Synthesis and radiofluorination of putative NMDA receptor ligands

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Ute Kronenberg

aus Leverkusen

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Berichterstatter:	Prof. Dr. H. H. Coenen
	Prof. Dr. A. G. Griesbeck
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Die vorliegende Arbeit wurde in der Zeit von Februar 2006 bis September 2010 am Institut für Nuklearchemie (INM-5) der Forschungszentrum Jülich GmbH unter der Anleitung von Herrn Prof. Dr. H. H. Coenen (Lehrstuhl für Nuklearchemie der Universität zu Köln) durchgeführt.

Zusammenfassung

Im Rahmen der vorliegenden Arbeit zur Synthese von Radioliganden für den NMDA Rezeptor wurde die organische Synthese von vier Vorläufern mit Amidinstruktur zur Markierung mit Fluor-18 sowie die zugehörigen inaktiven Fluorverbindungen als chromatographische Standards entwickelt und durchgeführt.

Die Synthese der Markierungsvorläufer folgte publizierten Reaktionswegen und gestaltete sich problemlos. Die für die Reaktion notwendigen Imidoester wurden in einer Pinner-Reaktion hergestellt. 2-Hydroxybenzylamin wurde aus dem korrespondierenden Nitril durch Reduktion unter Einsatz von Boran gewonnen. Seine Kopplung mit den Imidoestern geschah in DMF mit Hilfe von Triethylamin. Nach Kristallisation aus Methanol erhielt man die gewünschten Produkte in hoher Reinheit. Die cylische Verbindung wurde mittels Kopplung von 2-(Brommethyl)-benzonitril mit 2-Hydroxybenzylamin gewonnen und aus Methanol umkristallisiert.

Die Standardverbindungen wurden in einer Kopplungsreaktion von Imidoestern bzw. 2-(Brommethyl)-benzonitril und *ortho*-Fluorethoxybenzylamin erhalten. Letzteres wurde in einer vierstufigen Reaktion aus Salicylaldehyd hergestellt. Aufreinigung der Substanzen erfolgte mittels Säulenchromatographie oder durch Umkristallisation aus Ethanol und Wasser.

Die trägerarme Radiosynthese der gewünschten Fluorverbindungen wurde anhand der Modellverbindung *N*-(2-Fluorethoxybenzyl)-cinnamamidin getestet und optimiert. Die Reaktion mit 1-Brom-2-[^{18}F]fluorethan in DMF oder DMSO unter Benutzung von Natriumhydroxid als Base ergab das gewünschte Produkt in guten bis sehr guten Ausbeuten (78 % nach 30 Minuten bei 80 °C in DMSO). Als Alternative von Dibromethan zur Herstellung von 1-Brom-2-[^{18}F]fluorethan wurde auch Ethylenglykol-1,2-di(*p*-toluolsulfonat) getestet.

Das gewünschte [^{18}F]fluoralkylierte Cinnamamidin konnte mittels HPLC und DC eindeutig identifiziert werden. Das Produkt kann nun in ausreichenden Mengen zur Anwendung für *in vitro* und *in vivo* Tests produziert werden.

Erste Markierungsversuche am zyklischen Markierungsvorläufer verliefen nicht erfolgreich und erfordern weitere Untersuchungen.

Abstract

In the course of this work on the synthesis of radioligands for the NMDA receptor the authentic standards and labeling precursors of four compounds with an amidine structure was performed.

Synthesis of the precursors followed reaction conditions given in the literature and was successful. The imidoesters used for the synthesis were obtained from their nitriles in a Pinner synthesis, while 2-hydroxybenzylamine was synthesized in a reduction of 2-hydroxybenzonitrile using borane as a reducing agent. After a coupling reaction of the amine and the imidoester in DMF using triethylamine as base the precursors were obtained in good yields and purified by crystallization from methanol. The cyclic standard compound was synthesized directly from 2-(bromo-methyl)-benzonitrile and 2-hydroxybenzylamine in a ring closing reaction. Similar to the other precursors, crystallization from methanol produced a pure compound.

The authentic standards were synthesized starting from salicylaldehyde. In a four step synthesis the desired *ortho*-fluoroethoxybenzylamine was obtained in good yield. Coupling of the amine with the respective imidoester or in the case of the cyclic compound 2-(bromomethyl)-benzonitrile gave the desired product which was then purified by column chromatography or by crystallization from ethanol and water.

For the labeling procedure 1-bromo-2-[^{18}F]fluoroethane was synthesized following a previously published pathway starting from 1,2-dibromoethane. An alternative route of radiosynthesis for this prosthetic group was tested using ethyleneglycole-1,2-ditosylate.

The labeling reaction was performed on one of the precursors testing both DMF and DMSO as solvents and using NaOH as base. Yields of *N*-(2-fluoroethoxybenzyl)-cinnamamidine were about 78 % at 80 °C after 30 minutes in DMSO.

The desired product can now be synthesized in sufficient yields for *in vitro* and *in vivo* evaluation studies.

Labeling on the cyclic precursor was attempted utilizing DMSO as solvent, but no product could be found.

Contents

1 Introduction

1.1 Radionuclides in nuclear medicine

In the year 1895 Wilhelm Conrad Röntgen discovered the hitherto unknown X-rays and in 1901 was awarded the Nobel Prize for this discovery. Only a year later, in 1896, Henri Becquerel found that certain uranium-containing ores are able to blacken photographic plates. This was the first evidence for emission of energetic radiation from natural material and thus of radioactive decay.

At the same time Marie Curie was looking for a possible topic for her thesis and decided to focus on uranium and the then called “Becquerel rays”. The first work the Curies did was to develop a method for measuring and quantifying the newly discovered radiation. Using an electrometer they were able to measure the electric charges occuring with each radioactive decay. Marie Curie studied several uranium containing samples and discovered that pitchblende was four-fold more radioactive than uranium, which led to the correct deduction that it must contain at least one other radioactive substance.

The Curies isolated this radioactive material and thus discovered the element radium. They were able to extract 0.1 mg of radium chloride from several tons of pitchblende, and could determine the atomic weight of radium for the first time. Along with Becquerel, Pierre and Marie Curie received the Nobel Prize for Physics in 1903.

After the untimely death of her husband, Marie Curie continued their work, discovered other new elements, e.g. polonium, and eventually was also awarded the Nobel Prize for Chemistry in 1911 "in recognition of her services to the advancement of chemistry by the discovery of the elements radium and polonium, by the isolation of radium and the study of the nature and compounds of this remarkable element". [1]

The first practical application for radioactivity was established with the development of the radiotracer method by Friedrich Adolf Paneth and George de Hevesy in 1913. After first application of this principle to inorganic chemistry studies, De Hevesy extended the use to study metabolic processes in animals and humans. [2] De Hevesy’s work on the utilization of isotopic tracers and the recognition of the principle of the isotopes’ identical chemical and physiological properties led to the Nobel Prize for Chemistry 1943.

Invention and development of the cyclotron by Ernest Lawrence in 1930 established new methods for the production of artificial neutron-deficient radionuclides. For this discovery Lawrence was awarded the Nobel Prize for Physics in 1939.

In 1938 the German scientists Otto Hahn and Fritz Straßmann discovered the formation of “fission” products upon neutron irradiation of heavy elements. Later in the same year Lise Meitner provided a valid physical explanation of the fission process, which finally led to development and construction of the first nuclear reactor in 1942 by Enrico Fermi and Leo Szilard. Now a way to production of many neutron rich radionuclides was open. For his discoveries Otto Hahn was awarded the Nobel Prize for Chemistry in 1944. [3]

Today about 1600 artificial radionuclides are known. Due to constrictions to certain half-lives, type of radiation, energies of decay and a high (radio-) chemical purity, only a small portion of them are applicable in specific areas of life sciences. Positron (β^+) emitters and γ-radiating nuclides are specially utilized as tracers for *in vivo* diagnostics. Specific nuclides that show γ-decay with energies of 100 to 600 keV are used and allow detection of γ-radiation outside the body, while ensuring that the dose the patient receives is as small as possible. The corpuscular α- and β^--emitters are exclusively utilized for therapeutical purposes. Because of their high linear energy transfer, they cannot be detected outside the body, if no accompanying γ-radiation is present. [4,5]

Radioactive labeled biomolecules are important tools for the uncovering of physiological processes on the molecular level and enable monitoring of metabolism and not only the monitoring of morphological structures like computed (x-ray) tomography (CT) and magnetic resonance tomography (MRT or MRI) do. Positron emission tomography (PET) offers the possibility to quantify radioactivity and thus compute the amount of substance transported to different areas of the body.

While the spatial resolution of PET is lower then that of classic structural imaging techniques like MRI, the capability to use PET as a method of functional imaging makes it a useful tool in diagnosing diseases. Utilizing bio-mathematical models together with computer based analyses of time-dependent distribution of radioactivity it is possible to determine a large amount of metabolic parameters. Benefit of PET is that besides calculating parameters like enzyme concentration, receptor density or

rate of perfusion it is also possible to identify pharmacokinetics or pharmacodynamics.

In a case with no-carrier-added substances the radioactive tracer needs to be applied only in very small (pico- to nanomolar) quantities. Because of this, utilization *in vivo* is possible in an optimal scenario without influencing physiological processes.

There is a distinction between isotopic and analogous radiotracers, depending on whether the molecule is labeled using an isotopic or an analogous nuclide, which shows similar chemical properties. Especially with analogously labeled tracers, studies of their metabolism are mandatory When dealing with analogously-labeled tracers, the study of their metabolism is mandatory, since the chemically identical behavior of the labeled molecule has to be proven and fate of the radionuclide must be determined. [6]

1.2 Basic principles of positron emission tomography

Once it was established that the production of positron-emitting nuclides via cyclotrons was possible, using carbon-11, nitrogen-13 or oxygen-15 for analysis of biological and biochemical processes was a natural conclusion. During the 1930s they were employed for this purpose already; however, technical methods had not evolved far enough yet, and so further experiments could not be performed until computers, along with better detectors, were developed. When the development of detectors had advanced far enough that these radionuclides could be detected outside of the body, the use of positron emitters was introduced into biological and medical sciences. Modern day application of positron emitters is mainly PET, which makes use of the subsequent γ-radiation that occurs during decay of β^+-particles.

positron emission: $\mathrm{p}^+ \rightarrow \mathrm{n} + \beta^+ + \nu_\mathrm{e}$

electron caputure: $\mathrm{p}^+ + \mathrm{e}^- \rightarrow \mathrm{n} + \nu_\mathrm{e}$

Equation 1.1: Decay channels of proton rich nuclides

During β^+-decay a proton in the atomic nucleus is transformed into a positron, a neutrino and a neutron. Proton-rich nuclides show this kind of decay only, if the decay energy is above 1022 keV, since the energy equivalent of two electron masses is needed for the conversion. If the decay energy is lower, proton-rich nuclides decay via electron capture (EC), during which an electron from an inner atomic-shell reacts

with a proton of the atomic nucleus producing a neutron and a neutrino (cf. Equation 1.1). [7]

An emitted positron must lose its kinetic energy before annihilation with an electron from the surrounding material is possible. During this process a positronium is formed which is similar to a hydrogen atom. Like hydrogen a positronium can exist in either a singlet ($t_{½} = 1.25 \cdot 10^{-10}$s), or a triplet state ($t_{½} = 1.4 \cdot 10^{-7}$s). When annihilation finally occurs, either two (from the singlet) or three (from the triplet) γ-quanta are emitted. In the case of two quanta both carry energy of 511 keV and are being emitted in almost 180 degrees to each other. Since in condensed matter the pick-up process enables rapid transition to the singlet state, the triplet state can be neglected for the appliance with PET. [8]

