel outside the body where scintillation crystals can detect them. If two photons arrive at opposite detectors within a certain coincidence time, they will be registered as a valid event and the position where the annihilation took place can be located on the line between the detectors (Figure 1). This is one major advantage of PET because noise from incidental radiation is minimized. The spatial resolution of the images is high since it is just limited by the distance the positron needs to become thermalized. This is only few millimeters for suitable positron emitters.[1]

$$^{18}F \longrightarrow {}^{18}O + \beta^+ + \upsilon$$

Scheme 1: Decay of fluorine-18 by positron emission

Uncertainties based on adsorption and scattering radiation effects can be avoided by applying an attenuation correction, which is developed by an accurate independent transmission measurement. For this purpose a ^{68}Ge source is generally used before the real

tracer is injected. By this procedure, the distribution of radioactivity in the object tissues can be precisely quantified,[2] which is the most important advantage of this methodology and justifies the application of corpuscular emitters in the living body.

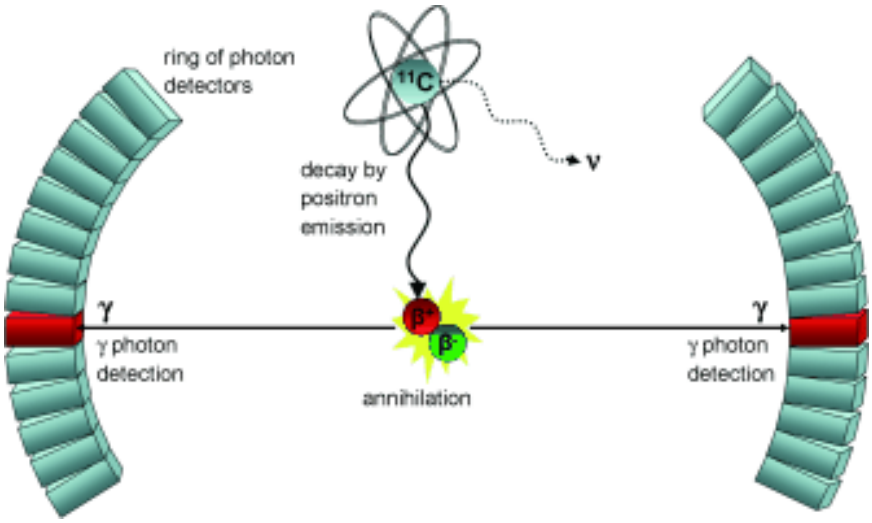


Figure 1: Schematic representation of the principle of PET. [3]

In order to gain quantitative medicinal relevant information from the distribution of the radioisotope in the body, the temporal and local change of the radiotracer has to be combined with a suitable bio-mathematical compartment model describing the pharmacokinetic behavior of the pharmaceutical.[4] In a typical molecular assay, a positron-labeled probe is injected intravenously, and PET scans provide measures of the tissue concentration of the probe and labeled products over time. These data are combined with a measure of the time course of the plasma probe concentration, representing its delivery to tissue, and processed with a compartmental model containing equations describing the temporal change in the individual compartments, transport and reaction processes the probe undergoes. The result is an image of the rate of the process under study.

Since some of the positron-emitting radionuclides are elements found in biomolecules (for example, C, N, and O) it is possible to label compounds of interest authentically, *i.e.* without a change of composition or structure interfering with their biological activity. Table 1 lists the most commonly used short-lived positron emitters, typically used nuclear reactions for its production, and its main physical-chemistry characteristics.[3] It can be noticed that the half-lives are quite small which is also an additional advantage since the patients are exposed to relatively low radiation doses and studies can be repeated in short time intervals if needed.

PET radionuclides can be produced with the help of a particle accelerator, generally a cyclotron. Radionuclides generated by this way show in general a high specific activity if stable material of the radionuclide produced is excluded. The term specific activity refers to the ratio of the amount of activity per mass of material. High specific activities allow to work with samples whose mass is so small that it can be neglected. Because of that it is possible to evaluate a biological process without disturbing it. Furthermore, toxicological and/or immunological effects are not an issue.

Table 1: Commonly used radionuclides in PET, their half-lives, production reactions, target materials, products and decay products[3]

Radionuclide	$T_{1/2}$ (min)	Nucl. Reaction	Target	Product	Decay product
^{11}C	20.4	$^{14}N(p,\alpha)^{11}C$	$N_2(+O_2)$	$[^{11}C]CO_2$	^{11}B
			$N_2(+H_2)$	$[^{11}C]CH_4$	
^{13}N	9.97	$^{16}O(p,\alpha)^{13}N$	H_2O	$[^{13}N]NO_x$	^{13}C
			H_2O + EtOH	$[^{13}N]NH_3$	
^{15}O	2.04	$^{15}N(d,n)^{15}O$	$N_2(+O_2)$	$[^{15}O]O_2$	^{15}N
^{18}F	110	$^{20}Ne(d,\alpha)^{18}F$	$Ne(+F_2)$	$[^{18}F]F_2$	^{18}O
		$^{18}O(p,n)^{18}F$	$[^{18}O]H_2O$	$[^{18}F]F^-$	
			$[^{18}O]O_2(+F_2)$	$[^{18}F]F_2$	

The short half-live of the above mentioned radionuclides requires the development of rapid synthetic methods for introducing them into the molecule of interest. The labeled compound has to be synthesized, purified, analyzed, and formulated usually within minutes. A timescale of roughly three isotope half-lives is loosely the limit for the total synthesis time allowed in order to obtain enough radiolabeled material for administration to a person undergoing the PET scan. Radiotracers should be designed in such a way that they arrive unchanged at the place of interest and provide a detailed picture of the targeted molecular structure or biological processes under study. Probes are synthesized with very low mass so as not to exert mass effects on the biological processes measured. Common tissue concentrations of PET probes are in the range of pico- to femtomoles per gram.[5] A number of modern PET facilities house cyclotrons (for radioisotope production), radiosynthetic laboratories, and PET scanners under one roof to allow efficient production and provision of short-lived PET probes to the scanner.

A further development in scanner technology has been the integration of PET with anatomical imaging techniques such as CT and MRI into one device. The combined PET/CT and PET/MRI techniques allow matching of functional information on the PET image to the detailed anatomical images from CT or MRI scans (Figure 2).

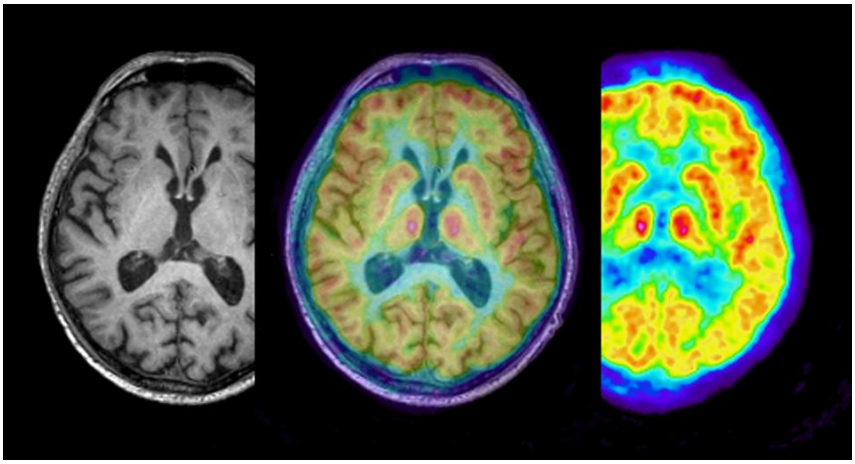


Figure 2: Images of the brain obtained using magnetic resonance tomography (left) and positron emission tomography (right) upon injection of [^{18}F]FDG which are combined in a fusion picture (middle). Image: Forschungszentrum Jülich[6]

PET has been widely used in oncology[7,8] for the diagnosis of tumors by looking at the accumulation and metabolism of certain PET probes within the tumor. In the field of cardiology,[9,10,11] PET has been developed as a clinical tool for myocardial perfusion imaging as a means of characterizing and diagnosing coronary heart disease. In neurology,[12,13,14] have been employed for the characterization of early stages of neurological disorders (such as Alzheimer's and Parkinson's disease), abnormal neurotransmitter activity, movement disorders, stroke, epilepsy, and neurooncology. It is expected that PET -as an in vivo pharmacological imaging tool- will play an increasingly important role in drug development.[15,16,17] It is anticipated that PET studies will improve the selection of potential drug candidates at an earlier stage of development, give a proof of principle or a greater understanding of a drugs mechanism of action, and an aid in guiding dose selection.[3]

1.2 ^{18}F-Radiofluorination of organic molecules

Fluorine-18 is the most often used radionuclide for diagnostics with PET. The relatively long half-life of ^{18}F ($T_{1/2}$ = 109.8 min) possess less constraints on synthesis time and permits longer imaging protocols to investigate processes of slower tracer kinetics up to about 6 h.[18] The low kinetic energy of the positron particle emitted by the ^{18}F during nuclear decay, with a

maximum energy of 650 keV (mean energy of 250 keV), and a maximum range in water of 2.4 mm (mean range 0.3 mm) prior to annihilation, translates into a high spatial image resolution.[19] The relatively long half-life of fluorine-18 also permits the distribution of ^{18}F-radiopharmaceuticals to clinical services located within a few hours of transport.

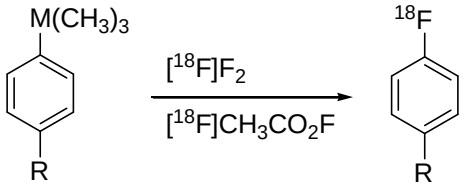
Most of the established synthetic methods for radiofluorination of organic molecules follow the general concepts of fluoro-organic chemistry like electrophilic and nucleophilic substitution. The short half-live, non-equimolar reactions with ^{18}F-reagents in the nanomolar range, and the limited availability of those reagents on a no-carrier-added (n.c.a.) scale, often limit labeling possibilities and require special techniques like labeling through secondary compounds. In addition, recently even an enzymatic fluorination method has been reported in order to achieve fast and specific labeling.[20]

1.2.1 Electrophilic fluorination

Direct electrophilic substitution is a strategy that has been established for the labeling of compounds with unsaturated bonds. The most common chemical form of the radioactive starting material is $[^{18}F]F_2$. Nowadays, $[^{18}F]F_2$ is generally produced in two steps by the $^{18}O(n,p)^{18}F$ nuclear reaction on $^{18}O_2$ in gaseous form followed by the addition of carrier F_2.[21] The addition of carrier leads to a low specific activity of the labeled products, furthermore, the maximum radiochemical yield (RCY) that can theoretically be obtained is 50% since only one atom of $[^{18}F]F_2$ reacts with the labeling precursor. Due to the high reactivity of molecular fluoride, reactions should be performed at low temperatures in order to reduce the formation of undesired side reactions. Other electrophilic reagents formed from $[^{18}F]F_2$ with a somewhat lower reactivity, like $[^{18}F]CH_3COOF$ and $[^{18}F]XeF_2$, have been also employed. Moreover, the electrophilic pathway is limited to relatively low amounts of radioactive product at elevated cost. Furthermore, the methodology for the generation of electrophilic radiofluorination species is not established in every PET centre.

In order increase the regioselectivity in arenes, demetallation reactions of organometallic precursors were introduced. Suitable organometallic precursors are aryltrimethyltin, aryltrimethylgermanium and aryltrimethylsilicon compounds.[22,23] Experiments performed on a series of *p*-substituted phenyl derivatives (CH_3O-, CH_3-, H-, F-, Br-, CF_3-, O_2N-), showed a decrease in the fluorodemetallation yield in the order Sn > Ge > Si, and with rings containing electron-withdrawing groups (Scheme 2). It was also observed that the only side-products which were formed from the corresponding metallated anisyl- and toluyl-compounds were 2-

fluoroanisole (< 16%) and benzylfluoride (< 5.5%). Moreover, no direct hydrogen substitution was observed with the other substrates.



R= OCH_3, CH_3, H, F, Br, CF_3, NO_2
M= Si, Ge, Sn

Scheme 2: Electrophilic radiosynthesis of [^{18}F]fluoroarenes *via* [^{18}F]fluorodemetallation reactions[23]

1.2.2 Nucleophilic substitution

Nucleophilic substitution with [^{18}F]fluoride is considered the most attractive method for the synthesis of ^{18}F-labeled radiopharmaceuticals, since [^{18}F]fluoride is rather easily available with both, high activity and specific activity through the ^{18}O(p,n)^{18}F reaction on ^{18}O-enriched water using a cyclotron. The [^{18}F]fluoride obtained in aqueous solution is poorly reactive due to high hydratation, for this reason, further treatment of the radioactive solution is necessary in order to enhance its reactivity.

The first step consist on the separation of the [^{18}F]fluoride from the relatively expensive ^{18}O-enriched water and solubilization in an organic solvent. This is generally achieved by adsorption of [^{18}F]fluoride onto an ion exchange resin allowing the recovery of the ^{18}O-enriched water, followed by elution of the radioactive species with a small volume of an aqueous weak base. The water is then removed by azeotropic evaporation with acetonitrile. Alternatively the [^{18}F]fluoride can electrochemically be isolated from the ^{18}O-enriched target water on an electrode in an electrochemical cell.[24] Rinsing of the electrode with a dry organic solvent is enough for drying and the [^{18}F]fluoride is then released into an aprotic reaction medium by inversion of the voltage in the cell.

Although alkali metal fluorides have traditionally been used for nucleophilic substitution, fluorination with these reagents is known to proceed only with strong activation and under vigorous conditions due to their limited solubility in organic solvents and low nucleophilicity. As an alternative, a "*naked*" fluoride ion, which is not solvated by bulky cations or solvent molecules, is usually used to improve these reactions. This is achieved using a phase transfer

catalyst (PTC) as anion activator in dipolar aprotic solvents. The most commonly employed anion activator system is the combination of the aminopolyether Kryptofix 2.2.2 as PTC and potassium carbonate as base. This system was originally developed for the radiosynthesis of 17-[^{18}F]fluoroheptadecanoic acid[25] and then directly transferred with great success to the radiosynthesis of 2-[^{18}F]fluorodeoxyglucose (FDG).[26] For base sensitive molecules tetrabutylammonium hydrogencarbonate or Kryptofix 2.2.2 in combination with a potassium oxalate/carbonate mixture have effectively been used.[27,28,29] The basic features of the principal radiofluorination methods have been already reviewed.[30]

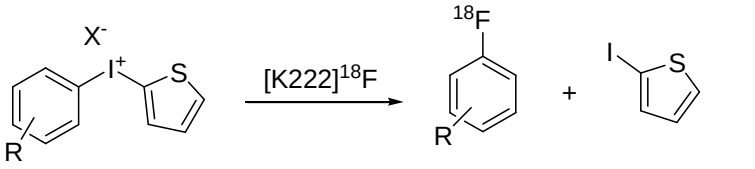
Radiofluorination by substitution can be performed both on aliphatic (S_N2) and aromatic compounds (S_NAr). The typical method for introducing [^{18}F]fluoride at a specific site of an aliphatic molecule is the nucleophilic displacement of the corresponding halides (Br, I) and sulphonyl esters such as triflate, mesylate, tosylate or nosylate. Typical media for ^{18}F-fluorination involve dipolar aprotic solvents, with acetonitrile the most used one, at temperatures around 80 °C. The basic character of the reaction mixture can play a role in possible competitive elimination reactions. Recently, Kim *et al.*[31] demonstrated the use of protic solvents with a very low pK_b value, such as tertiary alcohols, for the routine production of ^{18}F-tracers via nucleophilic reactions.

On the other hand, common leaving groups for S_NAr are F, NO_2 and NMe_3^+, with a pre-requisite of activation of the arene by electron withdrawing groups at the *ortho* or *para* position to the leaving group, such as carbonyl, CF_3, NO_2 or cyanide. Aromatic nucleophilic ^{18}F-fluorination reactions require higher temperatures than with aliphatic compounds. Therefore, solvents with higher boiling points like dimethylformamide (DMF) and dimethylsulfoxide (DMSO) are preferred. The application of this labeling methodology for the ^{18}F-fluorination of complex molecules has recently been reviewed by Ermert and Coenen.[32]

As mentioned above, synthetic methods for the preparation of [^{18}F]fluoroaromatic compounds rely on the use of activated aryl groups. This, however, limits the range of [^{18}F]fluoroaromatic compounds available from simple one-step methods to those arenes which bare electron-withdrawing substituents. Direct nucleophilic ^{18}F-fluorination of electron rich arenes for producing n.c.a. radiopharmaceuticals requires the generation of an electrophilic center.[33] The use of iodonium salts is an extremely useful alternative to achieve this goal even with electron-donating substituents in good RCY. The first use of iodonium

salts as a general route for the n.c.a. synthesis of non-activated [^{18}F]fluoroaromatic compounds with high specific activity was reported by Pike and Aigbirhio.[34]

The regioselectivity of this reaction was found to be controlled electronically as well as sterically *e.g*. by bulky *ortho* substituents (*ortho* effect). The *ortho* effect can be a dominant factor for the site of the preferential nucleophilic attack where the electron-rich ring is fluorinated. *Ortho*-substituted aryl fluorides can be selectively produced by using unsymmetric diaryl iodonium salts. The use of heteroaromatic iodonium salts containing the electron-rich 2-thienyl ring has been reported as efficient for completely directing the nucleophilic ^{18}F-substitution to the less electron-rich aryl ring (Scheme 3).[35]



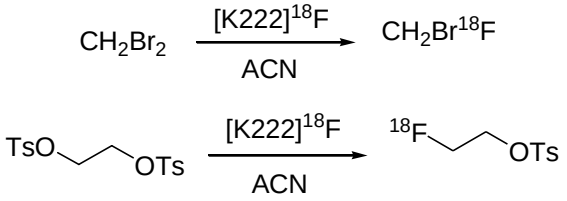
R= 2-OCH_3, 3-OCH_3, 4-OCH_3, 4-CH_3, 4-OBn, H, 4-I, 4-Br, 4-Cl
X= Br, I, OTs, OTf

Scheme 3: Nucleophilic substitution of heteroaromatic aryliodonium salts[35]

1.2.3 Labeling through secondary compounds

Not all fluorine-containing target radiotracers are amenable to direct single-step labeling with [^{18}F]fluoride ion; this is especially true for electron-rich arenes and for macromolecules. A useful strategy for obtaining ^{18}F-labeled tracers is to prepare secondary compound (a labeling agent) from [^{18}F]fluoride and use it for the introduction of fluorine-18 into the target. Such labeling agents may be aliphatic or aromatic and the targets complex molecules with low molecular weight or macromolecules.

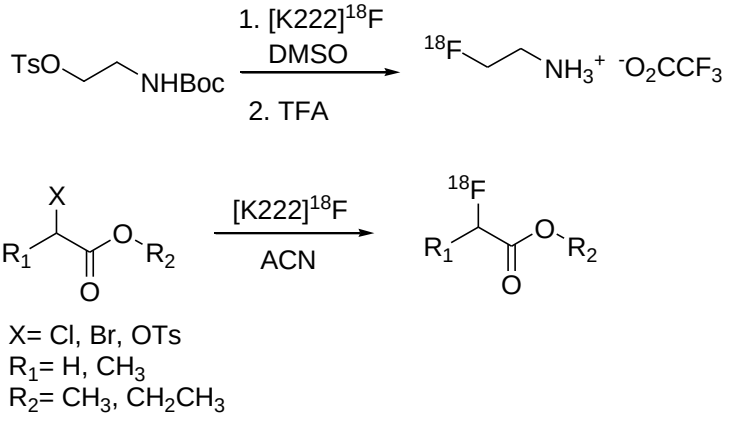
An important class of labeling agents are the functionalized straight chain aliphatic [^{18}F]fluorides, prepared from the reaction of [^{18}F]fluoride ion with α,ω-bifunctional agents (Scheme 4).[36,37,38] The most widely used are the short chain [^{18}F]ω-fluoroalkyl agents, especially [^{18}F]fluoromethyl bromide,[39,40] and 2-[^{18}F]fluoroethyl tosylate (Scheme 4).[41,42]



Scheme 4: Radiosynthesis of [^{18}F]fluoromethyl bromide[39] and 2-[^{18}F]fluoroethyl tosylate[41]

[^{18}F]Fluoromethyl bromide can be prepared from dibromomethane and purified by vapor phase transfer (in carrier nitrogen) through several Sep-Pak columns, before reaction with a precursor to obtain the target tracer by *O*- or *S*-alkylation (*N*-fluoromethyl compounds are generally unstable). The overall RCYs of these processes tend to be low [^{18}F]Fluoromethyl bromide may also be converted into a more reactive labeling agent, [^{18}F]fluoromethyl triflate, by passage over heated silver triflate.[43]

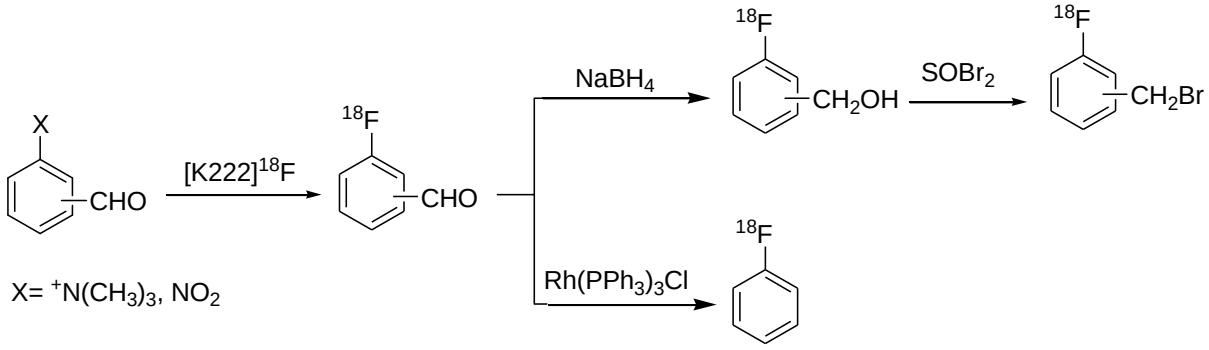
2-[^{18}F]Fluoroethyl tosylate has been successfully applied to the syntheses of several radiotracers. This labeling agent is rather more reactive than the alternative [^{18}F]fluoroethyl bromide and may be produced and used in situ.[44] The [^{18}F]fluoroalkylating agents are generally applied to the labeling of small molecules. In other hand, labeling agents for ^{18}F-fluoroamidation[45] and ^{18}F-fluoroacylation[46] may be prepared similarly and are generally applied to the labeling of peptides and proteins under mild conditions in aqueous solutions (Scheme 5).



Scheme 5: Radiosynthesis of intermediate labeling agents for ^{18}F-fluoroamidation[45] and ^{18}F-fluoroacylation[46]

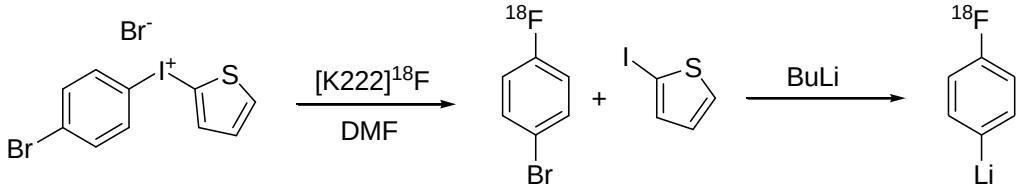
o- or *p*-[^{18}F]Fluorobenzaldehydes may be produced quite efficiently in one step by aromatic nucleophilic substitution in the corresponding benzaldehyde bearing a good leaving

group, such as NO_2 or Me_3N^+.[47,48] These aldehydes are very useful secondary compounds, since they can often be the first step in built-up syntheses of radiotracers. As depicted in Scheme 6, they may also serve as entries into other useful labeling agents such as the corresponding [^{18}F]fluorobenzyl halides, through their reduction and halogenation. Further, reductive decarbonylation leads to the corresponding [^{18}F]fluorophenyl compounds. A review regarding this labeling methodology has recently been published.[49]



Scheme 6: Radiosynthesis of *o*- or *p*-[^{18}F]fluorobenzaldehydes and derivatives as labeling agents[47]

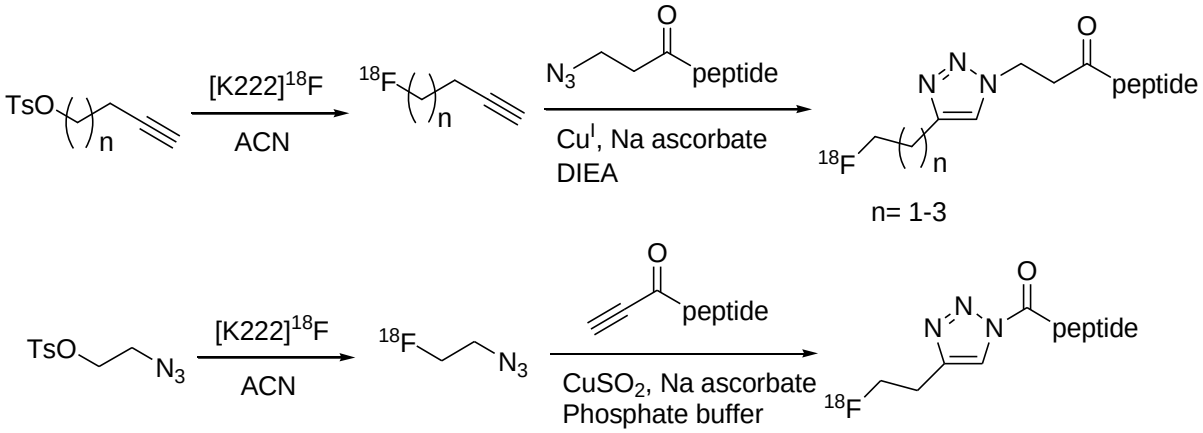
Other [^{18}F]fluoroarenes have also proven very versatile to serve as labeling agents. An example is *p*-[^{18}F]fluorobromobenzene,[50,51] which is *e.g.* efficiently produced by the reaction of bis(*p*-bromophenyl)iodonium bromide or its 2-thienyl analog with [^{18}F]fluoride ion (Scheme 7). This may be further converted into the useful synthon *p*-[^{18}F]fluorophenyllithium.



Scheme 7: Radiosynthesis of *p*-[^{18}F]fluorobromobenzene and *p*-[^{18}F]fluorophenyllithium[51]

"Click" chemistry, which utilizes Huisgen 1,3-dipolar cycloaddition of terminal alkynes and azides to form 1,2,3-triazoles, has recently also been adopted for the preparation of [^{18}F]fluoropeptides (Scheme 8).[52,53] The reaction requires a Cu^I catalyst, proceeds under mild conditions in aqueous media and requires virtually no protection of other functional groups. The use of the non-radioactive component of the reaction in excess compensates for moderate

reactivity. Thus, many labeling reactions can be completed within 20 min in moderate to high radiochemical yields.



Scheme 8: The two alternative onsets for Huisgen´s 1,3-dipolar cycloaddition for the preparation of [^{18}F]fluoropeptides[52,53]

1.2.4 Enzymatic fluorination

Fluorinase enzyme, from the bacterium *Streptomyces cattleya*, is a recent alternative method for the direct introduction of fluorine-18 into organic molecules. Developments by O'Hagan *et al*.[54,55] on the isolation and over-expression of the fluorination enzyme have led to its utilization for highly selective formation of C-F bonds and its application in ^{18}F-radiolabeling. Initial reports using the fluorinase enzyme gave a low RYC of 1%, but provided the proof-of-principle.[20] The introduction of a coupled-enzyme strategy brought a major improvement, with [^{18}F]-5′-fluoro-5′-deoxyadenine ([^{18}F]5′-FDA) being synthesized in 95% RCY within two hours.[56] Although the incubation times for these enzymatic reactions are rather long (2-4 h) rapid evolutionary techniques are expected to improve such enzymes and greatly enhance the reaction rates. The major limitation to this technique is the specificity of the fluorinase enzyme, which restricts its use as a general fluorination method. However, it is possible to label a range of derivatives using additional enzyme coupled systems and hydrolysis reactions. Enzymatic methods of radiolabeled compounds are particularly attractive because of their high chemo-, and enantio-specificity and the formation of few side products, which will simplify purification.

1.3 Amino acids

Amino acids serve many other essential functions in cells that are necessary for life but they mainly represent the building blocks of proteins. For this, they are linked together by peptide bonds to form the basic structure of proteins. However, owing to the many "side groups" that are part of the amino acids other sorts of bonds, *e.g.* disulfide and hydrogen bridges, may form between different amino acid units. These additional bonds twist and turn the protein into convoluted shapes that are unique to the protein and essential to its ability of performing certain functions in living organisms.[57]

The 20 amino acids commonly found in proteins are called "standard amino acids". All of them, except proline, have as common characteristics a free carboxylic acid and a free unsubstituted amino group attached to the α carbon atom. Figure 3 depicts their general structural formula. Due to the chiral center amino acids are D- or L-stereoisomers, except for glycine, which has none, and threonine and isoleucine, which each have two chiral centers. Generally, amino acids found in cellular proteins are L-stereoisomers.

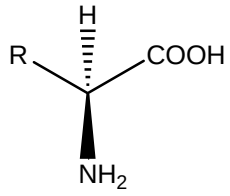


Figure 3: General structure of α-L-amino acids

Various ways of classifying the amino acids have been described, of which the most commonly used is based on the polarity of the R group. This leads to four classes of amino acids:

(1) Non-polar or hydrophobic R group: They have an equal number of amino and carboxyl groups and are neutral. These amino acids are hydrophobic and have no charge on the R group. The amino acids belonging to this group are alanine, valine, leucine, isoleucine, phenylalanine, glycine, tryptophan, methionine and proline.

(2) Neutral, uncharged, polar R group: These amino acids also do not have any charge on the R group but participate in hydrogen bonding of protein structure. The amino acids in this group are serine, threonine, tyrosine, cysteine, glutamine and aspargine.

(3) Positively charged R group: Polar amino acids with positive charge have more amino groups as compared to carboxyl groups making it basic. These are lysine, arginine and histidine.

(4) Negatively charged R groups: Polar amino acids with negative charge have more carboxyl groups than amino groups making them acidic. They are classified as dicarboxylic mono-amino acids. These are aspartic acid and glutamic acid.

1.4 Amino acid transport across the cell membrane

Amino acid transport across the membrane of cells has been extensively described by Pisitti and co-workers.[58] Although all amino acids can diffuse into cells, the main transport of amino acids into cells occurs through carrier mediated processes. The activity of the specific carrier is based on both binding affinity and the capacity of the carrier. The latter can also be expressed as the number of functional carriers per cell. Thus, an increase of amino acid transport into the cell can be due to either an increased affinity or an increased amount of carriers or a combination of both besides to an increased offer, *i.e.* concentration in the blood.

Three principal transport systems account for much of the amino acid uptake by mammalian cells (Figure 4). System A is Na^+ dependent. It transports most neutral amino acids and is often inhibited by the presence of intracellular substrates of this system, *i.e.* the presence of a certain intracellular amino acid inhibits the uptake into the cell of additional similar extracellular amino acids. System ASC is also Na^+ dependent and mainly acts as a carrier for the neutral amino acids, but with this carrier system the presence of neutral amino acids in the interior of the cell leads to stimulation of the transport of more amino acids into it. The affinity of the carrier protein increases once a sodium ion binds to the transporter. Subsequently, the amino acid will bind to the activated carrier forming an amino acid/sodium/co-transporter complex. A conformational change on the structure of this complex delivers both the amino acid and the sodium ion into the cell. The binding of the sodium ion is promoted by the electric potential of the membrane. This gradient is maintained by the glucose-dependent sodium/potassium adenosine triphosphatase ion transporter. Thus transporters A and ASC are energy consuming. Efflux of amino acids is unlikely to occur because of the inwardly directed sodium electrochemical gradient.

Amino acid transport by the sodium-independent transporter L is mainly dependent on the concentrations of the amino acid outside and inside the cell. The direction of the transport

is determined by this gradient. However, an amino acid can be transported against this gradient by a mechanism known as countertransport. This comprises the efflux by system L of another amino acid, whose gradient has been established by one (or more) of the sodium-dependent transport systems. The sodium-independent system L has a specificity for the branched and aromatic non-polar amino acids and uptake is subject to trans-stimulation by the intracellular substrates of this system. In general, the systems A and ASC are responsible for transport of the neutral amino acids, whereas system L transports the aromatic and the branched chain amino acids.

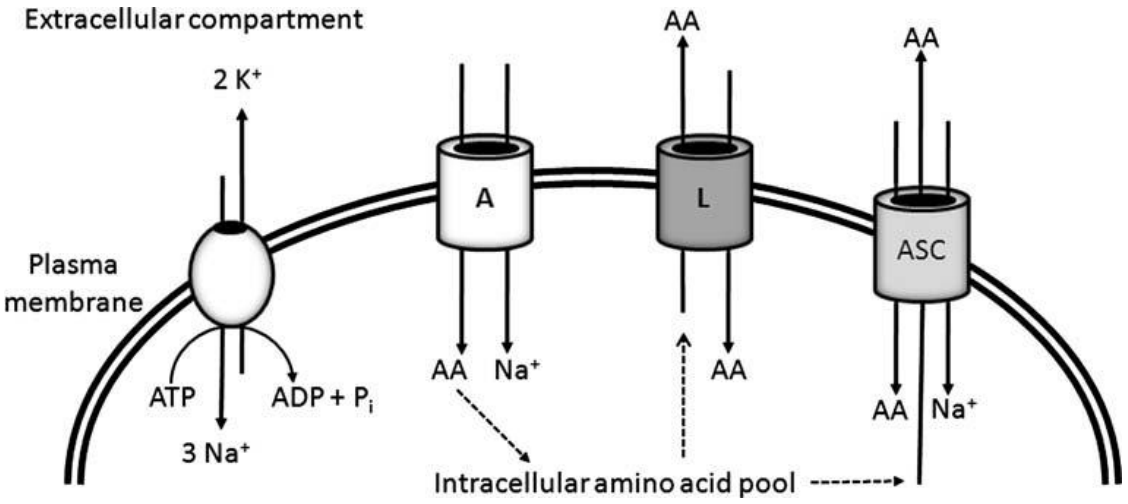


Figure 4: Schematic depiction of major amino acid transporter systems. The system A transporter cotransports one extracellular amino acid (AA) with one Na^+ into the cell. The system L transporter exchanges one AA from the extracellular compartment with one AA from the intracellular compartment and does not require Na^+. The system ASC transporter cotransports one extracellular AA with one Na^+ into the cell while transporting one intracellular AA out of the cell. The intracellular amino acid pool gradient is maintained by active transport by system A as well as further concentrative amino acid transporters. The sodium ion (Na^+) gradient is maintained by the Na^+, K^+-ATPase [59]

1.5 Tumor imaging with radiolabeled amino acids

Visualization of tumors with radiolabeled amino acids is based on surplus accumulation of the radiotracer in the diseased cell. The amino acid uptake is often increased in malignant tissue.[60,61] Tumor cells require amino acids for energy production, protein synthesis and cell duplication. Malignant tumors are characterized by a hypermetabolic state: not only glucose metabolism but also protein synthesis and amino acid transport are often enhanced in cancer

cells. Both A- and L-type amino acid transports have been shown to be up-regulated in tumor cells as compared with normal tissue.[62] Especially analogues of phenylalanine and tyrosine have proven to be useful for tumor imaging.[63]

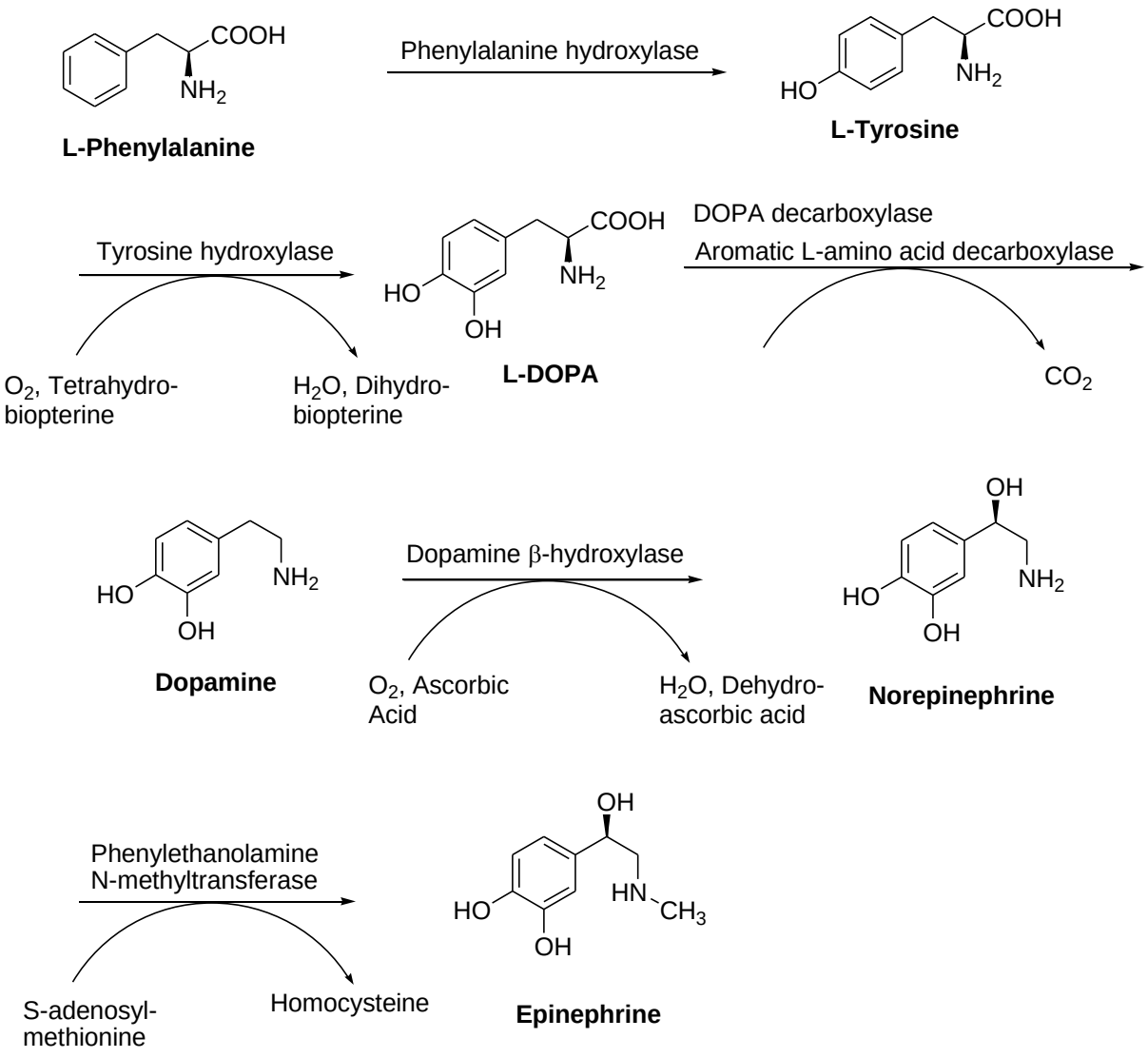
Several studies have described the application of radiolabeled amino acids or analogues for measurement of the protein synthesis rate (PSR) or solely the amino acid transport. For some applications, the use of amino acids analogues represents an advantage as compared to FDG. For instance, amino acids may help in imaging areas in which FDG imaging is limited, such as the brain (because of high background FDG uptake), or in differentiating tumorous from inflammatory lesions (because of high FDG uptake in macrophages, *e.g.*, after radiotherapy).

It has been suggested that amino acid transport may be more important for tumor imaging than the incorporation of amino acids into proteins.[64,65] The fraction of radiolabeled amino acid that is incorporated into proteins during the time of a PET study is small as compared to the total amount that is taken up by the cell. Therefore, it is unlikely that the incorporated fraction substantially contributes to the scintigraphic visualization of the tumor lesion. Consequently non-metabolisable synthetic amino acid analogues for the quantification of the amino acid transport rate have been developed.[64,66,67,68] With these analogues, release of radiolabeled metabolites from the cells back into the circulation does not occur. This enables quantification of the amino acid transport with relatively simple kinetic modeling. Moreover, amino acid transport is a relatively rapid process and, therefore, tumor imaging based on amino acid transport can be performed within 20 min post injection, whereas imaging of the rather slow PSR would require imaging for much extended times post injection. Thus, two kinds of amino acid-based PET tumor imaging can be distinguished: (1) that one based solely on amino acid transport and (2) that one based on amino acid transport and protein incorporation, *i.e.* also measuring the PSR.[69,70]

1.6 Synthesis of catecholamines from aromatic amino acids

Phenylalanine is the starting material for the synthesis of tyrosine in mammals. Conversion of phenylalanine into tyrosine is catalyzed by the enzyme phenylalanine hydroxylase (PH) which introduces a hydroxyl group in the position 4 of the aromatic ring of phenylalanine. Tyrosine again acts as precursor in the biosynthesis of catecholamines (CA). Dihydroxyphenylethylamine (dopamine, DA), norepinephrine (NE), and epinephrine (EP) are synthesized in the adrenal medulla and in adrenergic neurons in the central nervous system.

Biosynthesis of CA involves five enzymatic steps as shown in Scheme 9: phenylalanine is converted by PH in tyrosine; tyrosine hydroxylase (TH) catalyzes the conversion of tyrosine to dihydroxyphenylalanine (DOPA); DOPA is converted to DA by aromatic L-amino acid decarboxylase (AADC); dopamine β-hydroxylase (DBH) catalyzes the enzymatic reaction of DA to NE, and EP is synthesized from NE by phenylethanolamine N-methyltransferase (PNMT).[71]



Scheme 9: Synthesis of catecholamines from phenylalanine[71]

DA is produced in several areas of the brain, including the substantia nigra and the ventral tegmental area (VTA). The VTA is the origin of the dopaminergic cell bodies of the mesocorticolimbic DA system and is widely implicated in the drug and natural reward circuitry of the brain. In the terminal ends of neurons, DA is packed after synthesis into

vesicles, which are then released into the synapse in response to a presynaptic electrochemical action potential.

DA neurons are mainly present in the VTA of the midbrain, the substantia nigra, pars compacta, and the arcuate nucleus of the hypothalamus. DA has many functions in the brain, including important roles in behavior and cognition, voluntary movement, motivation, punishment and reward, inhibition of prolactin production (involved in lactation and sexual gratification), sleep, mood, attention, working memory, and learning.

Two major degradation pathways for DA exist. In most areas of the brain, including the basal ganglia, DA is either inactivated by reuptake via the DA transporter (DAT) or by the enzymatic breakdown by monoamine oxidase (MAOA and MAOB) into 3,4-dihydroxyphenylacetic acid. In the prefrontal cortex, however, there are very few DA transporter proteins, and DA is instead inactivated by reuptake via the NE transporter, presumably on neighboring NE neurons, rather than through enzymatic breakdown by catechol-O-methyl transferase (COMT) into 3-methoxytyramine.

1.7 Parkinson´s disease

Parkinson's disease (PD) is a progressive neurological disorder characterized by a large number of motor and non-motor features that can impact on function of the patient to a variable degree. Because there is no definitive physiological test for the diagnosis of PD, the disease must be diagnosed based on clinical criteria. Rest tremor, bradykinesia, rigidity and loss of postural reflexes are generally considered the cardinal signs of PD.[72] The primary symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia. Normally this involves insufficient formation and thus action of dopamine produced in the dopaminergic neurons of the midbrain (specifically the substantia nigra).

The pathological hallmark of PD is cell loss within the substantia nigra particularly affecting the ventral component of the pars compacta. By the time of death, this region of the brain has lost 50-70% of its neurons compared with the same region in unaffected individuals. The earliest documented pathological changes in PD have been observed in the medulla oblongata/pontine tegmentum and olfactory bulb. In these early stages patients are pre-symptomatic. As the disease advances the substantia nigra, areas of the midbrain and basal forebrain become involved. Finally, pathological changes appear in the neocortex.[73]

The standard symptomatic therapy of PD for more than 30 years has been DOPA. As it was mentioned before, this is the precursor of DA which is deficient in PD. DOPA is readily converted into dopamine by AADC. To reduce peripheral metabolism of DOPA, the drug is combined with a peripheral dopa decarboxylase inhibitor (*i.e.* carbidopa or benserazide). This increases the amount of DOPA that crosses the blood-brain barrier.[74]

1.8 [^{18}F]Fluoroaromatic amino acids

Many amino acids have been radiolabeled to study their potential imaging characteristics. These radiolabeled amino acids differ in ease of synthesis, biodistribution and formation of metabolites in vivo. To date, the most frequently used amino acid tracers for tumor diagnosis are [^{11}C-methyl]-L-methionine (MET) for PET and 3-[^{123}I]iodo-α-methyl-L-tyrosine (IMT) for single photon emission computed tomography (SPECT).[75] Due to the short physical half-life of the ^{11}C-label (20.4 min), MET PET remains restricted to a few PET centers with a cyclotron on site and could not be established in routine clinical practice despite of its compatibility with amino acid uptake kinetics and convincing first clinical results. IMT SPECT offers a more widespread application, but the spatial resolution of SPECT is considerably lower than that of PET.[76] Therefore, a number of attempts have been undertaken to label amino acids with fluorine-18.

1.8.1 6-[^{18}F]Fluoro-3,4-dihydroxy-L-phenylalanine

3,4-Dihydroxy-6-[^{18}F]fluoro-L-phenylalanine (6-[^{18}F]FDOPA) is a fluorinated analogue of the naturally-occurring L-3,4-dihydroxyphenylalanine (L-DOPA). It has been used extensively for evaluation of the dopaminergic system in the brain, particularly in Parkinson´s disease, as it is a substrate for the enzyme aromatic amino acid decarboxylase (AADC), normally found in high abundance in dopaminergic neurons. For this reason, under normal physiological conditions there is high uptake and retention of 6-[^{18}F]FDOPA in the substantia nigra and the striatum. Additionally, 6-[^{18}F]FDOPA also shows high uptake in neuroendocrine tumors (NET)[77] and some other tumors, such as cerebral gliomas, which may exhibit an increased accumulation due to amino acid transport.[78] The brain uptake of 6-[^{18}F]FDOPA in animal and humans can be decreased by other system L substrates such as L-phenylalanine, consistent with a 6-[^{18}F]FDOPA transport via system L.[79] However, accumulation of the radiotracer in non-NETs, except for cerebral gliomas, is less predictable, and the role of 6-[^{18}F]FDOPA seems to be limited.

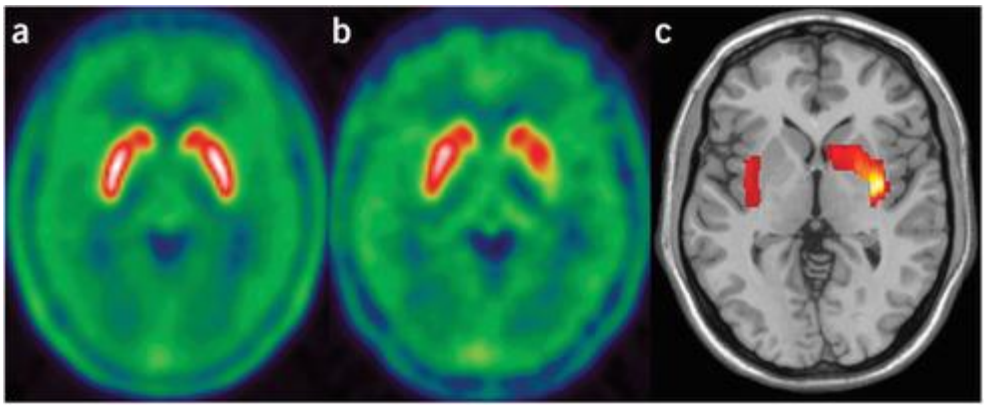
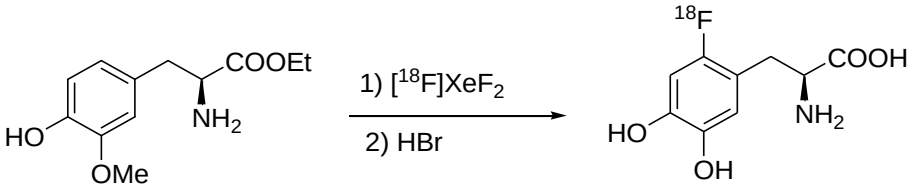


Figure 5: PET and MRI of PD-patient and control. (**a**) Control subjects shows high uptake of 6-[^{18}F]FDOPA (highest value in white) in the striatum. (**b**) Subject with Parkinson's disease where the uptake is unilaterally reduced (up to 70% below normal). (**c**) Analysis showing the difference in uptake between **a** and **b** (yellow represents the largest statistical difference and red the smallest one). The statistical map (c) is rendered over the corresponding MRI scan for anatomical localization.[80]

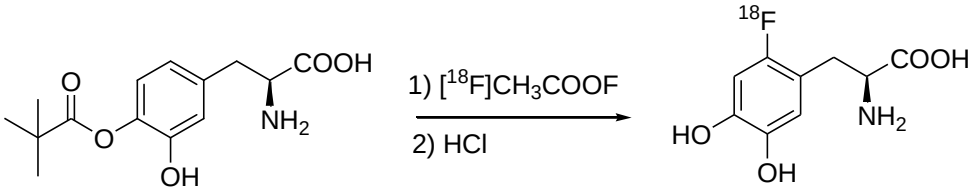
The first reported radiosynthesis of 6-[^{18}F]FDOPA is already 30 years old. It was performed through direct electrophilic labeling of 3-methoxy-L-DOPA ethyl ester using [^{18}F]XeF_2 as fluorinating agent and hydrogen fluoride as solvent followed by the cleavage of the methyl group with hydrobromic acid. Scheme 10 depicts the synthetic approach. This procedure achieved the desired amino acid in 1% radiochemical yield (RCY).[81] Using the same precursor but [^{18}F]acetyl hypofluorite as fluorinating agent the RYC was improved to 4%.[82]



Scheme 10: First reported electrophilic synthesis of 6-[^{18}F]FDOPA[81]

Several reports dealing with the direct electrophilic labeling of "naked" L-DOPA in different solvent systems were afterwards published. These experiments provided the desired compound in 3-8% RCY.[83,84,85] The major drawback of these procedures was the occurrence of all the others probable regioisomers which were not easy to separate from the 6-[^{18}F]FDOPA requiring complicate and time consuming HPLC procedures.

Further labeling attempts consisted in the labeling of other protected L-DOPA derivatives. Some of them had relative success, like the labeling of (*S*)-2-amino-3-(4-hydroxy-3-(pivaloyloxy)phenyl)propanoic acid which delivered the desired amino acid in 17% RCY after 60 min (Scheme 11).[86]

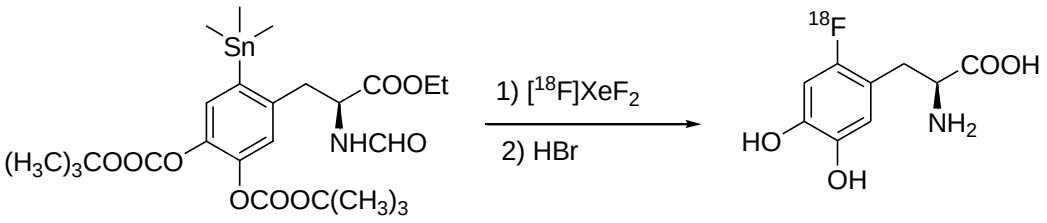


Scheme 11: Most efficient direct electrophilic synthesis of 6-[^{18}F]FDOPA[86]

However, as mentioned before, the use of direct electrophilic substitution is limited due to the low regioselectivity of the reaction. Efforts were focused to improve it by the use of a leaving group in the position 6 of the aromatic ring facilitating the regioselective introduction of the radioactive fluoride atom. The first regioselective synthesis of 6-[^{18}F]FDOPA following this approach was achieved via fluorodesilylation of 6-trimethylsilyl-3,4-dimethoxy-L-DOPA-ethylester. The precursor was labeled with [^{18}F]F_2 in a mixture of freon-11/CCl_4, subsequent hydrolysis with concentrated HBr followed by chromatographic purification yielding the radiotracer with a RCY of 8% in 60 min.[87] An improvement of the RYC was achieved when fluorodemercuration was performed instead of a fluorodesilylation. Here, the precursor (4,5-dimethoxy-2-((*S*)-3-methoxy-3-oxo-2-(2,2,2-trifluoroacetamido)propyl)phenyl)(oxo)(2,2,2-trifluoroacetyl) mercury was labeled at room temperature with [^{18}F]CH_3COOF in dichloromethane followed by cleavage of the protecting groups with hydrobromic acid. After chromatographic purification the 6-[^{18}F]FDOPA was obtained in a total time of 50 min.[88] The disadvantage of this procedure is the use of mercury derivative since the heavy metal is toxic.

In 1992 Namavari and co-workers published the synthesis that until now is the best alternative for the preparation of 6-[^{18}F]FDOPA via electrophilic substitution (Scheme 12). Toxic mercury was replaced by tin and the desired amino acid was obtained in 17% RCY.[89] de Vries *et. al.* adapted the fluorodestannylation method described by Namavari and coworkers and developed a simple, fully automated synthesis module for the routine clinical production of 6-[^{18}F]FDOPA.[90] This was prepared with both, high radiochemical yield 33 ± 4 %, and radiochemical purity > 99% in 45 min synthesis time. $CFCl_3$ was found to be a better solvent for the fluorodestannylation reaction than $CHCl_3$ or acetonitrile. Also in $CFCl_3$,

[^{18}F]F_2 was a superior fluorinating agent over [^{18}F]acetyl hypofluorite. However, $CFCl_3$ is not anymore available.



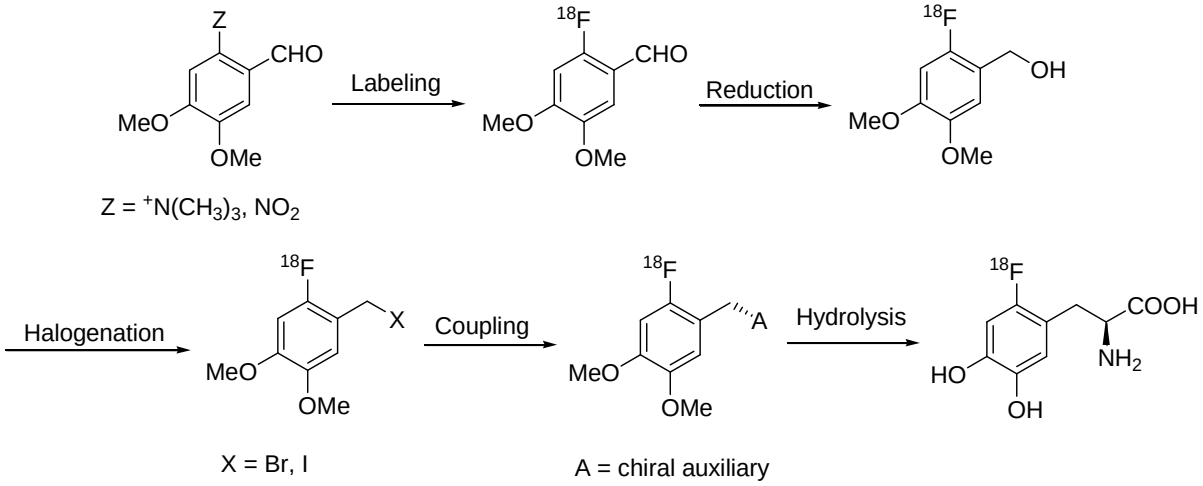
Scheme 12: Regioselective synthesis of 6-[^{18}F]FDOPA by electrophilic fluorodestannylation[89]

Nucleophilic approaches for the radiosynthesis of 6-[^{18}F]FDOPA started with substitution by [^{18}F]fluoride on small benzaldehyde derivatives with subsequent build-up reactions.[91] The first nucleophilic synthesis of 6-[^{18}F]FDOPA was based on the nucleophilic displacement of a nitro group of two commercially available substrates, 3,4-dimethoxy-2-nitrobenzaldehyde (nitroveratraldehyde) or 6-nitropiperonal by [^{18}F]fluoride.[92] Fluorination was conducted in presence of the aminopolyether Kryptofix 222 and potassium carbonate in DMSO. The condensation of the fluorinated aldehydes with phenyloxazolone and the subsequent hydrolysis with HI/P gave only a mixture of 6-(D, L) isomers after purification by HPLC. The racemic mixture was resolved on an analytical scale chiral column. The method, which requires 100 min (EOB) to be complete, produces 6-[^{18}F]FDOPA with 10% RCY, enantiomeric purity > 99%, and a specific activity of 44.4 GBq/µmol. Despite of formation of a racemic mixture this method was automated producing the desired L-amino acid after chiral HPLC in 2 h, with a RCY of 3-5%.[93] Further extensive efforts have been focused in the improvement of the nucleophilic substitution.[94,95,96]

In order to avoid the formation of racemates the introduction of a chiral group was envisioned. The general procedure relies on a built-up synthesis depicted in Scheme 13. After the ^{18}F-labeling step, the aldehyde functionality is reduced to the benzyl alcohol and afterwards converted to the benzyl halide derivative. The benzyl halide (generally bromide) is subsequently coupled to either a chiral auxiliary or to a prochiral auxiliary by chiral phase transfer alkylation. The last step consists in the hydrolysis of the protecting groups in acid medium.

The use of the glycine derivatives [(+)-2-hydroxypinanyl-3-idene]glycine *tert*-butyl ester and the analogous ethyl ester as chiral inductors provided the desired L-isomer in 75%.

Alkylation of the Schiff base was carried out with the lithium salt of 2,2,6,6-tetramethylpiperidine as base in anhydrous THF at -78°C. Following hydrolysis of the protecting groups with hydroxylamine and HI, the L-amino acid was obtained in 75% (*e.e.* 50%) with a 10% RCY within 120 min. [97,98]

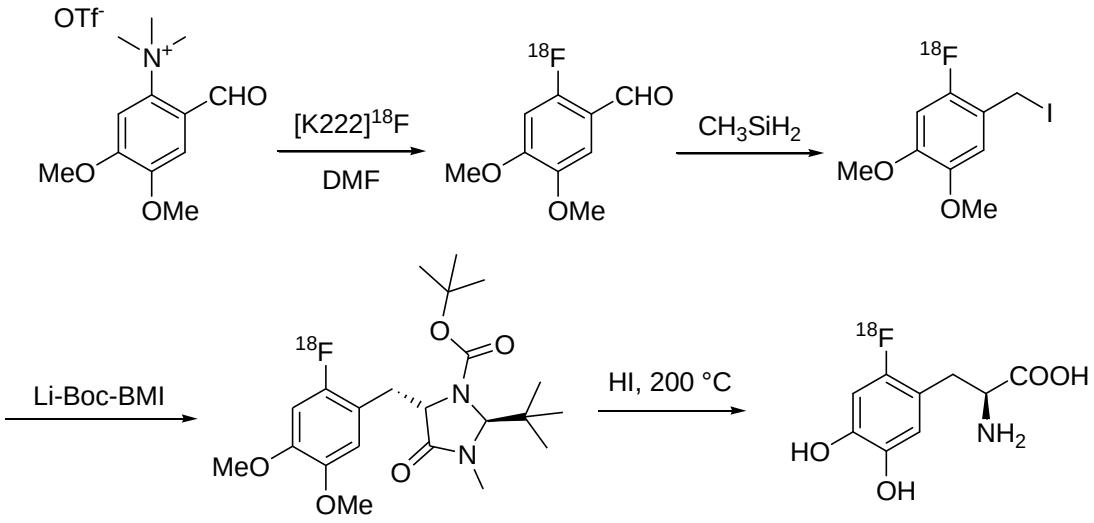


Scheme 13: General scheme of the nucleophilic built-up radiosynthesis of 6-[^{18}F]FDOPA

In 1993 Lemaire and coworkers[99] reported the synthesis of 6-[^{18}F]FDOPA using two different chiral inductors. 2-[^{18}F]Fluoro-4,5-dimethoxybenzyl bromide was added to the lithium enolates of 1-(*S*)-(-)camphor imine of *tert*-butyl glycinate and (*S*)-(-)-1-Boc-2-*tert*-butyl-3-methyl-4-imidazolidinone ((*S*)-Boc-BMI). After hydrolysis with HI and HPLC purification, the L-isomer of 6-[^{18}F]FDOPA was isolated in 5-10% RCY. The *e.e.* using the first chiral auxiliary was 83%, while 96% *e.e.* was obtained when the second one was used. The overall synthesis time was of 110 min.

3,5,6,7,8,8a-Hexahydro-7,7,8a-trimethyl-[6*S*-(6α,8α,8αβ)]-6,8-methano-2H-1,4-benzoxazino-2-one was investigated as chiral auxiliary. This procedure is slightly different to the previously described. A synthetic step is saved by direct condensation of the auxiliary with 3,4-dimethoxy-2-[^{18}F]fluorobenzaldehyde or 6-[^{18}F]fluoropiperonal in the presence of NaH providing the corresponding 3-[(2-[^{18}F]fluorophenyl)methylene]-3,5,6,7,8,8a-hexahydro-7,7,8a-trimethyl-[6S-(3Z,3α,6α,8α,8αβ)]-6,8-methano-2H-1,4-benzoxazin-2-one derivatives as a single stereoisomer. L-Selectride® promoted hydrogenation of the olefinic double bond of these derivatives, in presence of tertbutyl alcohol, generated the corresponding 3-[(2-[^{18}F]fluorophenyl)methyl]-3,5,6,7,8,8a-hexahydro-7,7,8a-trimethyl-[3S-(3α,6α,8α,8αβ)]-6,8-methano-2H-1,4-benzoxazin-2-one derivatives. Deprotection yielded 6-[^{18}F]FDOPA in 3% RCY, with an *e.e.* > 90% in a total synthesis time of 125 min.

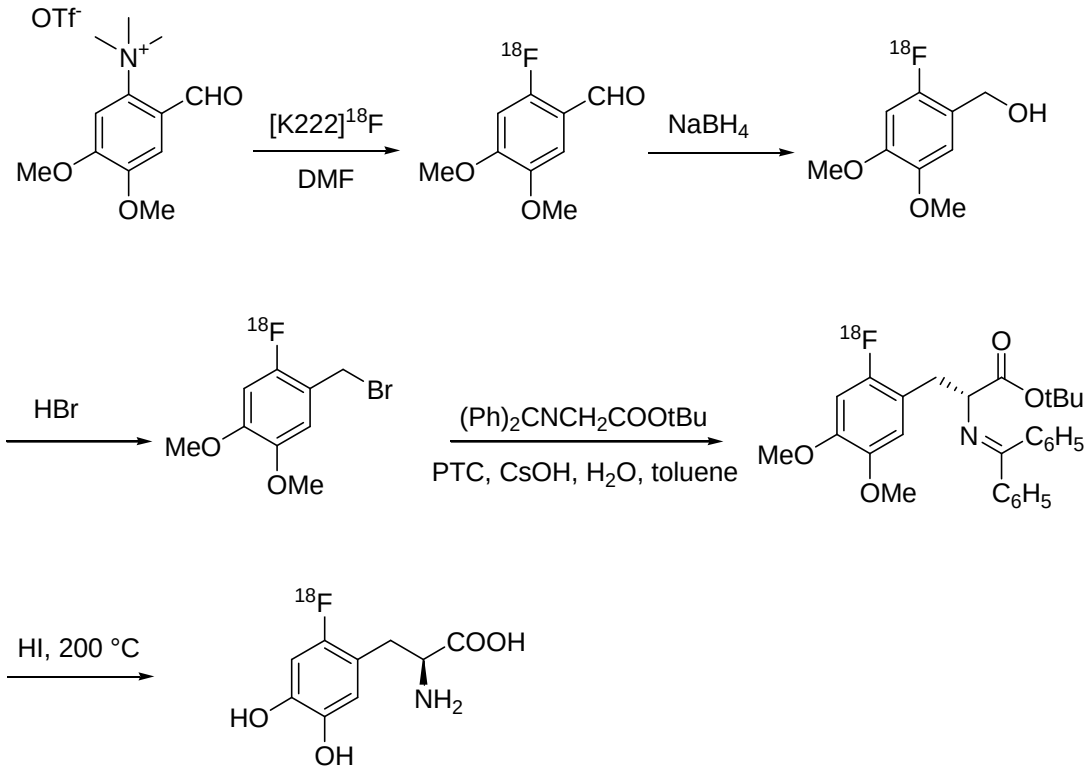
Another four-step synthesis and for long time the most successful among the nucleophilic built-up synthesis was reported by Lemaire and coworkers in 1993.[100] Trimethylammonium veratraldehyde triflate was used as precursor for the asymmetric synthesis of 6-[^{18}F]FDOPA. Diiodosilane was used to prepare the corresponding [^{18}F]fluorobenzyliodide. Akylation of (*S*)-Boc-BMI with this electrophilic agent, hydrolysis and purification by preparative HPLC provided 6-[^{18}F]FDOPA ready for human injection whit 23% RCY. The enantiomeric purity and the specific activity were above 96% and 37 GBq/µmol, respectively. The synthesis procedure was completed within 90 min (Scheme 14).



Scheme 14: Enantioselective nucleophilic built-up synthesis of n.c.a. 6-[^{18}F]FDOPA[100]

Further developments involved the use of phase-transfer chiral catalysts for the coupling of glycine derivatives with the benzyl bromide instead of the coupling with a chiral auxiliary (Scheme 14). A chiral quaternary ammonium salt derived from a Cinchona alkaloid served as phase-transfer catalyst for the enantioselective alkylation of a glycine derivative. The active methylene group of this Schiff-base substrate was deprotonated with cesium hydroxide and alkylated by the 2-[^{18}F]fluoro-4,5-dimethoxybenzyl halide (X = Br, I). The reaction proceeded with high yield (> 90%) at 0 °C or r.t. in solvents such as toluene or dichloromethane. After labelling, the labeled [^{18}F]fluoroveratraldehyde was trapped on a C18 cartridge and then converted on the cartridge into the corresponding benzyl halide derivatives by addition of aqueous sodium borohydride and gaseous hydrobromic or hydroiodic acid. Hydrolysis and purification by preparative HPLC gave 6-[^{18}F]FDOPA with 25-30% RCY (*e.e.* > 95%) within a synthesis time of 100 min.[101] A slightly modified automated synthesis using the concept described before has recently been reported. Using a home-made automatic synthesizer

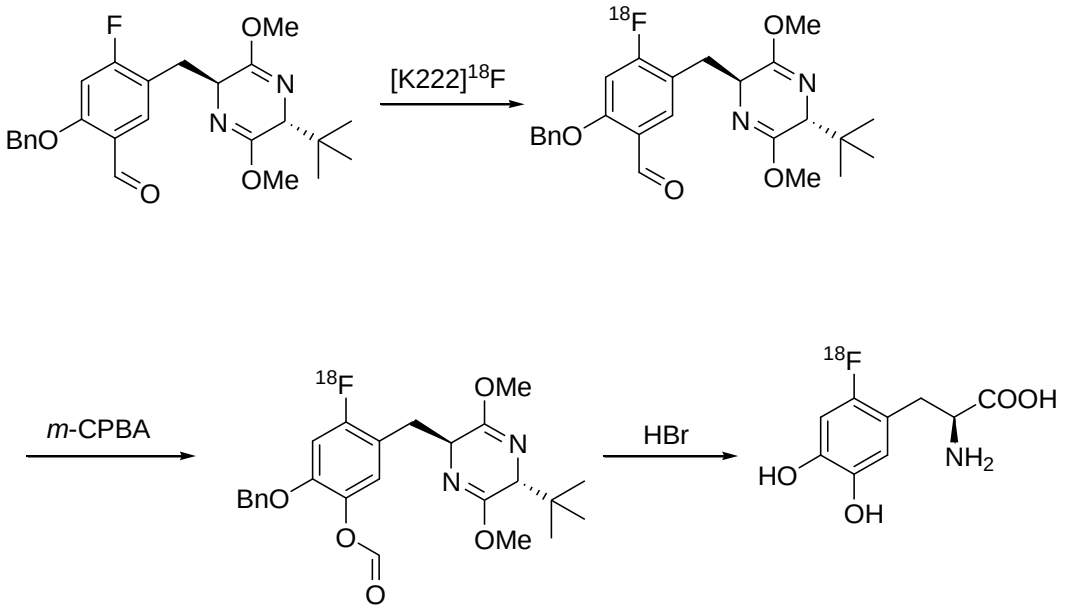
[^{18}F]FDOPA was produced with RCY of 20% within 120 min. Radiochemical purity and *e.e.* were both ≥ 95%. The specific activity obtained was approximately 50 GBq/mmol.[102]



Scheme 15: Most efficient nucleophilic built-up synthesis of n.c.a. 6-[^{18}F]FDOPA[101]

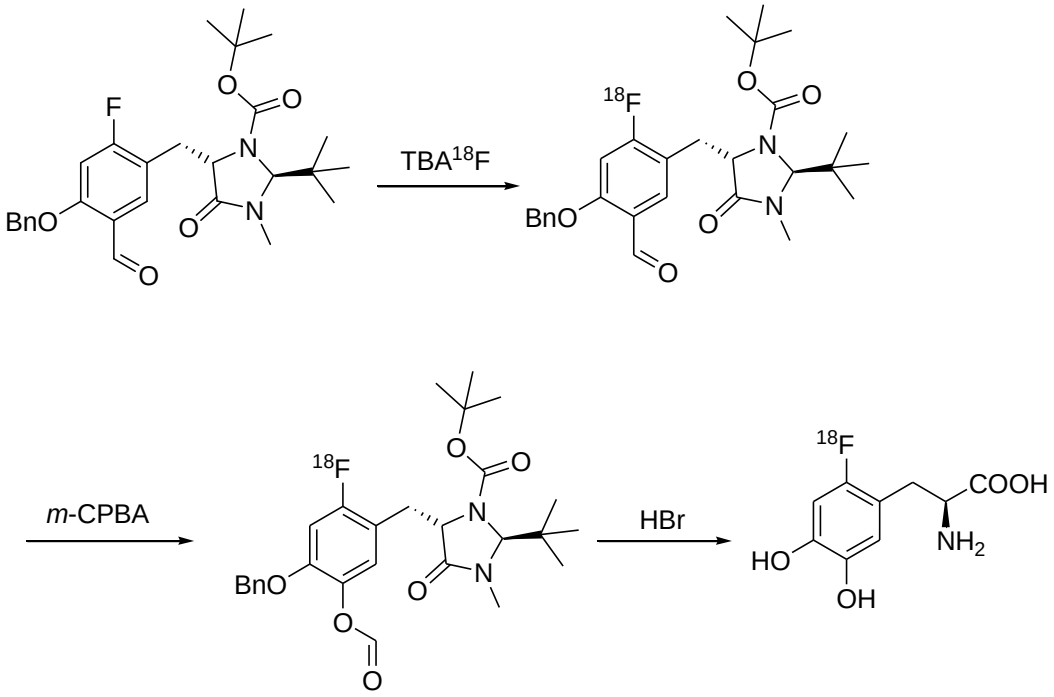
This approach yields the desired radiotracer at the no-carrier-added level and allowed large-scale productions with good chemical, radiochemical and enantiomerical purity. However, the built-up synthesis is still difficult to automate due to its complexity.

Independently of the previously described approaches a direct nucleophilic isotopic exchange reaction on a formyl-activated masked aromatic amino acid derivative was studied for improving the efficiency of the nucleophilic preparation of 6-[^{18}F]FDOPA. Here, the required carbon skeleton and the chiral center with the desired configuration were constructed before introduction of the [^{18}F]fluoride ion by use of a chiral auxiliary. The precursor undergoes an isotopic exchange with [^{18}F]fluoride followed by a Baeyer-Villiger oxidation and subsequent hydrolysis. In a first attempt the 2-(benzyloxy)-4-fluoro-5-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)benzaldehyde was used as starting material as depicted in Scheme 16.[103] Although this approach was suggested a long time ago upon first radiosynthesis of corresponding [^{18}F]fluoroaldehydes as one general possibility of their use no attempts for its realization were made so far.[104]



Scheme 16: Three-step c.a. synthesis of 6-[^{18}F]FDOPA by isotopic exchange using 2-(benzyloxy)-4-fluoro-5-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)benzaldehyde as labeling precursor[103]

Although the radiochemical yield of this procedure was similar to the n.c.a. built-up radiosynthesis reported by Lemaire, the *e.e.* was only 70%.

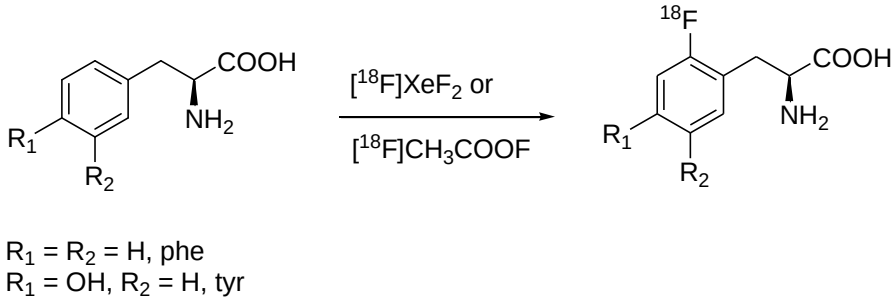


Scheme 17: Three-step c.a. synthesis of 6-[^{18}F]FDOPA by isotopic exchange using (2*S*,5*S*)-tert-butyl-5-(4-benzyloxy-2-fluoro-formylbenzyl)-2-tert-butyl-3-methyl-4-oxo-imidazolidine-1-carboxylate as labeling precursor[105]

Recently, using (2*S*,5*S*)-*tert*-butyl-5-(4-benzyloxy-2-fluoro-formylbenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate as precursor, the synthesis of 6-[^{18}F]F-DOPA was achieved with an acceptable radiochemical yield of 22 % and a high *e.e* of > 96%. (Scheme 17).[105] In spite of that, this method also presented some drawbacks, *e.g.* the synthesis of the labeling precursor could only be accomplished in eleven steps delivering a low yield of about 1%, thus limiting the optimization studies of the radiosynthesis. The formation of unidentified radioactive side-products during the isotopic exchange reaction was also reported, what requires further attention. In addition, the RCY of the hydrolysis step, although already acceptable, should be improved. This radiochemical approach will be discussed in more detail in Chapter 3 along the work presented here.

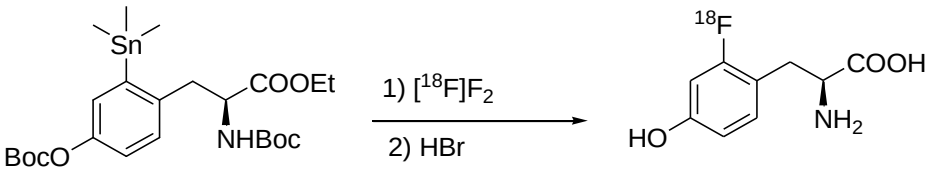
1.8.2 Radiosynthesis of 2-[^{18}F]fluoro-L-phenylalanine and 2-[^{18}F]fluoro-L-tyrosine

2-[^{18}F]Fluoro-L-phenylalanine (2-[^{18}F]Fphe) has proven to be a useful radiopharmaceutical for the study of neutral amino acid transport at the blood brain barrier *in vivo* in humans.[106] On the other hand, 2-[^{18}F]fluoro-L-tyrosine (2-[^{18}F]Ftyr), unlike other halogenated amino acids, is almost quantitatively incorporated into proteins lending it as interesting tracer for imaging of protein synthesis *in vivo*.[107] Since the accumulation of 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr enables to distinguish tumors from normal tissue, positron emission tomography studies of their uptake are of clinical value for the diagnosis of brain tumors.[108,109,110] Both radiotracers have been prepared by direct electrophilic synthesis with [^{18}F]F_2 and [^{18}F]CH_3COOF on phenylalanine and tyrosine, respectively, as well as on O-acetylated tyrosine. However, their radiochemical yield and positional isomeric purity were limited and demanded careful HPLC-separation (Scheme 18).[111]



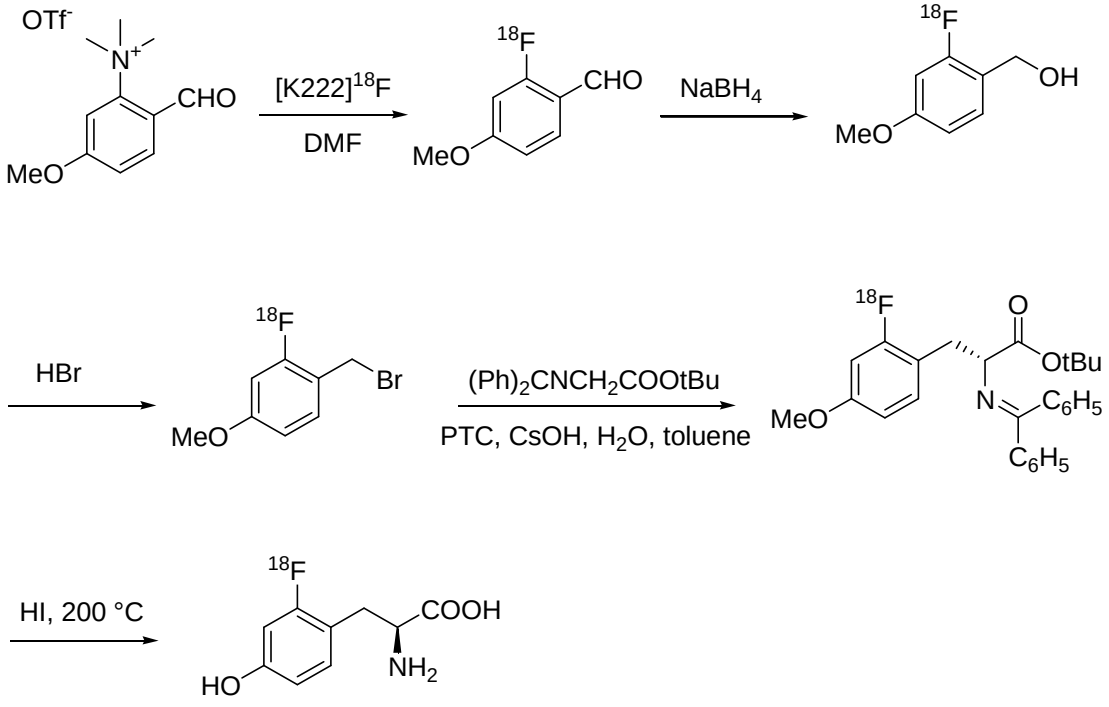
Scheme 18: Direct electrophilic synthesis of 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr using the respective "naked" amino acid as starting material[111]

As described above for 6-[^{18}F]FDOPA, here again radiofluorodemetallation and specially radiofluorodestannylation have been found most efficient labeling methods in order to achieve aromatic [^{18}F]fluoroamino acids in routine production. In the case of 2-[^{18}F]Ftyr, O,N-di-Boc-2-triethylstannyl-tyrosine ethyl ester has proven to a be suitable precursor for its radio-synthesis leading to an improved RCY of 21%.[112]



Scheme 19: Regioselective synthesis of 2-[^{18}F]Ftyr by electrophilic fluorodestannylation[112]

In the other hand, 2-[^{18}F]Ftyr has also been prepared in n.c.a. form in a multistep synthesis in similar way as 6-[^{18}F]FDOPA in five steps starting by nucleophilic ^{18}F-substitution of a small aldehyde as depicted in Scheme 20.[113]



Scheme 20: Build-up nucleophilic synthesis of n.c.a. 2-[^{18}F]Ftyr[113]

Again here, the problem with this synthetic approach is that despite the final product meets the quality control requirements and can be produced in amounts high enough for routine use in medical practice, the radiosynthesis is cumbersome to automate which hitherto limits a wider use of the radiotracer.

1.8.3 Radiosynthesis of 6-[^{18}F]fluoro-L-*m*-tyrosine

6-[^{18}F]Fluoro-L-*m*-tyrosine (6-[^{18}F]Fmtyr) has been employed as 6-[^{18}F]FDOPA-analogous imaging agent of the dopaminergic function in the brain by measuring the activity of the enzyme AADC in both animals as well as in humans using positron emission tomography (PET).[114] Historically the tracer of choice for such studies has been 6-[^{18}F]FDOPA.[115] However, 6-[^{18}F]FDOPA is metabolized in the periphery and central nervous system by the enzyme catecholamine O-methyltransferase (COMT) producing O-MeDOPA. This is also able to cross bidirectionally the blood-brain-barrier thus compromising the quality of the clinical image as well as making its quantitative analysis more complicated. Contrary to 6-[^{18}F]FDOPA, 6-[^{18}F]Fmtyr is not a substrate for COMT. Therefore the only radioactive species in the brain is the molecule of interest, providing a better signal/noise ratio and a clear diagnostic image while reducing the need for complex multicompartmental kinetic modeling.[116,117,118]

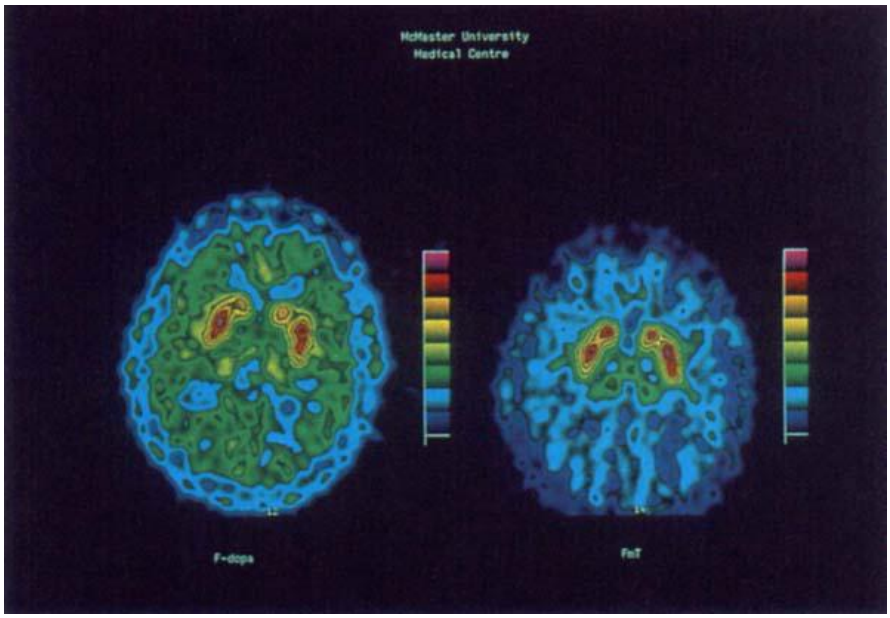


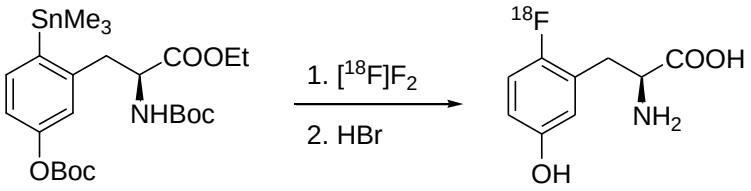
Figure 6: Distribution of radioactivity 90 min after the injection of 6-[^{18}F]FDOPA (left) or 6-[^{18}F]Fmtyr (right) in a patient with Parkinson's disease[119]

Recently several studies illustrate the capacity of 6-[^{18}F]Fmtyr for the assessment of the integrity of the presynaptic dopamine system. Studies dealing with the relationship of eating behavior and the dopamine synthesis capacity,[120] relationship of striatal dopamine synthesis capacity to age and cognition,[121] and for the evaluation of the effectiveness of gene therapy in primate models of PD[122] and PD patients[123] have been published.

Regarding the radiosynthesis of 6-[^{18}F]Fmtyr, DeJesus and coworkers reported its synthesis via direct electrophilic fluorination of L-*m*-tyrosine with [^{18}F]AcOF.[124,125] The radiochemical yield was 71 ± 5% (*n* = 3; decay correction based on [^{18}F]AcOF activity) after purification. The specific activity was 3.7-7.4 GBq/mmol (3.7-7.4 MBq)/mmol).

Due to the success of the fluorodemetallation approach 6-[^{18}F]Fmtyr has also been synthesized in two steps by electrophilic fluorodestannylation. Namavari and coworkers[126] reported the synthesis and ^{18}F-fluorination of *N*-trifluoroacetyl-3-acetyloxy-6-trimethyl-stannyl-L-phenylalanine ethyl ester followed by exhaustive deprotection of the acid, amine and phenol functionalities. The 6-[^{18}F]Fmtyr was obtained more than 99% chemically and radiochemically pure. The total synthesis time took 60 min, and the radiochemical yield was 17% of the ^{18}F activity recovered from the target.