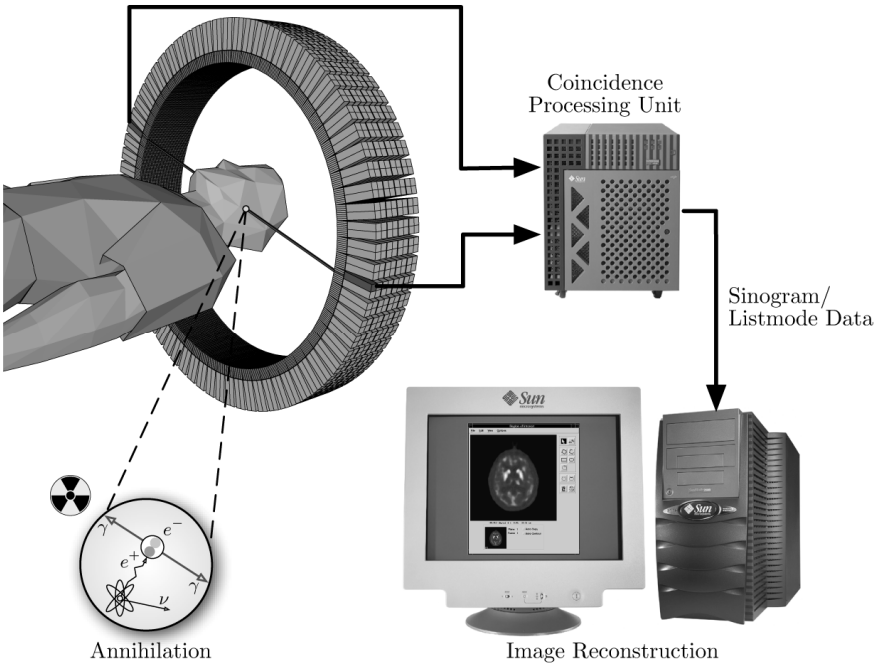


Figure 1.1: Scheme of a positron emission tomography: measurement of the radiation and computing of its position by coincidence measurement followed by computer based image reconstruction [9]

For detection purposes a circular detector array is applied. The detector material is generally either bismuthgermanat (BGO) or lutetiumsilica (LSO). The emitted quanta are detected by coincidence counting, which filters out all events that do not occur within 5 ns (coincidence window) at exactly opposite detectors.

Because of the emission of two γ-quanta in an angle of nearly 180 degrees and the detection with two diametric detectors, the counting efficiency is independent from the location of the decay between detectors. Absorption losses and scattering losses

can be calculated and corrected by utilizing a second measurement using an external radiation, thus enabling quantification of radioactivity.

Modern equipment offers resolutions down to 3-5 mm in routine diagnostic devices but reaches down to 1 mm in dedicated animal systems. Also a three dimensional acquisition of radioactivity distribution is possible. Even administering doses in the pico- and nanomolar range is possible due to the high sensitivity of equipment. [10, 11]

1.3 Radionuclides for labeling

Table 1.1: Important PET nuclides and their nuclear data [from 6, 12]

Nuclide	Half-life		Decay mode (%)			$E_{\beta+,max}$ [keV]
isotopic						
^{11}C	20.4	min	β^+ (99.8)	EC (0.2)		960
^{13}N	9.96	min	β^+ (100)			1190
^{15}O	2.03	min	β^+ (99.9)	EC (0.1)		1720
^{30}P	2.5	min	β^+ (99.8)	EC (0.2)		3250
analogue						
^{18}F	109.6	min	β^+ (97)	EC (3)		635
^{75}Br	98	min	β^+ (75.5)	EC (24.5)		1740
^{76}Br	16.1	h	β^+ (57)	EC (43)		3900
^{73}Se	7.1	h	β^+ (65)	EC (35)		1320
^{120}I	1.35	h	β^+ (64)	EC (36)		4100
^{124}I	4.18	d	β^+ (25)	EC (75)		2140
metallic						
^{64}Cu	12.7	h	β^+ (18)	β^- (37)	EC (45)	655
^{68}Ga	68.3	min	β^+ (90)	EC (10)		1900
^{82}Rb	1.3	min	β^+ (96)	EC (4)		3350
^{86}Y	14.7	h	β^+ (34)	EC (66)		1300
^{94m}Tc	52	min	β^+ (72)	EC (28)		2470

Nuclides with a median to short half-life are mostly applied for PET, since the amount of radiation applied to the patient should be kept to a minimum and the fast measurement with PET does not make the usage of longer lived nuclides necessary for better imaging.

Often used nuclides are the so called isotopic radionuclides (cf. Table 1.1). Labeling with these give an authentic tracer whose pharmacological properties are unchanged in comparison with the unlabeled molecule. Due to short half-life of most of these nuclides, they can only be applied, if the labeling synthesis is performed at high speed and a quick distribution in the body is given. From the authentic nuclides only carbon-11 shows properties that allow extended syntheses and examination times, but still oxygen-15 and nitrogen-13 are sometimes used for special purposes. [6]

Analogue tracers are labeled with a non-isotopic radionuclide, thus offering longer half lives. Nuclides whose electronic and steric properties will not change the characteristics of the molecule labeled with them are usually chosen. Most often nuclides are utilized, which offer longer half-life, thus enabling multi step syntheses and extended measurements. For example selenium-73 can be used as a structure analog for sulfur, due to its similar characteristics. An example is the labeling of methionine with selenium-73. [13, 14] Also iodine or bromine isotopes can often be used as analogues of a methyl group due to their similar spatial size. [15]

Metallic nuclides like copper-64 or gallium-68 have not been applied as tracers as much as the isotopic or analogue tracers have in the past. For example copper was mostly used as free ion ($[^{64}Cu]Cu^{2+}$) for the diagnosis of Wilson's Disease. Its possible use as a coordinated atom in an organic tracer molecule has been employed and explored more often in the last years. Research on this area of PET imaging is ongoing but has already led to new tracers offering new applications. [16]

Table 1.2: Physico-chemical data of hydrogen in comparison to fluorine [from 6, 17]

Physico-chemical properties	Hydrogen	Fluorine
Mass	1	19
Van-der-Waals-Radius [pm]	120	135
Electronegativity	2.2	4.1
C-X bond energy [kJ/mol]		
aliphatic	398	444
aromatic	460	523
Lipophilicity (ΔlogP)		
aliphatic	0.19	-0.51
aromatic	0.19	0.43

The most important analogue radionuclide today is fluorine-18 ($t_{½}$ = 109.6 min), whose isosteric and isoelectric properties often allow for the substitution of hydrogen atoms without altering the physico-chemical properties of a molecule too much. However its different C-X bond energy and higher electronegativity (cf. Table 1.2) change the dipole moment of the molecule, thus necessitating tests of the changed chemical and physiological behavior of a tracer upon labeling with it necessary. Thermodynamic stability of the carbon-fluorine bond is higher, so it can happen, that the bond cannot be broken within the metabolic processes and the molecule is more stable. On the other hand, fluorine is a good nucleofuge and the C-F bond may be less stable than the C-H bond of the corresponding authentic molecule, which underlines the importance of metabolic studies of the radiotracer or radioligand.

The changed chemical characteristics of an analog molecule (or an analog tracer) are what made e.g. 2-[^{18}F]fluor-2-deoxy-D-glucose ([^{18}F]FDG) such a successful tracer. Metabolization is stopped after phosphorylation (“metabolic trapping”) making further chemical reactions impossible and inhibiting removal from the labeled site, thus allowing application of a simple three compartement kinetic model. [18, 19]

Other advantages of fluorine-18 are the half-life of 109.6 min and the moderate β^+-energy, which helps to minimize radiation exposure and offers a good spatial resolution. The satellite concept allows distribution of fluorine-18 labeled components to smaller hospitals or universities that do not have a cyclotron on site, by producing fluorine-18 at a central production facility and delivering either the “raw” [^{18}F]fluoride or the final tracer, for example [^{18}F]FDG, to the site of usage.

1.4 Production of fluorine-18

Like other positron emitters fluorine-18 is produced at cyclotrons, but production at a reactor is also possible, though less favorable. Table 1.3 lists the most common methods of production.

A detailed knowledge of the excitation functions of nuclear reactions to fluorine-18 and potential byproducts is necessary to enable a clean production with high yields.

Generally the target material is determined by the main product form needed. For routine production of [^{18}F]fluoride the [^{18}O]H_2O-target has proven useful. Besides giving high yields, the enriched water can be separated from the produced [^{18}F]fluoride using a ion exchanger resin or electrochemical anodic absorption and be reused after a purification process. [20, 21]

Table 1.3: The most important production pathways for fluorine-18 [from 22, 23]

Reaction	$^{18}O(p,n)^{18}F$	$^{16}O(^{3}He,p)^{18}F$	$^{20}Ne(d,\alpha)^{18}F$	$^{18}O(p,n)^{18}F$ [c)]
Target	$[^{18}O]H_2O$ [a)]	H_2O	Ne (0,1-0.2%F_2) [b)]	$^{18}O_2$, Kr (1%F_2)
Particle energy [MeV]	16 → 3	36 → 0	14 → 0	16 → 3
Produced form of ^{18}F	$^{18}F^-_{aq}$	$^{18}F^-_{aq}$	$[^{18}F]F_2$	$[^{18}F]F_2$
Yield [GBq/µAh]	2.96	2.59	1.11	~1.85
Molar activity [MBq/µmol]	~600 · 10^4	~50 · 10^3	~100	~ 600

[a)] Ti-Target with Ti-window [b)] passivated Ni-Target [c)] two step process

An important factor in the favor of fluorine-18 is, that production is possible at smaller cyclotrons with a maximum proton energy of 17 or 11 MeV, which enables clinics to produce fluorine-18 as needed on site.

Production of electrophilic $[^{18}F]F_2$ is accomplished using $[^{20}Ne]$neon or $[^{18}O]$oxygen, but to obtain the product from the irradiated target, stable fluorine gas has to be utilized as carrier, which reduces specific activity greatly. [23]

A further seldom applied method of production is reactor based. Irradiation of a lithium-6 enriched target (LiOH or $LiCO_3$) with neutrons leads to a $^{6}Li(n,\alpha)^{3}H$ reaction. The produced tritons have an energy that is high enough to allow a consecutive nuclear reaction with oxygen-16 atoms present in the target material: ^{16}O (t,n) ^{18}F. Because the average cross section for the production of fluorine-18 over the effective range is only 87 mbarn the yield is not very high. The considerable amounts of tritium produced during the irradiation and the low specific activity obtained make this production pathway unprofitable and it is therefore not used often, but it offers a way of producing fluorine-18 when no cyclotron is available. [24]

1.5 Specific problems of reactions under no-carrier-added conditions

Radiopharmaceutical products are usually used in subnanomolar quantities, which make an induction of toxic or immunologic reactions almost impossible. The tracer is utilized in quantities that will not reach a saturation concentration, therefore it is very important to obtain the labeled products in high specific activities. This is only possible if the product is kept free of stable isotopes, which would dilute the specific activity of the radioactive product.

The possible states are differentiated as follows:

- carrier-free, c.f.
- no-carrier-added, n.c.a.
- carrier-added, c.a.

A carrier free substance can theoretically only be produced if no natural stable isotope of the used nuclide exists. Astatine is an example of radionuclides that lead to carrier free products. Syntheses utilizing nuclides for which at least one stable isotope exists lead to no-carrier-added products, since a dilution with the stable isotopes is practically inevitable. These are carried into the reaction from solvents, atmosphere, chemicals and for example target and reaction vessels.