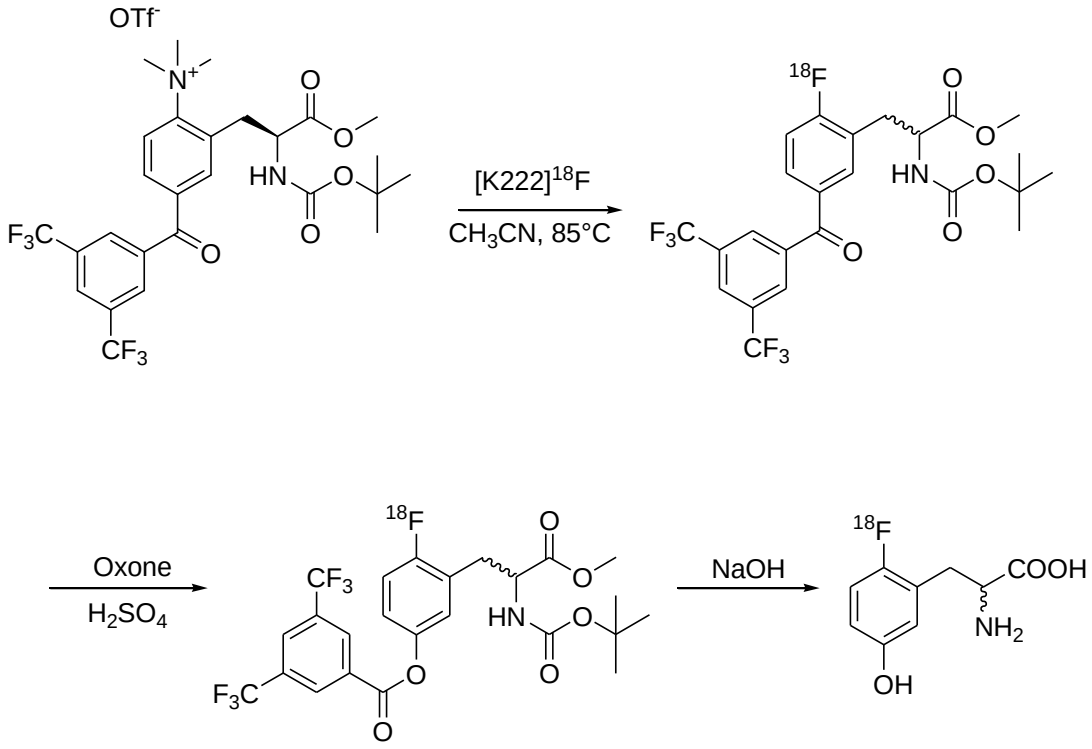
Scheme 21 depicts the synthesis of 6-[^{18}F]Fmtyr reported by VanBrocklin and coworkers. A new di-Boc protected precursor, *i.e. N-tert*-butoxycarbonyl-3- *tert*-butoxycarbonyloxy-6-trimethylstannyl-L-phenylalanine ethyl ester was employed, for the electrophilic fluorination.[127] This precursor was synthesized from L-*m*-tyrosine in four steps with an overall yield of 26-27%. This procedure saves 3 synthetic steps for the synthesis of the precursor compared with the synthesis of that reported by Namavari *et.al*.[126] The enatiomeric purity was > 95%. Decay-corrected radiochemical yields (*n* >6) for a two-pot method and one-pot method were 26 ± 3% and 25 ± 6%, respectively. For both methods, the chemical and radiochemical purities were > 96%, and the range of specific activities of 6-[^{18}F]Fmtyr was 28-74 MBq/μmol.



Scheme 21: Regioselective synthesis of 6-[^{18}F]Fmtyr by electrophilic fluorodestannylation[127]

An alternative procedure was reported Mulholland and coworkers for the radiosynthesis of this molecule (Scheme 22).[128] The labeling precursor in this case was a benzophenone derivative, in which the amino acid function is already included. Contrary to the previously presented 6-[^{18}F]FDOPA precursors for isotopic exchange nucleophilic radiofluorination (see Scheme 16 and 17), here the protected amino and carboxylic acid functional groups do not

form part of a cyclic compound. Unfortunately, the data related neither to RCY nor an enantiomeric purity was reported.



Scheme 22: N.c.a. nucleophilic synthesis of 6-[^{18}F]Fmtyr using (*S*)-4-(3,5-bis(trifluoromethyl)benzoyl)-2-(2-(*tert*-butoxycarbonylamino)-3-methoxy-3-oxo-propyl)-*N*,*N*,*N*-trimethylbenzenaminium triflate as labeling precursor[128]

2 AIMS AND SCOPE

In nuclear medicine diagnosis ^{18}F-labeled aromatic amino acids are widely employed as radiopharmaceuticals for *in vivo* imaging using PET. Up to now, routine preparation of [^{18}F]fluorophenyl amino acids is carried out via electrophilic labeling, preferably through fluorodestannylation reactions. The electrophilic approach is applicable because many of these compounds have a low toxicity and consequently they can be used in carrier-added form. However, since elemental [^{18}F]fluorine is needed, these methods are limited to low amounts of activity at high costs. On the other hand, present nucleophilic syntheses using the advantage of large scale production of [^{18}F]fluoride result either in insufficient enantiomeric purity or in a need of built-up syntheses that are difficult to automate

Recently a new nucleophilic isotopic exchange approach was developed allowing the radiosynthesis of 6-[^{18}F]fluoro-L-DOPA in three steps following the sequence ^{18}F-for-^{19}F exchange, Baeyer-Villiger oxidation, and hydrolysis using (2*S*,5*S*)-*tert*-butyl 5-(4-(benzyloxy)-2-fluoro-5-formylbenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate as precursor. However, the lack of an efficient synthesis of the labeling precursor has restricted the optimization of the radiosynthesis and broader application.

Therefore, the first objective here is to develop an efficient synthesis of the labeling precursor. Further goals include the optimization of every radiosynthetic step, involving the identification of side products and their mechanism of formation during the radiofluorination reaction. Furthermore, the study of the influence of the oxidizing agent on the Bayer-Villiger oxidation and the effect of the temperature and kind of mineral acid on the radiochemical yield of the hydrolysis reaction demand further exploration.

As an alternative a modified procedure in which a decarbonylation reaction should be performed instead of the Baeyer-Villiger oxidation in order to achieve the preparation of 2-[^{18}F]fluoro-L-tyrosine starting from the same precursor. Similarly a group of analogous fluoro-benzaldehyde precursors are envisaged for the radiosynthesis of 2-[^{18}F]fluoro-L-phenylalanine following this decarbonylation route. Following three derivatives were selected: (2*S*,5*S*)-*tert*-butyl 2-*tert*-butyl-5-(2-fluoro-5-formylbenzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate, (2*S*,5*S*)-benzyl 2-*tert*-butyl-5-(2-fluoro-5-formylbenzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate and 4-fluoro-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)benzaldehyde. Those will also enable to study the influence of the protecting group in the imidazolidinone ring and the kind of chiral auxiliary on the

radiochemical yield and the enantiomeric purity of the final product. The radiosynthetic procedure should be performed using both, conventional heating (oil bath) and microwave heating in order to compare the effect of the heating source on radiochemical yields and reaction times.

In the frame of the planned work, it is also intended to prepare 6-[^{18}F]fluoro-L-*m*-tyrosine using the oxidative pathway. Since fluoro-benzaldehydes which are not substituted with electron-donating groups are poorly directing their conversion into the respective phenol derivatives through Baeyer-Villiger oxidation, a series of fluoro-benzophenones will be synthesized to properly direct the reaction to the desired compound. This new series of precursors consists of (2*S*,5*S*)-*tert*-butyl 5-(5-acetyl-2-fluorobenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate, 1-(4-fluoro-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)phenyl)ethanone, (2*S*,5*S*)-*tert*-butyl 5-(5-(3,5-bis(trifluoromethyl)benzoyl)-2-fluorobenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate and (2*S*,5*S*)-*tert*-butyl 2-*tert*-butyl-5-(2-fluoro-5-(2,2,2-trifluoroacetyl)benzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate. With them it will be possible to study the effect of the activating group on the radiochemical yield of isotopic exchange reaction and the influence of the chiral group on the enantiomeric purity of the 6-[^{18}F]fluoro-L-*m*-tyrosine.

A major aim of the work present here is to achieve a deeper understanding regarding the isotopic exchange reaction on highly functionalized 4-fluoro-benzaldehyde and -benzophenone derivatives. To that end, an exhaustive study on this reaction is planned. Experimental parameters including concentration and kind of [^{18}F]fluoride anion activator, temperature, power of the microwave, reaction time, and nature of the carbonyl-activating group should be examined in order to determine their effect on the radiochemical yield and their role in the formation of further radiofluorinated non-wanted side products. Radioanalytical methods based on thin layer and high performance liquid chromatography using standard compounds must be developed in order to determine the radiochemical yields of products and side-products, their identity as well as radiochemical and enantiomerical purity.

Finally, it is planned to transfer the elaborated three-step radiosynthesis of 6-[^{18}F]fluoro-L-DOPA into an commercially available remote-controlled synthesis module. In order to achieve this goal the SynChrom R&D device of Raytest GmbH controlled by GINA SynChrom software will be used.

3 RESULTS AND DISCUSSIONS

3.1 Synthesis of precursors for the radiosynthesis of [^{18}F]fluorophenylamino acids

Preparation of suitable precursors for the radiosynthesis of aromatic [^{18}F]fluorophenyl-amino acids represent a major challenge for the development of these labeled compounds. As mentioned in the introduction of this work, most of the nucleophilic radiofluorinations of aromatic rings start with small aldehydes derivatives as labeling precursors. This is undesirable since the introduction of the radioactive fluorine atom at the beginning of the procedure leaves the construction of the enantioselective amino acid functionalities as part of the radiochemical pathway and generally these reactions are complex, multi-step procedures and consequently difficult to automate.

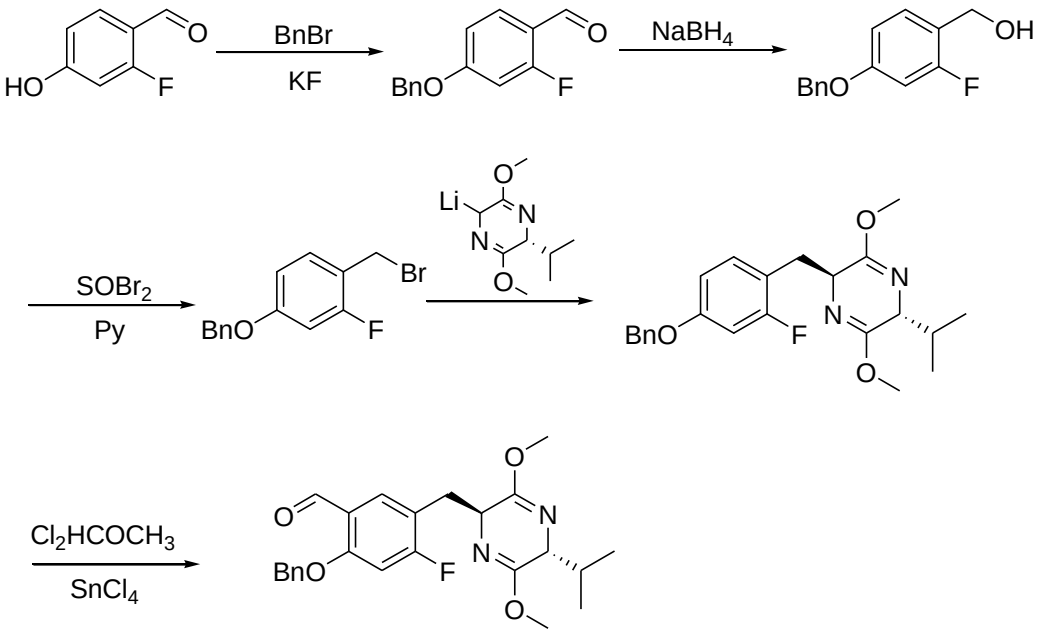
Due to the high electron density of the ring with the amino acids of interest, the precursor should contain an activating, electron withdrawing group located in *ortho* or *para* position relative to the leaving group. Additionally, in the ideal precursor, the L-amino acid function is already built in.

3.1.1 Earlier syntheses of precursors for the radiosynthesis of [^{18}F]fluorophenyl-amino acids

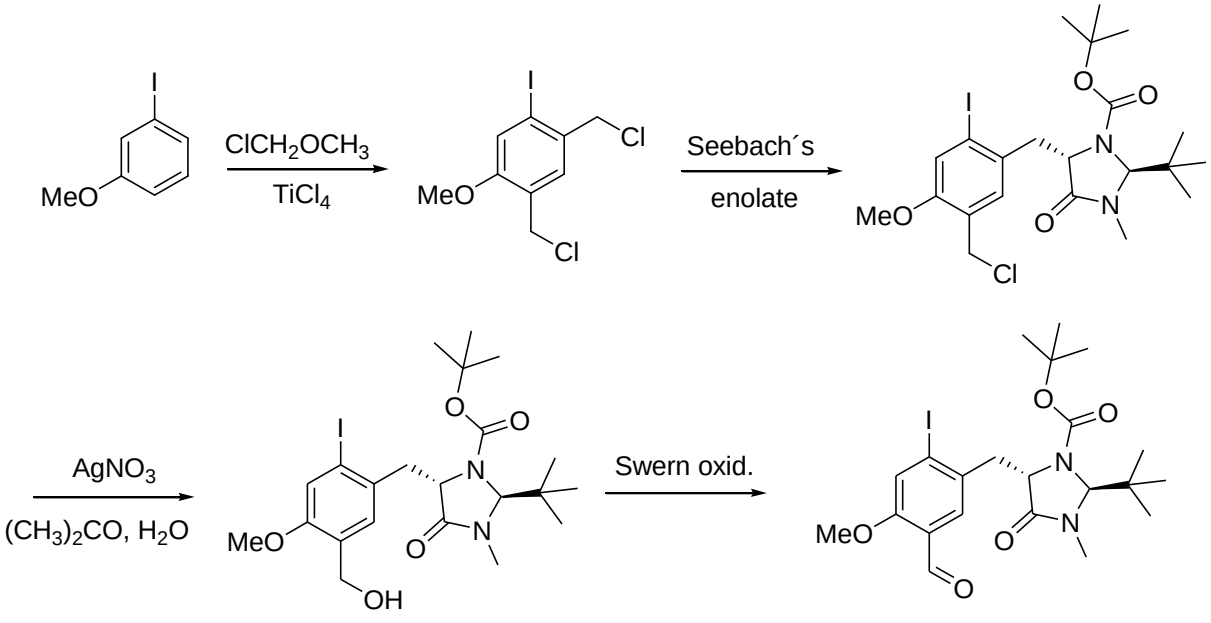
The synthetic route pursued by Tierling *et al.*[103] to attain this goal included the construction of the carbon skeleton of the phenylamino acid using the Schöllkopf's reagent (Scheme 23). By this way the chiral center with the desired configuration is built first, leaving the introduction of the activating formyl group for the last step. The disadvantage of this strategy is that the aromatic ring is not enough functionalized to allow an easy introduction of the activating formyl group. Moreover, the conditions of the reaction for the introduction of the formyl group are too harsh and probably led to decomposition of both starting material and products causing low yields.

On the other hand, Kuroda and coworkers[129] developed the synthesis of a labeling precursor choosing an imidazolidinone derivative as the masked amino acid functionality and iodide as the leaving group. The four-step synthesis starting from 3-iodoanisole furnishes the desired product with an overall yield of 18%. It is curious that, from the two chloromethyl groups in the first intermediate compound, the one adjacent to iodine is more reactive than the

other. However, in this procedure an appropriate substitution on the aromatic ring before the introduction of the chiral auxiliary led to a smooth conversion of the alcohol into the formyl group. Unfortunately, no ^{18}F-labeling results with this precursor have been reported so far.



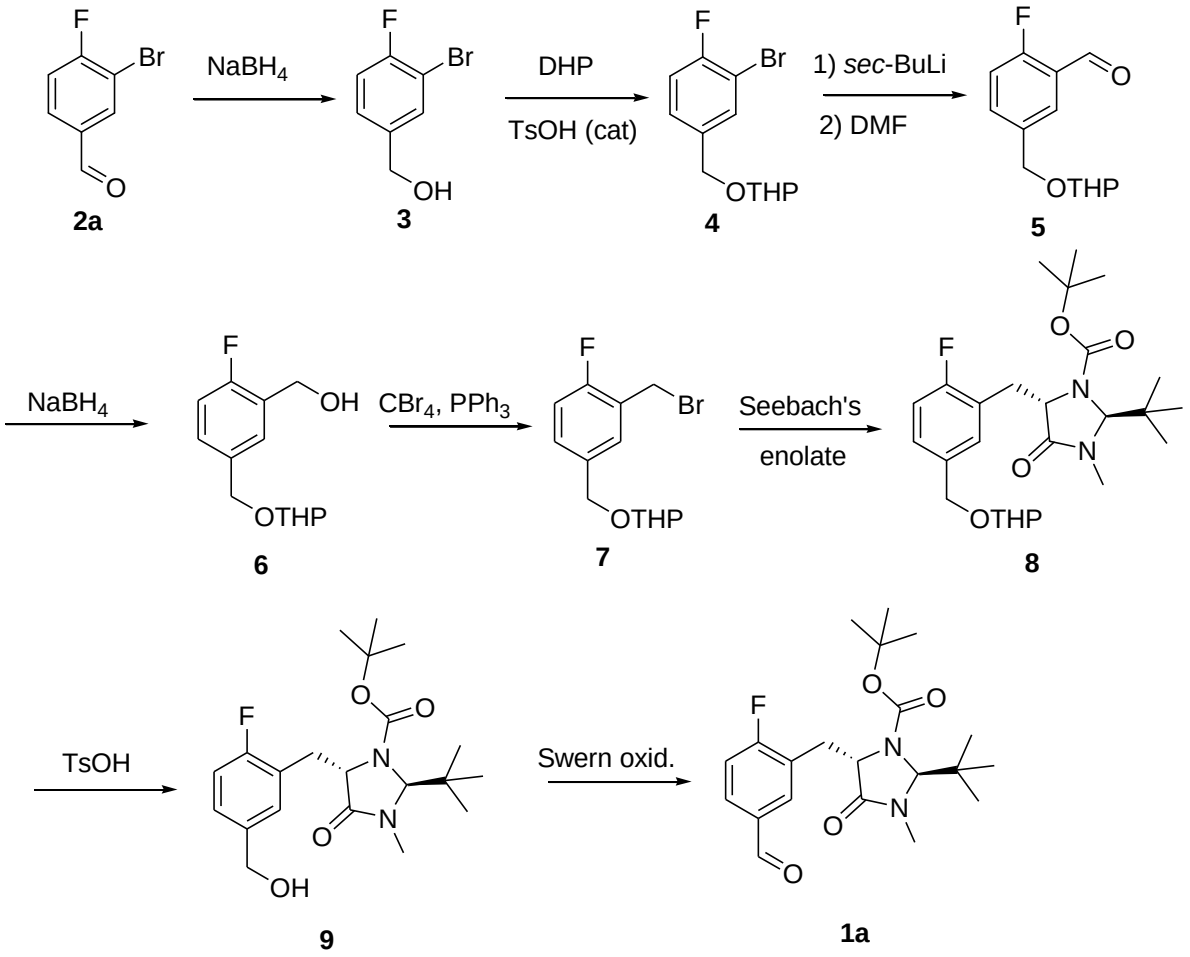
Scheme 23: Synthesis of "Schöllkopf"-precursor for the radiosynthesis of 6-[^{18}F]FDOPA by isotopic exchange according to Tierling *et al.*[103]



Scheme 24: Synthesis of "Seebach"-precursor for the radiosynthesis of 6-[^{18}F]FDOPA according to Kuroda *et al.*[129]

A further synthesis concept of Wagner *et al.*[105] integrates the advantages of the both concepts previously described. In the following paragraphs Wagner´s approach will be presented in detail for the example of the precursor **1a** and how a new and more efficient synthetic route was built here on these fundaments.

Compound **1a** was originally envisioned as potential precursor for the synthesis of 6-[^{18}F]Fmtyr,[130] due to its similarity with the 6-[^{18}F]FDOPA precursor, however, with a lower complexity, it became an important model compound. The optimized synthetic procedure for **1a** was supposed to be transferable to that of the 6-[^{18}F]FDOPA precursor **1b**.



Scheme 25: Synthesis of **1a**, precursor for the radiosynthesis 6-[^{18}F]Fmtyr by Wagner[130]

The eight-step procedure starts with the reduction of the carbonyl function of 3-bromo-4-fluorbenzaldehyde followed by the protection of the resulting alcohol as the tetrahydropyranylether (THP) derivative. Lithiation of the protected alcohol and quenching of the organometallic species with DMF yielded the *o*-fluorobenzaldehyde derivative **5**. The benzaldehyde was then reduced to the benzylalcohol with $NaBH_4$ and brominated with

CBr_4/PPh_3. Sequential treatment of the Seebach reagent ((*S*)-Boc-BMI) with lithium diisopropylamide and then with benzylbromide **7** led to the compound **8**. Acid deprotection of the THP group followed by a Swern oxidation of the resulting alcohol yield the precursor **1a** (Scheme 25).

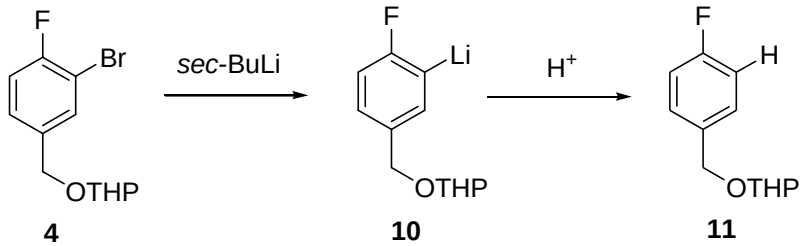
Although the precursor could be achieved using the described pathway, the overall yield was relatively low. The main cause was the low yields obtained in the formylation, bromination and alkylation steps which were lower than 30% for each of those.

3.1.2 Improvements of the existing synthetic procedure for precursors 1a and 1b

Improvement of the synthesis of precursor 1a

One of the goals of the present work was to optimize this synthetic pathway. To that purpose, a systematic study of these three critical reactions was performed.

According with the report of Wagner,[130] the formylation step yielded in average 26% of the desired product. This is a "one-pot two-step" reaction, starting with the formation of the lithiated species by the Br-for-Li exchange reaction. Then DMF is added and the reaction mixture warmed to room temperature. The TLC analysis of the crude reaction mixture besides a new spot belonging to the formylated product, showed a second one, which, due to the R_f value was presumed to be non-reacted starting material **4**. After chromatographic purification the supposed starting material was analyzed by ^{1}H-NMR. The analysis showed that this compound was the non-brominated derivative **11** and not **4** as assumed. This side compound is most probably generated by the hydrolysis of the organo-lithium species as depicted in Scheme 26.

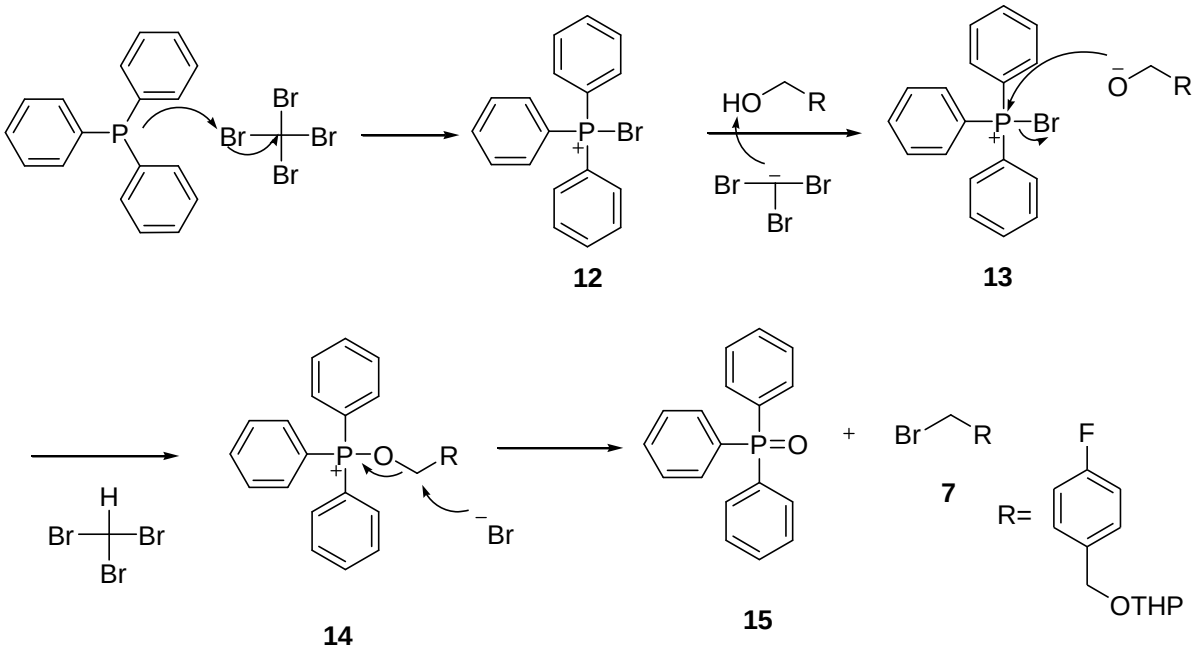


4 **10** **11**

Scheme 26: Side reaction of the formylation of **4** by lithiation yielding the non-brominated compound **11**

In order to avoid this side reaction, the water-free conditions were improved. Under strictly water-free conditions, the desired product **5** was obtained with a yield of 79%. This yield is in agreement with those previously reported for analogous compounds.[131]

Once the problems with the formylation were solved, the efforts were directed toward the optimization of the bromination reaction. Again here the yield was lower than 30%. The conversion of the benzylalcohol to the benzylbromide was earlier performed by Wagner *et al.*[105] using the Appel reaction.[132] This is a method for halogenation of alkylalcohols under mild conditions; however it can also be used for the bromination of benzylalcohols. The mechanism of the reaction involves the formation of a phosphonium salt pair **12**. Deprotonation of the alcohol, forming bromoform, yields an alkoxide ion pair. The nucleophilic displacement of the bromide by the alkoxide yields intermediate **14**. The bromide anion reacts by an S_N2 process forming the desired bromide **7** and triphenylphosphine oxide **15**. The formation of the stable oxide is the driving force of the reaction.[14]

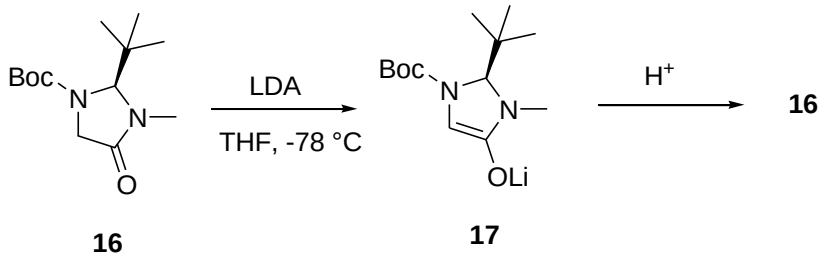


Scheme 27: Mechanisms of the Appel reaction for conversion of benzylalcohols into benzylbromides[132]

The reaction time and the temperature used by Wagner[130] were 0.5 h and 0 °C respectively. Under these conditions a complex mixture of products was obtained. In a first attempt to improve the yield, the temperature was increased to r.t. The experiment was not successful; a mixture of products with bigger complexity as before was obtained while the yield of the desired compound dropped to just 20%. Then, the conditions suggested by

Kocienski *et al.* [133] were applied. This time the reaction was cooled down to 0 °C, the triphenylphosphine was added portion wise and after the last addition the mixture was stirred for additional 5 min before quenching. The use of Kocienski experimental modification produced compound **7** in yields ranging from 51% to 64%. Additionally, exclusively the desired compound was formed, which facilitated the purification step. Despite the acceptable yield obtained in its preparation, the bromobenzyl derivative shows a further disadvantage, the compound is thermally unstable; particularly when a solution of it in chloroform of ethyl acetate was heated above 40 °C.

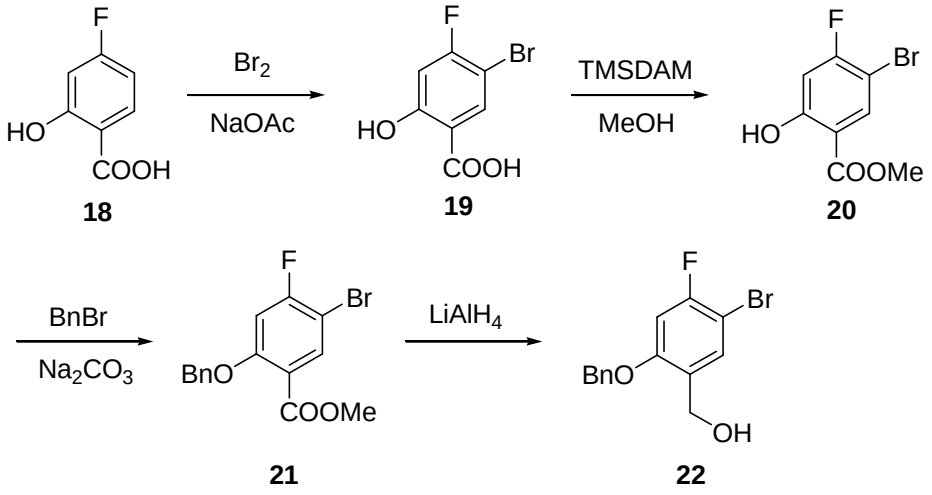
The next step of the optimization procedure was the alkylation of the benzylbromide **7** with the lithium enolate of the chiral auxiliary (*S*)-(-)-1-BOC-2-*tert*-butyl-3-methyl-4-imidazolidinone (Seebach's reagent) which before was producing compound **8** in low yield. It was observed that the conversion of the starting material, *i.e.* Seebach´s reagent, was very low and it could be recovered after chromatographic purification. The method, as well as in the case of formylation, is a "one-pot two-step" reaction which involved the formation of a nucleophilic species followed by quenching with an electrophile. Scheme 19 depicts both the formation of the Seebach´s enolate and its deactivation due to protonation. In order to increase the yield same measures to avoid moisture in the reaction medium were taken. Freshly prepared lithium diisopropylamide (LDA), product of the reaction of diisopropylamine and BuLi at -78 °C during 15 min, was used to generate the enolate derivative of Seebach reagent.[134] The use of commercially available LDA in contrast leads to poor yields (20-30 %). On the other hand, the synthesis of LDA at -78 °C provides better alkylation yields compared with LDA prepared at 0 °C. Under these conditions the yield of the coupling compound could be improved to 70%.



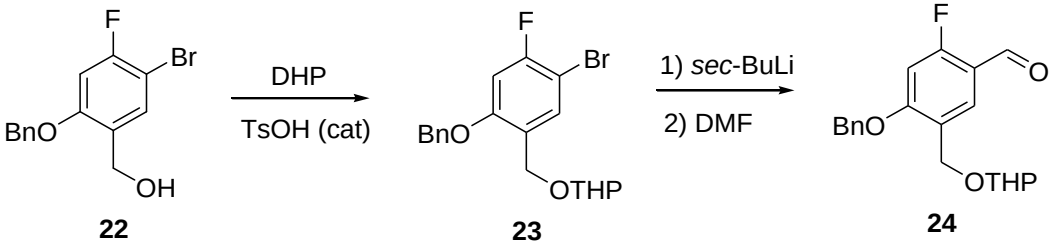
Scheme 28: Formation and side reaction of the enolate **17** with H^+.

Improvement of the synthesis of precursor 1b

Once the low yield reaction steps were optimized, the interest focused on the synthesis of the 6-[^{18}F]FDOPA precursor **1b**. Scheme 29 depicts the synthesis of the benzylalcohol **22** to be used as starting compound for the analogous synthesis (see Scheme 25, comp. **3**). The procedure starts with the bromination of 4-fluorosalicylic acid in ethanolic basic medium, followed by esterification with trimethylsilyldiazomethane (TMSDAM)/methanol. The phenol function was then protected as benzylether and finally the ester was reduced to the benzylalcohol with $LiAlH_4$, however, after the last reaction small amounts of the debrominated benzyl alcohol were also detected.



Scheme 29: Synthesis of the benzylalcohol **22** from 4-fluorosalicylic acid



Scheme 30: Synthesis of the aldehyde **24**

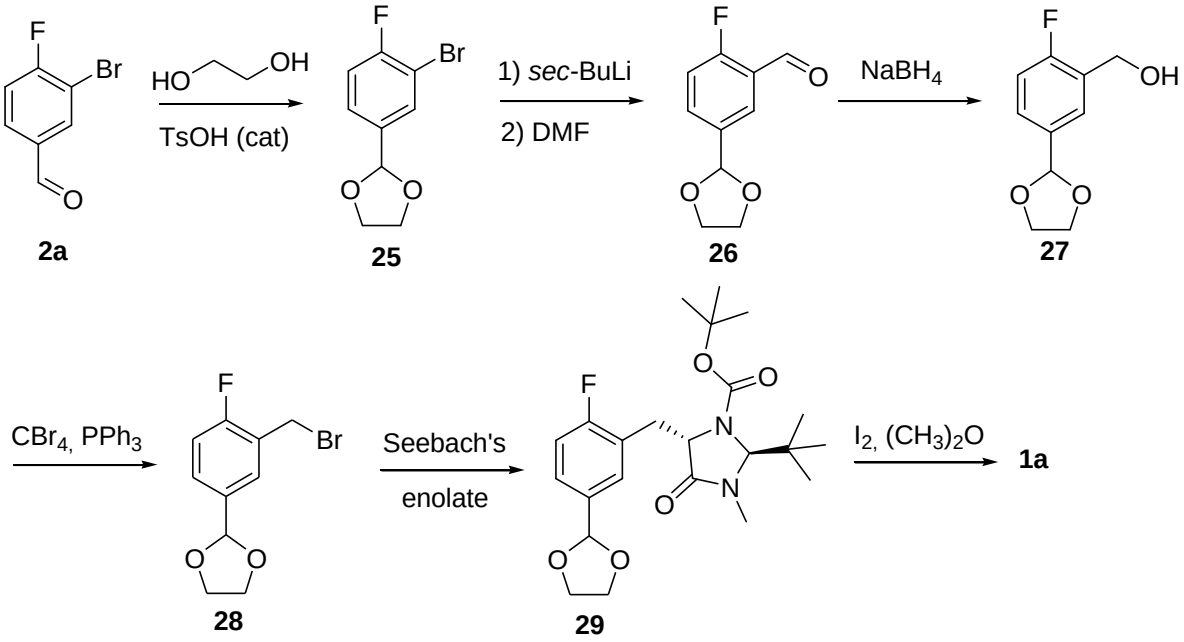
After the benzylalcohol was prepared, the intention was to follow the synthetic pathway as described by Wagner *et al.*[105] for compound **1a**. The alcohol **22** was protected without problems as tetrahydropyranylether (Scheme 30). However, the formylation reaction, although conducted under the optimized conditions, generated a complex mixture of products from where the desired compound was difficult to isolate. The yield of product **24** was 20%. Among others, the presence of different isomers of the desired product was observed which

demonstrates that the reaction was not regiospecific. After this drawback, efforts were focused to find an alternative milder and regioselective reaction.

3.1.3 New synthetic pathways for the preparation of labeling precursors

Synthesis of the precursor 1a via a dioxalane approach

Further experiments were performed in order to increase the efficiency of the synthesis of compound **1a** by shortening the number of steps involved. Although the sequence oxidation–THP protection as well as THP deprotection-reduction proceeds smoothly to afford the desired compounds in good yields, it also presents some drawbacks. First, the purification step of the protected alcohol **5** is complicate because of the desired compound and an impurity run closely together in the chromatography. Second, the deprotection reaction is time consuming; the reaction must be stirred overnight to afford the alcohol **9**. Finally in order to guarantee a high conversion yield of the alcohol to the carbonyl, fresh oxalyl chloride is needed. Otherwise the activation of the DMSO is not effective and a mixture of the desired carbonyl compound and the starting material is obtained.



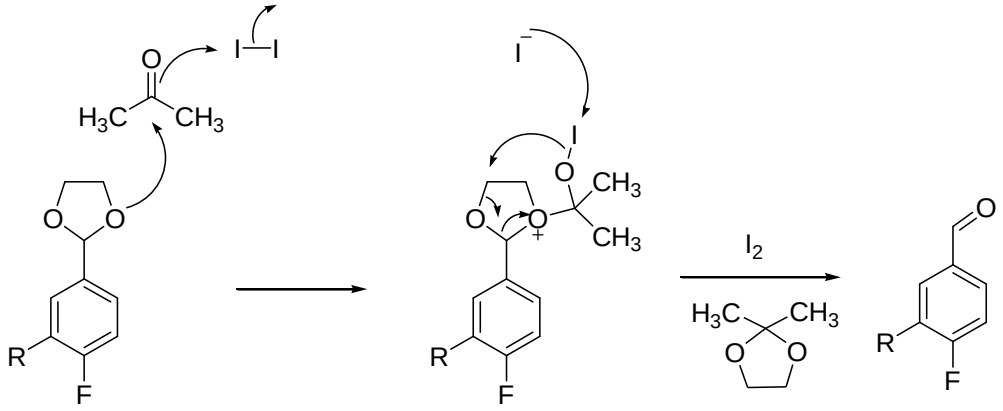
Scheme 31: Alternative synthesis of precursor **1a** via a dioxalane approach

Protection of the carbonyl group as the acetal derivative saves two synthetic steps. The acetal was prepared by condensation of 1,2-ethylene glycol with 3-bromo-4-fluorbenzaldehyde using toluene as solvent. Formylation via Br-Li exchange followed by

addition of DMF and subsequent reduction of the *o*-fluorobenzaldehyde **26** yielded the benzylalcohol **27** with a combined yield of 50% (Scheme 31).

Wagner explored also this strategy but was unsuccessful in achieving the bromobenzyl derivative **28**; however, the previous result obtained in this work for the Appel reaction encouraged us to try the conversion. Compound **28** was obtained with a yield of 64%. However, besides **28** the unprotected bromobenzylaldehyde was formed. The use of CBr_4 and PPh_3 has been pointed out as reagents for the hydrolysis of acetals.[135] In addition the bromobenzyl derivative **28**, analogously to compound **7**, evidenced thermal instability.

After coupling with Seebach reagent, the deprotection of the acetal was performed using the procedure proposed by Sun and coworkers.[136] The precursor **1a** was obtained in a yield of 94% after deprotection of **29** using catalytic amounts of I_2 in acetone under reflux conditions. A substrate exchange mechanism is proposed for this reaction. It is presumed that the deprotection initially involves a polarization of carbonyl group in acetone by molecular iodine. The possible mechanism is shown in the Scheme 32.



Scheme 32: Proposed mechanism for the removal of the acetal protecting group

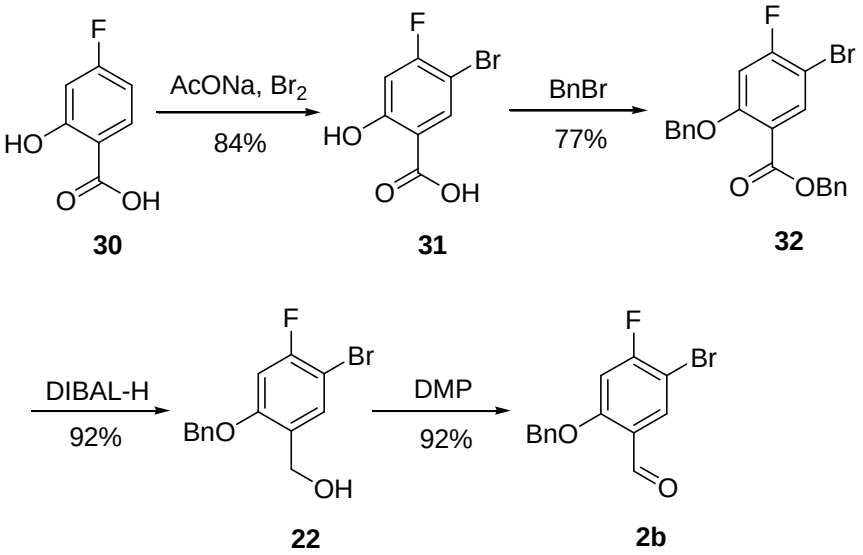
Synthesis of precursors 1a, 1b, 1c, and 1d via a dithiolane approach

Due to the success of the six-step approach for the synthesis of compound **1a**, it was decided to transfer this methodology to the preparation of the compound **1b** as well as to the novel (2*S*,5*S*)-*tert*-butyl-5-(5-acetyl-2-fluorobenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate **1c**, a promising precursor for the radiosynthesis of 6-[^{18}F]Fmtyr.

It was previously discussed that the use of the 1,3-dioxolane derivative showed moderate lability during the bromination reaction and besides of that, product **28** was not stable. In

order to prevent the incidense of these side reactions the protecting group was changed to the more stable 1,3-dithiolane.

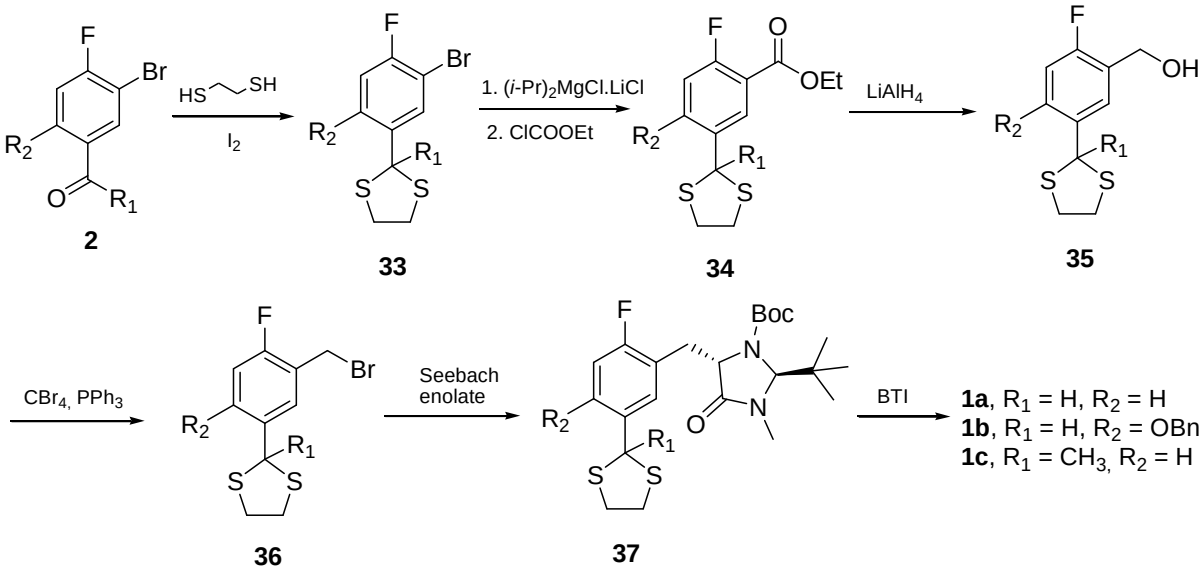
3-bromo-4-fluorobenzaldehyde **2a**, 2-(benzyloxy)-5-bromo-4-fluorobenzaldehyde **2b**, and 1-(3-bromo-4-fluorophenyl)ethanone **2c** were used as starting materials for precursors **1a**, **1b** and **1c**, respectively. The first and third are commercially available. The aldehyde **2b** was prepared as shown in scheme 24.



Scheme 33**:** Synthesis of 2-(benzyloxy)-5-bromo-4-fluorobenzaldehyde **2b**

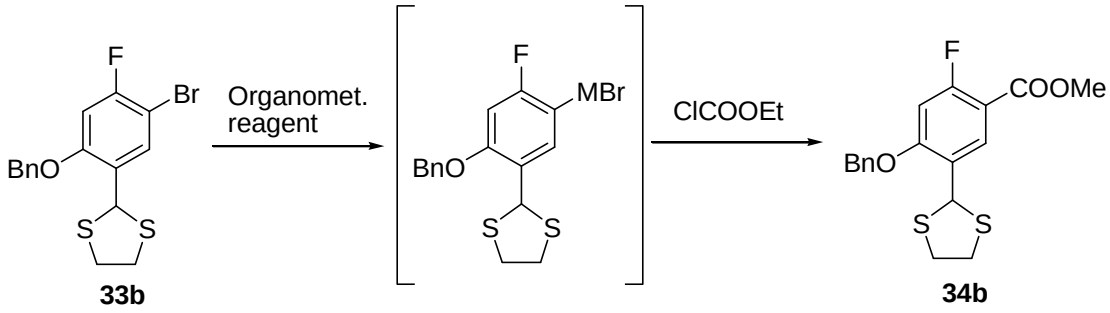
4-Fluorosalicylic acid was brominated in basic alcoholic medium using molecular bromine. The reaction produced the desired 3-bromo-4-fluoro-salicylic acid **30** in a yield of 82%. Benzylation of **31** with benzyl bromide in basic media yielded the compound **32** in 77%. The ester functionality of **32** was then reduced to the alcohol **22** with 92% yield using 2.1 equivalents of DIBAL-H. The first intention was to reduce the ester just to the corresponding aldehyde, however, after several trials using one equivalent of the reducing agent at different temperatures the result was always a mixture of 1:1 of the starting material and the benzyl alcohol derivative **22**. Red-Al-pyrrolidine, which has been earlier successfully employed in this kind of reduction,[137] produced similar results. Analogously as with compound **21** again here, the reduction of the ester to the alcohol using $LiAlH_4$, generates a mixture of the desired alcohol **22** plus the debrominated alcohol. However, oxidation of alcohol **22** with Dess-Martin periodinane (DMP) in dichloromethane at room temperature optimally provides exclusively the aldehyde **2b** in a yield of 92%.[138]

Scheme 34 resumes the general synthetic pathway for **1a**, **1b** and **1c**. As mentioned before, in the first step the carbonyl function was protected as 1,3-dithiolane derivative using the procedure described by Firouzabadi and coworkers.[139]



Scheme 34: General synthetic pathway to precursors **1a,b,c**

Due to the unsatisfactory results obtained for the formylation of **23**, compound **33b** was selected as substrate in order to find an alternative to the Br-for-Li exchange. Compound **33b** was reacted with $Li_2Cu(CN)Me_2$; a milder reagent than BuLi that has been used for the halogen-for-copper exchange reaction on aromatic aldehydes. In order to improve the homologation reaction, ethyl chloroformate was preferred as electrophile over DMF due to its higher electrophilic character (Scheme 35).



Scheme 35: Acylation reaction of **33b** with ethyl chloroformate

The reactions were performed following the procedure described by Kondo and coworkers.[140] When the Br-for-Cu exchange reaction was carried out at -40 ºC for 30 min the desired product **34b** was obtained in 15% yield. In spite of the low yield a positive aspect was that only the desired product was formed (Table 3, entry 1). Since the reaction showed the

desired regioselectivity, efforts were oriented to find optimal conditions for the conversion of compound **33b**. In a second attempt, the reaction time was extended to 1 h, but the desired compound was isolated again with a low yield only. When the exchange was conducted at r.t. no formation of the product was detected by TLC of the crude reaction mixture. This is probably due to decomposition of the organometallic complex because of the high temperature (Table 2, entries 2-3). An alternative copper reagent is $Neopent_2CuLi$, which has earlier been successfully used for the Br-for-Cu exchange reaction with diethyl 2-bromoterephthalate.[141] When $Neopent_2CuLi$ was reacted with **33b** at r.t. for 1 h and subsequently treated with ethyl chloroformate the desired product **34b** was obtained with a yield of 5% (Table 3, entry 4). The formation of lithiumcuprates through Br-for-Cu exchange proved to be regioselective, however, the conversion and therefore the yield of the desired compound were low. Generally, the exchange I-for-Cu is preferred over the Br-Cu exchange since iodine is a better leaving group than bromine for this kind of reactions. Based on these results a different option had to be explored since Li-for-Br exchange using *sec*-BuLi proved to be too reactive, while the Br-for-Cu exchange was too mild.

Table 2: Optimization of the reaction conditions for the acylation of **33b** with ethyl chloroformate

Entry	Reagent	Time h	Temp °C	Yield %
1	$Li_2Cu(CN)Me_2$	0.5	-40	15
2	$Li_2Cu(CN)Me_2$	1	0	5
3	$Li_2Cu(CN)Me_2$	0.5	r.t.	0
4	$Neopent_2CuLi$	1	r.t.	5
5	i-PrMgCl.LiCl	1	0	54
6	i-PrMgCl.LiCl	1	r.t.	30
7	i-Pr_2MgCl.LiCl	1	0	77

Krasovskiy and coworkers reported that *i*-PrMgCl·LiCl can be used for high yield preparations of functionalized arylmagnesium reagents starting from aryl bromides.[142] The best yield (54%) using this reagent was obtained when the Br-for-Mg exchange reaction was carried out at 0 ºC for 1 h (Table 2, entry 6). The addition of dioxane to a solution of *i*-PrMgCl·LiCl in THF displaces the anionic Schlenk equilibrium forming diisopropylmagnesium chloride lithium chloride complex (*i*-Pr_2MgCl·LiCl), this complex has shown exceptional reactivity for the conversion of aryl bromides into the corresponding

Grignard reagents.[143] Using *i*-$Pr_2MgCl{\cdot}LiCl$ under the conditions described before, compound **11** was achieved in 77% yield (Table 2, entry 7). Attempts to prepare the benzyl alcohol **12** directly by means of coupling of organo-magnesium species with *para*-formaldehyde provided the desired compound in poor yields of 10–20%.

Reduction of **34** with $LiAlH_4$ provided the benzyl alcohol **35** with around 92% yield after 1 h at room temperature. The conversion of the benzyl alcohol to the benzyl bromide was performed using the Appel reaction with good yields.[144] The stability of this group of benzyl bromides has been a problematic issue. Although the dioxalane protecting group should impart more stability, immediate decomposition was also observed when solutions of the benzyl bromide in dichloromethane or ethyl acetate were heated over room temperature. In order to avoid this problem, the reaction solvent CH_2Cl_2 was partially evaporated under *vacuo* without application of external heating.

After optimization, the alkylated product **37** was obtained in a yield range of 75-89 %. Deprotection of the 1,3-dithiolane following the procedure described by Languille and coworkers using Dess-Martin periodinane (DMP) regenerates the carbonyl functionality to afford the desired series of compounds **1** with yields of around 80%.[145] However, when commercial available DMP was used the reaction takes 3 h to be completed while with freshly prepared DMP only 0.5-1 h was required. Further experiments showed that the use of commercially available [bis(trifluoroacetoxy)iodo]benzene (BTI) generates the desired compounds **1** within 10 min in 91-93% yield.[146]

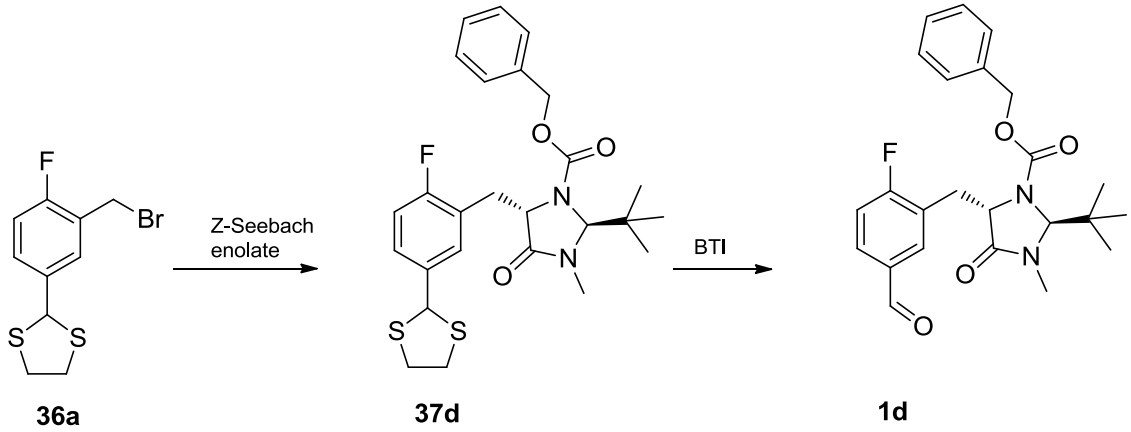
In summary, the synthesis of **1b** has been optimized. The 10 steps procedure achieved the desired compound with an overall yield of 19%. In addition, the derivatives **1a** and **1c** have been synthetized in 6 steps with overall yields of 41% and 48%, respectively. Table 3 lists the yields of the reaction steps to fluorobenzyloxoimidazolidinone derivatives **1a**, **1b** and **1c**.

Using the same synthetic strategy, and with the aim of studying the steric effect of bulky protecting groups over the RCY and the enantiomeric purity of 2-[^{18}F]Fphe, a precursor was prepared bearing a benzyloxycarbonyl protecting group instead of a *tert*-butoxycarbonyl (Boc) in position 1 of the imidazolidinone system. Scheme 36 depicts the synthesis of **1d**. For this purpose compound **36a** was coupled with the lithium enolate of the (*S*)-benzyl 2-tert-butyl-4-oxoimidazolidine-1-carboxylate ((*S*)-Z-BMI) in 78% yield and afterwards deprotected with BTI in order to get the desired compound in 90%.

Table 3: Yields of the reaction steps to fluorobenzyloxoimidazolidinone derivatives **1a**, **1b** and **1c**

Compound	a (%)	b (%)	c (%)
33	97	96	95
34	81	77	81
35	91	90	91
36	74	74	84
37	84	75	89
1	93	92	91
Overall yield	41	34[a]	48

[a]Including the synthesis of **2b** the overall yield for **1b** is 19 %.

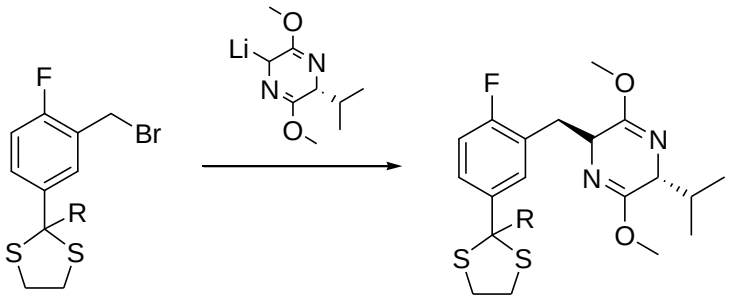


Scheme 36: Synthesis of precursor **1d** from compound **36a**

Synthesis of Schöllkopf-precursors 1e and 1f via a dioxalane approach

The previously developed synthetic approach provided not only the envisioned precursors **1** but also constitutes the basis for the preparation of other interesting derivatives. Compounds bearing Schöllkopf´s chiral auxiliary are also of interest since the hydrolysis can be achieved using milder conditions than those needed for Seebach´s derivatives. Moreover, this kind of labeling precursors has not been fully evaluated yet due to the lack of a reliable synthetic procedure.

The alkylation of the benzyl bromides **36a** and **36c** with Schöllkopf's derivatives generates the compounds **37e** and **37f**, respectively (Scheme 37).



36a, R= H
36c, R= CH_3

37e, R= H
37f, R= CH_3

Scheme 37: Alkylation of compounds **36** with Schöllkopf´s chiral auxiliary

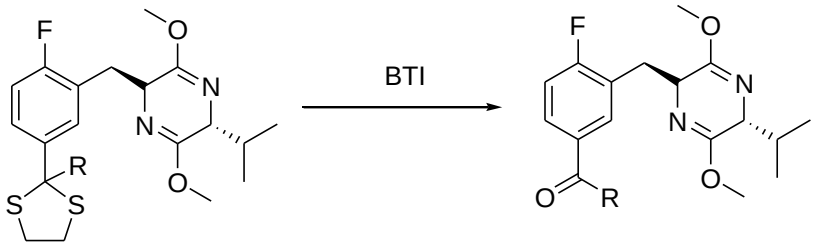
Attempts to cleavage the 1,3-dithiolane protecting group of **37f** using DMP, as it was described above for Seebach´s derivatives, were unsuccessful. Although the spot corresponding to the desired compound **1f** was seen in the TLC analysis of the reaction mixture after 10 min, it disappears with the advance of the time before the starting material is consumed. It seems that under these oxidative conditions the decomposition of the bislactimether occurs faster than the cleavage of the 1,3-dithiolane functionality. Further experiments following different procedures described in the literature were performed in order to achieve the desired deprotection. The reaction conditions and results are summarized in Table 4.

Table 4: Conditions and yields for the cleavage of the 1,3-dithiolane functionality of **37e**

Entry	**Solvent**	**Reagent[ref]**	**Temp ºC**	**Time min.**	**Yield %**
1	CH_2Cl_2	DMP[145]	r.t.	40	0
2	DMSO	I_2[147]	100	50	0
3	EtOH:H_2O	$AgNO_3$[148]	50	20	0
4	ACN:H_2O (3:1)	KBr, Oxone[149]	r.t.	60	0
5	ACN:H_2O (3:1)	KBr, Oxone[149]	r.t.	20	2
6	CH_2Cl_2	NBS+DMSO[150]	r.t.	20	0
7	CH_2Cl_2	*m*-CPBA+TFA[151]	r.t	30	0
8	ACN:H_2O (9:1)	BTI[146]	r.t.	10	70

The best results were achieved when BTI was used. The desired compound **1f** was isolated in 70% within 10 min reaction time. Analogously the aldehyde **1e** was produced with

76% yield (Scheme 38). These results were the reason why BTI was also tried for the deprotection of the precursors bearing Seebach´s chiral auxiliary.



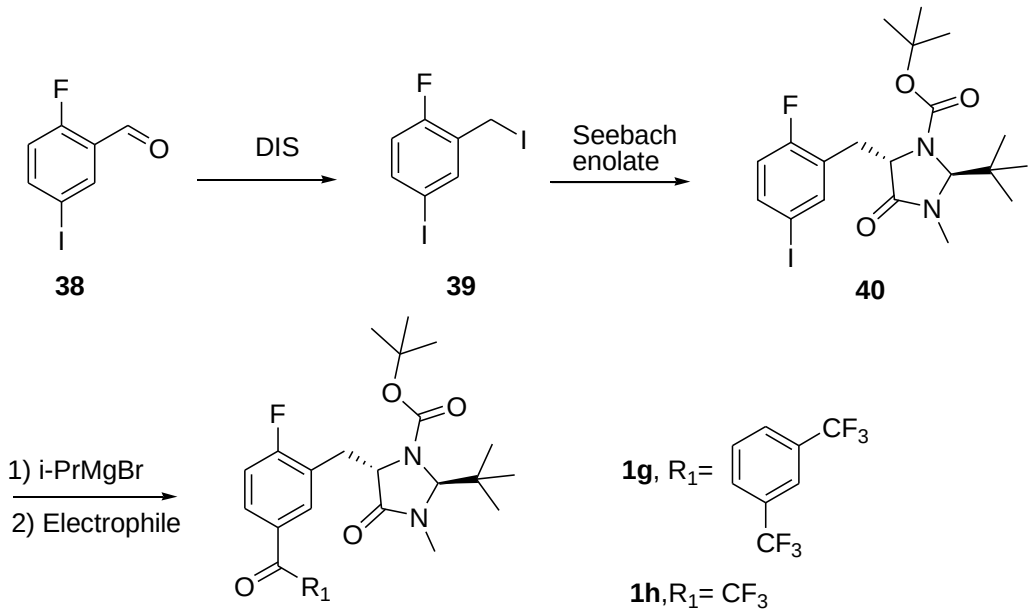
37e, R= H
37f, R= CH_3

1e, R= H
1f, R= CH_3

Scheme 38: Cleavage of the 1,3-dithiolane functionality of **37e** and **37f** with BTI

Synthesis of fluorophenone derivatives of Seebach-precursors 1g and 1h via a Grignard approach

As described before the synthesis of the precursor **1c** was performed starting from the commercially available 4-fluoro-3-bromoacetophenone in a six-step synthetic procedure with an overall yield of 46%. Due to the unavailability of analogues ketones derivatives and the success obtained with the halogen-for-magnesium exchange reaction a novel three-step synthetic pathway for precursors **1g** and **1h** was developed (Scheme 39).



38 **39** **40**

Scheme 39: General synthetic pathway to precursors **1g** and **1h**

The commercially available 2-fluoro-5-iodobenzaldehyde **38** was treated with an excess of diiodosilane (DIS) in order to produce the diiodo derivative **39**.[152] Using commercial DIS,

total conversion of the starting material was achieved after 8 h and 4 equivalents of DIS were needed. The use of freshly prepared DIS reduced both, the reaction time to 1.5 h and the amount of reagent needed to 1.5 equivalents.[153] In both cases the isolated yield of compound **39** was about 80%.

Freshly prepared lithium diisopropylamide (LDA) was used to generate the enolate derivative of Seebach´s reagent. This was coupled with compound **39** and the alkylated product **40** was obtained in 81% yield.

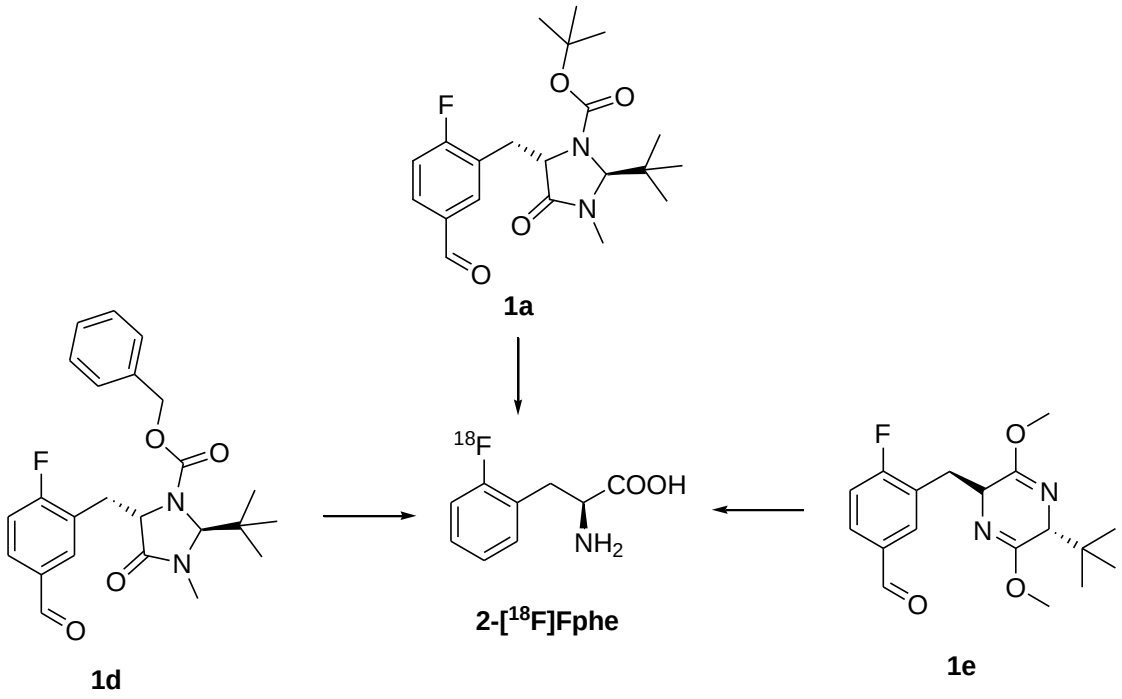
Finally, the precursor **1g** was obtained using a "one-pot two-step" reaction. In the first step compound **40** was reacted with *i*-PrMgBr in order to generate the magnesium derivative. The magnesium derivative was then acylated with 3,5-bis(trifluoromethyl)benzoyl chloride. The precursor **1g** was obtained with 84% yield. Following an analogous procedure, the organo-magnesium derivative was reacted alternatively with trifluoroacetic anhydride. In this case, the precursor **1h** was obtained with 62% yield.

3.1.4 Conclusions

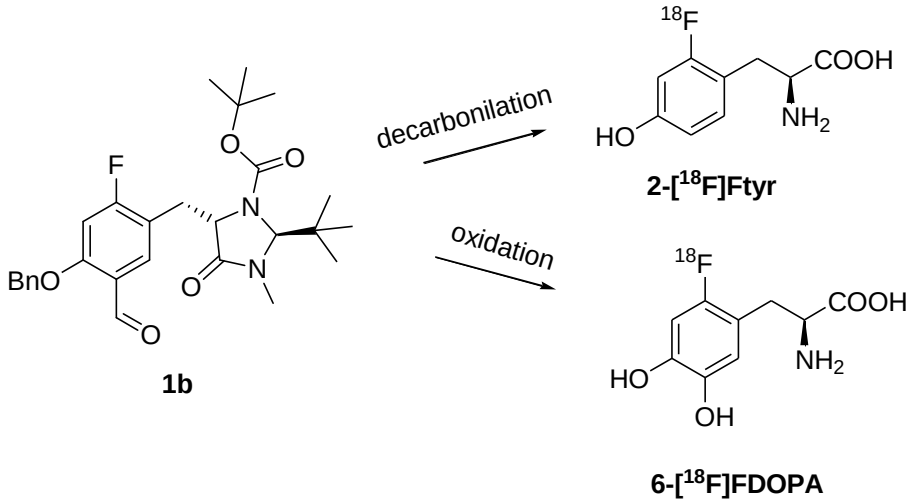
Two efficient synthetic approaches have been developed for the preparation of highly functionalized fluoro-benzaldehydes and ketones. The first approach involves 6 steps and is an optimized modification of the synthesis pursued by Wagner *et al*.[105] for the compounds **1a** and **1b**, in which two steps for **1a** and one for **1b** have been saved and the overall yields considerably increased from 4% for **1a** and 1% for **1b** to 41% and 19% respectively. The second approach is a novel three-step synthesis, which here has been used for the preparation of ketone precursors **1g** and **1h**. However, this attractive method can probably also be applied for the synthesis of aldehydes.

In total eight different precursors have been prepared and four [^{18}F]fluorophenylamino acids are supposed to be achievable from them. Precursors **1a**, **1d** and **1e** will be used for the radiosynthesis of 2-[^{18}F]F-phe via a decarbonylation reaction (Scheme 40).

As depicted in Scheme 41, the versatile precursor **1b** will be employed for the radiosynthesis of 2-[^{18}F]Ftyr via a decarbonylation reaction as well as for the radiosynthesis of 6-[^{18}F]FDOPA via Baeyer-Villiger oxidation.

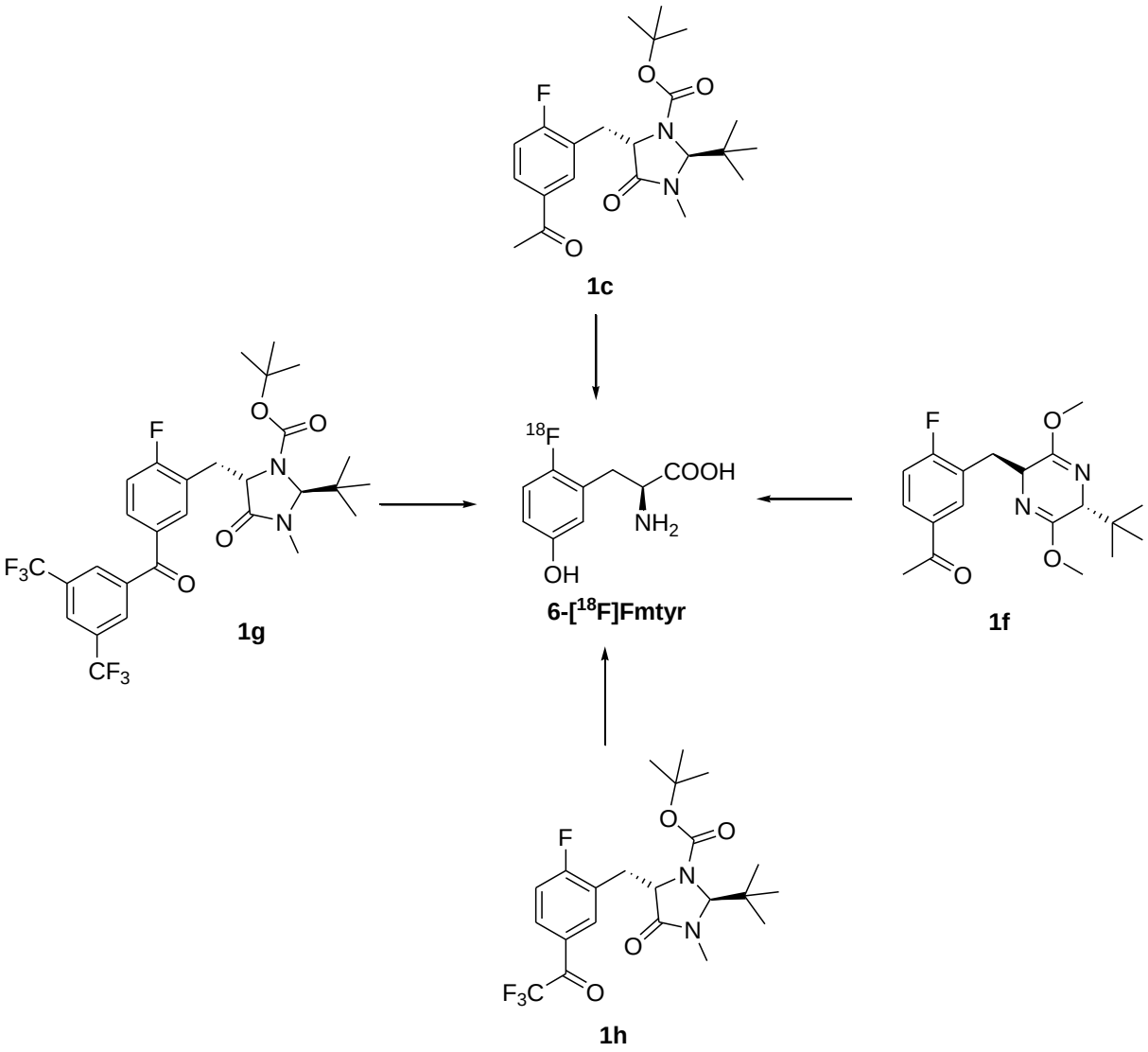


Scheme 40: Precursors **1a**, **1d** and **1e** for the radiosynthesis of 2-[^{18}F]Fphe



Scheme 41: Precursor **1b** for the radiosynthesis of 2-[^{18}F]Ftyr and 6-[^{18}F]FDOPA

The last four precursors enable the radiosynthesis of 6-[^{18}F]Fmtyr. It will be possible to comparatively evaluate the influence of the activating group in the labeling reaction as well as the influence of the chiral auxiliary in the enantiomeric purity (Scheme 42).

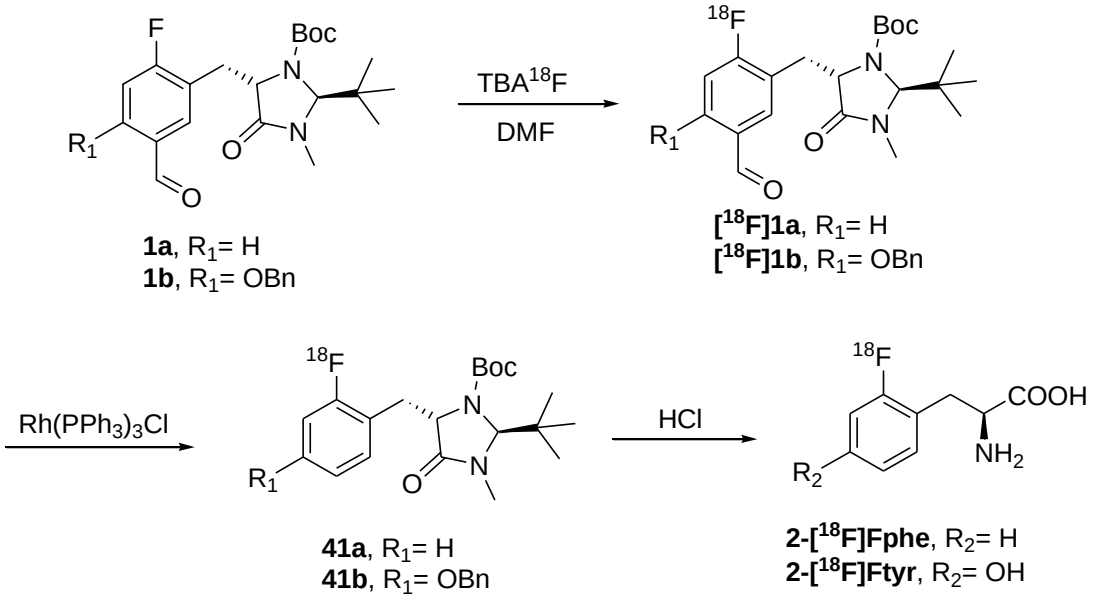


Scheme 42: Precursors **1c**, **1f**, **1g** and **1g** for the radiosynthesis of 6-[^{18}F]Fmtyr

3.2 Enantioselective radiosynthesis of 2-[^{18}F]fluoro-L-phenylalanine and 2-[^{18}F]fluoro-L-tyrosine by isotopic exchange

As mentioned in the introduction of this work, 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr are two radiopharmaceuticals that may be useful in the evaluation of the brain functions and have potential as diagnostics for tumors. In spite of the potential of these compounds the lack of a convenient radiosynthetic methodology for their preparation has limited a wider use in nuclear medicine practice.

The radiosynthetic approach pursued in this section is based on the procedure followed by Wagner *et al.*[105] for the synthesis of 6-[^{18}F]FDOPA. Scheme 43 depicts the radiosynthetic pathway. Different to the previous radiosynthesis a major modification has been done in the second step where instead of the Baeyer-Villiger oxidation a decarbonylation reaction using $Rh(PPh_3)_3Cl$ was performed, in order to obtain the target compounds.

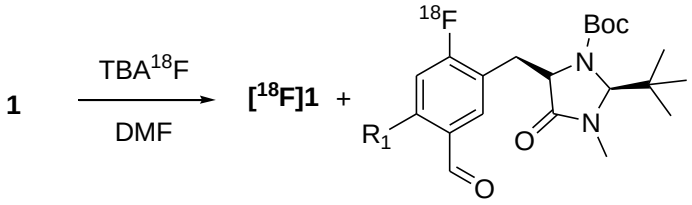


Scheme 43: General radiosynthetic pathway to 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr.

3.2.1 Radiofluorination

The first step of the radiosynthetic concept pursued here for labeling of the title compounds is an isotopic ^{18}F-for-^{19}F exchange. Previous work on nucleophilic aromatic ^{18}F-substitution of benzaldehydes using both, model compounds and complex precursors, have shown that the use of DMF as solvent and temperatures above 100 °C gave the best radiochemical yields.[105,154] Table 5 summarizes the results obtained with the optimization of

the labeling conditions for precursor **1a** under conventional heating. Entries 1 to 4 show that an increase of the concentration of the basic tetra-*n*-butylammonium hydrogen carbonate ($TBAHCO_3$) as anion activator led to higher labeling yields; but also the formation of an additional new product was evidenced by both TLC-UV and radio-TLC analyses. In order to identify the side product a cold experiment was carried out and the new compound was isolated and analyzed by NMR. The ^1H spectrum revealed that the new species was the diastereomer **42a** (Scheme 44), resulting from epimerization in position 5 of the imidazolidinone system. An increase of the temperature to 150 ºC (entry 5) did not provide a better RCY but a diastereomeric 1:1 mixture of the compounds **[^{18}F]1a** and **42a** within 10 min. Standard ^{18}F-labeling conditions using Kryptofix 2.2.2[25] (entry 6) provided the undesired diastereomer **42a** even as major product.



Scheme 44: Epimerization of **1a,b** during the isotopic exchange reaction.

Table 5: Influence of temperature, time and kind of anion activation (PTC) on the RCY of the isotopic exchange reaction on **1a**[a,b]

			10 min		20 min	
Entry	**PTC[c] [µmol]**	**Temp. °C**	**[^{18}F]1a (%)**	**42a (%)**	**[^{18}F]1a (%)**	**42a (%)**
1	$TBAHCO_3$ [2.3]	130	28	0	39	0
2	$TBAHCO_3$ [5.2]	130	51	6	57	7
3	$TBAHCO_3$ [8.5]	130	60	10	59	14
4	$TBAHCO_3$ [17.0]	130	61	17	52	30
5	$TBAHCO_3$ [5.2]	150	26	24	-	-
6	$[K222]_2CO_3$ [13.0]	130	26	40	14	50

[a]SD = ±5%. [b]1 mL DMF, 15 µmol **1a**, conv. heating (oil bath). [c]PTC = phase transfer catalyst for [^{18}F]fluorine activation.

Based on these results, the conditions described in entry 2 of Table 5 were selected for further experiments for the labeling of precursor **1a** under conventional heating.

Similarly, the isotopic exchange reaction with precursor **1b** generates the products **[^{18}F]1b** and **42b** following the trends previously described for the compound **1a**, *i.e.* the fluorine exchange yield increases with the increase of both, the concentration of the base and of the temperature, in detriment of the radiochemical purity. The best RCY of **[^{18}F]1b** was 59% while that of **42b** was 4%. The optimal results were obtained in this case under identical conditions as given in entry 2, besides that 6.4 μmol of $TBAHCO_3$ were employed.

It was also observed that the labeling reaction of precursors **1** is very sensitive to humidity. The RCY dropped dramatically when not freshly dried solvents (acetonitrile for TBA^{18}F drying process and DMF for labeling) were used. This phenomenon can be caused by formation of relative high concentrations of H^{18}F if, due to the low concentration of the base, the nucleophilic halide species cannot be effectively regenerated. However, deactivation of the base by neutralization not only led to the observed decrease of the RCY of the desired products [^{18}F]**1** but also the occurrence of diasteromers **42** was decreased.

In an attempt to improve the isotopic exchange process the reaction was performed using microwave heating. Microwave irradiation produces efficient internal heating by direct coupling of microwave energy with the molecules that are present in the reaction mixture creating an inverted temperature gradient compared to conventional thermal heating.[155] Due to these characteristics microwave heated reactions are faster and in some cases more selective and than those under conventional heating.

Figure 7 shows the RCY of compounds **[^{18}F]1a** and **42a** in dependence of the applied microwave power. It can be seen that the total RCY increases with power, however at above 45 W the undesired diastereomer **42a** starts to appear and its yield increases also with elevated power.

Table 6 compares the optimized results obtained with the synthesis of **[^{18}F]1a** and **[^{18}F]1b** under conventional and microwave heating. It can be noticed that either conventional or microwave heating produce a rather similar RCY. The main difference is the reaction time, where the microwave heated reaction is ten times faster than that with heating by oil bath. Increasing the RCY of the compounds **[^{18}F]1**, by extending the reaction times is limited due to the occurrence of the diastereomers **42.**

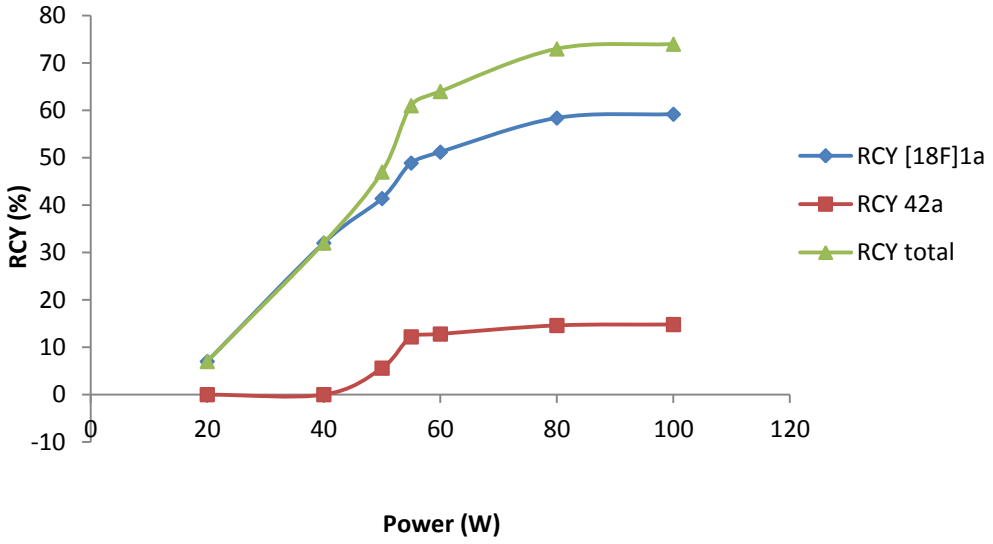


Figure 7: Dependence of the RCY of **[^{18}F]1a** and **42a** as function of the microwave power. 15 µmol **1a**, $TBAHCO_3$ 5.1 µmol, 1 mL DMF, 1 min

Table 6: Optimized RCY of labeling of **1a,b** by isotopic exchange using both conventional and microwave heating[a]

Precursor	Heating[b]	Time (min)	[^{18}F]1 (%)	4 2(%)
1a	CH	10	51	6
1a	MH	1	42	5
1b	CH	10	59	4
1b	MH	1	53	4

[a]SD = ±5% (CH), ±1% (MH). [b]CH: conventional heating (oil bath), MH: microwave heating.

3.2.2 Decarbonylation

The removal of the activating formyl group (Scheme 43) was performed with $Rh(PPh_3)_3Cl$ (Wilkinson´s catalyst). Using this reagent for decarbonylation, however, a stoichiometric reaction is required due to the formation of stable $Rh(PPh_3)_2(CO)Cl$ as secondary product. Optimization studies were carried out using compound **[^{18}F]1a** as starting material under similar conditions as those described by Plenevaux and co-workers for the decarbonylation of [^{18}F]fluorobenzaldehydes.[156] Dioxane was used as solvent and the reaction solution was magnetically stirred for 20 min in an oil bath at 150 ºC. Since the labeled

compound **[^{18}F]1a** is chemically indistinguishable from the precursor **1a** they can not be separated and the amount of the Rh complex was calculated as the molar equivalent of **1a**.

Figure 8 graphically depicts the obtained results. Using equimolar amounts of starting material and organometallic reagent 64 ± 5% of the desired compound **41a** was observed in radio-HPLC analysis (see experimental, System A) as well as 21 ± 7% of the starting material **[^{18}F]1a** and 15 ± 3% of an unknown compound. Increasing the amount of Wilkinson`s catalyst to 1.5 eq improved the conversion of the starting material to 99% and a RCY of 85 ± 8% of the desired compound **41a** (Table 7, entry 1). Further increase of the amount of catalyst did not cause any improvement, in constrast augmented the amount of the side product.

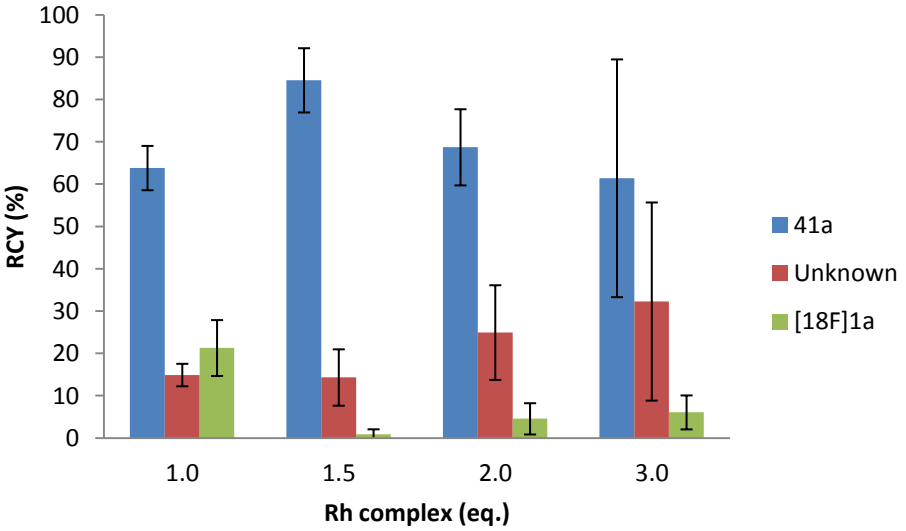


Figure 8: Radioactivity distribution of **[^{18}F]1a**, **41a** and an unknown compound as function of the amount of Rh complex. 15 µmol **[^{18}F]1a**, 1 mL dioxane, 20 min

In 2007 Shen and coworkers published a study dealing with the decarbonylation reaction of poly-substituted [^{18}F]fluorobenzaldehydes with $Rh(PPhe_3)_3Cl$.[11] Also there the formation of an unknown polar compound was observed besides the desired product. They found a relationship between the occurrence of the side product and the kind of solvent, pointing out benzonitrile as the medium of choice for this reaction. In fact using benzonitrile as solvent at 150 °C for the decarbonylation of **[^{18}F]1a** none of the unknown compound was detected while a mixture of the starting material and the desired compound **41a** in 40:60 proportion was provided after 20 min reaction time (Table 7, entry 2). In order to improve the conversion, the temperature was raisen to 180 °C. This time 15% of the unknown compound was detected

while the conversion of **[^{18}F]1a** was still incomplete (Table 7, entry 3). On the other hand, the use of DMF as solvent produced the unknown compound as major product (Table 7, entry 4).

Table 7: Solvent effect on the RCY of the decarbonylation reaction on **[^{18}F]1a**[a,b]

Entry	Solvent	Rh eq.	Temp °C	Unkn (%)	[^{18}F]1a (%)	41a (%)
1	Dioxane	1.5	150	14	1	85
2	Benzonitrile	2.0	150	-	40	60
3	Benzonitrile	2.0	180	15	18	67
4	DMF	2.0	150	62	16	22

[a]SD = ±5%. [b]15 µmol **[^{18}F]1a**, conventional heating, 20 min

In further experiments using dioxane as solvent was observed that a reaction time shorter than 20 min decreases the conversion yields of **[^{18}F]1a** while a longer reaction time increased the yield of the unknown product. Thus, the overall best conditions for decarbonylation were those ones described in entry 1 of the Table 7 with an almost quantitative conversion of **[^{18}F]1a** and in total only 15% of other labeled compounds. **41a** could easily be separated by use of a silica gel column and a solvent mixture of ethyl acetate in petroleum ether (30%) while the polar unknown compound remained attached on the stationary phase.

A microwave heated version of the decarbonylation reaction was also developed. In this case compound **[^{18}F]1b** was used for the optimization procedure and benzonitrile was preferred over dioxane as solvent since it shows better absorption of microwaves. The reaction was irradiated with 100 W during 50 s. Figure 9 shows the distribution of radioactivity among products formed as function of the amount of Wilkinson's catalyst. In contrast to the conventionally heated reaction both, the conversion of **[^{18}F]1b** and the yield of the desired compound **41b**, increased with the amount of the Rh complex.

This could be transferred to compound **1a** and when using 4 equivalents of the Rh complex the desired compounds **41a** and **41b** were obtained in yields of 83 ± 1% and 81 ± 1%, respectively. Although neither the yields nor the selectivity of the microwave heated reactions were better than those via conventional heating the reproducibility and especially the reaction time was again considerably improved by the change to microwave heating. Analogously as with conventional heating, here again an optimal reaction time (50 s) was found, with lower RCY at shorter times but increased formation of the unknown product above 50 s.