Certain reactions are only possible if carrier is added to the synthesis. The best known example are syntheses utilizing $[^{18}F]F_2$ that has to be produced using elemental carrier. Due to their low specific activity carrier added radiochemicals are not suitable for studies unless the corresponding natural compound is already present in the body in high concentrations and thus a higher amount of the stable product can be tolerated.

Specific activity A_S is defined as activity A per weight (*m* in gram). [7]

$$A_S = \frac{A}{m} \qquad \left[\frac{Bq}{g}\right] \qquad \textbf{Equation 1.2}$$

While activity A is defined as

$$A = \frac{dN}{dt} = N \cdot \lambda = \frac{N \cdot \ln 2}{T_{1/2}} \qquad [Bq] \qquad \textbf{Equation 1.3}$$

The combination of equation 1.2 and equation 1.3 necessarily leads to the conclusion that specific activity will decrease, if half-life ($T_{1/2}$) or mass become larger.

Fluorine-18 has a theoretical maximum molecular activity of $6.3 \cdot 10^{10}$ GBq/mol that can be calculated by adding the Avogadro constant into equation 1.3.

$$A_{S,max} = N_A \cdot \lambda = \frac{N_A \cdot \ln 2}{T_{1/2}} \left[\frac{1}{s \cdot mol}\right]$$

$$\Rightarrow \quad A_{S,max} = \frac{6{,}0221 \cdot 10^{23} \cdot \ln 2}{6586{,}2} \left[\frac{1}{s \cdot mol}\right] = 6{,}3378 \cdot 10^{19} \left[\frac{Bq}{mol}\right] \qquad \textbf{Equation 1.4}$$

This value of activity cannot be reached under practical conditions, since stable fluorine-19 cannot be excluded from the reaction. Thus the total number of atoms (N_A) is always larger than the number of radioactive atoms (N), which leads to A_S being smaller than $A_{S,max}$.

Nevertheless high specific activities can be reached for many radiolabeled compounds but require working with quantities within the subnanomolar range. When working with such small amounts, effects that do not influence reactions on macroscopic scales can be important factors that merit consideration. For example adsorption on walls of the reaction vessel or side reactions with even the smallest impurities in the chemicals can occur, since even miniscule impurities present within the labeling precursor will exceed the amount of the radionuclide.

In labeling reactions unexpected side reactions are often witnessed that normally would not occur or only occur in smaller percentages. This is due to the use of some of the reaction partners in a 10^6-fold excess. The concentration of non-radioactive reaction partners stays nearly constant through the reaction when compared to the radioactive compound, which is why the labeling reaction can be considered to be of pseudo-first order. Also due to the low amount of radioactive material it is statistically almost impossible that an already labeled molecule will undergo a second reaction with the radioactive nuclide.

Maximum yield is connected to the decay of the nuclide and therefore to its half-life, it will be lower the longer the duration of complete synthesis, since a higher amount of the product will have decayed. To gain good yields, it is important to let the labeling reaction take place as late as possible within a multi-step synthesis, as decay of the labeled product will be minimized that way. Additionally a fast labeling procedure and, if necessary after the labeling step, fast organic syntheses need to be employed, since it is of high importance to reduce overall reaction time.

For most radiochemical reactions reaction yields are given as radiochemical yield, which is decay corrected and not connected to the duration of the synthesis anymore.

Important criteria for a no carrier added synthesis are:

- use of proper material (chemicals, solvents and reaction vessels)
- short duration of synthesis
- labeling reaction utilized as late as possible within a multi-step synthesis

1.6 Labeling methods with fluorine-18

The most often used methods of synthesis for a fluorine-18 labeled molecule are

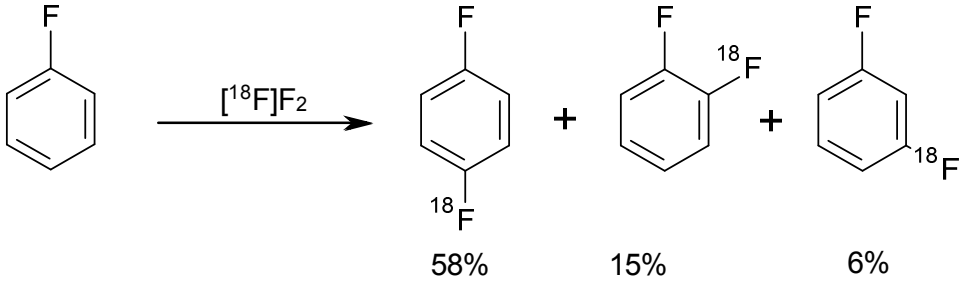
- direct labeling via electrophilic substitution
- direct labeling via nucleophilic substitution
- indirect labeling via synthons

1.6.1 Electrophilic substitution

Electrophilic substitution offers access to fluorine-18 labeled arenes and alkenes. Elemental fluorine ($[^{18}F]F_2$) is utilized most often but also the less reactive reagents acetylhypofluoride ($[^{18}F]CH_3COOF$) or xenondifluoride ($[^{18}F]XeF_2$) are applied, if milder reaction conditions are necessary. Because of its corrosiveness and extreme reactivity under normal conditions, fluorine needs to be handled carefully, which makes radiosyntheses with $[^{18}F]F_2$ very demanding.

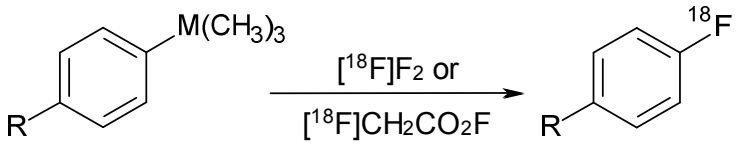
$[^{18}F]F_2$ can only be produced in good yields in carrier-added form (cf. above). Highest specific activities reached are at about 55 GBq/µmol, this is still 100-fold less than product yields of the no-carrier-added $[^{18}F]F^-$. [25, 26]

When using carrier-added $[^{18}F]F_2$, where each labeled molecule (which is only about every $10^{15\,th}$ molecule) will carry only one fluorine-18 atom, the radiochemical yield is limited to 50%.



Scheme 1.1: Yields of the electrophilic substitution of fluorobenzene [from 27]

As shown in Scheme 1.1 the high reactivity of $[^{18}F]F_2$ leads to mixture of products that further reduce the yield. The use of organometallic labeling precursors offers an alternative, by introducing a leaving group into the precursor and will give a lower amount of unwanted side reactions (cf. Scheme 1.2). [28]



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

Scheme 1.2: Electrophilic aromatic [^{18}F]fluorodemetallation [from 28]

For production of radiopharmaceuticals electrophilic fluorination can only be used if low specific activities can be tolerated, as with non toxic substances that can be used in high concentrations or if high concentrations of non-isotopic carrier are present within the body in any case. This is the case for [^{18}F]FDG where D-Glucose functions as non-isotopic carrier.

1.6.2 Nucleophilic fluorination

For synthesis of substances with high specific activity, only [^{18}F]Fluoride is practically useful, because a no-carrier-added synthesis is necessary.

Post production the fluoride is obtained in an aqueous solution and is highly solvated. ($\Delta_{Hydrate}$=506 kJ/mol) [29] In this state nucleophilic reactions are impossible which makes it necessary to remove the deactivating aqueous layer. This is generally done by azeotropic drying with acetonitrile. At the same time a soft cation is added to the fluoride, often Kryptofix 2.2.2®/K^+, an aminopolyether with potassium as ion, is applied. Under these conditions the potassium is coordinated by the aminoether and yields a soft ion (according to the HSAB-Principle). To generate a proton free medium a base is added, preferably a non-nucleophilic anion like CO_3^{2-}. If the precursor is labile to strong basic conditions, a K_2CO_3/$K_2C_2O_4$-mixture or the use of tetraalkyl ammonium salts is often helpful. [30, 31, 32, 33]

After drying of the [^{18}F]fluoride the reaction precursor, dissolved in a suitable polar aprotic solvent is added. Often used solvents are acetonitrile (MeCN), dimethyl sulfoxide (DMSO) und *N,N*-dimethylformamide (DMF). Alternatives to these classic conditions may be offered by the utilization of ionic liquids or tertiary alcohols, which are still in development. [34, 35]

While labeling under standard conditions with aprotic solvents, it is of utmost importance that no protic substances or metal cations are present, since both will

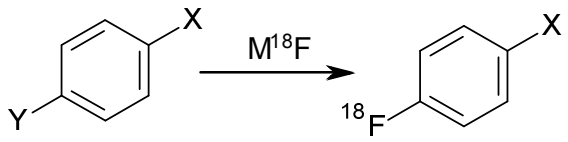
deactivate [^{18}F]fluoride due to the formation of hydrogen bonds or metal complexes, due to the high charge density of the fluorine anion.

Under the conditions given above, the reaction will be occurring under S_N2 conditions in aliphatic systems. This means a Walden inversion will take place. The precursor needs to be synthesized accordingly, if a stereospecific product is desired. Leaving groups for both aliphatic and aromatic reactions generally are halogens or tosylates/triflates. [36]

A general example is the synthesis of [^{18}F]FDG (cf. page 6), that is synthesized from a tetra-acetylated mannose triflate precursor. The conversion of the triflate into the labeled compound under polar aprotic conditions leads to an inversion of configuration, which subsequently gives the desired D-glucose derivate. [37]

During aliphatic reactions a β-elimination can happen, which will yield H^{18}F as a side product. Due to the high excess of base under which the labeling is conducted, the hydrogen fluoride will be converted back into the free ion and be available for further reactions.

For nucleophilic substitution reactions in the arene ring, an activating group is necessary, which needs to be positioned *ortho* or *para* to the labeling site. Electron withdrawing substituents are utilized whose –M and –I effects lower electron density at the target carbon atom. Often used activating groups are cyano and carbonyl groups. [38, 39]



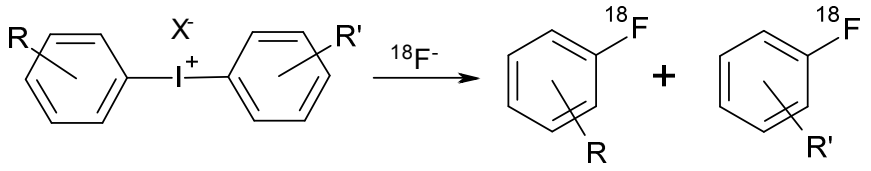
Y = NO_2, CN, CHO, RCO, COOR, Cl, Br, I
X = $(R_3)N^+$, NO_2, Br, Cl, I
M = [K⊂2.2.2]$^+$, R_4N^+, Cs^+, Rb^+

Scheme 1.3: Nucleophilic aromatic substitution with n.c.a. [^{18}F]fluoride [from 38]

Leaving groups for reactions on the aromatic ring are often trialkylated ammonium groups as well as aryliodinium salts (cf. below), which are the best nucleofuges. The choice of the counterion of the trialkylated ammonium group has a high impact on the radiochemical yields. Triflate ions are often used, while perchlorate and iodide are employed less often. [40] Further often utilized leaving groups are the nitro-group and

halogens, though the latter should not be applied unless low specific activities can be tolerated. [39]

If no strong activating substituents are present in the precursor molecule, utilization of the trimethylammonium group as leaving group will give significant amounts (about 80%) of [^{18}F]fluoromethane and thus lower the radiochemical yield of the [^{18}F]fluoroarene. Only by using strong activating groups like the nitro or cyano group this reaction can be prevented and better yields of the [^{18}F]fluoroarene are gained.



X$^-$ = I, Br, Triflat, Tosylat
R, R' = H, CH_3, C_4H_9, OCH_3, Cl, Br, I

Scheme 1.4: General principle of [^{18}F]fluorination with diphenyliodonium salt [from 42]

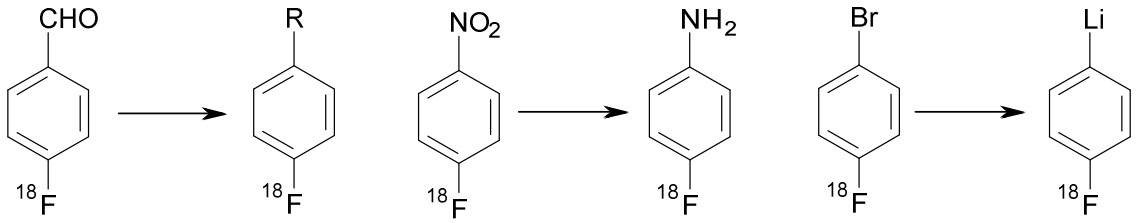
Non activated or only slightly activated molecules can be labeled employing the recently developed reaction with diphenyliodonium salts (cf. Scheme 1.4). Use of symmetric iodonium salts (R = R') leads to a single radioactive product with high radiochemical yields. [41] Asymmetric compounds (R ≠ R') will yield two different products, whose ratio will depend on the substituents present in the phenyl rings. Electron-withdrawing groups like halogens will favor the formation of the substituted [^{18}F]fluoroarene, while electron-donating groups like the methoxy-group will lead to the sole formation of [^{18}F]fluorobenzene (for R = OMe, R' = H, cf. Scheme 1.4). Counter ions used with diaryliodonium salts are generally triflate, tosylate or iodine and bromine. [42]

Another possibility to synthesize molecules that normally are not accessible through direct labeling is to insert an activating group into the arene ring, perform the labeling synthesis and then remove the activating group from the ring, giving the desired product. Examples are the simple removal of aldehyde groups using Wilkinson's catalyst (chlorotris(triphenylphosphine)rhodium(I)) or the hydrohalogenation of halogens with tributyltin hydride or sodium borohydride. The above mentioned reactions allow the synthesis of [^{18}F]fluoroarenes that normally would not be available due to the lack of an activating group in the ring. [43]

The often utilized Balz-Schiemann reaction is not useful in radiochemistry, since the counterion to the diazoniumion is tetrafluoroborate, which is used as donor of fluorine. Since only one of its fluorine atoms can be substituted with [^{18}F]fluorine maximal radiochemical yield is 25% and low specific activities will result. The use of a tetrachloroborate anion as an alternative has been suggested. This gives products with better specific activities, even though yields are not much higher, since the reaction mechanism follows a S_N1 pathway and side reactions with other nucleophiles present in the reaction mixture are common. [44]

1.6.3 Radiolabeling using synthons

If a direct labeling procedure is not possible under the conditions mentioned above, for example due to complex molecular structures, a labeling reaction using a pre-labeled synthon is a possible alternative. These mostly two step reactions consist of a first labeling reaction in which a smaller precursor molecule is labeled and then is coupled with a second precursor, giving the desired final product.



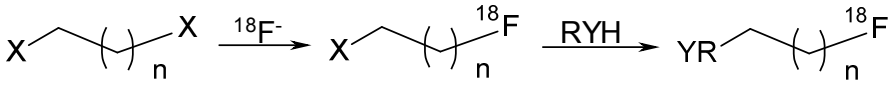
R = –CH_2OH, –CH_2X, –OH, –H

Scheme 1.5: Often utilized labeled synthons for further reaction [from 27]

There are two major methods for an indirect labeling reaction. Either one or two additional steps are added after the primary labeling reaction. Often the labeled synthon is directly reacted with a second compound and the final radioactive product is obtained, or the labeled precursor is transferred into a reactive synthon which is then coupled with the second precursor in a built-up synthesis (cf. Scheme 1.5).

To give good overall yields the reactions chosen for any indirect labeling with a labeled synthon must have a short reaction time with good to very good yields and a simple and fast way of obtaining the final product.

Good results with fast reaction conditions are found when using alkylating, acylating or amidating agents. For ^{18}F-fluoroalkylation a bifunctionalized alkane is labeled with [^{18}F]fluoride and then coupled with an amino, hydroxyl or thiol group. [45,46 47]



X = Tosylat, Triflat, Br, I Y = N, O, S R = Alkyl, Aryl

Scheme 1.6: ^{18}F-fluoroalkylation utilizing a functionalized alkane [45]

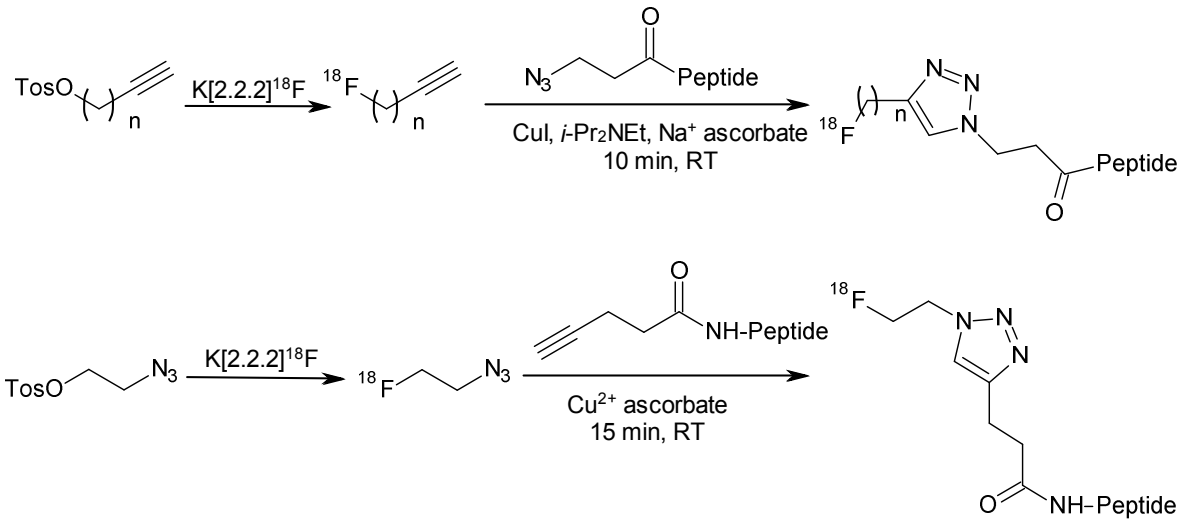
This reaction allows the synthesis of a wide range of radiopharmaceuticals, for example it has been used in the synthesis of dopaminergic or serotonergic receptor ligands. [48]

While the method introduced by Block *et al.* suggests [^{18}F]fluoroethyltosylate as the preferred labeling agent rather than 1-bromo-2[^{18}F]fluoroethane (BFE), recent publications reintroduced the latter as efficient labeling agent. [45, 49] While a distillation was previously required to separate labeled [^{18}F]BFE from its precursor, Comagic *et al.* introduced a chromatographic method utilizing solid phase exchange cartridges, which enables automation of the process. [49]

The similar acylation reaction using a previously ^{18}F-labeled ester can be performed in aqueous solutions, where the labeled agent is coupled with an H-acidic compound. Another possibility is the labeling of functionalized amines, which are subsequently coupled with esters to give the corresponding amide. Both reactions allow for the synthesis of labeled peptides or proteins, for example Biotin, Octreotide and insulin. [50, 51, 52]

A newly employed method of labeling peptides is the so called click-chemistry, a term introduced by K. Barry Sharpless that refers to a method of synthesis which utilizes modular building blocks that react readily to the desired product. [53] Of high interest for the synthesis of labeled compounds is the 1,3-dipolar Huisgen cycloaddition. While the uncatalyzed reaction needs harsh reaction conditions and gives a mixture of the 1,4- and the 1,5-disubstituted triazole product, the copper catalyzed variant allows reactions at room temperature and at moderate pH, giving only the 1,4-disubstituted triazole. [54,55]

This reaction has first been suggested for the synthesis of labeled peptides by two groups using different approaches. While Marik *et al.* used a labeled alkyn-synthon, Glaser *et al.* decided on the labeled azide, since alkynes are more readily available and thus easier to synthesize as the peptide-synthon. [56, 57]



Scheme 1.7: Radiosytheses utilizing click-chemistry as introduced by Marik *et al.* (upper scheme) and Glaser *et al.* (lower scheme) [56, 57]

Aside from click chemistry other new methods have recently found their way into radiochemistry, the use of an enzyme (fluorinase) is under development and even though reactions times are still longer than desired (1-4 hours) the use of a bio-catalyst opens possibilities for labeling under mild conditions with high chemo-selectivity. [58] For the labeling of peptides thiol labeling agents has been of great interest and a wide range of possible reagents is currently in development. Other possibilities include silicon, phosphor or boron carrying synthons which would offer easier preparation and hopefully the developement of labeling kits. [59, 60, 61]

1.7 Structure of the central nervous system (CNS)

Signal transduction in the nervous system is conducted via electrical or chemical processes. Neurons, the cells which enable regulation of our being, register a stimulus and react to it with an increased potential in their membranes, transporting the signal electrically from the dendrites towards the axons, the ends of the nerve cells.

To process the information reaching our brain in every single second of our life, millions of neurons are interacting with each other, transmitting and storing information. Both of these procedures do not only rely on signal transduction, but also on targeted potentiation or blocking of certain signals.

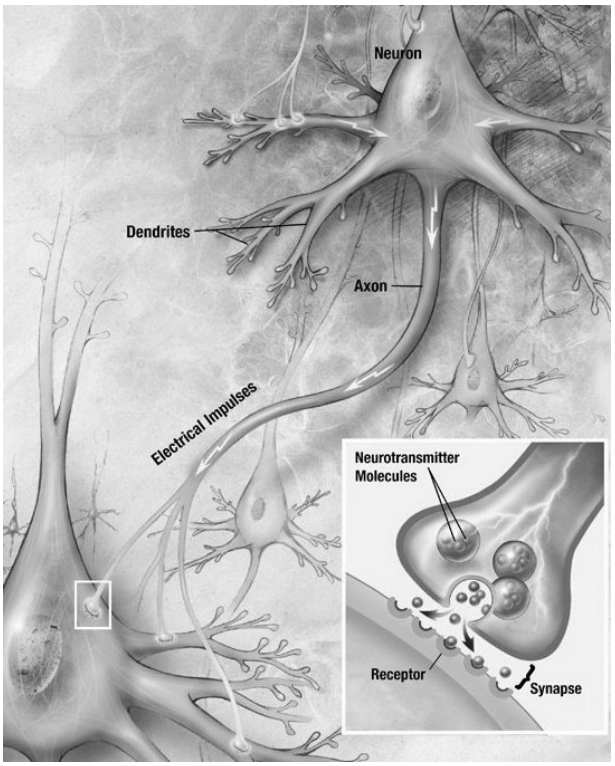


Figure 1.2: Signal transduction in the CNS via the axon and transmission to the next neuron at the synapse [62]

The junction between two nerve cells where an axon meets a dendrite is called a synapse. At the synapse the cells do not have direct contact, but rather are separated by the so called synaptic cleft. Signal transduction through the synaptic cleft proceeds by neurotransmitters, chemical compounds that are synthesized in the neuron and stored in vesicles in the axon terminal. If a stimulus arrives at the synaptic bouton, the vesicle merges with the cell membrane and sets the

neurotransmitter free (exocytosis). The neurotransmitter diffuses through the synaptic cleft towards the dendrite, where it binds to transmitter specific receptors and once again a membrane potential change occurs and the signal is further transported through the excited nerve cell. Generation of the potential can occur by different means. Either an ion channel opens, letting cations diffuse into the cell, generating a potential difference, or by secondary intracellular transmitter proteins.