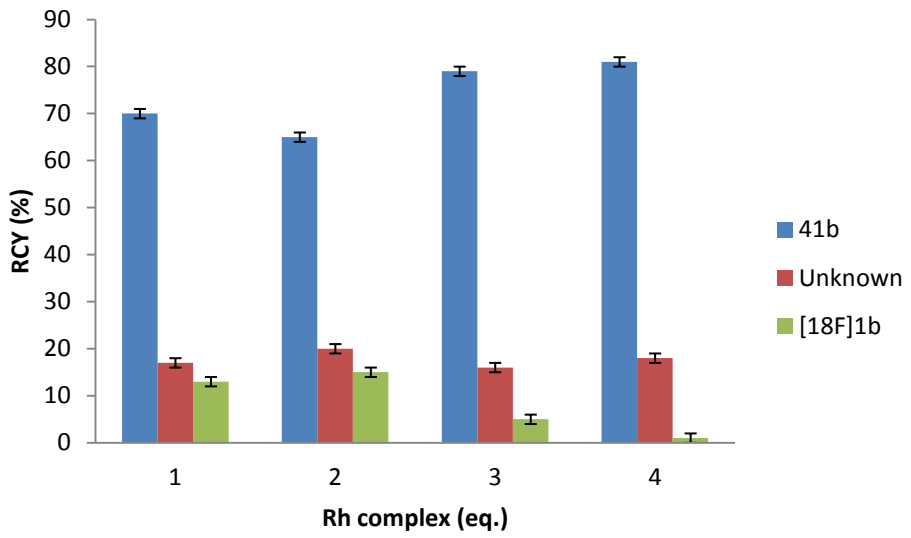


Figure 9: Radioactivity distribution of **[^{18}F]1b**, **41b** and unknown compound as function of the amount of Rh complex. 15 µmol **[^{18}F]1b**, 1 mL benzonitrile, 50 s

The decarbonylated products were purified using a silica gel cartridge. Due to the higher boiling point of benzonitrile, the solvent was not evaporated, in case of microwave heating, but the reaction mixture rather diluted with 5 % ethyl acetate in petroleum ether and then passed via syringe through the column where the reaction products were retained. A second portion of the same solvent mixture was used in order to assure the total elution of the benzonitrile. The desired compound **41b** was then eluted with a more polar solvent mixture as described above for the elution of **41a**.

3.2.3 Hydrolysis

In previous reports the hydrolysis of imidazolidinones has been carried out with concentrated HI or HBr and temperatures of 200 ºC and 150 ºC, respectively.[105,157] The need of such harsh conditions is due to the high stability of the amide bond.[158] In this work here hydrolysis with all typical mineral acids (HI, HBr and HCl) was performed resulting in quantitative hydrolysis yields at 190 ºC during 30 min no matter which acid was used. Several experiments were performed in order to achieve satisfying results with diluted acids, *e.g.* 1.0 to 7.5 N HCl, however, they were unsuccessful. Therefore for further experiments less hazardous concentrated HCl was used.

The microwave heated version of this reaction gave also quantitative yields while saving again reaction time when the temperature/time control of the device was used in order to keep

the temperature at 140 ºC for 20 min. The application of high power microwaves was avoided with HCl because of the rapid generation of overpressure which can lead to the explosion of the reaction vessel.

After the three steps the conventional heated reactions yielded the desired products 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr in 43% and 49% whereas 34% and 43% RCY, respectively, were obtained when microwave heating was applied, however, 38 min of total preparation time were saved with the latter. Thus considering the radioactive decay, the amounts of isolated radioactive products were directly comparable. Both products were obtained with good enantiomeric purity. The *e.e.* achieved for 2-[^{18}F]Fphe was 88% while an *e.e.* of 92% was obtained in the case of 2-[^{18}F]Ftyr.

3.2.4 Specific activity

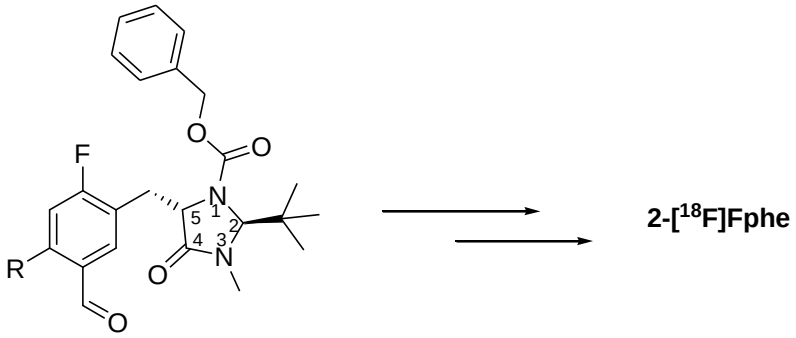
The specific activity obtained in isotopic exchange procedures is a function of both, the quantity of labeling precursor and starting [^{18}F]fluoride activity. The maximum amount of carrier that can be found in 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr preparations directly corresponds to the amount of precursor which in this work was 2.8 mg and 3.0 mg, respectively. Using the electrophilic radiofluorination by destannylation it has been reported that batches of activity of 1.41 GBq of 2-[^{18}F]Ftyr can be obtained with a specific activity of around 74 GBq/mmol,[41] which corresponds to a carrier content of approximately 3.8 mg. This amount is even bigger than the maximum amount of carrier with the isotopic exchange procedure described here under developmental conditions with a low starting radioactivity of about 300 MBq per experiment. Thus, in production runs starting with > 10 GBq of n.c.a. [^{18}F]fluoride it can be expected that the specific activity of the title compounds will be several times higher as compared to that achieved by electrophilic procedures.

3.2.5 Dependence of the enantiomeric purity of 2-[^{18}F]fluoro-L-phenylalanine as function of the protecting group of the imidazolidinone system

Achievement of high enantiomeric purity is an important goal in the development of multi-step radiosyntheses of [^{18}F]fluoroaromatic amino acids starting from nucleophilic [^{18}F]fluoride. Since the use of basic conditions are needed in the first substitution step in order to avoid inactivation of the radioactive nucleophile, chemical modifications in the structure of the precursor can be valuable for protection from epimerization.

The imidazolidinone system offers the possibility of a structural change by exchange of the protecting group in position 1 (Scheme 45). The idea was to use a more voluminous protecting group in order to introduce a steric hindrance of position 5 and in this way avoid the access of the base for H-abstraction during labeling.

Precursor **1d** bearing a bulky benzyloxycarbonyl group instead of *tert*-butoxycarbonyl protecting group was reacted under identical conditions as precursor **1a** using conventional heating. The radiochemical yields of all three reactions steps were similar to those obtained for precursor **1a**. After chiral HPLC (see experimental, System D) it was determined that the enantiomeric purity of the 2-[^{18}F]Fphe was 96 ± 3% which is comparable with the previous results.



1d

Scheme 45: Radiosynthesis of 2-[^{18}F]Fphe from precursor **1d**

Efforts to prepare derivatives of the precursor **1** bearing even bigger protecting groups were not successful. Therefore, a brief theoretical study on the influence of the protecting group on the space configuration of the precursor was then instead performed. Precursors **1a**, **1d** and the analogous compound, in which the imidazolidinone is protected with a subirane group, were selected for comparison. A molecular modeling calculation using the software Chem3D Pro (MM2 job) was run in order to find the configuration of least energy. The graphical results can be seen in Figure 10. No matter the size of the protecting group, the hydrogen atom in position 5 is always available for the reaction with the base. Thus, in the case of the bulky subirane protecting group, this proton is located in *anti*-configuration to it. From the experimental result and the configuration calculation it can be concluded that the size of the protecting group in position 1 has hardly an influence in the enantiomeric purity of the final product.

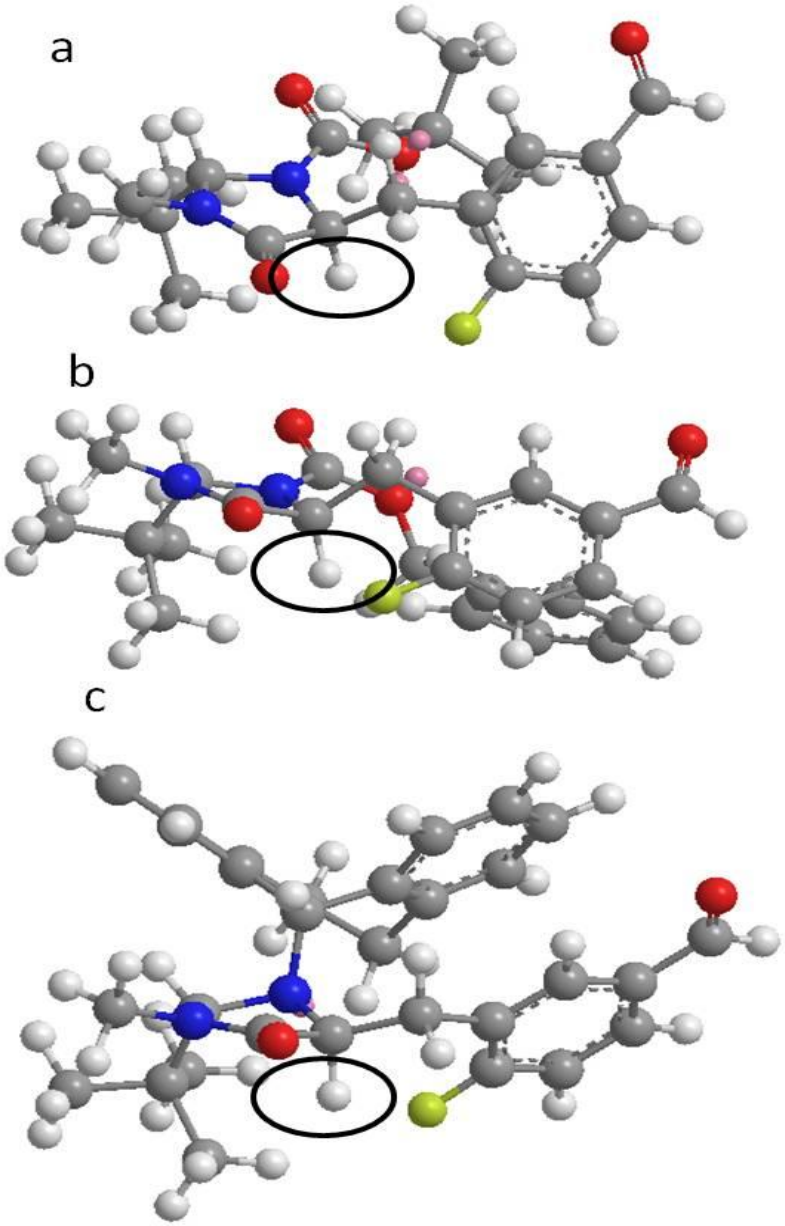
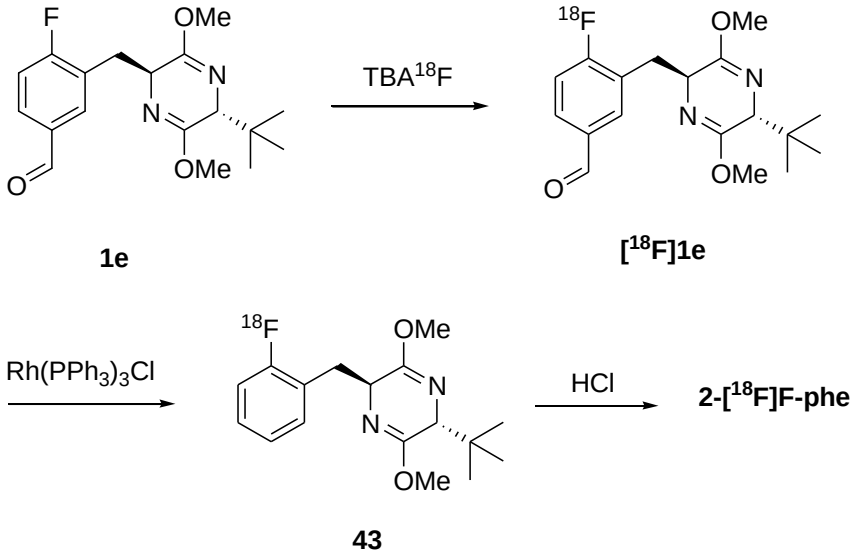


Figure 10: Graphical results of the MM2 energy minimization study. Energy-minimized structures of labeling precursors: a) **1a**, b) **1d**, c) **1** bearing a bulky subirane protecting group in position 1 of the imidazolidinone system. The acid proton in position 5 of the imidazolidinone ring is in every case enclosed within the elipse. It can be observed that the protecting groups have minimal screening effect over this proton and in the case of the subirane protecting group (c) it is located in *anti*-configuration to it.

3.2.6 Radiosynthesis of 2-[^{18}F]fluoro-L-phenylalanine using the alternative Schöllkopf-precursor 1e

It was mentioned before that precursors bearing Schöllkopf´s reagent were first used for the radiosynthesis of 6-[^{18}F]FDOPA, however the enantiomeric purity of the final product was not good enough in order to establish this approach as method of choice for a nucleophilic preparation of this important radiopharmaceutical. In that earlier work[103] the Kryptofix 2.2.2 carbonate/oxalate system was used for the labeling reaction. In the present work, it has been shown that the use of highly basic medium is inappropriate for the labeling of Seebach derivatives **1** due to diasteromerization of the precursor leading to the formation of the undesired enantiomer of the [^{18}F]fluorophenylamino acid. Because of Schöllkopf derivatives are easier to hydrolyze than the respective Seebach derivatives, it was interesting to re-evaluate this kind of compounds as precursors using $TBAHCO_3$ as base since the use of concentrated acid and/or high temperatures for the last hydrolysis step of the radiosynthesis could probably be avoided.

Unfortunately the precursor **1e** showed to be non-stable limiting the number of experiments which could be performed. However, using freshly prepared **1e**, it was possible to carry out the radiosynthesis using the same conditions used for the precursor **1a** (Scheme 46).



Scheme 46: Radiosynthesis of 2-[^{18}F]Fphe from precursor **1e**

The RCY for the isotopic exchange reaction using $TBAHCO_3$ as base was 20%. Only one product was distinguished in both, the TLC and radio-TLC analysis which seems to indicate

that diasteromerization of the precursor was not taking place. Decarbonylation gave similar results as with compound **[^{18}F]1a** with 80% RCY. Hydrolysis with concentrated HCl provided the desired 2-[^{18}F]Fphe in quantitative yield. Chiral HPLC analysis of the final product, however, showed to be an enantiomeric mixture containing 10% of the undesired 2-[^{18}F]F-D-phe.

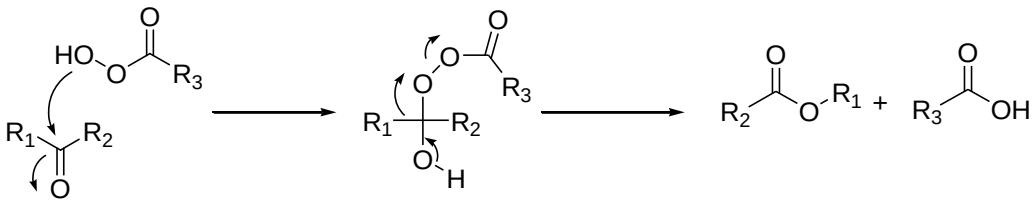
An exhaustive analysis of the formation of the undesired 2-[^{18}F]F-D-phe as function of the base concentration was not performed due to the limitations given by the decomposition of the precursor. It is not clear which processes are taking place, diasteromerization of the precursor or racemisation of the decarbonylated species during hydrolysis. However, the results showed that at least in the case of the radiosynthesis of 2-[^{18}F]Fphe under equal reaction conditions the precursor **1a** is better than the analogous **1e**.

Finally, it can be concluded that a new procedure for the radiosynthesis of 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr has been developed. The three-step approach can be performed either under conventional or microwave heating. It was also shown that the incidence of 2-[^{18}F]F-D-phe and 2-[^{18}F]F-D-tyr in the case of the Seebach´s derivatives occurs as consequence of the diastereomerization of the precursor during the nucleophilic exchange reaction with [^{18}F]fluoride and it happens independently of the size of the protecting group of the imidazolidinone system. Furthermore, this work is the first example of the radiosynthesis of ^{18}F-labeled aromatic amino acids via a decarbonylation reaction with $Rh(PPh_3)_3Cl$.

3.3 Enantioselective radiosynthesis of 6-[^{18}F]fluoro-L-*m*-tyrosine by isotopic exchange

6-[^{18}F]Fluoro-L-*m*-tyrosine (6-[^{18}F]Fmtyr) is an imaging agent of high interest for measuring the activity of the enzyme AADC. However, a wider application of this probe in nuclear medicine practice again has been restricted by the lack of an efficient radiosynthetic procedure. Due to the success achieved with the three-step radiochemical synthesis of 6-[^{18}F]FDOPA,[105] 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr, it was decided to adapt this pathway in order to prepare 6-[^{18}F]Fmtyr following the reaction sequence: ^{18}F-for-^{19}F exchange, Baeyer-Villiger oxidation, and acid hydrolysis.

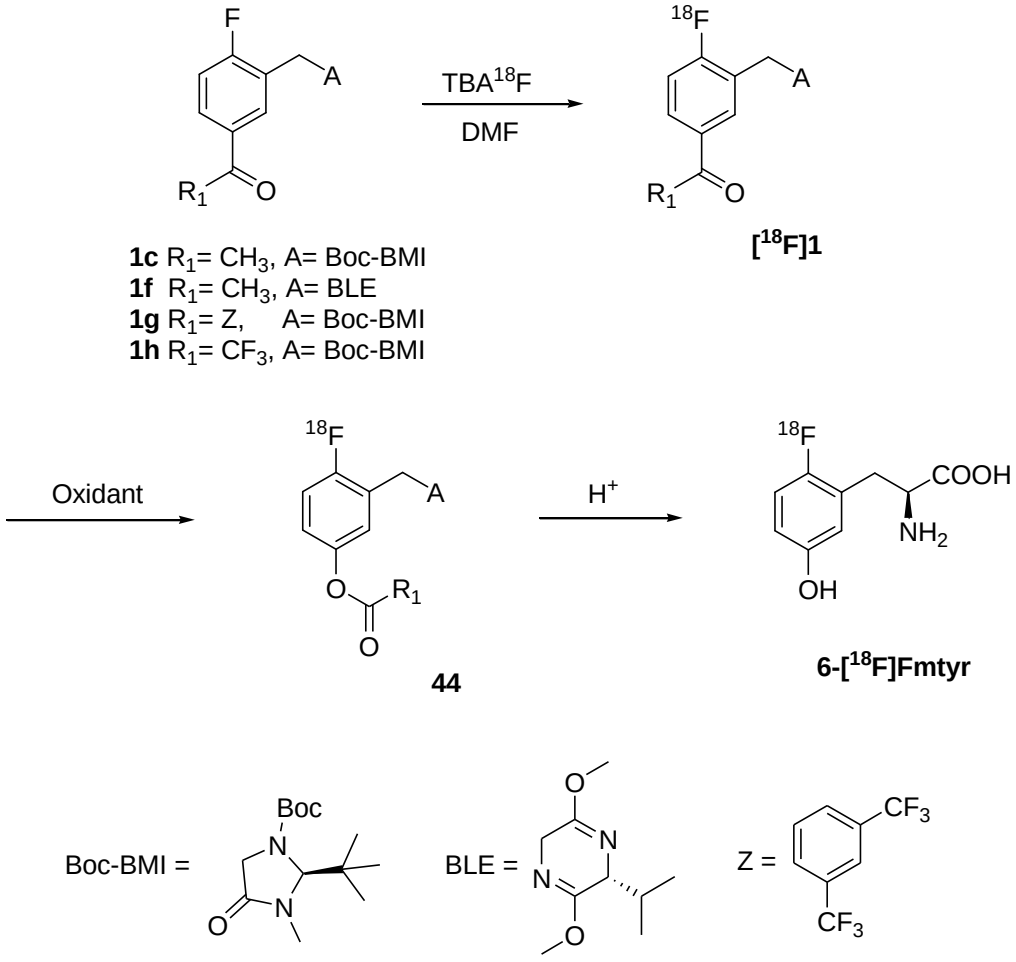
4-Fluorobenzaldehydes derivatives have successfully been employed as precursors for the preparation of 6-[^{18}F]FDOPA,[105] 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr. However, the use of the aldehyde **1a** for the radiosynthesis of 6-[^{18}F]Fmtyr is not appropriate because regioselectivity problems can arise during the oxidation step. As depicted by Scheme 47, the Baeyer-Villiger oxidation mechanism involves first the addition of a peroxy acid to the carbonyl group of the substrate followed by a concerted migration of one of the substituents to the oxygen with formation of the carboxylic acid derived from the peroxy acid. Therefore, two different products can be generated from benzaldehydes, either benzoic acids or phenyl formates. The direction of reaction is largely determined by the electron density of the phenyl ring: electron donating substituents favor ring migration to yield the phenyl formate whereas electron withdrawing substituents favor hydrogen migration to yield benzoic acid.[159] Thus, oxidation of 3-bromo-4-fluorobenzaldehyde leads to the aromatic carboxylic acid and not to the 3-bromo-4-fluorophenyl formate.[160]



Scheme 47: General mechanism of the Baeyer-Villiger oxidation

In order to avoid regioselectivity problems it was decided to include a directing group in the structure of the precursor for the oxidation. Asymmetric ketones undergo Baeyer-Villiger reaction following a well known migration pattern, where an aryl group migrates to the oxygen atom with preference over a methyl substituent (which has the lowest migration speed

among the ketones). In the light of this fact, the phenone derivatives **1c**, **1f**, **1g** and **1h** were employed as labeling precursors (Scheme 48).

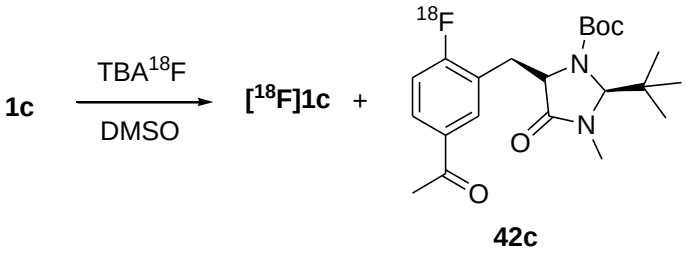


Scheme 48: General radiosynthetic pathway to 6-[^{18}F]Fmtyr by isotopic exchange

3.3.1 Radiofluorination

Table 8 lists the results obtained with labeling of precursor **1c**. In the previous section it was shown that the use of DMF as solvent provided good labeling results, however in this case, attempts to carry out the reaction using DMF as reaction medium showed no formation of the desired labeled product (Table 8, entries 1-2). The change of solvent to DMSO provided better results. After 10 min reaction time at 130 °C the desired labeling product **[^{18}F]1c** was obtained in 5% RCY. When the temperature was raised to 160 °C the RCY increased to 16%, however, at higher temperatures diasteromerization of the labeling precursor takes place without a further increase in the yield of the desired compound (Table 8, entry 5). Analogously to the aldehyde derivatives **1a** and **1b** an increase of the basic anion

activator concentration led to a bigger epimerization rate; as well as the use of kryptate/K_2CO_3 gave the undesired diastereoisomer as major product (Table 8, entries 6-8).



Scheme 49: Epimerization of **1c** during the isotopic exchange reaction

For further experiments which were carried out, therefore the conditions were used as described in entry 4 of Table 8. Again here the avoidance of humidity plays an important role. As well as for the analogous aldehyde precursors **1a** and **1b** just freshly dried acetronitrile should be used for the drying process of [^{18}F]fluoride as well as dry DMSO for the labeling reaction.

Table 8: Influence of temperature, time and kind of anion activation on the RCY of the isotopic exchange reaction on **1c**[a,b]

				10 min		20 min	
Entry	**Solvent**	**PTC[c] [µmol]**	**Temp. °C**	**[^{18}F]1c (%)**	**42c (%)**	**[^{18}F]1c (%)**	**42c (%)**
1	DMF	$TBAHCO_3$ [7.7]	130	0	0	0	0
2	DMF	$TBAHCO_3$ [7.7]	150	0	0	0	0
3	DMSO	$TBAHCO_3$ [7.7]	130	5	0	7	0
4	DMSO	$TBAHCO_3$ [7.7]	160	16	0	18	0
5	DMSO	$TBAHCO_3$ [7.7]	180	15	2	16	3
6	DMSO	$TBAHCO_3$ [10.3]	160	14	6	16	7
7	DMSO	$TBAHCO_3$ [13.0]	160	21	12	20	13
8	DMSO	$[K222]_2CO_3$ [13.0]	160	4	27	5	40

[a]SD = ±3%. [b]1 mL solvent, 15 µmol **1c**, conv. heating. [c]PTC = phase transfer catalyst for [^{18}F]fluoride activation.

Labeling of the bislactimether derivative **1f** (see Scheme 48) under the conditions mentioned above produced similar results. The desired labeled compound **[^{18}F]1f** was obtained in 15 ± 3% RCY. Following the same trend as with precursor **1c**, the RCY increased

with the anion activatior concentration arriving to an average of 30% when 17 µmol of $TBAHCO_3$ were employed for the reaction. However, contrary to **[^{18}F]1c** both radio-TLC and TLC-UV analyses showed a single spot with a R_f value consistent with precursor **1f**.

Due to the success of previous labeling experiments using microwave heating, this method was also tried using precursor **1c**. The best RYC (16 ± 2%) of the desired labeled compound **[^{18}F]1c** was obtained when the reaction mixture was irradiated with 110 W microwaves during 1 min. Either longer irradiations times or higher power induced diastereomerization of the precursor while the RYC did not improve.

Compared to aldehyde **1a**, the activation of the isotopic exchange reaction in the acetophenone derivative **1c** was weaker. On the other hand, it was also observed that precursor **1c** tolerates better the basic conditions, because no diastereomerization was observed when 7.7 µmol of $TBAHCO_3$ of base were used for the reaction in contrast to the maximum tolerance of the aldehyde of 5.2 µmol. However, this tendence can probably be attributed to deprotonation of the acidic α carbonyl methyl group more than steric or electronic effects of the ketone that avoids the side reaction. Deprotonation with subsequent deactivation of the base can also be affecting the isotopic exchange process because of the impossibility to regenerate the nucleophilic [^{18}F]fluoride in case of protonation. Besides of that the enol would not be able to withdraw electrons from the aromatic ring.

In order to corroborate the hypothesis formulated above a precursor without enolizable protons was radiolabeled. Precursor **1g** is a benzophenone derivative analogous to Mulholland´s ketone.[128] Instead of an individual protecting group for the amine and for the acid, **1g** is bearing an imidazolidinone system. ^{19}F-for-^{18}F exchange with **1g** was performed using microwave heating, after 1 min of reaction the desired compound was obtained with 30 ± 4% RYC. Increase of the RCY confirms that in the case of the acetophenone **1c** the α-carbonyl acid protons could be the origin of a negative influence on the isotopic exchange reaction.

Based on the previous experimental results a new precursor was designed where the second phenone substituent was a trifluoromethyl group (**1h**). The intention was to create a stronger imbalance in the electronic cloud of the aromatic ring due to the high electrophilic character of the trifluoromethyl group and by this way to accomplish a better activation for the isotopic exchange. Labeling of precursor **1h** using 7.7 µmol of $TBAHCO_3$, DMSO as

solvent and heating by 110 W microwaves for 1 min in fact generated the desired compound **[^{18}F]1h** in 40 ± 5% RCY.

3.3.2 Baeyer-Villiger oxidation and hydrolysis

The second step in the radiochemical pathway to 6-[^{18}F]Fmtyr is the conversion of the radiofluorinated precursor into the respective phenylester. In a cold experiment, the Baeyer-Villiger oxidation of **1c** was performed using 4 equivalents *m*-CPBA and an excess trifluoroacetic acid in chloroform at 60 °C. After 20 min the reaction was allowed to cool to room temperature, the reaction mixture was neutralized and extracted with dichloromethane, the crude product purified by flash chromatography and the isolated product analysed by ^{1}H-NMR. The spectrum showed the signals expected for compound **44c** with the exception of the nine protons singlet belonging to the Boc protecting group. The hydrolysis of the corresponding imidazolidinone in acid medium led to the formation of Fmtyr. This result was confirmed by HPLC analysis through the comparison with a standard sample.

Those preliminary results encouraged us to try the oxidation of the labeled compound **[^{18}F]1c**. Table 8 lists the RCY of 6-[^{18}F]Fmtyr obtained using different oxidizing agents after hydrolysis with concentrated hydroiodic acid. RCY of 6-[^{18}F]Fmtyr reflected the yield obtained in the oxidation reaction since the hydrolysis produced quantitative results. All the reactions were carried out using chloroform as solvent and were stoped after 30 min. When *m*-CPBA (Table 9, entry 1) was used as oxidant only 13% RCY of 6-[^{18}F]Fmtyr was quantified by analytic HPLC. Moreover, after hydrolysis solid residues, probably belonging to the organic acid, remained in the reaction vial hindering the dilution and subsequent transfer of the final product into the semi-preparative HPLC system.

Table 9: Optimization of the reaction conditions for the oxidation of **[^{18}F]1c** and RCY of 6-[^{18}F]Fmtyr after acid hydrolysis[a]

Entry	Oxidant	Yield (%)[b]
1	*m*-CPBA[b]	13
2	CH_3COOOH[c]	68
3	CF_3COOOH[c]	81

[a]SD = ±5%.[b] 4 mol eq, [c] 8 mol eq, 1 mL $CHCl_3$, 60 °C, conventional heating 30 min.

In order to avoid the solubility problems observed with *m*-CPBA, it was replaced with water-soluble peracetic acid. Peracetic acid is an established reagent for the Baeyer-Villiger oxidation, thus, it is commercially available as a 40% solution in acetic acid. When the reaction of compound **[^{18}F]1c** was performed using 8 eq. excess of this reagent, the RCY of 6-[^{18}F]Fmtyr after hydrolysis was increased to 68% (Table 9, entry 2). Besides of the improvement of the RCY, in this case a second advantage was that a homogeneous solution of the final product was achieved, which can easily be transferred into the semi-preparative column.

Trifluoroperacetic acid is an alternative oxidant that has shown great activity in Baeyer-Villiger reactions. Since the earlier preparation of trifluoroperoxyacetic acid from trifluoroacetic anhydride and concentrated hydrogen peroxide[161] is not possible any more as concentrated hydrogen peroxide is not commercially available any longer, some alternative procedures using different sources of H_2O_2 have been developed.[162,163] In order to achieve the oxidation of compound **[^{18}F]1c**, here trifluoroperacetic acid was generated *in situ* by the reaction of trifluoroacetic anhydride with sodium percarbonate. After hydrolysis of the intermediate **44c** obtained by this oxidation method the desired 6-[^{18}F]Fmtyr was obtained with RCY of 81 ± 5% (Table 9, entry 3). Use of this oxidation reagent not only increased the RCY in comparison to the other two discussed above, but it has also the advantage that the remaining reactive as well as the formed trifluoroacetic acid can be removed at the end of reaction by simple distillation while sodium carbonate is neutralized by the acid used for oxidation. Additionally the salt formed by neutralization of the percarbonate raises the boiling point of the reaction mixture for hydrolysis, which, considering the high temperatures needed for the reaction, constitutes a further advantage.

Oxidation under the above optimized conditions and subsequent hydrolysis with concentrated HCl of the bislactimether derivative **[^{18}F]1f** delivers the desired labeled amino acid in 73% RCY. Taking advantage of the higher stability of precursor **1f** when compared to the aldehyde **1e**, experiments were performed to show the dependence of the enantiomeric purity of the final preparation as function of the amount of $TBAHCO_3$ used for labeling. Chiral HPLC analysis (see experimental, System D) of the final product generated from the labeling of **1f** with different amounts of anion activator clearly evidenced a dependence of the enantiomeric purity on its concentration. As depicted in Figure 11, also with bislactimether derivatives the decrease of the enantiomeric purity is totally influenced by the diastereomerization of precursor **1f** during the isotopic exchange reaction.

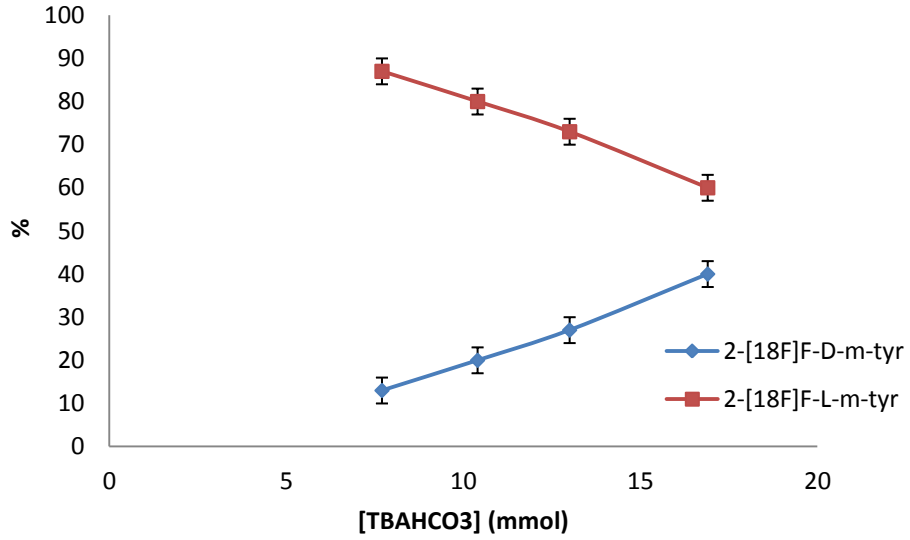


Figure 11: Radioactivity distribution of 6-[^{18}F]F-L-*m*tyr and 6-[^{18}F]F-D-*m*tyr as function of the $TBAHCO_3$ concentration used for the isotopic exchange reaction on precursor **1f**

From this results it is also clear that precursors bearing the Schöllkopf´s bislactimether are more sensitive to the conditions of the ^{18}F-for-^{19}F exchange than those formed with Seebach´s imidazolidinone system, since preparations of 2-[^{18}F]Fphe and 6-[^{18}F]Fmtyr from **1e** and **1f**, respectively, showed a higher fraction of the undesired D-enantiomer than those from **1a** and **1c**.

The Baeyer-Villiger oxidation of compound **[^{18}F]1g** with trifluoroperoxyacetic acid, followed by hydrolysis of the protecting groups produced the desired amino acid in an average RCY of 18%. The HPLC analysis of the oxidation reaction mixture showed that the conversion of the starting material **[^{18}F]1g** was low (46 ± 5%) and multiple new labeled compounds were observed. The low reactivity of compound **[^{18}F]1g** can probably be attributed to steric effects by both phenyl groups of the carbonyl function plus the imidazolidinone ring impeding the effective approach of the oxidant. Additionally, in spite of the two electron withdrawing trifluoromethyl groups attached to the secondary phenyl ring, it seems that the reaction is also not regioselective since the RCY of the desired amino acid is only 40% of the converted products. Oxidation of **[^{18}F]1g** with 4 eq. *m*-CPBA in $CHCl_3$ at 60 °C did not provide any of the desired [^{18}F]fluoroamino acid at all.

Previous work dealing with the radiosynthesis of 4-[^{18}F]fluorophenol starting from benzophenones derivatives *via* Baeyer-Villiger oxidation, reported a good or even excellent

RCY.[164] In order to achieve the desired compound, the benzophenone derivatives were treated with peracetic acid (generated *in situ* from a mixture of acetic anhydride and hydrogen peroxide) in acetic acid at 80 °C. Therefore, compound **[^{18}F]1g** was reacted under similar conditions using a commercially available solution of peracetic acid in acetic acid (40%), however, the radiochemical yield of the desired product was only 14%.

Table 10 summarizes the conditions for the oxidation reaction of **[^{18}F]1h** and the RCY of 6-[^{18}F]Fmtyr obtained after hydrolysis. Treatment of **[^{18}F]1h** with peracetic acid and subsequent hydrolysis afforded the desired 6-[^{18}F]Fmtyr in 33% RCY (entry 1). Oxidation followed by acid hydrolysis of **[^{18}F]1h** provided a better radiochemical yield than that achieved with **[^{18}F]1g**. However, it is still lower than that obtained after oxidation and hydrolysis of **[^{18}F]1c** and **[^{18}F]1f**.

Table 10: Conditions for the Baeyer-Villiger oxidation of **[^{18}F]1h** and RCY of 6-[^{18}F]Fmtyr obtained after hydrolysis

Entry	Oxidant agent	Solvent	Temp °C	RCY (%)
1	CF_3COOOH[b]	$CHCl_3$	60	33±5
2	*m*-CPBA[a]	$CHCl_3$	reflux	-
3	*m*-CPBA[a]	CH_2Cl_2/CF_3CH_2OH	r.t.	-
4	*m*-CPBA[a]	CH_2Cl_2/CF_3CH_2OH	reflux	5
5	CH_3COOOH[b]	ACN	60	-
6	CF_3COOOH[b]	CF_3CH_2OH	60	-

[a] 4 mol eq, [b] 8 mol eq, 1 mL solvent, 30 min

The use of peracetic acid as oxidant for Bayer-Villiger reaction on trifluoroacetophenone has been previously reported.[161] In this earlier work phenyl trifluoroacetate was obtained with 5% yield after a reaction time of 5 h. A further paper from Kitazume and Kataoka[165] reported 93% yield of the same compound after 1 h reaction time using *m*-CPBA as oxidazing agent in refluxing $CHCl_3$. Oxidation of **[^{18}F]1h** using these conditions followed by acid hydrolysis did however not produce any 6-[^{18}F]Fmtyr (entry 2). More recently Kobayashi and coworkers[166] obtained 87% yield of phenyl trifluoroacetate after 10 min reaction at r.t.. The oxidant was again *m*-CPBA, while a mixture of CH_2Cl_2 and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) was used as solvent containing additionally phosphate buffer (pH 7,6) to catalyze the reaction. The use of similar conditions for the oxidation of **[^{18}F]1h** was not successful (entry 3). 6-

[^{18}F]Fmtyr was obtained in 5% RCY when the temperature of the reaction was increased to 60 °C (entry 4). Further experiments using trifluoroperacetic acid in acetronitrile or peracetic acid in trifluoroethanol did not provided any 6-[^{18}F]Fmtyr (entries 5 and 6).

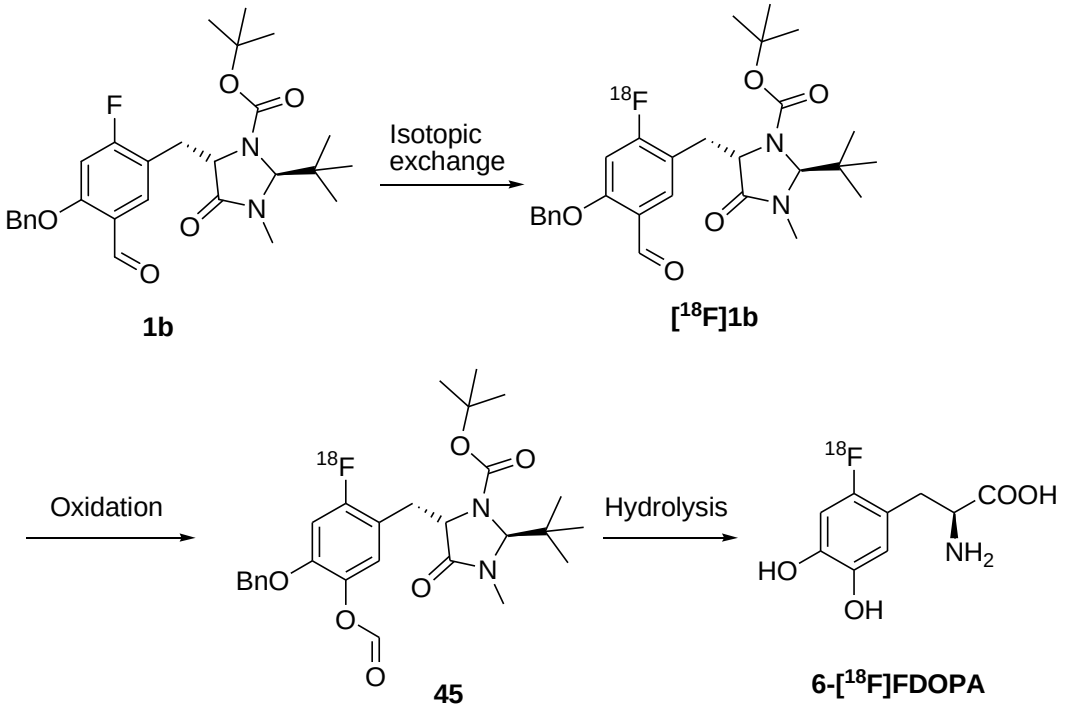
A new nucleophilic synthesis of 6-[^{18}F]Fmtyr by isotopic exchange has been developed. The three-step radiosynthetic procedure leads to the desired amino acid in ca. 13% overall radiochemical yield with high enantiomeric purity of > 93% when compound **1c** is used as precursor. It was shown that analogously to precursor **1c**, compound **1f** suffered epimerization during the isotopic exchange. A comparable overall radiochemical yield of 11% of 6-[^{18}F]Fmtyr was achieved with precursor **1f** while the enantiomeric purity in this case was only 87%.

Precursors **1g** and **1h** showed relative high radiochemical yield of the ^{18}F-for-^{19}F substitution, with 30% and 40% respectively. However, it was not possible to match the RCY for the Baeyer-Villiger oxidation obtained for the acetophenone derivatives **[^{18}F]1c** and **[^{18}F]1f**. 6-[^{18}F]Fmtyr was obtained from **1g** and **1h** with overall RCY of only 6% and 13%, respectively. However, the enantiomeric purity of the final product using any of both precursor was >98%. Thus, further efforts of the optimization of the Baeyer-Villiger reaction of these precursors seem to be justified.

3.4 Enantioselective radiosynthesis of 6-[^{18}F]fluoro-DOPA by isotopic exchange

6-[^{18}F]Fluoro-DOPA (6-[^{18}F]FDOPA) is one of the few established radiopharmaceuticals for diagnostic imaging using PET. In spite of that 6-[^{18}F]FDOPA has been widely used for human studies, is still considered in a development stage since its availability is limited due to radiosynthetic constraints.[18] As described in the introduction of this work (see Chapter 1.8.1), today electrophilic methods are generally applied to produce 6-[^{18}F]FDOPA,[90] however, with the disadvantages of relatively low specific activity and batch yields in addition to a limited availability of electrophilic radiofluorination established in many PET centers. Although nucleophilic procedures were developed for routine production of 6-[^{18}F]FDOPA, they are difficult to implement because of the complexity of the reactions involved in built-up syntheses.[101,102]

Following the compromising concept as discussed above, Wagner *et al.*[105] presented an interesting radiochemical pathway for the radiosynthesis of c.a. 6-[^{18}F]FDOPA by isotopic exchange. Scheme 50 depicts the general pathway for this radiosynthesis. In the work presented here, all steps have been optimized, as was done for the precursor (see Chapter 3.1.3.2).



Scheme 50: Three-step radiosynthesis of 6-[^{18}F]FDOPA by isotopic exchange[105]

Here, the results achieved are summarized for the optimization of every individual reaction together with preliminary results of the automation of this procedure using a commercially available synthesis module.

3.4.1 Radiofluorination

The results concerning the first step, which is identical with the radiosynthesis of 2-[^{18}F]Ftyr were reported above (see Chapter 3.2.1). As discussed there the isotopic exchange reaction on precursor **1b** generates the products **[^{18}F]1b** and **42b**. The best RCY of **[^{18}F]1b** was 59% while that of **42b** was 4% with 15 µmol of precursor, 6.4 µmol of $TBAHCO_3$ and 1 mL of DMF at 130 °C for 10 min as optimal reaction conditions.