Depending on the receptor type, either an ion channel is opened or closed, or a change of confirmation of a membrane protein will lead to cascade of internal signal transduction, which then can also lead to the opening of ion channels. Calcium, sodium, potassium cations or chloride anions can diffuse through the ion channels into the cell and generate a potential change that will lead to the generation of a signal and its electric transport towards the next synapse.

Unlike ion channel receptors, which regulate the influx or efflux of ions, so called G-protein coupled receptors work by generating a signal through the release of cyclic adenosine monophosphate (c-AMP) or cyclic guanosine monophosphate (c-GMP) as first signaling molecule of a complex cascade inside the receptive cell.

Receptors also occur presynaptic on axons (so called autoreceptors), where they are important to the regulating system of the synapse. Aside from the compounds that normally act as neurotransmitters, other endogenous compounds (for example hormones) and artificial molecules such as synthesized receptor ligands (like many CNS active pharmaceuticals) influence receptor function. Both functions, as agonist or antagonist, are possible. So called agonists are substances which stimulate the receptor, while substances which block it are called antagonists.

Every axon contains a neurotransmitter specific for this type of cell. For regulation after release into the synaptic cleft the neurotransmitter can be removed by reuptake or metabolic elimination which causes resynthesis in the releasing cell. Important neurotransmitters are acetylcholine, amino acids (for example γ-amino-butyric acid or glutamate, the salt of glutaminic acid), monoamines (serotonine, dopamine) and oligopeptides.

1.8 Ionotropic glutamate receptors

The glutamate receptor is primarily expressed in the central nervous system. In the brain it is mainly situated on membranes in the cerebellum and hippocampus. It is the most important excitatory neurotransmitter in the mammalian brain. It probably plays an important role in learning and memory processes and is one of the receptors involved with neurodegenerative diseases. [63]

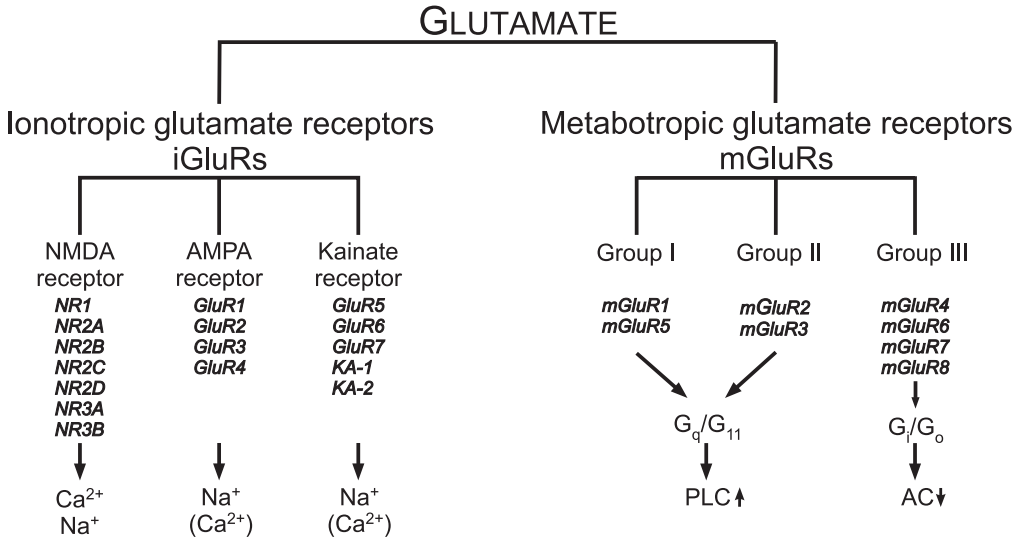


Figure 1.3: Overview of the different glutamate receptors [64]
↑PLC indicates activation of phospholipase C
↓AC indicates inhibition of adenylate cyclase

Depending on the method of signal transduction glutamate receptors are divided into metabotropic and ionotropic receptors. The metabotropic glutamate receptors have an important role in regulation of the glutamatergic receptor system by acting as modulators of the ionotropic glutamate receptors. Ionotropic receptors are subdivided into NMDA, AMPA or Kainate receptors, after their respective agonists *N*-methyl-D-aspartat (NMDA), α-amino-3-hydroxy-5-methylisoxazol-4-propionsäure (AMPA) and kainate.

Composition of the receptor assembly in the cell wall differs from that of other known ionotropic receptor types such as the acetylcholine or GABA receptor. The ionotropic glutamate receptor consists of four subunits that combine into the working receptor. Each subunit contains four membrane domains (MD), only three of which truly pass through the membrane. The second MD forms an intracellular loop. In consequence this leads to each subunit displaying an intracellular carboxyl ending (cf. Figure 1.4). [64]

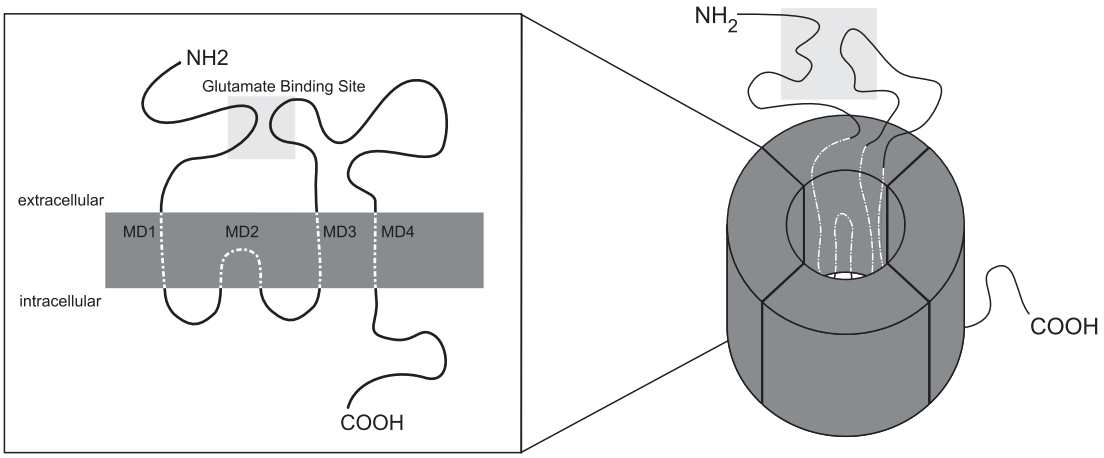
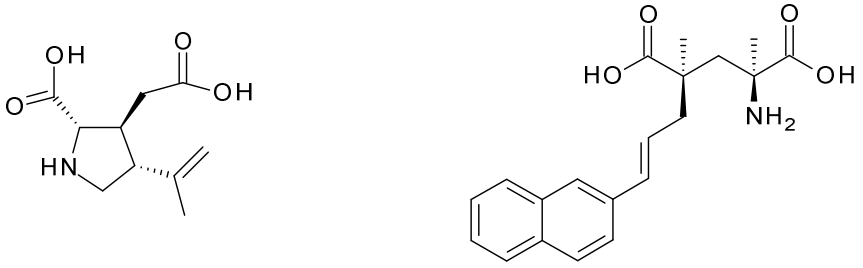


Figure 1.4: Possible build of a glutamate receptor

1.8.1 Kainate receptors

Kainate receptors have two kinds of subtypes which are identified as GluR5 through GluR7 and KA-1 and KA-2. The actual receptor is build either solely from subtypes of the GluR5 to 7 kind, or is a combination of the former with KA-1 or KA-2 units. The latter cannot give functioning receptors in a homomeric arrangement. [64]



Kainic acid **LY339434**

Figure 1.5: Examples of selective agonists of the Kainate receptor

Due to the lack of selective agonists the kainate receptor for a long time has not been as thoroughly examined as the AMPA and NMDA receptors. Only after the synthesis of selective agonists was successful, research in that area was brought forward.

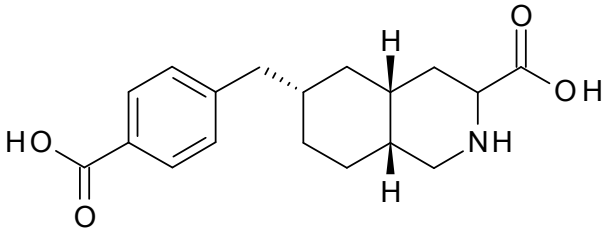


Figure 1.6: Structure of the GluR5 selective antagonist LY382884 [65]

Using agonists for the AMPA receptor as model compounds, a series of competitive and non competitive ligands was synthesized. Several antagonists were specially developed for the GluR5 subtype. Development of allosteric ligands is hoped to lead to further insight into the kainate receptors.

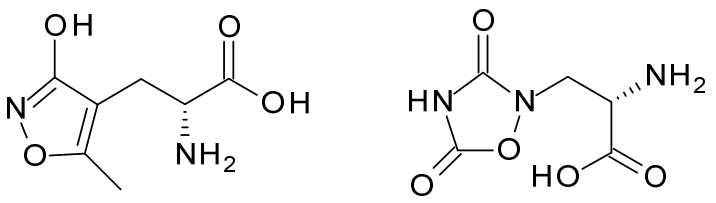
1.8.2 AMPA receptors

AMPA receptors are divided into subtypes named GluR1 to 4. The receptor consists of four subunits which are heteromerically arranged. All subunits can exist in different spliced versions. During gene transcription mRNA is edited, so that two alternate varieties of the protein will be generated. Generally these differ only in a few amino acids. With AMPA receptors RNA editing generally occurs between the 3rd and 4th transmembrane domain (between MD3 and MD4) and on the C-terminal end (cf. Figure 1.4). One important change made by this version of alternate splicing is the existence of the so called Flip and Flop versions of the subunit. Both are differently folded versions of the protein, which results from the change of several amino acids in the protein structure in the area between the third and forth membrane domain.

While the Flip version is predominant in the prenatal brain, the Flop variant only develops postnatally and reaches equivalent levels in the adult brain. Both version show different properties in desensitization and behavior towards allosteric modulators, which leads to the assumption that interaction between these modulators and the receptor occur in the area that has been modified. [64, 66, 67] Pharmacologic and kinetic properties of both variants are so distinct that different receptor ligands exist for each of them.

1.8.2.1 AMPA receptor agonists

Most agonists developed for the AMPA receptor were similar in structure to the original agonist AMPA itself. Different agonists show different rates of desensitization. While glutamate and AMPA lead to a fast reaction of the receptor, weaker agonists do not lead to complete desensitization.