Since a high enantiomeric purity of 6-[^{18}F]FDOPA is required for its application in medicine it is pertinent to repeat that the isotopic exchange reaction is the critical step in order to achieve this objective. Once again: the structure of precursor **1b** contains an acidic proton in position 5 of the imidazolidinone. The isotopic exchange, however, requires the use of a base to activate the nucleophilic species by avoiding the formation of H^{18}F. The base, in this case $TBAHCO_3$, is able to remove the acidic proton from **1b** generating a planar carbanion. The carbanion can get re-protonated, but two protonation sites are now available the first regenerating **1b** and the second producing diasteromer **42b**. After oxidation and hydrolysis **42b** leads to the formation of non-wanted 6-[^{18}F]F-D-DOPA. This process, like any other chemical reaction, depends on the concentration of the reactants and the temperature. For this reason, it is necessary to keep the concentration of $TBAHCO_3$ as low as possible.

3.4.2 Baeyer-Villiger oxidation

The Baeyer-Villiger oxidation is applicable for the generation of a phenol from **[^{18}F]1b** because the substrate is substituted with an electron-donor benzyloxy group. Previous work[105] reported the use of *m*-CPBA as oxidizing agent in dichloromethane as solvent. Although the reaction produced the desired compound in a good RCY after 20 min, a solid residue remained in the reaction vessel after hydrolysis that hinders the transfer of the reation mixture into the HPLC system. Alternatively, the Baeyer-Villiger oxidation can be performed either with trifluoroperacetic acid, generated *in situ* from sodium percarbonate and trifluoroacetic anhydride[163] or commercially available 40% solution of peracetic acid/acetic acid, as shown for the radiosynthesis of 6-[^{18}F]Fmtyr (see Chapter 3.3.2). The oxidation of **[^{18}F]1b** was performed using 8 equivalents of the oxidizing agent, calculated from the amount of precursor

in 1 mL chloroform at 60 °C for 10 min. With these reagents, either trifluoroperacetic acid or peracetic acid, a radiochemical yield of 81 ± 5% was achieved, which is comparable with that obtained with *m*-CPBA but the reaction time could be reduced to the half. Finally trifloroperacetic acid is preferable over peracetic acid since reactions with the first result in a cleaner product and its excess as well as the formed trifluoroacetic acid can be easily removed under *vacuo*.

3.4.3 Hydrolysis

Seebach´s chiral auxiliary has been early employed by Lemaire *et al.*[100] in a nucleophilic build-up synthesis of 6-[^{18}F]FDOPA. The hydrolysis reaction was carried out with concentrated HI at 200 ºC achieving a quantitative RCY. Wagner *et al.*[105] reported that hydrolysis under similar conditions led to a poor RCY due to the formation of several side products. Treatment with HBr at the same temperature did not cause any improvement. In the latter publication it was proposed that an oxidation of HI and HBr to elemental iodine and bromine, respectively, could probably be taking place due to the excess of *m*-CPBA. Finally as optimal condition the hydrolysis of the oxidazed intermediate was performed with concentrated HBr at 150 °C for 30 min and the desired product was obtained in 53% RCY.

In the present work it was observed that under those conditions only a low conversion of the starting material is achieved. When the hydrolysis was, however, carried out in a heavy-wall borosilicate glass tube closed with a Teflon screw cap (pressure tube) and the temperature raised to 200 °C, a quantitative radiochemical yield was obtained in spite of the excess of *m*-CPBA in the reaction medium. Moreover, when the oxidation step was performed with trifluoroperacetic acid it could be distilled off prior to hydrolysis, thus diminishing the risk of oxidation of the mineral acid. Analogously to the hydrolysis of the other [^{18}F]fluoroamino acids presented in this work here, concentrated HCl provided a quantitative RCY. Figure 12 depicts a comparison between the HPLC analysis of a standard sample of 6-L-FDOPA and the radio-HPLC analysis of the crude product after the hydrolysis reaction. From the simple comparison of both chromatograms it is easy to identify 6-[^{18}F]F-L-DOPA.

Thus, in total the nucleophilic radiosynthesis of 6-[^{18}F]FDOPA by isotopic exchange has been optimized. The three-step radiosynthetic procedure leads to the desired amino acid in ca. 40% overall radiochemical yield with a high enantiomeric purity of > 96%. The increase of the overall RCY from 22% reported by Wagner *et al*.[105] is basically due to the improved RCY of the hydrolysis reaction. Furthermore, a better understanding of the isotopic exchange

reaction has also been accomplished. The side product-A, referred to by Wagner and coworkers,[105] was here identified as diasmeromer **42b** being the product of epimerization of position 5 of the imidazolidinone system due to the basic conditions and high temperature during the isotopic-exchange reaction. The Baeyer-Villiger oxidation was also optimized: employment of trifluoroperacetic acid allowed to reduce the time of the reaction from 20 min to 10 min, whereas the remaining peracid and the trifluoroacetic acid formed were easily removed at the end of the reaction by distillation while addition of HCl used for hydrolysis neutralized sodium carbonate.

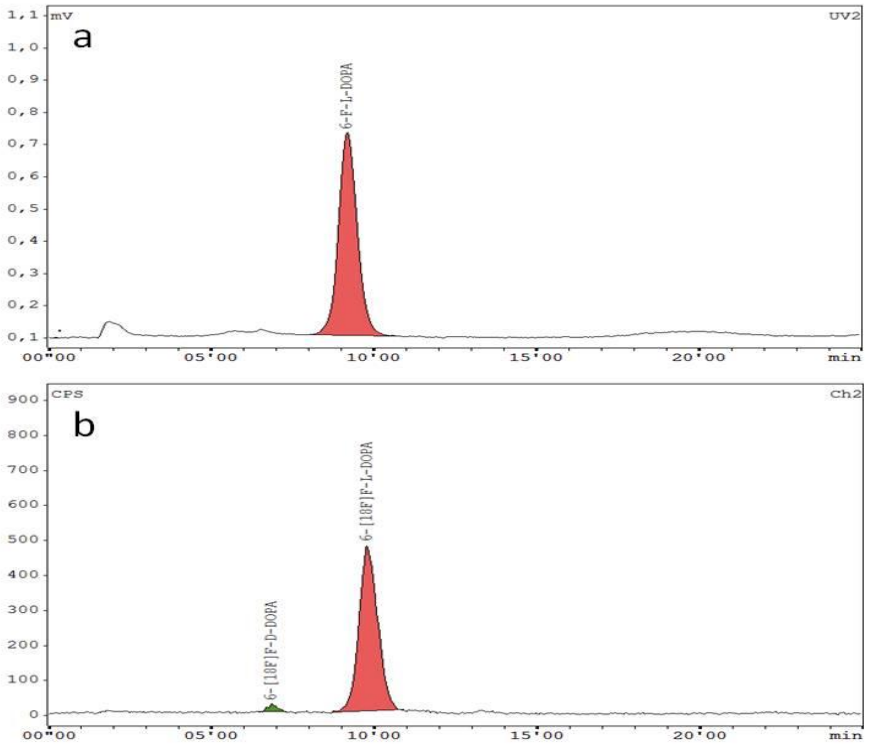


Figure 12: HPLC analysis of a standard sample of 6-L-FDOPA and the crude product after the hydrolysis reaction. a) UV-chromatogram of a standard solution of 6-L-FDOPA. b) Radio-chromatogram of a sample of 6-[^{18}F]FDOPA after hydrolysis

3.4.4 Preliminary attempts to automate the radiosynthesis of 6-[^{18}F]fluoro-L-DOPA

In order to transfer the elaborated three-step radiosynthesis of 6-[^{18}F]FDOPA into an automated synthesizer the SynChrom R&D device of Raytest GmbH controlled by GINA SynChrom software was chosen. Figure 13 depicts the scheme of the technical concept of this synthesis module.

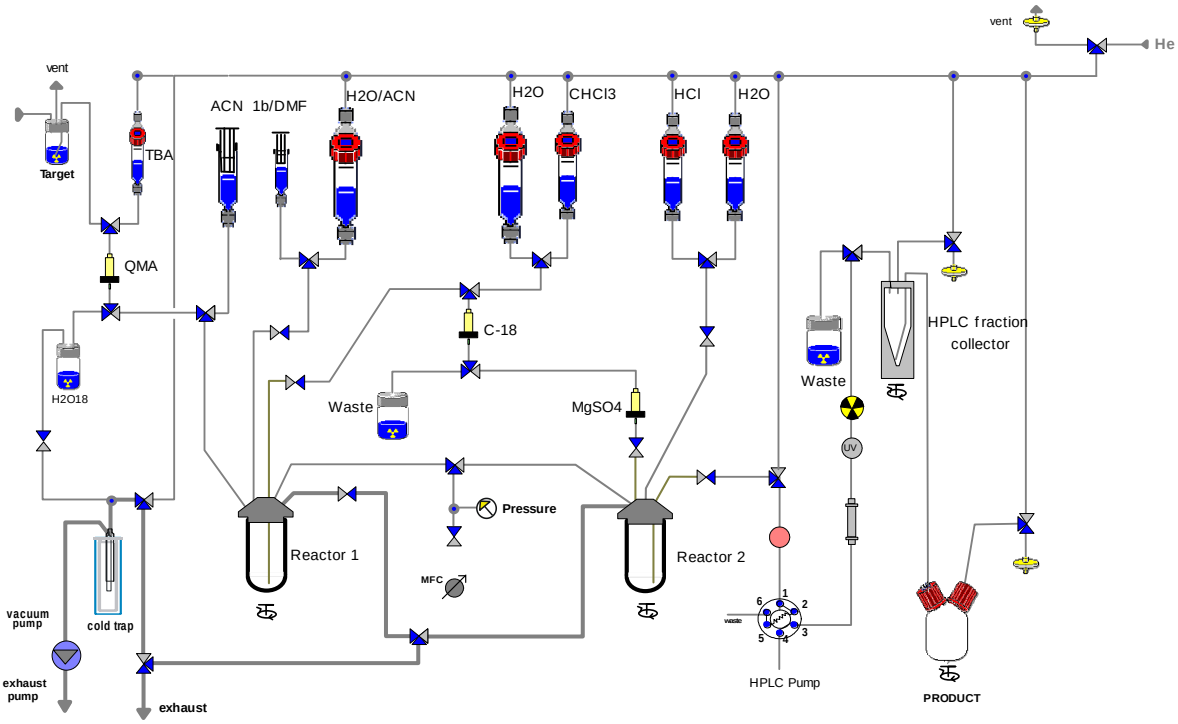


Figure 13: Schematic representation of the 6-[^{18}F]FDOPA synthesis module set-up

The optimization experiments described above were performed using [^{18}F]fluoride which was separated from the enriched water by electrochemic adsorption (see experimental). However, for the remote controlled radiosynthesis the [^{18}F]fluoride was directly transferred from the cyclotron target into the synthesis module. For this reason, the first step toward automation of this method was to perform the separation of the enriched water. Usually this process is carried out by ion exchange chromatography, specifically with a cartridge containing an anion exchange resin (QMA light). Generally, once the radioactive species is trapped on the cartridge its elution is achieved using a solution of the PTC system in water or acetronitrile. Since it was not possible to elute the [^{18}F]fluoride with only 6.4 µmol of $TBAHCO_3$ and an increase of the concentration of $TBAHCO_3$ was undesirable because of the occurrence of the side product **42b**, it was decided to add a second tetra-*n*-butyl ammonium

salt containing a non-nucleophilic anion in order to facilitate the elution. A mixture of $TBAHCO_3$ and tetra-*n*-butyl ammonium mesylate (TBAMs) in water was used for this purpose. With this eluent 92 ± 5% of the activity attached on the cartridge was recovered. Freshly dried acetonitrile was added to it and the resulting mixture distilled azeotropically to dryness. Control experiments showed that, as expected, the addition of TBAMs has not influence on the RCY of the isotopic exchange reaction. Moreover, when the ^{18}F-for-^{19}F exchange reaction was performed in the presence of TBAMs only, no formation of **[^{18}F]1b** was detected.

During the automation of the isotopic exchange reaction it was also observed that when the solution of precursor **1b** in DMF was stored in the glass container provided with the synthesis device before the labeling reaction, the compound **[^{18}F]1b** was not formed. Since it was already known that the presence of moisture could deactivate the isotopic exchange reaction, the glass containers for acetronile and the solution of the precursor were meticulously dried. After that, the formation of the desired compound was achieved, however, with a low RCY of only ≈ 10%. An alternative and more practical solution was found when both, the glass container housing the acetronitrile as well as the one for the precursor solution were replaced with disposable syringes. In this way the moisture holded by the glass containers and their connectors was avoided while saving time in exhaustive cleaning and drying procedures before every radiosynthesis. The syringe contents were dispensed into the reactor 1 using vacuum. Radiochemical yields between 40% and 50% were obtained by employing this method.

After the isotopic exchange reaction, it was necessary to remove the polar solvent and the non-reacted [^{18}F]fluoride from **[^{18}F]1b**. This step was achieved by solid-phase extraction (SPE). A previous report[105] suggested the addition of water to the reaction mixture, but it produced precipitation of the organic compounds leading to the obstruction of tubing and valves. A 4:1 mixture of water and acetonitrile resulted to be more proper to that end. **[^{18}F]1b** was retained on the SPE cartridge and the water-soluble impurities washed out. Afterward the desired compound was eluted with chloroform and the eluate dried online over a column packed with of magnesium sulfate.

The online drying procedure presented two advantages, first it saved time by avoiding azeotropic distillation and second the reactor 2 (see Figure 13) had not to be used and could be prepared for the Baeyer-Villiger oxidation reaction, *i.e.* the oxidation agent could be loaded in reactor 2 before starting the radiosynthesis. Pre-loading of the oxidation agent was a

major achievement since it made feasible the generation of trifluoroperacetic acid from a solution of trifluoroacetic anhydride and solid sodium percarbonate. The dry solution containing **[^{18}F]1b** was thus conducted into the reactor 2 and the reaction mixture was then heated at 60 °C for 10 min. After that the reaction mixture was evaporated to dryness.

The last step of the radiosynthesis also proved to be the most difficult one to automate. Hydrolysis of the imidazolidinone group requires a high temperature. Along the elaboration in the present work it turned out that optimal conditions included heating of compound **45** in concentrated hydrochloric acid at 200 °C for 30 min. This generates a pressure of approximately 8 bar in an 18 mL closed reaction vessel. This high pressure did not represent any problem during optimization studies since a pressure tube was used. However, the specifications of the valves of the synthesis module, especially those directly attached to reactor 2, guarantee proper function only up to 3 bar. In a first experiment the hydrolysis reaction was performed at 180 °C, due to safety reasons, however, it resulted in broken valves and leaking of the reaction mixture.

Thus, the automatic radiosynthesis of 6-[^{18}F]FDOPA became a technical challenge rather than a chemical one. Since the original valves were not able to manage the pressure generated during the hydrolysis and the dimensions of commercially available high pressure valves are too big to be easily installed in the device, the effort was directed toward the protection of the existing ones. The reaction temperature was reduced to 170 °C and the tubing distribution modified in order to permit the transport of a He stream against every valve connected directly with the reactor 2, in such a way reducing the pressure difference between both sides. Application of these measures allowed to perform the reaction. However, in the future the synthesis device should technically be improved because during some experiments small leakage of the reaction mixture was observed. However, HPLC analysis of a sample taken from reactor 2 after hydrolysis provided proof-of-principle of the formation of 6-FDOPA (Figure 14).

Although further work is still needed, especially in order to overcome the technical problems described above, the results shown here demonstrate that the three-step radiochemical synthesis is feasible to be performed in an automated remote-controlled synthesis device.

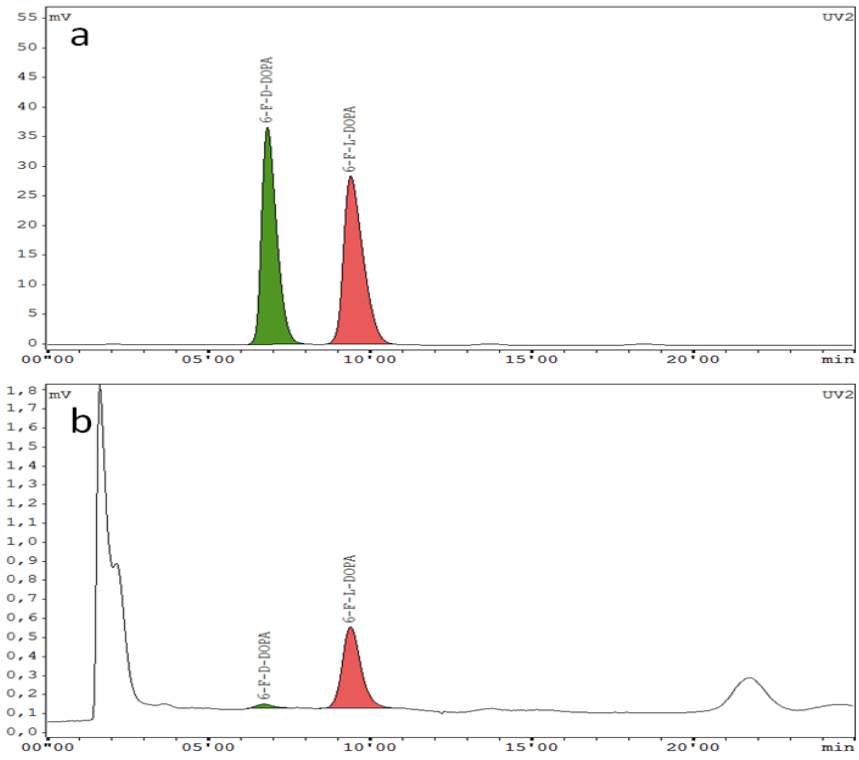


Figure 14: HPLC analysis of a standard sample of 6-D,L-FDOPA and the crude product after automatic synthesis. a) UV-chromatogram of a standard solution of 6-D,L-FDOPA. b) UV-chromatogram of a sample from reactor 2 after the hydrolysis reaction

4 EXPERIMENTAL

4.1 General techniques

All reactions sensitive to humidity were carried out under argon atmosphere, and prior to use, the reaction flasks were dried over night in an oven at 95 °C. All liquids sensitive to humidity were transferred into the reaction flask, equipped with a septum, through syringes. All reaction mixtures were magnetically stirred.

Spectrometric devices

^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a on an Inova 400 MHz spectrometer (Varian, Germany) using $CDCl_3$. All shifts are given in ppm using as reference the signal of $CDCl_3$. HRMS spectra were obtained on an FTICR 'LTQ FT Ultra' (Thermo Fisher Scientific, Germany).

Microwave device

Microwave heated experiments were performed using a CEM Discover (Matthews, USA) single-mode microwave reactor system.

Preparative chromatography and analytic thin layer chromatography

Flash chromatography was performed following the procedure proposed by Still[167] on silica gel (Merck 60 mesh for flash chromatography). Thin layer chromatography (TLC) was performed on precoated plates of silica gel 60 F_{254} (Merck, Germany) and the compounds were detected at 254 nm.

4.2 Reagents and Solvents

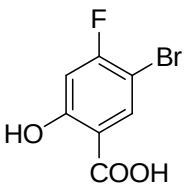
Dry solvents, dichloromethane, tetrahydrofurane, dioxane and methanol were purchased from Fluka. Ethylether, acetone, *N*,*N*-dimethyformamide, benzonitrile and acetonitrile were purchased from Aldrich, Germany Ethylether, petroleum ether, acetonitrile (HPLC grade), ethylacetate and ethanol were purchased from Merck. All solvents were used without further purification.

3-Bromo-4-fluorobenzaldehyde (**2a**), 1-(3-bromo-4-fluorophenyl)ethanone (**2c**), isopropylmagnesium chloride lithium chloride complex, [bis(trifluoroacetoxy)iodo]benzene, lithium

aluminium hydride, diisobutylaluminium hydride, carbontetrabromide, trifluoroacetic acid, trifluoroacetic anhydride, triphenylphosphine, diisopropylamine, *(S)*-Z-BMI, butyllithium, Wilkinson's catalyst, 2-fluoro-5-iodobenzaldehyde (**38**), isopropylmagnesium bromide, 3,5-bis(trifluoromethyl)-benzoyl chloride, potasium carbonate, sodium sulfate, sodium thiosulfate, magnesium sulfate, 2-fluoro-L,D-phenylalanine, ammonium chloride, sodium hydroxide, sodium chloride and Dess-Martin periodinane were purchased from Aldrich. Sulfuric acid, iodine, sodium acetate, benzylbromide, (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine, 1,2-ethanedithiol and ethylchloroformate from Fluka, and tributylammonium hydroxide, molecular bromine, *(S)*-BocBMI, hydroiodic and hydrobromic acids were acquired from Merck, Germany. 4-Fluorosalicilyc acid (**30**) was acquired from Alfa Aesar GmbH & Co KG. 2-Fluoro-L-tyrosine, 6-fluoro-L-*m*-tyrosine and 6-fluoro-L-DOPA were acquired from ABX. Hydrochloric acid and sodium hydrogencarbonate were purchased from KMF Laborchemie Handels GmbH. Diiodosilane was prepared as described by Keinan and Perez.[153]

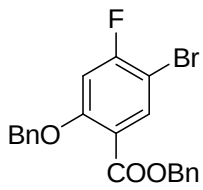
4.3 Synthesis of Seebach-precursors via a dithiolane approach

5-Bromo-4-fluoro-2-hydroxybenzoic acid (31)



4-fluorosalicilyc acid (1.00 g, 6.4 mmol) was dissolved in methanol (10 mL). Sodium acetate (2.20 g, 26.88 mmol) was added and the mixture was cooled to -78 °C. Molecular bromine (0.33 mL, 6.4 mmol) dissolved in 10 mL of methanol was slowly added and the mixture was allowed to warm to room temperature. After 5 h the solvent was removed under vacuum and the remnants treated with a solution of 10% HCl. The residue was filtered under vacuum, washed with water and dissolved in EtOAc. After drying with Na_2SO_4 the solvent was removed *in vacuo* to give 1.23 g (82%) of the desired product **31**. M.p. 205-207 °C (Lit. 203-205 °C[168])

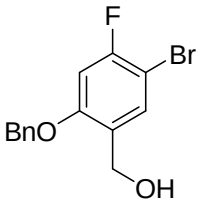
Benzyl 2-(benzyloxy)-5-bromo-4-fluorobenzoate (32)



K_2CO_3 (7.06 g, 51.08 mmol) and benzylbromide (3.34 mL, 28.08 mmol) were added to a solution of the acid **31** (3.00 g, 12.77 mmol) in acetone (100 mL). The mixture was stirred under reflux. After 3 h the heating was suspended and the reaction was cooled down to room temperature. Water was added and the mixture extracted with CH_2Cl_2 (3 x 30 mL). The organic fractions were combined and washed with brine. The organic layer was separated, dried over Na_2SO_4 and the solvent removed under vacuum. The crude product was purified by flash chromatography (5%

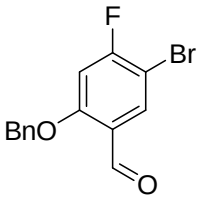
EtOAc/petroleum ether) giving 4.08 g of **32** (77%). R_f= 0.33 (5% EtOAc/petroleum ether); M.p. 77 – 78 °C. ^{1}H NMR ($CDCl_3$) δ 8.07 (d, *J*= 8.0, 1H), 7.41-7.29 (m, 10H), 6.79 (d, *J*= 10.4 Hz, 1H), 5.17 (s, 2H), 5.31(s, 2H); ^{13}C NMR ($CDCl_3$) δ 164.1, 161.9 (*J*= 253.4 Hz), 159.2 (*J*= 10.4 Hz), 136.6 (*J*= 2.5 Hz) 135.7, 135.4, 128.7, 128.6, 128.3, 128.24, 128.18, 127.1, 118.0 (*J*= 3.4 Hz), 102.8 (*J*= 26.2 Hz), 99.3 (*J*= 22.0), 71.2, 67.1; ^{19}F NMR ($CDCl_3$) δ -97.7; HRMS $C_{21}H_{17}BrFO_3$ m/z $[M + H]^+$ calculated 415.0345, found 415.0341.

(2-(Benzyloxy)-5-bromo-4-fluorophenyl)methanol (22)



3.06 g (7.36 mmol) of the ester **32** were dissolved in dry dichloromethane (50 ml) and cooled to 0 °C. 16.19 mL (1.0 M, 16.19 mmol) of a solution of DIBAL was added dropwise. The reaction was stirred during 10 min at 0 °C and then was allowed to warm to room temperature. After 30 min the reaction mixture was quenched with water. The organic layer was separated and the aqueous phase extracted with dichloromethane, the organic fractions were combined and washed with brine, separated and dried over Na_2SO_4. The solvent was removed and the crude product purified by flash chromatography (15% AcOEt/petroleum ether) to yield the product **22** in 90%. R_f= 0.60 (20 % EtOAc/petroleum ether); M.p. 100-101 °C. ^{1}H NMR ($CDCl_3$) δ 7.47 (d, *J*= 7.8, 1H), 7.31-7.38 (m, 5H), 6.73 (d, *J*= 10.2 Hz, 1H), 5.09 (s, 2H), 4.65 (s, 2H), 2.08 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 158.9 (*J*= 245.7 Hz), 156.4 (*J*= 8.5 Hz), 135.6 (*J*= 2.5 Hz), 132.4, 128.8, 128.4, 127.3, 127.1 (*J*= 4.2 Hz), 101.3 (*J*= 27.0 Hz), 99.1 (*J*= 21.1Hz), 70.7, 60.6; ^{19}F NMR ($CDCl_3$) δ -105.9; E.A. calculated C: 54.04, H: 3.89; found C: 53.6 H: 4.24.

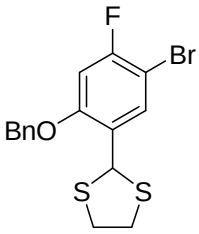
2-(Benzyloxy)-5-bromo-4-fluorobenzaldehyde (2b)



DMP 1.50 g (3.42 mmol) was added to a solution of the alcohol **22** (0.97 g, 3.13 mmol) in methylene chloride (20 mL) under stirring. After 20 min the reaction mixture was diluted with 50 mL of ether and poured into saturated aqueous $NaHCO_3$ containing a seven fold excess of $Na_2S_2O_3$. The mixture was stirred to dissolve the solid, and the layers were separated. The organic layer was washed with water, separated, dried over Na_2SO_4, filtrated and the solvent evaporated *in vacuo*. The crude product was purified by flash chromatography (5% EtOAc/petroleum ether) giving the desired compound in 95% of yield. R_f= 0.45 (5% EtOAc/petroleum ether); M.p. 89-91 °C. ^{1}H NMR ($CDCl_3$) δ 10.31 (s, 1H), 7.98 (d, *J*= 8.2, 1H), 7.31-7.38 (m, 5H), 6.78 (d, *J*= 9.9 Hz, 1H), 5.10 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 186.9, 163.3 (*J*= 255.9 Hz), 161.4 (*J*= 10.1 Hz), 134.8, 133.5 (*J*= 3.4 Hz), 128.9, 128.7, 127.4, 122.9 (*J*= 3.4 Hz), 102.4 (*J*= 26.2 Hz), 101.1 (*J*= 22.0

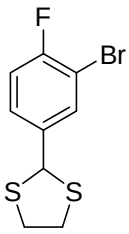
Hz), 71.2; ^{19}F NMR ($CDCl_3$) δ -93.6; HRMS $C_{14}H_{11}BrFO_2$ m/z $[M + H]^+$ calculated 308.9926, found 308.9905.

2-(2-(Benzyloxy)-5-bromo-4-fluorophenyl)-1,3-dithiolane (33b)



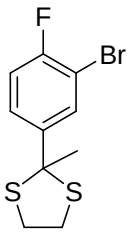
1,2-Ethanedithiol (0.29 mL, 3.55 mmol) was added to a solution of **2b** (1.00 g, 3.23 mmol) and iodine (0.082 g, 0.32 mmol) in dichloromethane (10 mL) and the mixture was stirred at room temperature. After 1 h the reaction was quenched with NaS_2O_3 (0.1 M, 25 mL) and NaOH (10 %, 25 mL). Then 25 mL of dichloromethane were added and the organic layer was separated, dried with $MgSO_4$ and filtered. The solvent was removed *in vacuo* and the crude product purified by flash chromatography (3% AcOEt/petroleum ether) giving 1.21 g of **33b** (97%). R_f= 0.49 (% EtOAc/petroleum ether); M.p. 117-118 °C. ^{1}H NMR ($CDCl_3$) δ 7.82 (d, *J*= 8.0, 1H), 7.14-7.39 (m, 5H), 6.63 (d, *J*= 10.2 Hz, 1H), 5.03 (s, 2H), 5.90 (s, 1H), 3.25 (m, 2H), 3.32 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.9 (*J*= 247.4 Hz), 156.8 (*J*= 8.9 Hz), 136.8, 133.3, 129.9, 129.4, 128.4, 129.2 (*J*= 3.2 Hz), 102.4 (*J*= 27.0 Hz), 100.3 (*J*= 21.1 Hz), 72.0, 49.4, 40.5; ^{19}F NMR ($CDCl_3$) δ -105.9; HRMS $C_{16}H_{15}BrFOS_2$ m/z $[M + H]^+$ calculated 384.9732, found 384.9726.

2-(3-Bromo-4-fluorophenyl)-1,3-dithiolane (33a)



Compound **33a** was prepared following the procedure described for compound **33b**. Yield 97%; R_f= 0.51 (5% EtOAc/petroleum ether); M.p. 51-53 °C. ^{1}H NMR ($CDCl_3$) δ 7.71 (dd, *J*= 7.2 Hz, *J*= 2.2 Hz, 1H), 7.39 (ddd, *J*= 8.5 Hz, *J*= 4.5 Hz, *J*= 2.3 Hz, 1H), 7.03 (t, *J*= 8.2 Hz, 1H), 5.53 (s, 1H), 3.47 (m, 2H), 3.34 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.6 (*J*= 248.3 Hz), 138.1 (*J*= 3.4 Hz), 133.0, 128.6 (*J*= 7.6 Hz), 116.3 (*J*= 22.8 Hz), 108.9 (*J*= 22.0 Hz), 54.8, 40.3; ^{19}F NMR ($CDCl_3$) δ -108.4.; HRMS $C_9H_9BrFS_2$ m/z $[M + H]^+$ calculated 278.9313, found 278.9133.

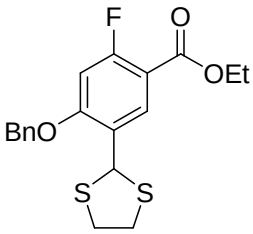
2-(3-Bromo-4-fluorophenyl)-2-methyl-1,3-dithiolane (33c)



1,3-Ethanedithiol (0.435 mL, 5.07 mmol) was added to a solution of 1-(3-bromo-4-fluorophenyl)ethanone (1.00 g, 4.60 mmol) and iodine (0.12 g, 0.46 mmol) in dichloromethane (10 mL) and the mixture was stirred under reflux. After 3.5 h the reaction was cooled to room temperature and quenched with NaS_2O_3 (0.1 M, 25 mL) and NaOH (10%, 25 mL). Then 25 mL of dichloromethane were added and the organic layer was separated, dried with $MgSO_4$ and filtered. The solvent was removed

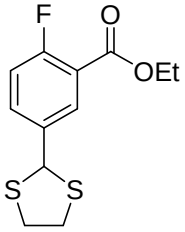
in vacuo and the crude product purified by flash chromatography (3% AcOEt/petroleum ether) to give **33c** (95%) as a pale yellow oil. R_f= 0.35 (3% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.94 (dd, *J*= 6.4 Hz, *J*= 2.6 Hz, 1H), 7.65 (ddd, *J*= 8.8 Hz, *J*= 4.5 Hz, *J*= 2.5 Hz, 1H), 7.02 (t, *J*= 8.5 Hz, 1H), 3.29-3.49 (m, 4H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 157.9 (*J*= 248.8 Hz), 143.8 (*J*= 3.3 Hz), 132.1, 127.7 (*J*= 7.6 Hz), 115.7 (*J*= 22.8 Hz), 108.3 (*J*= 24.7), 67.4, 40.5, 33.6; ^{19}F NMR ($CDCl_3$) δ -110.1; HRMS $C_{10}H_{11}BrFS_2$ m/z $[M + H]^+$ calculated 292.9470, found 292.9466.

Ethyl 4-(benzyloxy)-5-(1,3-dithiolan-2-yl)-2-fluorobenzoate (34b)



Dioxane (0.3 mL) was added to a solution of isopropylmagnesium chloride lithium chloride complex in THF (1.3 M, 3.14 mL 4.08 mmol) and the mixture was cooled to 0 °C. After 5 min 1.21 g (3.14 mmol) of compound **33b** was added and the mixture stirred during 1.0 h at 0 °C. Then 0.61 mL (6.28 mmol) of ethylchloroformate was added and the mixture was allowed to reach room temperature. The solution was stirred for 2 h and then quenched with saturated aqueous NH_4Cl solution (4 mL). The reaction mixture was partitioned in H_2O:ether (30:30) the organic layer separated and the aqueous phase was extracted with ether (2 x 20 mL), the organic fractions were combined dried with Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by flash chromatography (10% AcOEt/petroleum ether) yielding compound **34b** (77%). R_f= 0.39 (10% EtOAc/petroleum ether); M.p. 83-85 °C. ^{1}H NMR ($CDCl_3$) δ 8.29 (d, *J*= 8.4, 1H), 7.29-7.42 (m, 5H), 6.63 (d, *J*= 12.1 Hz, 1H), 5.13 (s, 2H), 4.35 (q, *J*= 7.1 Hz, 2H), 5.95 (s, 2H), 3.30 (m, 2H), 3.38 (m, 2H), 1.36 (s, *J*= 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 164.1 (*J*= 4.2 Hz), 162.7 (*J*= 260.9 Hz), 159.8 (*J*= 10.1 Hz), 135.4, 131.6 (*J*= 2.5 Hz), 128.7, 128.4, 127.3, 126.5 (*J*= 3.4 Hz), 110.7 (*J*= 9.3 Hz), 100.8 (*J*= 27.9 Hz), 70.9, 61.0, 48.4, 39.3, 14.3; ^{19}F NMR ($CDCl_3$) δ -106.4; HRMS $C_{19}H_{20}FO_3S_2$ m/z $[M + H]^+$ calculated 379.0838, found 379.0830.

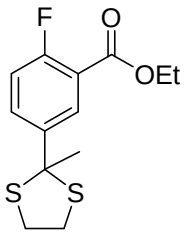
Ethyl 5-(1,3-dithiolan-2-yl)-2-fluorobenzoate (34a)



Compound **34a** was prepared following the procedure described for compound **34b**. Yield 81%; R_f= 0.42 (10% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.67 (ddd, *J*= 8.4 Hz, *J*= 4.4 Hz, *J*= 2.6 Hz, 1H), 7.99 (dd, *J*= 6.8 Hz, *J*= 2.4 Hz, 1H), 7.04 (dd, *J*= 8.7 Hz, *J*= 10.2 Hz, 1H), 4.35 (q, *J*= 7.1 Hz, 2H), 5.59 (s, 1H), 3.47 (m, 2H), 3.34 (m, 2H), 2.12, 1.37 (t, *J*= 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 164.0 (*J*= 4.2 Hz), 161.4 (*J*= 260.9 Hz), 136.5 (*J*= 3.4 Hz), 133.9 (*J*= 9.3

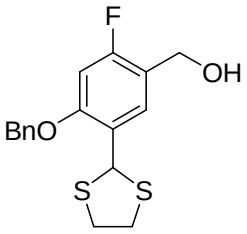
Hz), 131.5, 118.7 (*J*= 10.1 Hz), 117.2 (*J*= 22.8 Hz), 61.4, 55.0, 40.3, 14.2; ^{19}F NMR ($CDCl_3$) δ -110.5. HRMS $C_{12}H_{14}FO_2S_2$ m/z $[M + H]^+$ calculated 273.0419, found 273.0414.

Ethyl 2-fluoro-5-(2-methyl-1,3-dithiolan-2-yl)benzoate (34c)



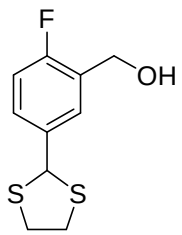
Compound **34c** was prepared following the procedure described for compound **34b**. Yield 81%; R_f= 0.57 (10 % EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 8.26 (dd, *J*= 6.8 Hz, *J*= 2.7 Hz, 1H), 7.91 (ddd, *J*= 8.8 Hz, *J*= 4.5 Hz, *J*= 2.7 Hz, 1H), 7.04 (dd, *J*= 8.8 Hz, *J*= 10.0 Hz, 1H), 4.38 (q, *J*= 7.2 Hz, 2H), 3.31 – 3.50 (m, 4H), 2.12 (s, 3H), 1.38 (t, *J*= 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 164.4 (*J*= 9 Hz), 160.8 (*J*= 261.3 Hz), 142.1 (*J*= 3.8 Hz), 133.2 (*J*= 9.5 Hz), 130.3, 118.1 (*J*= 10.0 Hz), 116.5 (*J*= 22.5 Hz), 67.5, 61.4, 40.5, 33.5, 14.3; ^{19}F NMR ($CDCl_3$) δ -112.2. HRMS $C_{13}H_{16}FO_2S_2$ m/z $[M + H]^+$ calculated 287.0576, found 287.0570.

(4-(Benzyloxy)-5-(1,3-dithiolan-2-yl)-2-fluorophenyl)methanol (35b)



1.21 mL of $LiAlH_4$ in dry THF (2.0 M, 2.42 mmol) was added dropwise to a solution of the ester **34b** (0.81 g, 2.42 mmol) dissolved in dry THF (25 ml). The reaction was stirred for 1 h at room temperature. The mixture was quenched with water and acidified with concentrated H_2SO_4 until the solid was dissolved. The mixture was extracted with CH_2Cl_2, the organic phase separated and dried over Na_2SO_4. The solvent was removed and the crude purified by flash chromatography (25 % AcOEt/petroleum ether) yielded product **35b** in 90%. R_f= 0.44 (30 % EtOAc/petroleum ether); M.p. 114-117 °C. ^{1}H NMR ($CDCl_3$) δ 7.73 (d, *J*= 8.6, 1H), 7.32 – 7.45 (m, 5H), 6.58 (d, *J*= 11.5 Hz, 1H), 5.06 (s, 2H), 4.63 (s, 2H), 6.00 (s, 1H) 3.28 (m, 2H), 3.37 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 160.6 (*J*= 247.4 Hz), 156.1 (*J*= 10.1 Hz), 136.0, 129.2 (*J*= 5.9 Hz), 128.6, 128.1, 127.2, 125.8 (*J*= 3.4 Hz), 119.5 (*J*= 15.2 Hz), 100.1 (*J*= 26.2 Hz), 70.6, 59.2 (*J*= 4.0 Hz), 48.6, 39.4; ^{19}F NMR ($CDCl_3$) δ -117.8; E.A. calculated C: 60.69, H: 5.09, S: 19.06; found C: 60.4, H: 5.45, S: 21.1.

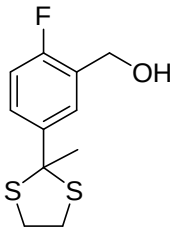
(5-(1,3-Dithiolan-2-yl)-2-fluorophenyl)methanol (35a)



Compound **35a** was prepared following the procedure described for compound **35b**. Yield 91%; R_f= 0.40 (20% EtOAc/petroleum ether); M.p. 60-61 °C. ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 1H), 7.53 (dd, *J*= 6.9 Hz, *J*= 1.9 Hz, 1H), 6.92 (“t”, *J*= 9.0 Hz, 1H), 4.67 (s, 2H), 5.56 (s, 1H) 3.44 (m, 2H), 3.30 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 160.0 (*J*= 247.4 Hz), 136.2 (*J*= 3.4 Hz), 128.78

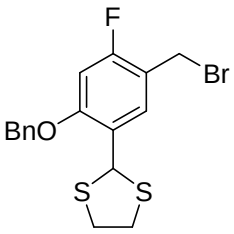
(*J*= 14.3 Hz), 128.76, 127.8 (*J*= 15.2 Hz), 115.2 (*J*= 22.0 Hz), 59.2 (*J*= 4.2 Hz), 55.5, 40.2; ^{19}F NMR ($CDCl_3$) δ -120.6; E.A. calculated C: 52.15, H: 4.81, S: 27.84; found C: 52.0, H: 4.95, S: 28.5.

(2-Fluoro-5-(2-methyl-1,3-dithiolan-2-yl)phenyl)methanol (35c)



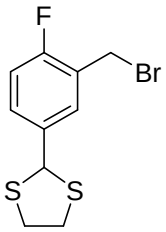
Compound **35c** was prepared following the procedure described for compound **34b**. Yield 91%; R_f= 0.52 (20% EtOAc/petroleum ether). ^{1}H NMR ($CDCl_3$) δ 7.79 (dd, *J*= 7.0 Hz, *J*= 2.5 Hz, 1H), 7.66 (ddd, *J*= 8.2 Hz, *J*= 4.9 Hz, *J*= 2.7 Hz, 1H), 6.95 ("t", *J*= 9.0 Hz, 1H), 4,73 (s, 2H), 3.32 – 3.44 (m, 4H), 2.12 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 159.5 (*J*= 247.2 Hz), 142.0 (*J*= 3.8 Hz), 128.0 (*J*= 8.2 Hz), 127.9, 127.0 (*J*= 14.5 Hz), 114.7 (*J*= 21.5 Hz), 67.9, 59.5 (*J*= 3.9 Hz), 40.4, 33.9; ^{19}F NMR ($CDCl_3$) δ -112.4; E.A. calculated C: 54.07, H: 5.36, S: 26.3; found C: 53.9, H: 5.84, S: 26.3.

2-(2-(Benzyloxy)-5-(bromomethyl)-4-fluorophenyl)-1,3-dithiolane (36b)



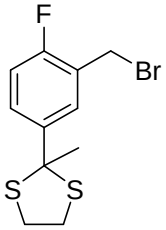
A magnetically stirred solution of the alcohol **35b** (1.25 g, 3.71 mmol) and 1.37 g (4.09 mmol) of carbontetrabromide in 10 mL of dichloromethane was cooled to 0 °C. A solution of triphenylphosphine (1.46 g, 5.57 mmol) in 5 mL of dichloromethane was added dropwise. After the addition the mixture was stirred for further 5 min, whereupon the solvent was partially removed *in vacuo* at room temperature. Ether (20 mL) was added and the mixture filtered. The filter cake was washed with ether (2 x 20 mL). The combined filtrates and washings were concentrated and the residue purified via flash chromatography giving 1.10 g (74%) of the bromide as a white solid. R_f= 0.46 (3% EtOAc/petroleum ether); M.p. 114-115 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (d, *J*= 8.7, 1H), 7.31-7.44 (m, 5H), 6.61 (d, *J*= 11.2 Hz, 1H), 5.09 (s, 2H), 4.48 (s, 2H), 5.97 (s, 1H) 3.40 - 3.27 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 160.7 (*J*= 250.9 Hz), 156.9 (*J*= 9.8 Hz), 135.7, 130.5 (*J*= 4.9 Hz), 128.7, 128.3, 127.2, 126.5 (*J*= 3.5 Hz), 116.6 (*J*= 14.9 Hz), 100.3 (*J*= 25.6 Hz), 70.7, 48.5, 39.3, 29.3 (*J*= 3.6 Hz); ^{19}F NMR ($CDCl_3$) δ -114.6; HRMS $C_{17}H_{17}BrFOS_2$ $[M + H]^+$ calculated 298.9888, found 298.9708.