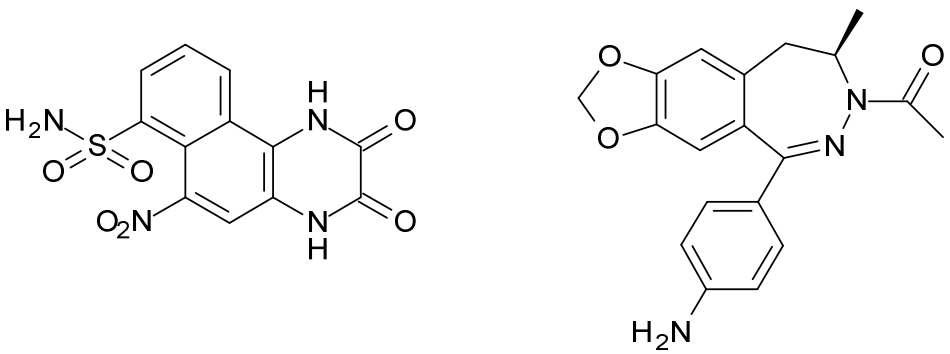


***R*-AMPA** **L-Quisqualic acid**

Figure 1.7: Examples of AMPA receptor agonists

1.8.2.2 AMPA receptor antagonists

Both competitive and non-competitive antagonists for the AMPA receptor are under development. Starting from quinoxaline derivatives like NBQX a wide range of potential competitive antagonists have been designed. Their poor solubility in water, however restricts their application so far. Non-competitive antagonists are also called negative allosteric modulators. They do not bind at the same binding site as the agonist and competitive antagonists. Design of competitive antagonists started from 2,3-benzodiazepines, followed by phthalazines. [68] Their selectivity towards specific subunits is not very high and research for more selective antagonists is ongoing.



a) NBQX **b) *(R)*-LY300164**

Figure 1.8: Examples of a) competitive and b) non-competitive AMPA-antagonists

1.8.3 NMDA receptors

Subunits of the NMDA receptor are called NR1, NR2A to D and NR3A and B (cf. Figure 1.3). A functioning receptor again consists of four units, two of which must be NR1 subunits. The other two may either be NR2 or NR3 units. [64] The agonist glutamate binds to the NR2 subunit, while the necessary co-agonist glycine has its binding site on the NR1 unit. [69] In order to activate the receptor, glycine has to be

present when glutamate binds. It has been reasoned that in order to enable fast excitation, the glycine binding site is almost constantly occupied with glycine. [70]

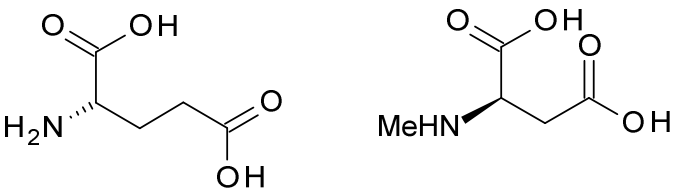
The NMDA channel is permeable by Na^+ and K^+ ions, but also by Ca^{2+}, which other glutamatergic receptors are not permeable to. In state of rest the receptor is blocked with Mg^{2+}, but the block is removed once depolarization of the receptor membrane occurs (meaning a change of the resting potential of the cell). [71]

The NMDA receptor plays an important role in memory and learning, its wide distribution in the mammalian brain hints to its importance. While receptors that are prevalent prenatally contain NR2B units, in the postnatal brain receptors containing NR2A units increase and are the most prevalent in adult brains. At the same time receptors containing only NR2B units can be mainly found in the forebrain, accompanied by such containing one NR2A and one NR2B unit, the latter probably is the major receptor in the adult forebrain. [72]

The fact that the distribution and composition of the receptor changes postnatally indicates that it has a role in learning and memory. The model currently used to explain how our brain stores information is called Long Term Potentiation (LTP) and consists of the principle that synapses which get activated more often form a stronger pathway and durably increase the effect of synaptic transmission. [73] Research conducted so far points towards NMDA receptors playing an important role in LPT and thus in forming our memory. This leads to the conclusion, that malfunctions of the receptor may be occurring in dementia connected illnesses like Alzheimer's. [74] The mapping of NMDA receptors would enable to better understand and influence the function of the receptor.

1.8.3.1 NMDA receptor agonists

The two most important agonists of the NMDA receptor are the salts of glutamic acid, which dissociates in the body to glutamate, and *N*-methyl-D-aspartate (NMDA), the structures of which are shown in Figure 1.9. Most other important agonists are based on these structures, but there has been no completely new approach towards the synthesis of NMDA agonists during the past years.

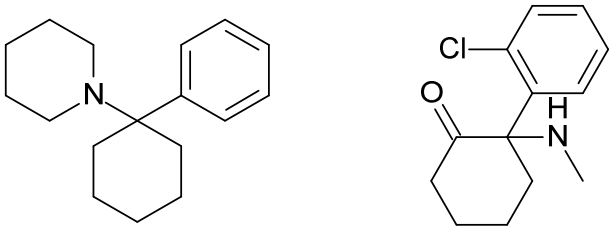


L-glutamic acid **N-methyl-D-aspartate (NMDA)**

Figure 1.9: Structure of the two most important NMDA receptor agonists

1.8.3.2 NMDA receptor antagonists

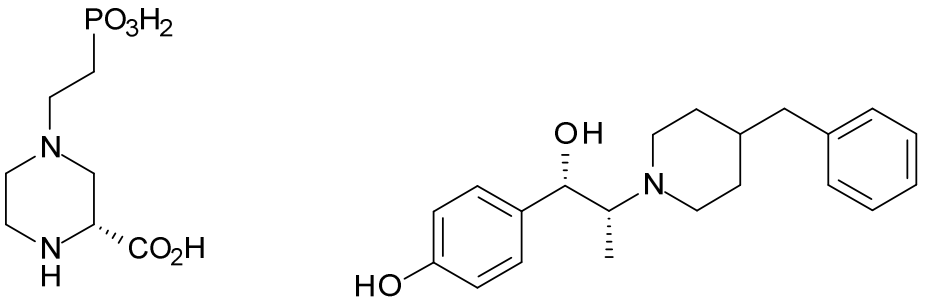
Antagonists of the NMDA receptor show great influence on the psychological behavior of the user, an example being two compounds well-known under their aliases Angel Dust and Special K.



PCP (Angel Dust) **Ketamine (Special K)**

Figure 1.10: NMDA receptor antagonists that are misused as street drugs

Both are considered dissociative drugs, which induce a state in which the conscious mind is disconnected from the rest of the brain. While a lot of other drugs also show similar effects dissociative drugs also produce effect like sensory deprivation, hallucinations as well as dream-like states and trances. [75] Similar effects can be witnessed with most of the NMDA receptor antagonists, which makes clinical application difficult. On the other hand both, ketamine and PCP, show effects on other receptors and are not selective for the NMDA receptor only, which may explain the dire effects they have. Abuse of both drugs leads to cognitive impairment (e.g. confusion, forgetfulness and difficulty to concentrate) and possibly cell death, resulting in lasting memory problems.

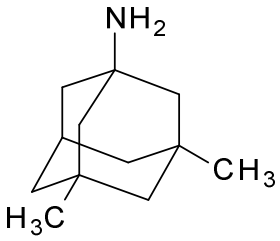


a) CCP **b) Ifenprodil**

Figure 1.11: Examples of a) competitive and b) non-competitive antagonists of NMDA receptors

Most antagonists that have been developed during the last years are either selective for the NR2A (for example CCP) or the NR2B (for example Ifenprodil) subtype (cf. Figure 1.11), while only few compounds exist that show selectivity for NR2C or NR2D. This is due to the higher interest in NR2A and NR2B selective antagonists, since these subtypes are more common and more important in mammalian brain.

A different method of antagonism, called uncompetitive or functional antagonism, is witnessed with the so called channel blockers, molecules which do not associate with the receptor but block the receptor channel itself. One of these channel blockers, memantine, has already found application in patients with Alzheimer's disease and is currently in research for other applications. [76]



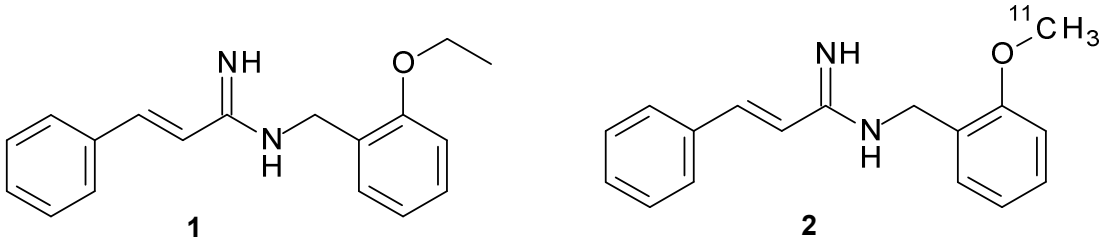
Memantine

Figure 1.12: Memantine, an NMDA channel blocker

1.9 NMDA receptor ligands with amidine structure

The development of radioligands for studying the human brain by means of emission tomographic methods is of high interest. Especially those for the NR2B sub-system of the NMDA receptor family have come into focus during the last years. In 2003 a Merck group published a series of new compounds with benzamidine structure that act as antagonists of the NMDA receptor with a very promising affinity (low k_i-values), allegedly high metabolic stability and a good permeability into the brain. [77, 78] First radiolabeled NMDA receptor ligands with amidine structure were synthesized in 2005 and 2006. [79, 80] Labeling of these compounds was performed using tritium, fluorine-18 or carbon-11. [79, 80] While good selectivity was shown *in vitro*, no data is known on the *in vivo* evaluation of those radiolabeled compounds, besides for the carbon-11 derivatives, which exhibited a high metabolization rate *in vivo*. [81]

Compound **1**, depicted in Figure 1.13, had previously been published and had shown a very good k_i-value of 0.5 nM. [77] The k_i-value of compound **2** showed equally low values and suggests labeling of the compound achievable. While substance **2** was originally labeled using the [^{11}C]methyl group, the [^{18}F]fluoroethyl group would offer a good alternative for labeling with fluorine-18. The attributes of these two groups are similar enough that steric and electric properties of the molecule would be changed as little as possible. Radiolabeling of the compound could be performed indirectly via 2-[^{18}F]fluoro-1-bromoethane. This type of labeling via a prosthetic group seems promising, as the structure of compound **1** is similar to the planned labeled product.



1 **2**

k_i = 0.5 nM **k_i = 0.7 nM**

Figure 1.13: Cinnamamidines synthesized by Curtis *et al.* (**1**) and Thominiaux *et al.* (**2**) [77, 80]

In 2006 a publication by Årstad *et al.* gave first *in vivo* results for carbon-11 labeled compounds including derivative **2**, which showed low metabolic stability of most of the benzamidine compounds while k_i-values were good (0.7 nM for **2**) and brain permeability was promising. [81]

When a publication by Nguyen *et al.* in 2007 first set a spotlight on cyclic amidines, it was shown that those compounds promise better stability *in vivo* and at the same time have similar k_i values to the benzamidine compounds. [82]

Central functional groups in these compounds are two structures that have been under research for more than a hundred years: salicylates and amidines.

Willow bark had been known to reduce fevers and ease pain since at least the 5th century B.C., but it was not until 1826 that Johann Andreas Buchner was able to extract the compound that caused the activity of the bark: salicylic acid. By the end of the 19th century the development of the new compound into a useable drug had been accomplished, when Bayer introduced Aspirin® to the market. Since then a wide range of salicyl derivatives have been under research for different uses. Pharmaceutical chemistry would be different without it. Many structures derived from natural compounds contain an aromatic ring that carries a hydroxyl group in 2-position to an aldehyde, carboxylic acid or methylamine moiety, for example, thus making salicylates an important synthon for organic synthesis.

Discovery of the mechanism of how acetylsalicylic acid works in the human body was published in 1971 by Robert John Vane. [83] In 1982 Vane received the Nobel Prize for Physiology or Medicine for the discovery how acetylsalicylic acid inhibits prostaglandin synthesis.

While salicyl compounds have been under constant investigation, amidines have not been in the focus of research until the end of the 19th century, when Adolf Pinner described the synthesis of imidates. [84] His work was the first to describe the synthesis of this functional group and while it may not be one of the most important groups in organic chemistry, it can be applied as useful intermediate in many multi step syntheses. [85] Amidines can generally be easily synthesized from the nitril by reacting it in a suitable solvent (benzene, chloroform, ether or ethanol) at 0 °C with HCl gas. The imidate that will form can either be separated as the imidoester or reacted with anhydrous ammonia or ammonium cloride to give the amidine as hydrochloric salt, while reaction with water will give the ester. [85] A wide range of amidines is contrivable and the Pinner synthesis has found wide use especially in modern day chemistry with the commercial availability of hydrogen chloride gas.