2-(3-(Bromomethyl)-4-fluorophenyl)-1,3-dithiolane (36a)



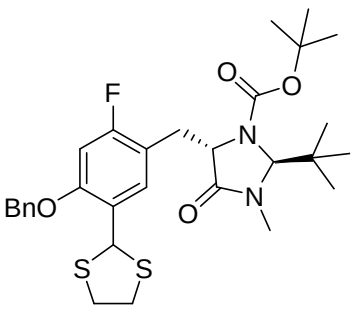
Compound **36a** was prepared following the procedure described for compound **36b**. Yield 74%; R_f= 0.49 (3% EtOAc/petroleum ether), M.p. 63-64 °C; ^{1}H NMR ($CDCl_3$) δ 7.59 (dd, *J*= 7.1 Hz, *J*= 2.4 Hz, 1H), 7.50 (ddd, *J*= 8.4 Hz, *J*= 4.8 Hz, *J*= 2.3 Hz, 1H), 7.04 ("t", *J*= 9.0 Hz, 1H), 5.63 (s, 1H), 4.53 (s, 2H) 3.60 - 3.33 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 160.2 (*J*= 249.7 Hz), 136.7 (*J*= 3.6 Hz), 130.8 (*J*= 3.5 Hz), 130.2 (*J*= 8.4 Hz), 125.1 (*J*= 14.9 Hz), 115.9 (*J*= 21.7 Hz), 55.3, 40.3, 25.5 (*J*= 4.4 Hz); ^{19}F NMR ($CDCl_3$) δ -117.7; HRMS $C_{10}H_{10}FS_2$ $[M + H - HBr]^+$ calculated 213.0208, found 213.0203.

2-(3-(Bromomethyl)-4-fluorophenyl)-2-methyl-1,3-dithiolane (36c)



Compound **36c** was prepared following the procedure described for compound **36b**. Yield 84%; R_f= 0.51 (3% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.76 (dd, *J*= 7.1 Hz, *J*= 2.1 Hz, 1H), 7.66 (ddd, *J*= 8.0 Hz, *J*= 4.5 Hz, *J*= 2.5 Hz, 1H), 6.95 ("t", *J*= 7.5 Hz, 1H), 4,49 (s, 2H), 3.51-3.28 (m, 4H), 2.01 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 159.6 (*J*= 250.9 Hz), 142.4 (*J*= 3.4 Hz), 129.8 (*J*= 3.2 Hz), 128.3 (*J*= 8.2 Hz), 124.3 (*J*= 14.6 Hz), 115.2 (*J*= 21.5 Hz), 67.7, 40.5, 33.7, 25.9 (*J*= 4.3 Hz); ^{19}F NMR ($CDCl_3$) δ -119.5; HRMS $C_{22}H_{24}BrF_2S_4$ $[2M + H - HBr]^+$ calculated 532.9912, found 532.9908.

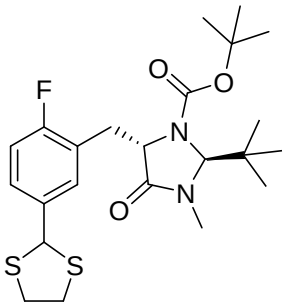
(2*S*,5*S*)-*tert*-Butyl 5-(4-(benzyloxy)-5-(1,3-dithiolan-2-yl)-2-fluorobenzyl)-2-tert-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (37b)



A stirred solution of diisopropylamine (0.47 mL, 3.30 mmol) in dry THF (2 mL) was cooled down to -78 °C, then BuLi (2.5 M/hexane, 1.32 mL, 3.30 mmol) was added dropwise. The resulting solution was stirred during 15 min. A solution of (*S*)-Boc-BMI (0.71 g, 2.75 mmol) in dry THF (2 mL) was added slowly and stirring was maintained for 40 min. The benzyl bromide **36b** (1.10 g, 2.75 mmol) in THF (3 mL) was then added. The reaction mixture was allowed to warm to room temperature and stirring was continued over 3 h. The reaction was quenched with saturated aqueous ammonium chloride (10 mL), 30 mL of water were added and the reaction was extracted with CH_2Cl_2 (3 x 25 mL). The organic layer was dried over sodium sulfate and evaporated giving the crude product

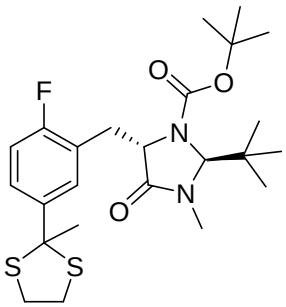
which was purified by flash chromatography (20% EtOAc/petroleum ether) (1.19 g, 75%, pale yellow solid). R_f= 0.42 (25 % EtOAc/petroleum ether); M.p. 117-118 °C. ^{1}H NMR ($CDCl_3$) δ 7.37 (d, *J*= 7.2, 1H), 7.32-7.45 (m, 5H), 6.54 (d, *J*= 11.5 Hz, 1H), 4.95 (br, H), 5.03 (s, 2H), 4.30 (br, 1H), 5.99 (s, 1H), 3.29 (m, 2H), 3.41 (m, 2H), 2.89 (s, 3H), 1.34 (s, 9H), 3.18 (d, *J*= 15.8 Hz, 1H), 3.60 (br, 1H), 0.95 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.5, 160.9 (*J*= 246.6 Hz), 154.8 (*J*= 10.1 Hz), 152.8 (br), 136.3, 129.3 (*J*= 4.3 Hz), 128.6, 128.0, 127.2, 125.0 (br), 115.1 (*J*= 16.0 Hz), 100.0 (*J*= 27.0 Hz), 81.0, 80.9, 70.5, 58.9 (*J*= 4.0 Hz), 48.7, 40.9, 39.4, 39.4, 39.2, 32.3, 28.0, 26.5, 26.6; ^{19}F NMR ($CDCl_3$) δ -113.6; HRMS $C_{30}H_{40}FN_2O_4S_2$ $[M + H]^+$ calculated 575.2414, found 575.2413.

(2*S*,5*S*)-*tert*-Butyl 5-(5-(1,3-dithiolan-2-yl)-2-fluorobenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (37a)



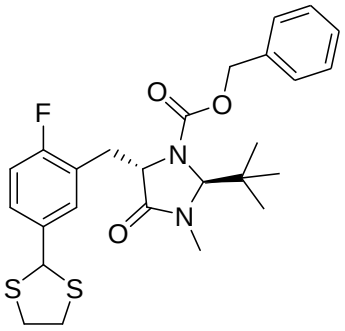
Compound **37a** was prepared following the procedure described for compound **37b**. Yield 84%; R_f= 0.33 (25% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.19-7.26 (m, 2H), 6.87 ("t", *J*= 9.3 Hz, 1H), 4.90 (br, 1H), 4.32 (br, 1H), 5.52 (s, 1H), 3.70 (br, 1H), 3.24 (d, *J*= 15.1 Hz, 1H), 3.46 (m, 2H), 3.30 (m, 2H), 2.88 (s, 3H), 1.33 (s, 9H), 0.93 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.5, 160.7 (*J*= 247.4 Hz), 152.7 (br), 135.2 (br), 129.7, 127.5 (*J*= 8.7 Hz), 123.8 (*J*= 15.8 Hz), 115.0 (*J*= 23.4 Hz), 81.2, 81.0, 58.8, 55.9, 40.9, 40.0, 40.1, 32.2, 28.0, 27.0 (br), 26.5; ^{19}F NMR ($CDCl_3$) δ -116.3; HRMS $C_{23}H_{34}FN_2O_3S_2$ m/z $[M + H]^+$ calculated 469.1995, found 469.1994.

(2*S*,5*S*)-*tert*-Butyl 2-*tert*-butyl-5-(2-fluoro-5-(2-methyl-1,3-dithio-lan-2-yl)benzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (37c)



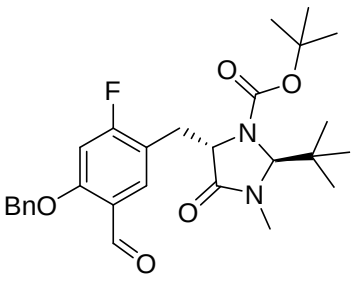
Compound **37c** was prepared following the procedure described for compound **37b**. Yield 89%; R_f= 0.52 (25% EtOAc/petroleum ether); M.p. 103-105 °C; ^{1}H NMR ($CDCl_3$) δ 7.51 (ddd, *J*= 8.6 Hz, *J*= 4.9 Hz, *J*= 2.7 Hz, 1H), 7.37 (dd, *J*= 7.0 Hz, *J*= 2.3 Hz, 1H), 6.88 ("t", *J*= 9.4 Hz, 1H), 5.04 (br, 1H), 4.33 (br, 1H) 3.25-3.41 (m, 4H), 3.27 (br, 2H), 2.03 (s, 3H), 2.97 (s, 3H), 1.25 (s, 9H), 0.96 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.6, 154.9(*J*= 248.6 Hz), 152.9 (br), 141.6 (br), 127.7 (br), 126.3 (*J*= 8.0 Hz), 123.0 (*J*= 15.1 Hz), 114.5 (*J*= 23.1 Hz), 81.0, 80.9, 68.6 (br), 58.2 (br), 40.8, 40.0, 39.9 (br), 33.7 (br), 32.4, 27.9, 26.7 (br), 26.5; ^{19}F NMR ($CDCl_3$) δ -118.5. HRMS $C_{24}H_{36}FN_2O_3S_2$ m/z $[M + H]^+$ calculated 483.2151, found 483.2148.

(2*S*,5*S*)-Benzyl 5-(5-(1,3-dithiolan-2-yl)-2-fluorobenzyl)-2-tert-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (37d)



A stirred solution of diisopropylamine (0.27 mL, 2.68 mmol) in dry THF (2 mL) was cooled down to -78 °C, then BuLi (2.5 M/hexane, 1.10 mL, 2.68 mmol) was added dropwise. The resulting solution was stirred during 15 min. A solution of (*S*)-Z-BMI (0.65 g, 2.23 mmol) in dry THF (2 mL) was added slowly and stirring was maintained for 40 min. The benzyl bromide **36a** (0.66 g, 2.23 mmol) in THF (2 mL) was then added. The reaction mixture was allowed to warm to room temperature and stirring was continued over 3 h. The reaction was quenched with saturated aqueous ammonium chloride (10 mL). Then 30 mL of water were added and the reaction was extracted with CH_2Cl_2 (3 x 25 mL). The organic layer was dried over sodium sulfate and evaporated giving the crude product which was purified by flash chromatography (20% EtOAc/petroleum ether) (0.87 g, 78%, pale yellow solid). R_f= 0.30 (25% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.39-7.29 (m, 7H), 6.93 (“t”, *J*= 9.3 Hz, 1H), 5.55 (s, 1H), 5.31-4.99 (dd, *J*= 53.7 Hz, *J*= 12.0 Hz, 2H), 4.87 (br, 1H), 4.44 (br, 1H), 3.79 (m, 1H), 3.57-3.32 (m, 4H), 3.16 (m, 1H), 2.90 (s, 3H), 0.95 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.9, 165.0, 135.8, 131.0 (*J*= 4.8 Hz), 132.5, 130.9, 128.5, 128.3, 128.1, 128.0, 115.2 (*J*= 23.7 Hz), 81.2, 67.5, 59.6, 55.61, 41.0, 40.26, 40.25, 32.2, 26.5; ^{19}F NMR ($CDCl_3$) δ -116.9;

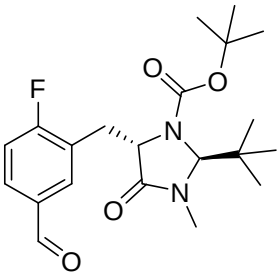
(2*S*,5*S*)-*tert*-Butyl 5-(4-(benzyloxy)-2-fluoro-5-formylbenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (1b)



[Bis(trifluoroacetoxy)iodo]benzene (1.37 g, 3.09 mmol) was added in one portion to 1.19 g of **37b** (2.06 mmol) dissolved in 10 mL of 9:1 MeOH:H_2O. The reaction mixture was stirred at room temperature during 5 min. The reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL). The product was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. Purification by flash chromatography (30% EtOAc/petroleum ether) yields the desired aldehyde **1b** in 92%. R_f= 0.42 (25% EtOAc/petroleum ether); M.p. 90-92 °C; ^{1}H NMR ($CDCl_3$) δ 10.36 (s, 1H), 7.51 (d, *J*= 8.8, 1H), 7.32-7.39 (m, 5H), 6.67 (d, *J*= 11.5 Hz, 1H), 4.88 (br, 1H), 5.10 (s, 2H), 4.29 (br, 1H), 2.98 (s, 3H), 3.28 (d, *J*= 14.9 Hz, 1H), 3.60 (br, 1H), 1.33 (s, 9H), 0.95 (s, 9H);

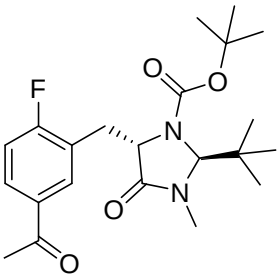
^{13}C NMR ($CDCl_3$) δ 187.9, 171.7, 165.8 (*J*= 256.7, Hz), 160.9 (*J*= 11.0 Hz), 152.5 (br), 135.4, 130.1 (*J*= 8.4 Hz), 128.8, 128.4, 127.2, 121.4 (br), 117.0 (*J*= 18.1 Hz), 100.9 (*J*= 27.0 Hz), 81.2, 81.1, 70.7, 58.4 (br), 40.9, 32.1, 29.7, 27.9, 26.5; ^{19}F NMR ($CDCl_3$) δ -101.2; HRMS $C_{28}H_{36}FN_2O_5$ $[M + H]^+$ calculated 499.2608, found 499.2602.

(2*S*,5*S*)-*tert*-Butyl 2-*tert*-butyl-5-(2-fluoro-5-formylbenzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (1a)



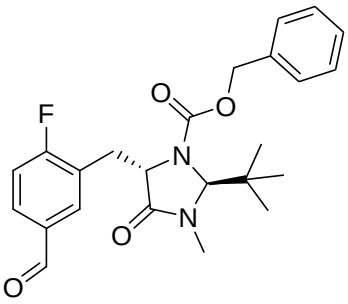
Compound **1a** was prepared following the procedure described for compound **1b**. Yield 93%; R_f= 0.34 (25% EtOAc/petroleum ether); M.p. 92-93 °C; ^{1}H NMR ($CDCl_3$) δ 9.81 (s, H), 7.69 (m, 1H), 7.58 (dd, *J*= 7.0 Hz, *J*= 1.4 Hz, 1H), 7.10 (“t”, *J*= 9.2 Hz, 1H), 4.84 (br, 1H), 4.33 (br, 1H), 3.65 (br, 1H), 3.41 (d, *J*= 15.2 Hz, 1H), 2.92 (s, 3H), 1.34 (s, 9H), 0.94 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 190.5, 171.4, 165.0 (*J*= 257.0 Hz), 152.5 (br), 132.5, 132.3 (*J*= 6.2 Hz), 130.2 (*J*= 10.0 Hz), 125.4 (*J*= 16.7 Hz), 116.2 (*J*= 24.4 Hz), 81.3, 81.1, 58.5, 40.9, 32.2, 28.0, 27.6 (br), 26.5; ^{19}F NMR ($CDCl_3$) δ -104.3; HRMS $C_{21}H_{30}FN_2O_4$ m/z $[M + H]^+$ calculated 393.2190, found 393.2183.

(2*S*,5*S*)-*tert*-Butyl 5-(5-acetyl-2-fluorobenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (1c)



Compound **1c** was prepared following the procedure described for compound **1b**. Yield 91%; R_f= 0.29 (25% EtOAc/petroleum ether); M.p. 76-78 °C; ^{1}H NMR ($CDCl_3$) δ 7.76 (ddd, *J*= 8.4 Hz, *J*= 5.2 Hz, *J*= 2.4 Hz, 1H), 7.66 (dd, *J*= 7.4 Hz, *J*= 2.3 Hz, 1H), 6.88 (“t”, *J*= 8.7 Hz, 1H), 4.85 (br, 1H), 4.34 (br, 1H), 3.68 (br, 1H), 3.38 (d, *J*= 14 Hz, 1H), 2.94 (s, 3H), 2.52 (s, 3H), 1.33 (s, 9H), 0.96 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 196.6, 171.6, 164.3(*J*= 255.7 Hz), 152.7 (br), 130.9 (*J*= 5.4 Hz), 128.8 (*J*= 9.0 Hz), 124.3 (*J*= 14.4 Hz), 115.5, 113.0 (*J*= 3.0 Hz), 81.3, 81.1, 58.5, 40.9, 32.2, 27.9, 26.6, 26.5; ^{19}F NMR ($CDCl_3$) δ -107.4. HRMS $C_{22}H_{32}FN_2O_4$ m/z $[M + H]^+$ calculated 469.1995, found 469.1994.

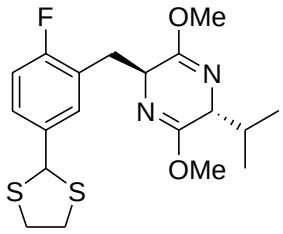
(2*S*,5*S*)-Benzyl 2-*tert*-butyl-5-(2-fluoro-5-formylbenzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (1d)



Compound **1d** was prepared following the procedure described for compound **1b**. Yield 90%; R_f= 0.22 (25% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 9.86 (s, 1H), 7.79-7.65 (m, 2H), 7.14 ("t", *J*= 9.5 Hz, 1H), 5.30-4.96 (dd, *J* = 56.3, *J*= 12.0) 4.89 (br, 1H), 4.48 (br, 1H), 3.80 (m, 1H), 3.47 (m, 1H), 2.93 (s, 3H), 0.96 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 190.6, 171.0, 165.0, 135.5, 133.4 (*J*= 6.2 Hz), 132.5, 130.0 (*J*= 10.0 Hz), 128.7, 128.6, 128.4, 128.2 (*J*= 16.7 Hz), 116.0 (*J*= 24.4 Hz), 81.2, 67.6, 58.1, 40.9, 32.0, 26.4; ^{19}F NMR ($CDCl_3$) δ -105.4.

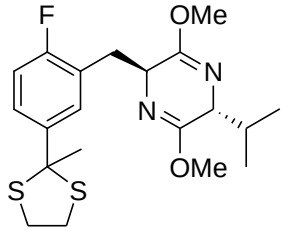
4.4 Synthesys of Schöllkopf-precursors via a dithiolane approach

(2*S*,5*R*)-2-(5-(1,3-Dithiolan-2-yl)-2-fluorobenzyl)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazine (37e)



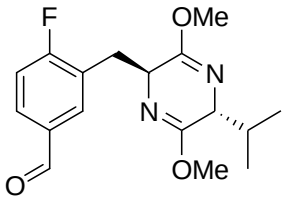
(*R*)-2,5-Dihydro-3,6-dimethoxy-2-isopropylpyrazine (0.23 ml, 1.23 mmol) was dissolved in 2 ml of dry THF. The solution was cooled down to -78 °C and BuLi (2.5 M/hexane, 0.49 ml, 1.23 mmol) was added under stirring. After 15 min, compound **36a** (0.36 g, 1.23 mmol) in 2 mL THF was added and the reaction mixture was allowed to warm to room temperature and stirring was continued over 3 h. The reaction was quenched with water (20 mL) and extracted with ether (3x15 mL). The organic extracts were combined, dried over sodium sulfate and the solvent evaporated giving the crude product which was purified by flash chromatography (5% EtOAc/petroleum ether) (0.35 g, 71%, colorless oil); R_f= 0.45 (10% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.63 (m, 2H), 6.93 ("t", *J*= 9.2 Hz, 1H), 5.60 (s, 1H), 4.35 (m, 1H), 3.75 (s, 3H), 3.70 (s, 3H), 3.47 (t, *J*= 2.3 Hz, 1H), 3.34-3.49 (m, 4H), 2.97-3.29 (m, 2H), 2.23 (m, 1H), 1.01 (d, *J*= 6.8 Hz, 3H), 0.66 (d, *J*= 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 162.5 (*J*= 196.3 Hz), 162.4, 135.1 (*J*= 3.8 Hz), 131.9 (*J*= 4.9 Hz), 127.7 (*J*= 8.4 Hz), 124.7 (*J*= 16.4 Hz), 115.3 (*J*= 23.3 Hz), 60.3, 55.8, 52.5, 40.1, 33.9, 33.2, 31.2, 26.5, 19.0, 16.5; ^{19}F NMR ($CDCl_3$) δ -117.9.

(2*S*,5*R*)-2-(2-Fluoro-5-(2-methyl-1,3-dithiolan-2-yl)benzyl)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (37f)



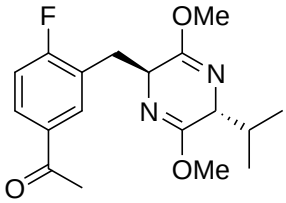
Compound **37f** was prepared following the procedure described for compound **37e**. Yield 77%; R_f= 0.55 (10% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.58 (m, 1H), 6.93 ("t", *J*= 9.2 Hz, 1H), 4.40 (m, 1H), 3.78 (s, 3H), 3.73 (s, 3H), 3.54-3.23 (br, 6H), 3.11-3.02 (m, 1H), 2.18 (m, 1H), 2.11 (s, 3H), 0.98 (d, *J*= 6.8 Hz, 3H), 0.64 (d, *J*= 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 163.0, 162.4, 131.2, 130.9 (*J*= 3.2 Hz), 130.0 (*J*= 9.6 Hz), 126.8 (*J*= 8.2 Hz), 114.3 (*J*= 23.0 Hz), 60.1, 56.1, 52.5, 40.3, 33.9, 33.1, 31.0, 19.1, 16.4; ^{19}F NMR ($CDCl_3$) δ -119.5.

4-fluoro-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)benzaldehyde (1e)



Compound **1e** was prepared following the procedure described for compound **1b**. Yield 71%; R_f= 0.34 (5 % EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 9.95 (s, H), 7.76 (m, 1H), 7.17 ("t", *J*= 8.5 Hz, 1H), 4.36 (m, 1H), 3.77 (s, 3H), 3.67 (s, 3H), 3.57 (t, *J*= 3.4 Hz, 1H), 3.04-3.40 (m, 2H), 2.20 (m, 1H), 1.00 (d, *J*= 6.8 Hz, 3H), 0.67 (d, *J*= 6.8 Hz, 3H);

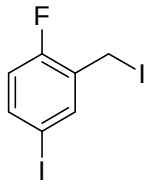
1-(4-fluoro-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)phenyl)ethanone (1f)



Compound **1f** was prepared following the procedure described for compound **1b**. Yield 70%; R_f= 0.30 (10% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.84 (m, 1H), 7.10 ("t", *J*= 9.1 Hz, 1H), 4.34 (m, 1H), 3.77 (s, 3H), 3.69 (s, 3H), 3.41 (t, *J*= 3.4 Hz, 1H), 3.03-3.35 (m, 2H), 2.59 (s, 3H), 2.18 (m, 1H), 0.99 (d, *J*= 6.8 Hz, 3H), 0.66 (d, *J*= 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 196.2, 167.0 (*J*= 253.1 Hz), 162.4, 133.2 (*J*= 6.5 Hz), 132.9 (*J*= 3.2 Hz), 129.0 (*J*= 9.6 Hz), 125.2 (*J*= 16.8 Hz), 115.3 (*J*= 23.6 Hz), 60.5, 55.8, 52.5, 33.2, 31.5, 26.5, 19.0, 16.5; ^{19}F NMR ($CDCl_3$)δ -109.1.

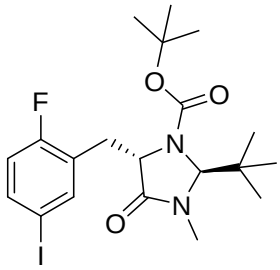
4.5 Synthesis of fluorophenone derivatives of Seebach-precursors 1g and 1h via a Grignard approach

1-Fluoro-4-iodo-2-(iodomethyl)benzene (39)



2-Fluoro-5-iodobenzaldehyde (1.00 g, 4.0 mmol) was dissolved in 25 mL dichloromethane. Diiodosilane (0.6 mL, 6.0 mmol) was added and the mixture stirred at roon temperature during 1.5 h. The reaction was quenched with a saturated solution of $NaHCO_3$ (20 mL) and 10 % aqueous $Na_2S_2O_3$ (20 mL). The organic phase was separated, dried over Na_2SO_4 and the solvent removed *in vacuo*. The residue was purified by flash chromatography (1% AcOEt:P.E.). The desired product was obtained as white solid in 80% yield. R_f= 0.50 (1% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.70 (dd, *J*= 7.0 Hz, *J*= 2.4 Hz, 1H), 7.57 (ddd, *J*= 8.6 Hz, *J*= 4.8 Hz, *J*= 2.3 Hz, 1H), 6.82 (“t”, *J*= 8.8 Hz, 1H), 4,37 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 162.8 (*J*= 249.6 Hz), 139.3 (*J*= 3.1 Hz), 138.7 (*J*= 8.0 Hz), 129.4 (*J*= 15.6 Hz), 118.1 (*J*= 21.9 Hz), 87.1 (*J*= 3.7 Hz); ^{19}F NMR ($CDCl_3$) δ -117.3.

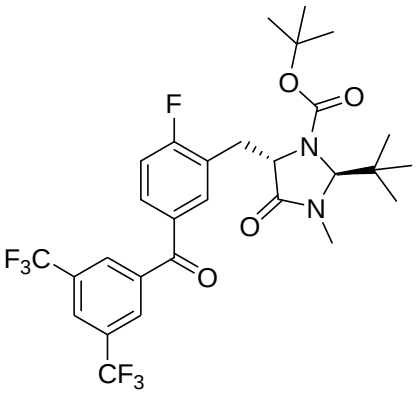
(2*S*,5*S*)-*tert*-Butyl 2-*tert*-butyl-5-(2-fluoro-5-iodobenzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (40)



A stirred solution of diisopropylamine (0.27 mL, 1.87 mmol) in dry THF (1.0 mL) was cooled down to -78 °C, then BuLi (2.5 M/hexane, 0.75 mL, 1.87 mmol) was added dropwise. The resulting solution was stirred during 15 min. A solution of (*S*)-Boc-BMI (0.40 g, 1.56 mmol) in dry THF (1.5 mL) was added slowly and stirring was maintained for 40 min. The benzyl iodide **39** (0.56 g, 1.56 mmol) in THF (2.0 mL) was then added. The reaction mixture was allowed to warm to room temperature and stirring was continued over 3 h. The reaction was quenched with saturated aqueous ammonium chloride (10 mL), 30 mL of water were added, and the reaction was extracted with ether (3 x 25 mL). The organic layer was dried over sodium sulfate and evaporated to give the crude product which was purified by flash chromatography (20% EtOAc/petroleum ether) (0.62 g, 81%, white solid). R_f= 0.48 (20% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.44 (m, 1H), 7.38 (dd, *J*= 6.8 Hz, *J*= 2.2 Hz, 1H), 6.78 (“t”, *J*= 8.6 Hz, 1H), 4.86 (s, 1H), 4.33 (br, 1H), 3.65 (br, 1H), 3.30 (dd, *J*= 15.1 Hz, *J*= 2.4 1H), 2.97 (s, 3H), 1.44 (s, 9H), 0.99 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.4, 163.7 (*J*= 246.3 Hz), 152.0, 139.4 (*J*= 4.1 Hz), 136.8 (*J*= 8.1 Hz),

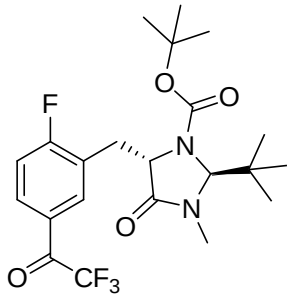
126.6 (*J*= 16.1 Hz), 117.4 (*J*= 24.0 Hz), 86.6 (*J*= 3.7 Hz), 81.3, 81.2, 58.8, 41.0, 32.0, 28.1, 26.6; ^{19}F NMR ($CDCl_3$) δ -116.1.

(2*S*,5*S*)-tert-Butyl 5-(5-(3,5-bis(trifluoromethyl)benzoyl)-2-fluorobenzyl)-2-tert-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (1g)



Isopropylmagnesium bromide (2.9 M, 0.42 mL, 1.22 mmol) was placed through syringe in a dry and argon flushed round bottom flask, 1.0 mL of dry THF and the solution was cooled to 0 °C. A solution of compound **40** (0.30 g, 0.61 mmol) in 1.0 mL dry THF was added and the mixture stirred during 1.0 h at 0 °C. Then, 0.23 mL (1.22 mmol) of 3,5-bis(trifluoromethyl)-benzoyl chloride was added and the mixture was allowed to reach room temperature. The reaction mixture was stirred for 2 h and was then quenched with saturated aqueous NH_4Cl solution (4 mL). The mixture was partitioned in H_2O:ether (30:30) the organic layer separated and the aqueous phase was extracted with ether (2 x 20 mL), the organic fractions were combined, dried with Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (25% AcOEt/petroleum ether) yielding compound **1g** (84%). R_f= 0.29 (10% EtOAc/petroleum ether). R_f= 0.74 (25% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 8.24 (br, 2H), 8.13 (br, 1H), 7.64 (m, 2H), 7.18 ("t", *J*= 8.6 Hz, 1H), 4.98 (s, 1H), 4.40 (m, 1H), 3.60 (m, 2H), 2.98 (s, 3H), 1.41 (s, 9H), 1.01 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 192.3, 171.6, 163.0, 139.4, 133.2 (*J*= 6.1 Hz), 132.4, 131.6 (*J*= 3.2 Hz), 130.6 (*J*= 9.7 Hz), 129.7 (br), 125.6 (br), 125.4 (*J*= 11.0 Hz), 120.2, 115.8 (*J*= 23.9 Hz), 81.4, 81.1, 58.3, 40.9, 31.9, 28.0, 26.5; ^{19}F NMR ($CDCl_3$) δ -105.9, -62.8.

(2*S*,5*S*)-*tert*-Butyl 2-*tert*-butyl-5-(2-fluoro-5-(2,2,2-trifluoroacetyl)benzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (1h).



Compound **1h** was prepared following the procedure described for compound **1g** but using trifluoroacetic anhydride. Yield 62%. R_f= 0.32 (25 % EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.40 (m, 1H), 7.16 (m, 2H), 4.85 (s, 1H), 4.36 (m, 1H), 3.75 (m, 1H), 3.01 (m, 1H), 2.90 (s, 3H), 1.16 (s, 9H), 0.94 (s, 9H); ^{13}C NMR ($CDCl_3$) δ; ^{19}F NMR ($CDCl_3$)δ -82.8, -116.3.

4.6 Radiochemistry

4.6.1 Preparation of tetrabutylammonium [^{18}F]fluoride

N.c.a. [^{18}F]fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction with bombardment of an isotopically enriched [^{18}O]water target with 17 MeV protons at the JSW cyclotron BC 1710 (FZ Jülich).[169] The produced ^{18}F-fluoride was isolated from the irradiated water through electrochemically supported adsorption on a Sigradur-Anode (HTW Hochtemperatur-Werkstoffe GmbH) and desorption into 500 mL of pentadistilled water after recovery of the ^{18}O enriched water.[24] An aliquot of the [^{18}F]fluoride solution was added to 17.5-130 µL (2.6-17.0 µmol) of a 0.13 M tetrabutylammonium bicarbonate solution ($TBAHCO_3$). The mixture was diluted with 1.0 mL of dry acetonitrile and transferred by syringe into a reaction flask. The solvent was evaporated under a stream of nitrogen at 80 °C and 650 mbar. The azeotropic evaporation was repeated twice with 1.0 mL of acetonitrile and afterwards the vial was evacuated for 5 min at 20-30 mbar.

4.6.2 General procedure for the radiosynthesis of 2-[^{18}F]fluoro-L-phenylalanine and 2-[^{18}F]fluoro-L-tyrosine under conventional heating

A solution of 10-15 µmol of the corresponding amino acid-precursor in 1.0 mL DMF was added to the dried residue of TBA^{18}F. The mixture was heated at 130 ºC for 10 min. After labeling, the DMF solution was diluted with water (15 mL), and passed through a pre-conditioned LiChrolut RP-18e cartridge (Merck, Germany). The product was eluted from the cartridge with 2.0 mL acetonitrile and then the solvent evaporated at 80 °C and 650 mbar. A solution of 1.5 eq. of Wilkinson´s catalyst in 1 mL dioxane was added to the dry residue and the mixture was stirred for 20 min at 150 ºC. The dioxane was distilled off, the residue suspended in 1 mL of a solution of ethyl acetate in petroleum ether (30% AcOEt/P.E.) and then filtered through a silica gel plug (650 mg silica gel in a 3 mL polyethylene filtration tube). The reaction vial was washed with an extra portion of the solution (1 mL) and the compound was then eluted with 4 mL of the same mixture. The solvent mixture was evaporated and 250 µL of hydrochloric acid were added and the reaction was heated to 200 °C and stirred for 30 minutes.

4.6.3 General procedure for the radiosynthesis of 2-[^{18}F]fluoro-L-phenylalanine and 2-[^{18}F]fluoro-L-tyrosine under microwave heating

A solution of 10-15 µmol of the corresponding amino acid-precursor in 1.0 mL DMF was added to the dried residue of TBA^{18}F. The mixture was irradiated with 50 W microwaves during 1 min. The purification of the labeled compound was carried out using the same procedure described above. A solution of 4.0 eq. of Wilkinson´s catalyst in 1 mL benzonitrile was added and the mixture was irradiated with 100 W microwaves during 50 s. The reaction mixture was diluted with 10 mL of a solution of ethyl acetate in petroleum ether (5% AcOEt/P.E.) and then passed through a silica gel column (see above). The desired compound was then eluted from the column with 5 mL of a solution of 30% AcOEt/P.E. After the solvent mixture was evaporated, 250 µL of hydrochloric acid were added and the reaction mixture was heated with microwaves to 140 ºC for 20 min (Ramp: 150 W, 15 s, Hold: 20 min).

4.6.4 General procedure for the radiosynthesis of 6-[^{18}F]fluoro-L-*m*-tyrosine

A solution of 10-15 µmol of the precursor in 1.0 mL DMSO was added to the dried residue of TBA^{18}F. The mixture was heated at 160 ºC for 10 min. After labeling, the DMSO solution was diluted with water (10 mL), and passed through a pre-conditioned LiChrolut RP-18e cartridge; the product was eluted from the cartridge with 2.0 mL chloroform and the eluate was dried using a column packed with Na_2SO_4. 45 µmol of the oxidant agent was added, and the mixture was stirred for 30 min at 60 ºC. After the reaction was completed the chloroform was evaporated at 60 °C and 750 mbar. 1 mL of hydrochloric acid (32 %) was added to the residue and the solution was heated at 200 ºC for 30 min.

4.6.5 General procedure for the radiosynthesis of 6-[^{18}F]fluoro-L-DOPA

A solution of 10-15 µmol of the precursor in 1.0 mL DMF was added to the dried residue of TBA^{18}F. The mixture was heated at 130 ºC for 10 min. After labeling, the DMF solution was diluted with water (10 mL), and passed through a pre-conditioned LiChrolut RP-18e cartridge; the product was eluted from the cartridge with 2.0 mL chloroform and the eluate was dried using a column packed with Na_2SO_4. 45 µmol of the oxidant agent was added, and the mixture was stirred for 10 min at 60 ºC. After the reaction was completed the chloroform was evaporated at 60 °C and 750 mbar. 1 mL of hydrochloric acid (32 %) was added to the residue and the solution was heated at 200 ºC for 30 min.

4.6.6 Automated radiosynthesis of 6-[^{18}F]FDOPA

Synthesis device

The automated device used for a remote-controlled radiosynthesis of 6-[^{18}F]FDOPA device was a SynChrom R&D (Raytest GmbH, Germany) controlled by GINA SynChrom software. For a schematic sketch of the set-up see Figure 13.

[^{18}F]Fluoride fixation and desorption

[^{18}F]Fluoride was separated from the irradiated ^{18}O-enriched water by an anion exchange resin (QMA light). The elution of the activity was achieved with 0.3 ml of a mixture of tetra-*n*-butyl ammonium hydrogencarbonate (6.4 µmol) and tetra-*n*-butyl ammonium mesylate (25 µmol) in water. Azeotropic drying was then repeatedly performed with acetonitrile (DNA quality, 3 x 1 mL).

Radiofluorination

(2*S*,5*S*)-*tert*-Butyl 5-(4-(benzyloxy)-2-fluoro-5-formylbenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate **1b** (5 mg, 14.8 mmol) dissolved in 1 ml of dry DMF was added to the TBA^{18}F residue and heated at 130 °C for 10 min. Afterwards, the solution was diluted with 10 mL of a mixture 4:1 of water and acetonitrile.

Purification of the intermediate compound [^{18}F]1b

The solution containing **[^{18}F]1b** was passed through a C-18 cartridge and afterward the cartridge was washed with 10 mL water. The desired compound was eluted with 1.5 mL chloroform and the eluate was driven through a column containing 700 mg of sodium sulfate.

Baeyer-Villiger oxidation and Hydrolysis

The dry solution was transferred to the second reactor where 120 mg of sodium percarbonate and 100 µL of trifluoroacetic anhydride in 0.5 mL dry chloroform were previously loaded. The reaction mixture was then heated at 60 °C for 10 min. After the time was over the reaction mixture was evaporated to dryness at the under a stream He at 650 mbar. Concentrated hydrochloric acid (1 mL) was added to the dry residue and the reaction mixture heated to 170 °C for 30 min. Afterward the mixture was cooled down to 40 °C and diluted with 1 mL water.

4.6.7 Radioanalytic procedures

The analysis and isolation of the ^{18}F-labeled products was performed by either thin layer chromatography (TLC) or high-performance liquid chromatography (HPLC).

Radio-thin layer chromatography

Analytical radio-thin layer chromatography (radio-TLC) was carried out in order to determine the RCY of the products of the isotopic exchange reaction. This was performed on precoated plates of silica gel 60 F_{254}. In practice, 2 µl of the sample solution was applied on the TLC plates and developed in the appropriate solvent mixture. The [^{18}F]fluorine could easily be identified since, under the elution conditions, it remained on the origin of the TLC plate. Radioactivity on the TLC plate was detected simultaneously using an Instant Imager™ (Packard Instruments). The RCY then was calculated dividing the activity of the compound of interest by the total activity deposited on the TLC plate, multiplied by 100.

Table 11: R_f –values and eluent system of the compounds labeled by isotopic exchange

Compound	Solvent system (% AcOEt/P.E.)	R_f value
[^{18}F]1a	25	0.34
42a	25	0.28
[^{18}F]1b	25	0.42
42b	25	0.36
[^{18}F]1c	25	0.29
45c	25	0.25
[^{18}F]1d	30	0.35
42d	30	0.28
[^{18}F]1e	5	0.34
[^{18}F]1f	10	0.30
[^{18}F]1g	10	0.29
[^{18}F]1h	25	0.35

Radio-high performance liquid chromatography

High performance liquid chromatography (HPLC) separations were achieved with a Knauer pump, a Knauer K-2500 UV/VIS detector, two manual Rheodyne injectors (20 µL loop), the first located before the chromatography column and the second after it, and an NaI(Tl) well-type scintillation detector (EG&G Ortec; model 276 Photomultiplier Base) with an ACE Mate Amplifier and BIAS supply (Ortec) for radioactivity detection. Data acquisition and interpretation were performed with Gina software (Raytest Germany).

In practice, the radioactive sample was injected into the column using the first injector, and after the elution of the last radioactive component three aliquots of the sample were introduced into the system through the second injector. The RCY of the product was determined using the Equation 1 by multiplying the activity of the desired compound (A_p), by 100 and the correction factor for decay (λ is the decay constant of fluorine-18 and "t" is the time difference between the retention time of the peak of interest and the standard), and dividing by the total activity of the sample (A_0).

$$RCY[\%] = \frac{A_p \times e^{-\lambda t} \times 100}{A_0}$$

Equation 1: Determination of the RCY

HPLC-Systems:

A: Analysis of ^{18}F-labelled compound was performed with a reverse-phase Kromasil 100-5 C18 column (250-4.6 mm; CS Chromatographieservice GmbH). Elution was performed at a constant flow rate of 1 mL/min with acetonitrile:water (70:30).

B: Analysis was performed with an analytic reverse-phase Synergi 4µ Hydro-RP 80A column (250-4.6 mm; Phenomenex). The mobile phase was aqueous acetic acid (0.1%) used at a flow rate of 1 mL/min.

C: Preparative separations were carried out with a Synergi 4µ Hydro-RP 80A column (250 - 10 mm; Phenomenex). The mobile phase was aqueous ethanol (2%), and the flow rate was 4 mL/min.

D: The enantiomeric purity of the ^{18}F-labeled compounds was determined by HPLC using a Crownpak CR (1) 5µ column (150-4 mm; Daicel Chemical Industries) and aqueous

$HClO_4$ (0.025 M) as eluent at a flow rate of 1 mL/min. Peak recording was achieved by UV detection at 261 nm.

Table 12: k´-values of the samples analyzed by HPLC

Compound/HPLC system	**A**	**B**	**C**	**D (L/D enant.)**
[^{18}F]1a	3.7	-	-	-
[^{18}F]1b	11.2	-	-	-
[^{18}F]1c	3.8	-	-	-
[^{18}F]1g	25	-	-	-
[^{18}F]1h	2.7	-	-	-
41a	4.7	-	-	-
41b	14.3	-	-	-
44c	4.2	-	-	-
2-[^{18}F]Fphe	-	5.4	5.1	6.0/4.4
2-[^{18}F]Ftyr	-	3.0	2.7	5.2/3.6
6-[^{18}F]Fmtyr	-	6.3	6.0	6.8/4.3
6-[^{18}F]FDOPA	-	5.4	5.1	6.0/4.4

5 SUMMARY

Aromatic [^{18}F]fluoroamino acids have been developed as potential probes for the diagnosis of different pathologies in nuclear medicine. The most representative of this class of compounds is 6-[^{18}F]fluoro-L-DOPA (6-[^{18}F]FDOPA), one of the few established radio-pharmaceuticals for positron emission tomography (PET), which is routinely used for the diagnosis of central motor disorders and also of brain and peripheral endocrine tumors. On the other hand, there are some other aromatic [^{18}F]fluoroamino acids which have already preliminary been tested that showed also interesting properties as diagnostic tracers. 2-[^{18}F]Fluoro-L-phenylalanine (2-[^{18}F]Fphe) has proven to be a useful radiopharmaceutical for the study of neutral amino acid transport at the blood brain barrier *in vivo* in humans. 2-[^{18}F]Fluoro-L-tyrosine (2-[^{18}F]Ftyr), unlike other halogenated amino acids, is almost quantitatively incorporated into proteins lending it as interesting tracer for imaging of protein synthesis *in vivo*. Thus the accumulation of 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr enables to distinguish tumors from normal tissue, and positron emission tomography studies of their uptake are of special clinical value for the diagnosis of brain tumors. 6-[^{18}F]Fluoro-L-*m*-tyrosine (6-[^{18}F]Fmtyr) is another interesting compound that has potential as analogous imaging agent of 6-[^{18}F]FDOPA measuring the dopaminergic function in the brain in both animals as well as humans using PET.