2 Aims and scope

The special role of fluorine-18 in radiopharmaceutical chemistry has been demonstrated by many examples. Meanwhile numerous radioligands exist for *in vivo* studies of the various receptor systems, but only few suitable ones are known for the glutamatergic receptor system. The radiosynthesis of a n.c.a. receptor ligand labeled with fluorine-18 therefore is of high interest to enable further studies on glutamate receptors.

As described in the introduction several organic molecules with a benzamide structure have been proven to be good ligands for the NMDA receptor. While the selected lead structure had already been labeled using carbon-11, in the scope of this work labeling with fluorine -18 is planned in order to enable extended studies. Based on the lead structure, compounds were therefore chosen as target molecules, which can be radiofluorinated with high specific activity for possible use as *in vivo* radioligands of NMDA receptors.

All molecules show similar structures, three of them (**3**, **4**, **5**, see Figure 2.1) differing only in the kind of the spacer between the phenyl ring and the amidine. The forth compound **6** shows a cyclic amidine structure that promised better *in vivo* stability.

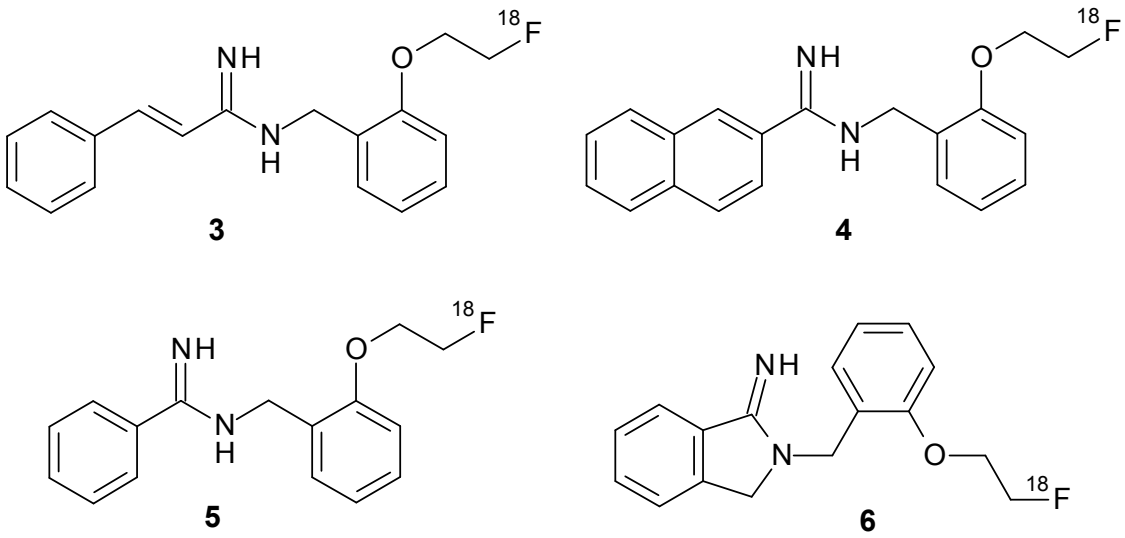


Figure 2.1: Radiolabeled benzamidines that are to be synthesized in the scope of this work

The first task to fulfill is the synthesis of the labeling precursors and of the authentic target compounds to be used as chromatographic standards for identification of the

no-carrier-added labeled compounds. Synthesis of both can partially follow routes of production described in the literature. Accordingly, it is planned to synthesize 2-hydroxybenzylamine and react it with a selected imidoester in a base assisted coupling reaction to form the desired amides. The imdoesters are planned to be obtain from the respective nitriles. The cyclic compound **6** should be synthesized from benzylamine and 2-(bromomethyl)-benzonitrile.

The authentic standard compounds have to be produced by alkylation of the corresponding labeling precursor previously synthesized. Introduction of 1-bromo-2-fluoroethane into the molecule should be attempted by using a base and possibly sodium iodide as co-reagents.

Once both compounds (precursor and the fluoro-compound) have been synthesized in pure form, the ^{18}F-labeling reaction on the precursor can be established. Labeling reactions of phenols with 1-bromo-2-[^{18}F]fluoroethane have been described in literature and it is planned to follow those generally. A possible alternative to a labeling reaction starting from a bihaloalkane is the use of double functionalized glycols, especially ethylenglycole-1,2-ditosylate.

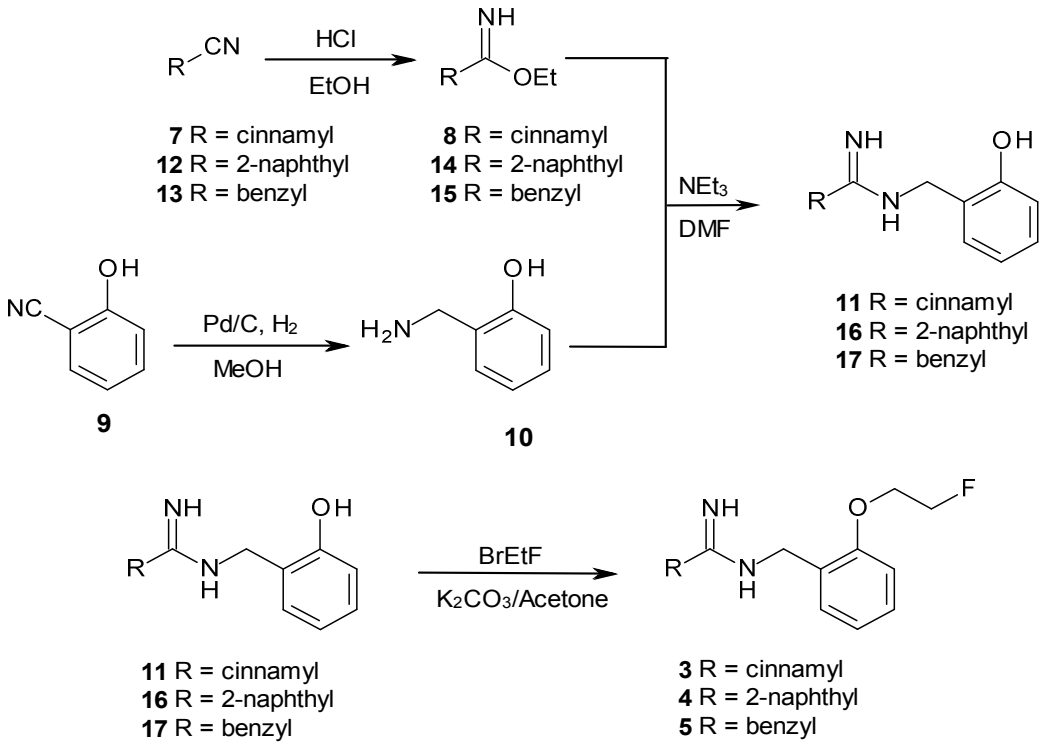
Optimization of the labeling reaction needs to be performed once it has been confirmed that the chosen method of labeling is generally feasible. Optimization with respect to time, solvent and temperature will be of foremost importance. Further optimization will comprise the concentration of precursor and base, as well as a variation of the base used.

In order to analyze the labeled products and to determine their radiochemical yield it is necessary to establish chromatographic systems for both (radio-) thin layer chromatography and (radio-) high performance liquid chromatography (HPLC). For determination of the specific activity of the labeled compounds the detection limit of the HPLC has to be determined of the corresponding authentic standards.

3 Results and discussion

3.1 Organic synthesis of precursors and fluoro-compounds

While several compounds with benzamidine structure were to be synthesized in the course of this work, a model compound was chosen to establish the reaction conditions. Cinnamylnitril **7** was deemed a good choice, since several publications about its synthesis and chemical and metabolic properties and those of similar compounds have been published. [79, 80, 81] The course of reaction for synthesis of those compounds was originally planned as according to Scheme 3.1.

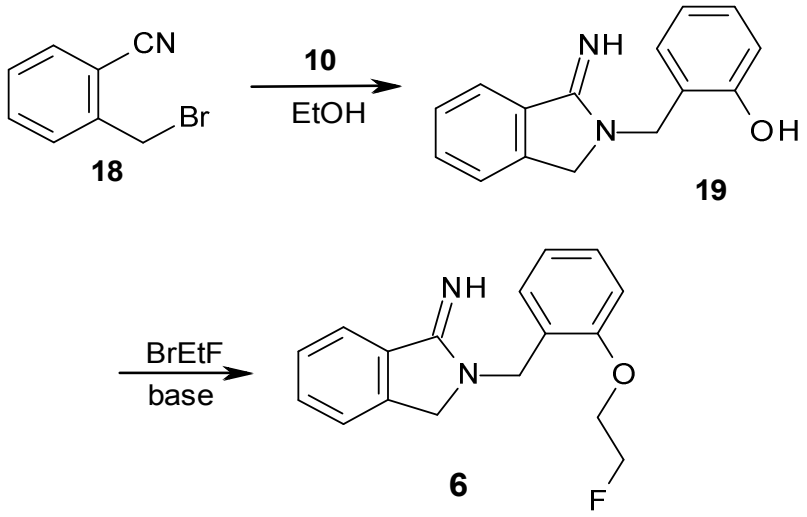


Scheme 3.1: Concept of reaction path for the non-cyclic labeling standards **3** – **5**

For the model compound synthesis of the authentic standard **3** started with a Pinner reaction in ethanol to form the imidoester **8**, this was coupled with salicylamine **10**, obtained by reduction of 2-hydroxybenzonitrile, to give the labeling precursor **11**. This was then to be alkylated to give the final product **3**.

Other compounds synthesized were the labeling precursors **16**, **17** and **19** and the corresponding standard compounds **4**, **5** and **6** (cf. Scheme 3.1 and Scheme 3.2). With these additional compounds the structure was only slightly modified. While

compound **17** lacks the spacer between the phenyl ring and the amidine, in **16** a naphthyl group is introduced that stabilizes the linker and adds steric hindering. All these structures are known to be metabolically unstable, [81] what lead to the introduction of a different molecule into this work, the cyclic compound **6**.



Scheme 3.2: Reaction pathways for the cyclic compound **6** synthesized in this work

The labeling precursor for this molecule is compound **19**, a cyclic amidine that is not synthesized in a Pinner like reaction but in a simple condensation reaction with the hydroxybenzylamine.

Cyclic amidines have been evaluated by Nguyen *et al.* and showed very promising results. [82] The plasma half-life for one these compounds (**20**, cf. Figure 3.1) was measured and reported to be about 2 hours. This compound was subsequently chosen as model for the synthesis of cyclic amidine **6**.

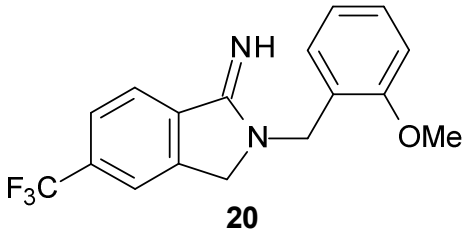
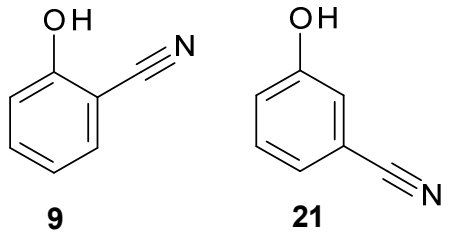


Figure 3.1: Lead structure for the cyclic amidine

3.1.1 Synthesis of 2-hydroxybenzylamine

Synthesis of 2-hydroxybenzylamine **10** was previously described in various publications. [86, 87, 88] In those studies synthesis was performed using either the salicyloxime or the salicylamide, which are commercially available or easy to synthesize. The pure compound is also available commercially, but prices are very high (1 g > 150 Euro) and due to the amount needed in the course of this work, it was decided to synthesize **10**.