In spite of all the possible applications of these compounds their availability so far has been limited due to radiosynthetic constraints. Two general pathways have been developed for the radiofluorination of arene-derivatives of high electron density. The electrophilic approach is applicable because many of those compounds have a low toxicity and therefore they can be used in c.a. form. Due to the necessary generation of elemental [^{18}F]fluorine for this approach, however, the currently used methods for routine preparation are limited to rather low amounts of activity at high costs. Alternatively developed nucleophilic syntheses using the advantage of large scale production of [^{18}F]fluoride, however, result either in insufficient enantiomeric purity or in need of multi-step syntheses that are difficult to automate, due to their complexity.

Recently, a nucleophilic isotopic exchange approach made the radiosynthesis of 6-[^{18}F]FDOPA available in three steps. In this radiochemical process the precursor (2*S*,5*S*)-*tert*-butyl 5-(4-(benzyloxy)-2-fluoro-5-formylbenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (**1b**) was radiofluorinated by an isotopic exchange reaction. Subsequently the formyl group was converted into the formate derivative by Baeyer-Villiger oxidation. Finally,

an acid hydrolysis allowed the cleavage of the protecting groups of the catechol function and unmasked the amino acid producing 6-[^{18}F]FDOPA.

In the present work this three-step procedure has been optimized allowing not only an improved radiosynthesis of 6-[^{18}F]FDOPA but also, starting from the identical precursor, 2-[^{18}F]Ftyr could be prepared by a modified procedure in which a decarbonylation reaction was performed instead of the Baeyer-Villiger oxidation. Additionally, using analogous precursors, 6-[^{18}F]Fmtyr was prepared via the oxidative approach while 2-[^{18}F]Fphe was obtained, again by the decarbonylation route. The specific activity of the radiotracers prepared in this work under developmental conditions was already as high as compared to that achieved by electrophilic procedures and can be expected to increase several times under production conditions with high starting activity of [^{18}F]fluoride.

A modified efficient synthetic approach was employed for the preparation of highly functionalized fluoro-benzaldehydes while a new one was developed for the preparation of fluoro-benzophenones, which were used as labeling precursors. The first involved six steps and allowed the synthesis of the already reported (2*S*,5*S*)-*tert*-butyl 2-*tert*-butyl-5-(2-fluoro-5-formylbenzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (**1a**) and **1b** in overall yields of 41% and 19%, respectively. Additionally, using the same synthetic approach the new precursors (2*S*,5*S*)-*tert*-butyl 5-(5-acetyl-2-fluorobenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (**1c**), (2*S*,5*S*)-benzyl 2-*tert*-butyl-5-(2-fluoro-5-formylbenzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (**1d**), 4-fluoro-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)benzaldehyde (**1e**) and 1-(4-fluoro-3-(((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)methyl)phenyl)ethanone (**1f**) could also successfully be prepared in yields of 48%, 37%, 27% and 32%, respectively.

Preparation of precursor **1b** required the prior synthesis of 2-(benzyloxy)-5-bromo-4-fluorobenzaldehyde, which was accomplished in four steps. On the other hand, the synthesis of compounds **1a**, **1d**, and **1e** started from 3-bromo-4-fluorobenzaldehyde while that of **1c** and **1f** required 1-(3-bromo-4-fluorophenyl)ethanone; both those starting materials were commercially available.

In the first step, the carbonyl function of those starting compounds was protected as the 1,3-dithiolane derivatives **33**. From those, compounds **34** were achieved using a “two-step one-pot” coupling reaction by a Br-for-Mg exchange between **33** and a diisopropylmagnesium chloride lithium chloride complex followed by coupling with ethyl chloroformate. Reduction

of **34** with $LiAlH_4$ provided the benzyl alcohols **35** in yields of about 92%. The conversion of the benzyl alcohols to the benzyl bromides **36** was performed using the Appel reaction, again with good yields of 74% to 84%. Freshly prepared lithium diisopropylamide (LDA) generated the enolate derivative of the chiral auxiliaries (*S*)-(-)-1-BOC-2-*tert*-butyl-3-methyl-imidazolidin-4-one (Seebach´s reagent) and (*S*)-(-)-1-Z-2-*tert*-butyl-3-methyl-imidazolidin-4-one while butyl-lithium was used with (*R*)-2,5 dihydro-3,6-dimethoxy-2-isopropylpyrazine. Afterwards they were coupled to the corresponding benzyl bromides producing the alkylated products **37** in a range of 70-89% yield. By deprotection of the 1,3-dithiolane with [bis(trifluoroacetoxy)iodo]benzene the desired compounds **1a-f** were achieved.

The second approach, which was developed here for the preparation of the ketone precursors (2*S*,5*S*)-*tert*-butyl 5-(5-(3,5-bis(trifluoromethyl)benzoyl)-2-fluorobenzyl)-2-*tert*-butyl-3-methyl-4-oxoimidazolidine-1-carboxylate (**1g**) and (2*S*,5*S*)-*tert*-butyl 2-*tert*-butyl-5-(2-fluoro-5-(2,2,2-trifluoroacetyl)benzyl)-3-methyl-4-oxoimidazolidine-1-carboxylate (**1h**) represents a novel three-step synthesis. In summary, the procedure went like this: commercially available 2-fluoro-5-iodobenzaldehyde **38** was treated with an excess of diiodosilane yielding the diiodo derivative **39** with 80%. Freshly prepared lithium diisopropylamide (LDA) was used here again to generate the enolate derivative of Seebach´s reagent. This was coupled with compound **39** achieving product **40** in 81% yield. From this, precursors **1g** and **1h** were obtained using a "one-pot two-step" reaction. In the first step compound **40** was reacted with *i*-PrMgBr in order to generate the magnesium derivative. This was then acylated with 3,5-bis(trifluoromethyl)benzoyl chloride producing **1g** in 84% yield. Alternatively the organo-metallic species was reacted with trifluoroacetic anhydride generating **1h** in 62% yield. The overall yields of **1g** and **1h** were 54% and 40%, respectively.

The radiosynthesis of 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr was achieved using precursors **1a** and **1b**, respectively. Optimization studies on the isotopic exchange reaction using conventional heating (oil bath) showed that an increase of the concentration of the basic $TBAHCO_3$ anion activator led to higher labeling yields; but also to the formation of additional new products which were identified as diasteromers of the desired compounds **[^{18}F]1a** and **[^{18}F]1b**. An increase of the temperature from 130 to 150 ºC did not provide a better RCY but increased the production of the diastereomer while standard ^{18}F-labeling conditions using Kryptofix 2.2.2 provided the undesired compound even as major product. The use of microwave heating for isotopic exchange delivered similar RCY than those obtained with conventional heating but saving 9 min reaction time. It was observed that an elevated power of the microwave

produced higher RCY of the desired product but also of the non-wanted diastereomer. Additionally, reaction times under 10 min and 50 s for conventional and microwave heating, respectively, produced low RCY of both the desired compounds and the diastereomers, while extended reaction times increased only the production of the diastereomers.

After optimization of the three steps of the radiosynthetic pathway the conventional heated reactions yielded the desired products 2-[^{18}F]Fphe and 2-[^{18}F]Ftyr in 43% and 49%, whereas 34% and 43% RCY were obtained, respectively, when microwave heating was applied. However, 38 min of total preparation time were saved with the latter. Thus considering the radioactive decay, the amounts of isolated radioactive products obtained with both methods were directly comparable. Both products were obtained with good enantiomeric purity. The *e.e.* achieved for 2-[^{18}F]Fphe was 88% while an *e.e.* of 92% was obtained in the case of 2-[^{18}F]Ftyr. It was also shown that the incidence of 2-[^{18}F]F-D-phe and 2-[^{18}F]F-D-tyr in the case of the Seebach´s derivatives occurred as consequence of the diastereomerization of the precursors during the nucleophilic exchange reaction with [^{18}F]fluoride. Experiments performed using the bulky precursor **1d** showed that diastereomerization occurred independently of the size of the protecting group of the imidazolidinone system. Radiosynthesis of 2-[^{18}F]Fphe starting from the labeling precursor **1e** resulted only in a low RCY; moreover, the instability of this precursor limited the number of experiments which could be performed.

A new nucleophilic synthesis of 6-[^{18}F]Fmtyr by isotopic exchange was also developed. In this case, DMSO resulted to be more suitable than DMF for the ^{18}F-for-^{19}F exchange reaction. Also a higher temperature was required for labeling of the precursor **1c**, while the RCY was low compared to the analogous aldehyde **1a**. The three-step radiosynthetic procedure led to the desired amino acid in 13% overall radiochemical yield with a high enantiomeric purity of > 93% when the Seebach-precursor **1c** was employed. A comparable overall radiochemical yield of 11% of 6-[^{18}F]Fmtyr was achieved with the Schöllkopf-precursor **1f** while the enantiomeric purity in this case was only 87%. These results demonstrated that Schöllkopf´s derivatives were more sensitive to the labeling conditions than Seebach´s ones. Precursors **1g** and **1h** in contrast to **1c** and **1f** showed a relative high radiochemical yield of the ^{18}F-for-^{19}F substitution, with 30% and 40% respectively, but it was not possible to match the RCY of the Baeyer-Villiger oxidation obtained with the acetophenone derivatives **[^{18}F]1c** and **[^{18}F]1f**. Therefore, 6-[^{18}F]Fmtyr was obtained from **1g** and **1h** with an overall RCY of only 6% and 13%, respectively. However, the enantiomeric

purity of the final product using any of the latter precursors was > 98%. Thus, further efforts of the optimization of the Baeyer-Villiger reaction of these precursors seem to be justified.

The nucleophilic radiosynthesis of 6-[^{18}F]FDOPA by isotopic exchange could also be improved. In comparison to the overall RCY of 22% reported earlier the here optimized three-step radiosynthetic procedure led to the desired amino acid in approximately 40% overall radiochemical yield with high a enantiomeric purity of > 96%. This was largely due to the increased RCY of the hydrolysis reaction. Furthermore, a better understanding of the isotopic exchange reaction was attained. The side product-A, as referred to by Wagner *et al*., was identified as diasmeromer **42b** being produced by the epimerization of position 5 of the imidazolidinone system due to the basic conditions and high temperature during the isotopic exchange reaction. Further, the Baeyer-Villiger oxidation was also optimized by the use of trifluoroperacetic acid which reduced the time of reaction from 20 min to 10 min.

Preliminary studies on the automation of the radiosynthesis of 6-[^{18}F]FDOPA using a commercially available remote-controlled synthesis module showed that this is principally feasible for the three-step radiochemical procedure as developed here. However, further work is still needed, especially in order to overcome the technical problems (high pressure) originated from the harsh conditions needed to hydrolyze the imidazolidinone group.

6 REFERENCES

1 Paans, A. M. J.; vanWaarde, A.; Elsinga, P. H.; Willemsen, A. T. M.; Vaalburg, W. *Methods* **2002**, *27*, 195.

2 Herzog, H. *Radiochim. Acta* **2002**, *89*, 203.

3 Miller, P. W.; Long, N. J.; Vilar, R.; Gee, A. D. *Agew. Chem. Int. Ed.* **2008**, *47*, 8998.

4 Wienhard, K.; Wagner, R.; Heiss, W. D. in *PET*, Springer Verlag, Berlin, 1989.

5 Phelps, M. E. *Proc. Natl. Acad. Sci. USA.* **2000**, *97*, 9226.

6 http://www.fz-juelich.de/portal/forschung/highlights/9komma4. September 15th, 2010.

7 Wood, K. A.; Hoskin, P. J.; Saunders, M. I. *Clin. Oncol*. **2007**, *19*, 237.

8 Weber, W. A. *J. Clin. Oncol*. **2006**, *24*, 3282.

9 Schwaiger, M.; Ziegler, S.; Nekolla, S. G. *J. Nucl. Med*. **2005**, *46*, 1664.

10 Di Carli, M. F.; Dorbala, S.; Meserve, J.; El Fakhri, G.; Sitek, A.; Moore, S. C. *J. Nucl. Med*. **2007**, *48*, 783.

11 Dekemp, R. A.; Yoshinaga, K.; Beanlands, R. S. B. *J. Nucl. Cardiol*. **2007**, *14*, 380.

12 Cai, L. S.; Innis, R. B.; Pike, V. W. *Curr. Med. Chem*. **2007**, *14*, 19.

13 Cohen, R. M. *Mol. Imaging Biol*. **2007**, *9*, 204.

14 Nordberg, A. *Lancet Neurol*. **2004**, *3*, 519.

15 Wang, J. L.; Maurer, L. *Curr. Top. Med. Chem*. **2005**, *5*, 1053.

16 Gee, A. D. *Br. Med. Bull*. **2003**, *65*, 169.

17 Lever, J. R. *Curr. Pharm. Des*. 2007, *13*, 33.

18 Coenen, H. H.; Elsinga, P. H.; Iwata, R.; Kilbourn, M. R.; Pillai, M. R. A.; Rajan, M. G. R.; Wagner Jr., H. N.; Zaknun J. J. *Nucl. Med. Biol*. **2010**, *37*, 727.

19 Levin, C. S.; Hoffman, E. J. *Phys. Med. Biol*. **1999**, *44*, 781.

20 Martarello, L.; Schaffrath, C.; Deng, H.; Gee, A. D.; Lockhart, A.; O'Hagan, D. *J. Label. Compd. Radiopharm.* **2003**, *46*, 1181.

21 Hess, E.; Blessing, G.; Coenen; H. H.; Qaim, S. M *Appl. Rad. Isot*. **2000**, *52*, 1431.

22 Adam, M. J.; Abeysekaera, B. F.; Ruth, T. J. *J. Label. Comp. Radiopharm*. **1984**, *21*, 1227.

23 Coenen, H. H.; Moerlein, S. M. *J. Fluorine Chem*. **1987**, *36*, 63.

24 Hamacher, K.; Hirschfelder, T.; Coenen, H. H. *Appl. Radiat. Isot.* **2002**, *56*, 519.

25 Coenen, H. H.; Klatte, B.; Knöchel, A.; Schüller M.; Stöcklin, G. *J. Label. Compd. Radiopharm.*, 1986, **23**, 455.

26 Hamacher, K.; Coenen, H. H.; Stöcklin, G. *J. Nucl. Med.* **1986**, *27*, 235.

27 Kiesewetter, D. O.; Eckelman, W. C.; Cohen, R. M.; Finn, R. D; Larson, S. M. *Appl. Radiat. Isot.* **1993**, *44*, 1015.

28 Chi, D.; Kilbourn, M. R.; Katzenellenbogen, J. A.; Brodack, J. W.; Welch, M. J. *Appl. Radiat. Isot.* **1986**, *37*, 1173.

29 Katsifis, A.; Hamacher, K.; Schnitter, J.; Stöcklin, G. *Appl. Radiat. Isot.* **1993**, *44*, 1015.

30 Coenen, H. H. in PET Chemistry – The Driving force in Molecular Imaging. Springer, Berlin, 2007, 15-50.

31 Kim, D. W.; Ahn, D. S.; Oh, Y. H.; Lee, S.; Kil, H. S.; Oh, S. J.; Lee, S. J.; Kim, J. S.; Ryu, J. S.; Moon, D. H.; Chi, D. Y. *J. Am. Chem. Soc.* **2006**, ***128***, 16394.

32 Ermert, J.; Coenen, H. H. *Curr. Radiopharm*. **2010**, *3*, 109.

33 Coenen, H. H.; Ermert, J. *Curr. Radiopharm*. **2010**, *3*, 163.

34 Pike, V. W.; Aigbirhio, F. I. *J. Chem. Soc. Chem. Commun.* **1995**, 2215.

35 Ross, T. L.; Ermert, J.; Hocke, C.; Coenen, H. H. *J. Am. Chem. Soc.* **2007**, *129*, 8018.

36 Shiue, C.-Y.; Bai, L.-Q.; Teng, R. R.; Wolf, A. P. *J. Label. Compd. Radiopharm*. **1987**, *24*, 55.

37 Zheng, L.; Berridge, M. S. *Appl. Radiat. Isot*. **2000**, *52*, 55.

38 Wilson, A. A.; DaSilva, J. N.; Houle, S. *Appl. Radiat. Isot*. **1995**, *46*, 765.

39 Coenen, H. H.; Colosimo, M.; Schüller, M.; Stöcklin, G. *J. Label. Compd. Radiopharm*. **1986**, *23*, 587.

40 Bergman, J.; Eskola, O.; Lehikoinen, P.; Solin, O. *Appl. Radiat. Isot*. **2001**, *54*, 927.

41 Block, D.; Coenen, H. H.; Stöcklin, G. *J. Label. Compd. Radiopharm*. **1987**, *24*, 1029.

42 Chi, D. Y.; Kilbourn, M. R.; Katzenellenbogen, J. A.; Welch, M. J. *J. Org. Chem*. **1987**, *52*, 658.

43 Iwata, R.; Pascali, C.; Bogni, A.; Furumoto, S.; Terasaki, K.; Yanai, K.Appl. Radiat. Isot. 2002, 57, 347.

44 Chin, F. T.; Morse, C. L.; Shetty, H. U.; Pike, V. W. *J. Label. Compd. Radiopharm*. **2006**, *49*, 17.

45 Jelinski, M.; Hamacher, K.; Coenen, H. H. *J. Label. Compd. Radiopharm*. **2002**, *45*, 217.

46 Block, D.; Coenen, H. H.; Stöcklin, G. *J. Label. Compd. Radiopharm*. **1988**, *25*, 185.

47 Wadsak, W.; Wirl-Sagadin, B.; Mitterhauser, M.; Mien, L. K.; Ettlinger, D. E.; Keppler, B.; Dudczak, R. K.; Kletter, K. *Appl. Radiat. Isot*. **2006**, *64*, 355.

48 Al-Labadi, A.; Zeller, K. P.; Machulla, H.-J. *J. Radioanal. Nucl. Chem*. **2006**, *270*, 313.

49 Ermert, J.; Coenen, H. H. *Curr. Radiopharm*. **2010**, *3*, 127.

50 Ross, T.; Ermert, J.; Coenen, H. H. *J. Label. Compd. Radiopharm*. **2005**, *48 (Suppl. 1)*, S153.

51 Ludwig, T.; Gail, R.; Ermert, J.; Coenen, H. H. in *Synthesis and Applications of Isotopically Labelled Compounds 7*. (Eds.: U. Pleiss , R. Voges), **2001**, 358–361.

52 Marik, J.; Sutcliffe, J. L. *Tetrahedron Lett*. **2006**, *47*, 6681.

53 Glaser, M.; Årstad, E. *Bioconjugate Chem*. **2007**, *18*, 989.

54 O'Hagan, D.; Schaffrath, C.; Cobb, S.L.; Hamilton, J.T.G.; Murphy, C.D. *Nature*, **2002**, *416*, 279.

55 Schaffrath, C.; Deng, H.; O'Hagan, D. *FEBS Lett.* **2003**, *547*, 111.

56 Deng, H.; Cobb, S.L.; Gee, A.D.; Lockhart, A.; Martarello, L.; McGlinchey, R.P.; O'Hagan, D.; Onega, M. *Chem. Commun.* **2006**, 652.

57 Stryer, L. *Biochemistry*. 4th ed. New York, NY: WH Freeman; 1995.

58 Souba, W. W.; Pacitti, A. J. *J. Parenter. Enteral Nutr*. **1992**, *16*, 569.

59 McConathy, J.; Goodman, M. M. *Cancer Metastasis Rev*. **2008**, *27*, 555.

60 Busch, H.; Davis, J. R.; Honig, G. R.; Anderson, D. C.; Nair, P. V.; Nyhan, W. L. *Cancer Res.* **1959**, *19*, 1030.

61 Isselbacher, K. J. *N. Engl. J. Med.* **1972**, *286*, 929.

62 Saier, M. H. Jr.; Daniels, G. A.; Boerner, P.; Lin. J. *J. Membr. Biol*. **1988**, *104*, 1.

63 Jager, P. L.; Vaalburg, W.; Pruim, J.; de Vries, E. G.; Langen, K. J.; Piers, D. A. *J. Nucl. Med*. **2001**, *42*, 432.

64 Wienhard, K.; Herholz, K.; Coenen, H. H.; Rudolf, J.; Kling, P.; Stöcklin, G.; Heiss, W. D. *J. Nucl. Med*. **1991**, *32*, 1338.

65 Miyagawa, T.; Oku, T.; Uehara, H.; Desai, R.; Beattie, B; Tjuvajev, J.; Blasberg, R. *J. Cereb. Blood Flow Metab.* **1998**, *18*, 500.

66 Shoup, T. M.; Olson, J.; Hoffman, J. M.; Votaw, J.; Eshima, D.; Eshima, L.; Camp, V. M.; Stabin, M.; Votaw, D.; Goodman, M. M. *J. Nucl. Med.* **1999**, *40*, 331.

67 Uehara, H.; Miyagawa, T.; Tjuvajev, J.; Joshi, R.; Beattie, B.; Oku, T.; Finn, R.; Blasberg, R. *J. Cereb. Blood Flow Metab.* **1997**, *17*, 1239.

68 Wester, H.-J.; Herz, M.; Weber, W.; Heiss, P.; Senekowitsch-Schmidtke, R.; Schwaiger, M.; Stöcklin, G. *J. Nucl. Med.* **1999**, *40*, 205.

69 Laverman, P.; Boerman, O. C.; Corstens, F. H. M.; Oyen, W. J. G. *Eur. J. Nucl. Med*. **2002**, *29*, 681.

70 Coenen, H. H., in *PET Studies on Amino Acid Metabolism and Protein Synthesis*, ed. Mazoyer, B. M.; Heiss, W. D.; Comar, D., Kluwer Academic Publishers, Dordrecht, 1993, pp. 109-129.

71 Joh, T. H.; Hwang, O. Annals of the New York Academy of Sciences. **1987**, 493, 342.

72 Jankovic, J. J. Neurol. Neurosurg. Psychiatry. **2008**, 79, 368.

73 Davie, C. A. *Br. Med. Bull*. **2008**, *86*, 109.

74 Parkinson´s disease. "Symptomatic pharmacological therapy in Parkinson's disease". The National Collaborating Centre for Chronic Conditions, ed. **2006**. London: Royal College of Physicians. ISBN 1-86016-283-5.

75 Jager, P. L.; Vaalburg, W.; Pruim, J.; de Vries, E. G.; Langen, K. J.; Piers, D. A. *J. Nucl. Med.* **2001**, *42*, 432.

76 Langen, K.J.; Pauleit, D.; Coenen, H. H. *Nucl. Med. Biol*. **2002**, *29*, 625.

77 Minn, H.; Kauhanen, S.; Seppänen, M.; Neuutila, P. *J. Nucl. Med.* **2009**, *50*, 1916.

78 Seibyl, J. P.; Chen, W.; Silverman, D. H. *Semin. Nucl. Med.* **2007**, *37*, 440.

79 McConathy, J.; Goodman, M. M. *Cancer Metastasis Rev*. **2008**, *27*, 555.

80 Obeso, J. A.; Rodriguez-Oroz, M. C.; Goetz, C. G.; Marin, C.; Kordower, J. H.; Rodriguez, M.; Hirsch, E. C.; Farrer, M.; Schapira, A. H.V.; Halliday, G. *Nature Med*. **2010**, *16*, 653.

81 Firnau, G.; Chirakal, R.; Sood, S.; Garnett, S. *Can. J. Chem*. **1980**, *58*, 1449.

82 Chiraka, R. Firnau, F; Schrobilgen, G. J.; McKay, J.; Garnett, E.S. *Int. J. Appl. Radiat. Isot.* **1984**, *35*, 401.

83 Firnau, G.; Chirakal, R.; Garnett, E. S. *J. Nucl. Med.* **1984**, *25*, 1228.

84 Chirakal, R.; Firnau, G.; Garnett, E. S. *J. Nucl. Med.* **1986**, *27*, 417.

85 Coenen, H. H.; Franken, K.; Kling, P.; Stöcklin, G. *Int. J. Radiat. Appl. Instrum. Part A.* **1988**, *39*, 1243.

86 Ishiwata, K.; Ishii, S. I.; Senda, M.; Tsuchiya, K.; Tomimoto, K. *Appl. Radiat. Isot*. **1993**, *44*, 755.

87 Diksic, M.; Farrokhzad, S. *J. Nucl. Med*. **1985**, *26*, 1314.

88 Luxen, A.; Barrio, J. R.; Bida, G. T.; Satyamurthy, N. *J. Label. Compds. Radiopharm*. **1986**, *23*, 1066.

89 Navamari, M.; Bishop, A.; Satyamurthy, N.; Bida, G.; Barrio, J. R. *Appl. Radiat. Isot.* **1992**, *43*, 989.

90 de Vries, E. F. J.; Luurtsema, G.; Brüssermann, M.; Elsinga P. H.; Vaalburg, W. *Appl. Radiat. Isot.* **1999**, *51*, 389.

91 Lasne, M. C.; Perrio, C.; Rouden, J; Barré, L.; Roeda, D.; Dollé, F.; Crouzel, C. *Top Curr Chem.* **2002**, *222*, 201.

92 Lemaire, C.; Guillaume, M.; Cantineau, R.; Christiaens, L. *J. Nucl. Med.* **1990**, *31*, 1247.

93 Reddy, G. N.; Haeberli, M.; Beer H. -F.; Schubiger, A. P. *Appl. Radiat. Isot.* **1993**, *44*, 645.

94 Ding, Y. S.; Fowler, j. S.; Gatley, S. J.; Dewey, S. L.; Wolf, A. P. Schlyer, D. J. *J. Med. Chem.* **1991**, *34*, 861.

95 Al-Labadi, A.; Zeller, K. P.; Machulla, H. J. *J. Radioanal. Nucl. Chem.* **2006**, *270*, 313.

96 Al-Labadi, A.; Zeller, K. P.; Machulla, H. J. *Radiochim. Acta* **2006**, *94*, 142.

97 Ding, Y. S.; Fowler, C. Y.; Wolf, A. P.; Plenevaux, A. *J. Fluorine Chem.* **1990**, *48*, 189.

98 Lemaire, C.; Guillaume, M.; Cantineau, R.; Plenevaux, A. *Appl. Radiat. Isot.* **1991**, *42*, 629.

99 Lemaire, C.; Plenevaux, A.; Cantineau, R.; Christaens, L.; Guillaume, M.; Comar, D. *Appl. Radiat. Isot*. **1993**, *44*, 747.

100 Lemaire, C.; Damhaut, P.; Plenevaux, A.; Comar, D. *J. Nuc. Med*. **1994**, *35*, 1996.

101 Lemaire, C.; Gillet, S.; Guillout, S.; Plenevaux, A.; Aerts, J.; Luxen, A. *Eur. J. Org. Chem.* **2004**, 2899.

102 Shen, B.; Ehrlichmann, W.; Uebele, M.; Machulla, H. J.; Reisch, G. *Appl. Radiat. Isot*. **2009**, *67*, 1650.

103 Tierling, T.; Hamacher, K.; Coenen, H. H. *J. Label. Compds. Radiopharm*. **2001**, *44 (suppl)*, S146.

104 Chakraborty, P. K.; Kilbourn, M. R. *Appl. Radiat. Isot*. **1991**, *42*, 673.

105 Wagner, F. M.; Ermert, J.; Coenen, H. H. *J. Nucl. Med*. **2009**, *50*, 1724.

106 Ito, H.; Hatazawa, J.; Murakami, M.; Miura, S.; Iida, H.; Bloomfield, P. M.; Kanno, I.; Fukuda H.; Uemura, K. *J. Nucl. Med*. **1995**, *35*, 1232.

107 Coenen, H. H.; Kling P.; Stöcklin, G.; *J. Nucl. Med*. **1989**, *30*, 1367.

108 Wienhard, K.; Herholz, K.; Coenen, H. H.; Rudolf, J.; Kling, P.; Stöcklin, G.; Heiss, W. D. *J. Nucl. Med*. **1991**, *32*, 1338.

109 Kubota, K.; Ishiwata, K.; Kubota, R.; Yamada, S.; Takahashi, J.; Abe, Y.; Fukuda H.; Ido, T. *J. Nuc. Med*. **1996**, *37*, 320.

110 Rutten, I.; Cabay, J.; Withofs, N.; Lemaire, C.; Aerts, J.; Baart V.; Hustinx, R. *J. Nucl. Med.* **2007**, *48*, 720.

111 Coenen, H. H.; Franken, K.; Kling, P.; Stöcklin, G. *Appl. Radiat. Isot*. **1988**, *39*, 1243.

112 Hess, E.; Sichler, S.; Kluge, A.; Coenen, H. H. *Appl. Radiat. Isot.* **2002**, *57*, 185.

113 Lemaire, C.; Gillet S.; Kameda, M. *J. Label. Compd. Radiopharm*. **2001**, *44(suppl)*, S857.

114 DeJesus O.; Endres C.J.; Shelton S.; Nickles J.; Holden J. *J. Nucl. Med*. **1997**, *38*, 630.

115 Garnett, E. S.; Firnau, G.; Nahmias, C. Nature, **1983**, *305*, 137.

116 Barrio, J. R.; Huang, S. C.; Yu, D. C.; Melega, W.P.; Quintana, J.; Cherry, S. R.; Jacobson, A.; Namavari, M.; Satyamurthy, N.; Phelps, M. E. *J. Cereb. Blood Flow Metab*. **1996**, 16, 667.

117 Melega, W. P.; Perlmutter, M. M.; Luxen, A.; Nissenson, C. H.; Grafton, S. T.; Huang, S. C.; Phelps, M. E.; Barrio, J. R. *J. Neurochem*. **1989**, *53*, 311.

118 Jordan, S.; Eberling, J. L.; Bankiewicz, K. S.; Rosenberg, D.; Coxson, P. G.; VanBrocklin, H. F.; O'Neil, J. P.; Emborg, M. E.; Jagust, W. *J. Brain Res.* **1997**, *750*, 264.

119 Nahmias, C.; Wahl, L.; Chirakal, R.; Firnau, G.; Garnett, E. S. *Movement Disorders*. **1995**, *3*, 298.

120 Wilcox, C. E.; Braskie, M. N.; Kluth, J. T.; Jagust, W. J. *J. Obes*. **2010**.

121 Braskie, M. N.; Wilcox, C. E.; Landau, S. M.; O'Neil, J. P.; Baker, S. L; Madison, C. M.; Kluth, J. T.; Jagust, W. J. *J. Neurosci*. **2008**, *28*, 14320.

122 Christine, C. W.; Starr, P. A.; Larson, P. S.; Eberling, J. L.; Jagust, W. J.; Hawkins, R. A.; VanBrocklin, H. F.; Wright, J. F.; Bankiewicz, K. S.; Aminoff, M. J. *Neurology*. **2009**, *73*, 1662.

123 Muramatsu, S.; Fujimoto, K.; Kato, S.; Mizukami, H.; Asari, S.; Ikeguchi, K.; Kawakami, T.; Urabe, M.; Kume, A.; Sato, T.; Watanabe, E.; Ozawa K. Nakano I. *Mol Ther*. **2010**, *18*, 1731.

124 DeJesus, O. T.; Mukherjee, J.; Appelman, E. H. *J. Label. Comp. Radiopharm*. **1989**, *26*, 133.

125 DeJesus, O. T.; Sunderland, J. J.; Nickles, J. R.; Mukherjee, J.; Appelman, E. H. *Int. J. Rad Appl. Instrum. A*. **1990**, *41*, 433.

126 Namavari, M.; Satyamurthy N.; Phelps M.; Barrio J. *Appl. Radiat. Isot*. **1993**, *44*, 527.

127 VanBrocklin, H. F. ; Blagoev, M. ; Hoepping, A. ; O'Neil, J. P. ; Klose, M. ; Schubiger, P. A.; Ametamey, S. *Appl Radiat Isot.* **2004**, *61*, 1289.

128 Mulholland; G. K.; O'Donnell, M. J.; Chin, F. T.; Delgado, F. *Patent WO02/44144*. **2002**.

129 Kuroda, C.; Ochi, A.; Nakane, N.; Umeyama, T.; Muto, N.; Niimura, N.; Teramoto, Y.; Nogami, H.; Reddy, G. *Bull Chem Soc Jpn*. **2000**, *73*, 417–422.

130 Wagner, F. M. *Dissertation*. Forschungszentrum Jülich, **2008**.

131 Monclus, M.; Masson, C.; Luxen, A. *J. Fluor. Chem.* **1995**, *70*, 39-43.

132 Appel, R. *Angew. Chem. Int. Ed*. **1975**, *14*, 801.

133 Kocienski P.J.; Cernigliaro G.; Feldstein. *J. Org. Chem*. **1977**, *42*, 353.

134 Fitzi, R.; Seebach, D. *Tetrahedron* **1988**, *44*, 5277.

135 Johnstone, C.; Kerr, W. J.; Scott, J. S. *J. Chem. Soc., Chem. Commun.***1996**, 341.

136 Sun J.; Dong Y.; Cao L.; Wang X.; Wang S.; Hu Y. J. Org. Chem. **2004**, *69*, 8932.

137 Ahrendt, K.; Bergman, R.; Ellman, J. *Org Lett*. **2003**, *5*, 1301,

138 Dess, D. B.; Martin J. C. *J. Org. Chem.*, **1983**, *48*, 4155**.**

139 Firouzabadi, H.; Iranpoor, N.; Hazarkhani, H. *J. Org. Chem*., **2001**, *66*, 7527

140 Kondo, Y.; Matsudaira, T.; Sato, J.; Murata, N.; Sakamoto, T. *Angew. Chem. Int. Ed. Engl*. 1996, 35, 736.

141 Piazza, C.; Knochel, P. *Angew. Chem. Int. Ed*. **2002**, *41*, 3263.

142 Krasovskiy, A.; Knochel, P. *Angew. Chem. Int. Ed*., **2004**, *43*, 3333.

143 Krasovskiy, A.; Straub, B.; Knochel, P. *Angew. Chem. Int. Ed*., **2006**, *45*, 159.

144 Appel, R. *Angew. Chem. Int. Ed*. **1975**, *14*, 801.

145 Languille, N.; Dakin, L.; Panek, J. *Org. Lett.* **2003**, *5*, 575.

146 Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *3*, 287.

147 Chattopadhhyaya, J. B.; Rama Rao, A. V. *Tetrahedron Lett*. **1973**, 3735

148 Reece, C. A.; Rodin, J. O.; Brownlee R. G.; Duncan, W. G.; Silverstein, R. N. *Tetrahedron*. **1968**, 24, 4249.

149 Desai, U. V; Pore, D. M.; Tamhankar, B. V.; Jadhav, S. A.; Wadgaonkar, P. P. *Tetrahedron Lett.* **2006**, *47*, 8559-8561.

150 Iranpoor, N.; Firouzabadi, H.; Shaterian, H. R. *Tetrahedron Lett.* **2003**, *44*, 4769.

151 Cossy, J. *Synthesis*. **1987**, 1113.

152 Keinan, E.; Perez, D.; Sahai, M.; Shvily, R. *J. Org. Chem*. **1990**, *55*, 2927.

153 Keinan, E.; Perez, D. *J. Org. Chem*. **1987**, *52*, 4846.

154 Shen, B.; Löffler, D.; Zeller, K. P.; Übele, M.; Reischl G.; Machulla, H. J. *Appl. Radiat. Isot.* **2007**, *65*, 1227.

155 Kappe, C. O. *Angew. Chem. Int. Ed*., **2004**, *43*, 6250.

156 Plenevaux, A.; Lemaire, C.; Palmer, A. J.; Damhaut P.; Comar, D. *Appl. Radiat. Isot.* **1992**, *43*, 1035.

157 Lemaire, C.; Damhaut, P.; Plenevaux, A.; Comar, D. *J. Nucl. Chem.*, **1994**, *35*, 1996.

158 Seebach, D.; Sting, A. R.; Hoffmann, M. *Angew. Chem. Int. Ed. Engl.*, **1996**, *35*, 2708.

159 Renz, M.; Meunier, B. *Eur. J. Org. Chem*. **1999**, 737.

160 Adejare, A.; Shen, J.; Ogunbadeniyi, A. M. *J. Fluorine Chem*. **2000**, *105*, 107.

161 Smissman, E. E.; Li, J. P.; Israili, Z. H. *J. Org. Chem.*, **1968**, *33*, 4231.

162 Cooper, M. S.; Heaney, H.; Newbold, A. J.; Sanderson, W. *Synlett* **1990**, 533.

163 Kang, H. J.; Jeong H. S. *Bull. Korean Chem. Soc.* **1995**, *17*, 5.

164 Ludwig, T.; Ermert, J.; Coenen, H. H. *Nuc. Med. Biol*. **2002**, *29*, 255.

165 Kitazume, T.; Kataoka, J. *J. Fluorine Chem*. **1996**, *80*, 157.

166 Kobayashi, S.; Tanaka, H.; Amii, H.; Uneyama, K. *Tetrahedron* **2003**, *59*, 1547.

167 Still, W.; Kahn, M.; Mitra, A.J. *J. Org. Chem.*, **1978**, *43*, 2923.

168 Shiraishi, M.; Hashiguchi, S.; Watanabe, T. Patent Number; 5.270.308, **1993**.

169 Qaim, S. M.; Clark, J. C.; Crouzel, C.; Guillaume, M.; Helmecke, H. J.; Nebeling, B.; Pike, V. W.; Stöcklin, G. in *Radiopharmaceuticals for PET*, ed. Stöcklin G. and Pike, V. W. Kluwer Academic Publishers, Dordrecht, **1993**, pp. 15–26.

ABBREVIATIONS

(*S*)-Boc-BMI	(*S*)-(-)-1-Boc-2-*tert*-butyl-3-methyl-4-imidazolidinone
(*S*)-Z-BMI	(*S*)-benzyl 2-tert-butyl-4-oxoimidazolidine-1-carboxylate
2-[^{18}F]Fphe	2-[^{18}F]fluoro-L-phenylalanine
2-[^{18}F]Ftyr	2-[^{18}F]fluoro-L-tyrosine
6-[^{18}F]FDOPA	3,4-Dihydroxy-6-[^{18}F]fluoro-L-phenylalanine
6-[^{18}F]Fmtyr	6-[^{18}F]Fluoro-L-*m*-tyrosine
AA	amino acid
AADC	aromatic L-amino acid decarboxylase
ACN	acetonitrile
Bq	Becquerel
BTI	[bis(trifluoroacetoxy)iodo]benzene
BuLi	butyllithium
c.a.	carrier added
CA	catecholamines
CH	conventional heating
CNS	central nervous system
COMT	catechol-O-methyl transferase
d	doublet (NMR)
DA	dopamine
DBH	dopamine β-hydroxylase
DEA	diethylamine
DIBAL-H	diisobutylaluminium hydride
DIPA	diisopropylamine
DIS	diiodosilane
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
DOPA	dihydroxyphenylalanine
e.e.	enantiomeric excess
EOB	end of bombardment
EP	epinephrine
FDG	2-[^{18}F]fluorodeoxyglucose

HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HPLC	high performance liquid chromatography
IMT	3-[^{123}I]iodo-α-methyl-L-tyrosine
Kryptofix 2.2.2	4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane
LDA	lithium diisopropylamine
m	multiplet (NMR)
M.p.	melting point
MAOA	monoamine oxidase
m-CPBA	*m*-chloroperoxybenzoic acid
MET	[^{11}C-methyl]-L-methionine
MH	microwave heating
MRI	magnetic resonance imaging
n.c.a.	no-carrier-added
NE	norepinephrine
NET	neuroendocrine tumors
NMR	nuclear magnetic resonance
PD	Parkinson´s disease
PET	positron emission tomography
PH	phenylalanine hydroxylase
PNMT	phenylethanolamine N-methyltransferase
PSR	protein synthesis
q	quartet (NMR)
RCY	radiochemical yield
s	singlet (NMR)
SPECT	single photon emission computed tomography
t	triplet (NMR)
TBA	tetrabutylammonium
TFA	trifluoroacetic acid
THP	tetrahydropyranylether
TLC	thin layer chromatography
TMSDAM	trimethylsilyldiazomethane
US	ultrasound
VTA	ventral tegmental area

ACKOWLEDGMENTS

I would like to express my gratitude to Prof. Dr. H. H. Coenen for offering me an interesting thesis theme, excellent work conditions and for his constant support. It has been a wonderful experience to be part of the prestigious radiopharmaceutical group of Forschungszentrum Jülich.

I thank my supervisor, Priv-Doz. Dr. Johannes Ermert for his support, confidence and friendship.

I want to thank Dr. S. Willbold, Dr. D. Hofmann and Dr. M. Holschbach for recording the spectroscopic data.

I would like to thank the team of the baby cyclotron for the production of the [^{18}F]fluoride as well as all other colleagues of the INM-5 for the good work atmosphere.

My appreciation goes also to Ms. Alexia Giannaki and my wife Janine Winkler for the help regarding digital format of this work.

Finally, I would like to thank my family and friends, fortunately they are so many, that it would be impossible to write their names in this page, but certainly I thank God for bringing them into my way.

Ich versichere, dass ich die von mir vorgelegte Dissertation selbstständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit − einschließlich Tabellen, Karten und Abbildungen −, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.
Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Professor H. H. Coenen betreut worden.

Teilpublikationen:

Castillo Meleán, J.; Ermert, J.; Coenen, H. H. Efficient synthesis of fluorobenzyloxoimidazolidinone derivatives; precursors for the radiosynthesis of [^{18}F]fluorophenylamino acids. *Tetrahedron*, **2010**, *66*, 9996.

Castillo Meleán, J.; Ermert, J.; Coenen, H. H. Enantiospecific synthesis of 2-[^{18}F]fluoro-L-phenylalanine and 2-[^{18}F]fluoro-L-tyrosine by isotopic exchange. *Org. Bio. Chem.* **2010**, DOI: 10.1039/c0ob00440e

Jülich, den 02.12.2010 Johnny Castillo Meleán

LEBENSLAUF

Name, Vorname:	Castillo Meleán, Johnny.
Geburtsdatum:	04.05.1977.
Geburtsort:	Caracas, Venezuela.
Staatsangehörigkeit:	venezolanisch.
Familienstand:	verheiratet.
Adresse:	Römerstr. 2B. 52428, Jülich, Deutschland.
E-mail	jcmleo@hotmail.com - j.castillo@fz-juelich.de
Telefon:	++49 163-364 03 32.

STUDIUM

Promotion:	Universität zu Köln. Köln, Deutschland. April 2008 – Dezember 2010.
Doktorarbeit:	Radiosynthesis of [^{18}F]fluorophenyl-L-amino acids by isotopic exchange on carbonyl-activated precursors
Master:	Fachhochschule Aachen. Abteilung Jülich, Deutschland. März 2006 – Februar 2008.
Akademischer Grad:	Master of Sciences in Nuclear Applications.
Masterarbeit:	Preliminary study of the nucleophilic synthesis of 6-[^{18}F]fluoro-m-tyrosine. Optimization of the synthesis of the precursor and labeling.
Diplom:	Universidad Simón Bolívar. Caracas, Venezuela. September 1994 - Mai 2001.
Akademischer Grad:	Diplom Chemiker.
Diplomarbeit:	Study of the palladium catalyzed cross coupling reaction between α-bromocinamates and organotin compounds.

BERUFSERFAHRUNG

Radiochemiker. Centro Diagnóstico Docente (CDD). Caracas, Venezuela.
Oktober 2003 - März 2006.

Laborausbilder. Universidad Simón Bolívar. Caracas, Venezuela.
Mai 2002 - Dezember 2004.

Stabilitäts- und Entwicklungsanalytiker. Lab. Oftalmi. C.A. Caracas, Venezuela.
Mai 2001 - Mai 2002.

Johnny Castillo Meleán