In addition to having the target compound carry the hydroxyl-group in 2-position (**10**), the compound carrying the hydroxyl-group in 3-position (**22**) was to be synthesized. The variation of the position and the subsequent change of k_i-values were considered to be of interest. This plan was not followed when synthesis of the 2-hydroxy compounds proved more demanding than expected and at the same time the 3-hydroxy compound was expected to be metabolically less stable. While establishing the reaction pathways still a method of synthesis was searched that could be used for both compounds. Since neither 3-hydroxybenzylamide nor 3-hydroxybenzylaldoxime are commercially available, the benzonitrile **21** was chosen as starting compound (cf. Figure 3.2).



9 **21**

Figure 3.2: Benzonitriles for the synthesis of benzylamines

A common method for the reduction of nitrile groups is the use of hydrogen and palladium on active charcoal (Pd/C) in methanol. The reagent is dissolved in methanol and Pd/C is added, and the reaction is either done under hydrogen atmosphere or under argon atmosphere with the addition of ammonium formate. [89] Under these conditions the formate decomposes into hydrogen, carbon monoxide and ammonia and thus acts as a hydrogen donor. Since this method of reduction is easy and generally gives excellent yields, it was the first choice.

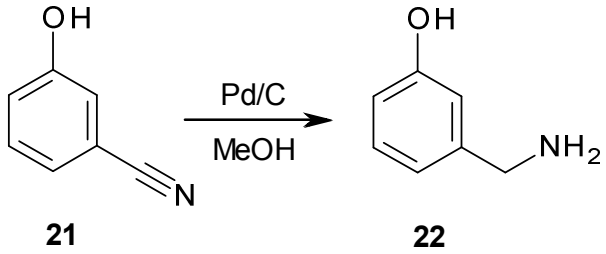
Reaction with methanol as solvent, using a hydrogen gas atmosphere and 2-hydroxybenzonitrile **9** as reagent, did not give the desired product, even though

reaction was complete and a control by thin layer chromatography (TLC) showed only a single product. An analysis with mass spectroscopy proved that dibenzylamine was the sole product. Further methods of synthesis utilizing a Pd/C system, but using ethanol instead of methanol as solvent or adding ammonium formate also gave no product. Analysis with TLC once again showed one product and no traces of reagent left, but mass spectroscopy confirmed the product to be dibenzylamine.

Literature indicates other options for synthesis: the use of complex metal hydrides, like lithium aluminium hydride (LAH), or borane (in either dimethyl sulfide or tetrahydrofuran) as reducing agents. [90, 91, 92]

LAH is often utilized in today's chemistry as a useful and widely applicable reagent for hydration reactions. Depending on reaction conditions it can be used to reduce esters or carboxylic acids to primary alcohols (offering a feasible alternative to the Bouveault-Blanc reduction) or nitriles to the corresponding amine. During this work syntheses with LAH were done both with and without the addition of aluminium chloride, the latter improving the formation of free aluminium hydride in the solution. Both reactions showed a product in TLC control, but mass spectroscopy showed that a different product than the desired one was obtained.

At this point a problem with analysis was presumed and therefore the compound was acquired commercially and tested with mass spectroscopy. The method of analysis was slightly changed and synthesis of the amine was repeated using the conditions with Pd/C in methanol and hydrogen gas (cf. Scheme 3.3).

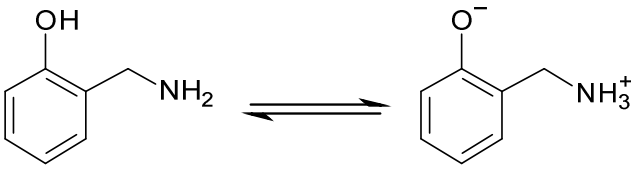


21 **22**

Scheme 3.3: Synthesis of 3-hydroxybenzylamine (**22**)

Surprisingly the 2-hydroxy compound did not yield any product, while the 3-hydroxybenzylamine (**22**) could be isolated in very good to quantitative yields. It remains unclear why the reduction of **9** was not successful, but several further attempts and control by mass spectroscopy confirmed that the dimer was the sole product formed.

As alternative method of synthesis a reaction path using borane in THF was developed. This reaction pathway provided the product **10** while offering simple reaction conditions and short reaction times. The reagent was dissolved in THF and reacted with borane under reflux. After 3 hours reaction was stopped by adding aqueous HCl and the product was extracted from the solution. [92] These reaction conditions have been established by Brown *et al.* as simple and rapid giving excellent yields for a wide range of nitriles. While the reaction proceeds only slowly if borane is added in equimolar amounts or at lower temperatures (0 °C), an excess of borane and higher temperatures give the desired product in good yields after reaction times of a few hours. Reduction is selective for the nitrile even in the presence of (less reactive) functional groups. [93]



Scheme 3.4: Possible intramolecular formation of a salt.

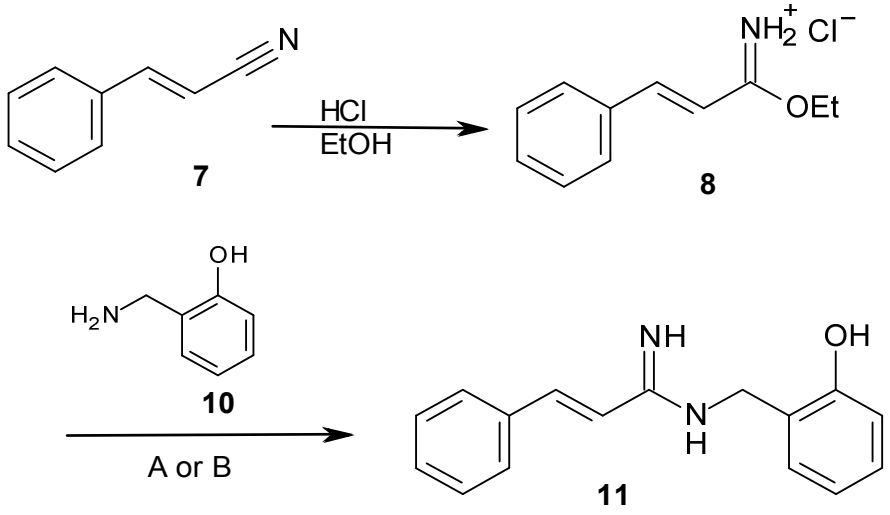
Thus, the desired product **10** was obtained, though yields varied greatly and were less than expected (41 - 69 %). A possible explanation for this could be the intramolecular formation of a salt (cf. Scheme 3.4). Since the product had to be extracted from an aqueous solution, it can be assumed that a part of the salt remained in the aqueous layer and could not be extracted with an organic solvent. To prevent this, the solution was set to both acidic and basic pH values and extraction was done, but at neither pH = 1 nor pH = 10 were better yields found.

3.1.2 Synthesis of precursors

For the preparation of the model compound **3** the cinnamyl precursor **11** was synthesized in a 2-step reaction, starting from (E)-cinnamyl nitrile **7**, following instructions published by Thominiaux *et al.*. [80] In addition to this compound the three other labeling precursors **16**, **17** and **19** were synthesized as described by Årstad *et al.* and Nguyen *et al.*. [81, 82] Further as an alternative to indirect labeling via prosthetic groups an attempt at synthesizing a precursor for direct labeling was made as described in chapter 3.1.2.4.

3.1.2.1 Synthesis of *N*-(2-hydroxybenzyl)cinnamamidin (11)

As first step in the synthesis of the cinnamyl precursor the nitrile **7** was transformed to the corresponding imidoester **10** by a Pinner synthesis (see Scheme 3.5). The reagent was reacted in ethanol at 0 °C with hydrogen chloride gas. Monitoring by TLC showed completion after 18 hours reaction time. The reaction was left standing at room temperature, which allowed a colorless to cream colored precipitate to be formed. This generally took between one and two weeks and is the usual method of synthesis for a Pinner reaction.



Scheme 3.5: Method of synthesis of *N*-(2-hydroxybenzyl)cinnamamidin (**11**), A: NEt_3, DMF; B: $NaOCH_3$, MeOH

The solid was isolated from the solvent by filtration. After drying, the ethyl cinnamimidate hydrochloride was obtained in excellent yields (>90 %). No formation of the ortho ester was observed, even though it is a known side reaction, if ethanol is used as a solvent. [86]

The obtained product **8** was further reacted without purification and coupled with 2-hydroxybenzylamine (**10**) in DMF with triethylamine as base to give *N*-(2-hydroxybenzyl)cinnamamidin (**11**) in a wide range of yields (22 - 77 %; method A, see Scheme 3.5).

Method B for the synthesis of **11** was published by Årstad *et al.*, who used sodium methanolate as base. [81] For this reaction the 2-hydroxybenzylamine **10** was dissolved in methanol and solid sodium methanolate was added. After addition of the ester the reaction was stirred at room temperature until control by TLC confirmed the completion of the reaction. After 12 to 18 hours of reaction time the reaction was

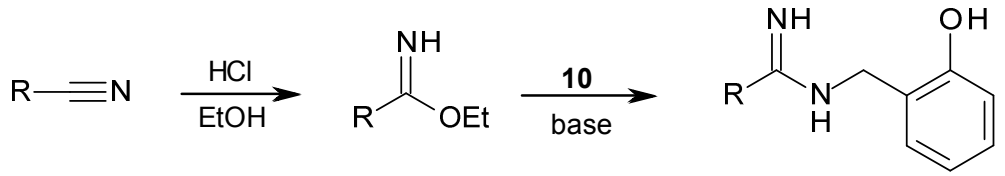
quenched and the solvent was evaporated *in vacuo*. After removal of the solvent the raw product was obtained as a light orange to cream colored solid in quantitative yields and purified by crystallization.

Both methods of reaction were employed and the product was used for further reaction if mass spectrometry confirmed that the product was sufficiently pure (> 95 % by mass).

Only if the product was to be applied as labeling precursor was a second crystallization performed. This lowered the yields significantly but gave a perfectly clean product (> 99 % by HPLC) that formed light yellow crystals.

3.1.2.2 Synthesis of *N*-(2-hydroxybenzyl)naphthylamidin (16) and *N*-(2-hydroxybenzyl)benzylamidin (17)

In order to synthesize the naphthyl and phenyl compounds the respective imidoesters were formed in a pinner synthesis that followed the conditions given in chapter 3.1.2.1. Both imidoesters were obtained in excellent yields above 90 % and used without further purification.



R = 2-naphthyl **12**
R = phenyl **13**

R = 2-naphthyl **14**
R = phenyl **15**

R = 2-naphthyl **16**
R = phenyl **17**

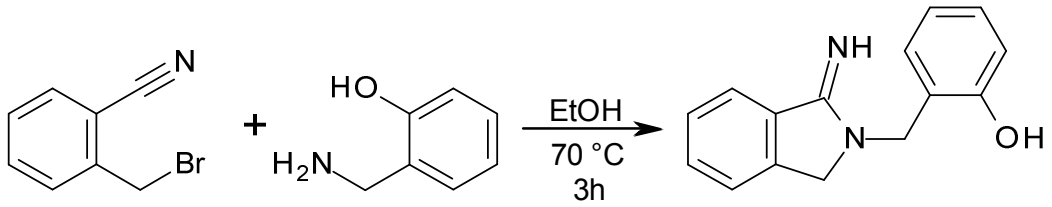
Scheme 3.6: Synthesis of precursors **16** and **17**

For synthesis of the compounds **16** and **17** method A using NEt_3 as described in chapter 3.1.2.1 was applied (cf. Scheme 3.6). Surprisingly no product could be obtained with both compounds following these instructions. Significantly, no precipitate formed when the imidoester was stirred with NEt_3. Even though it is not to be expected, one must consider whether or not the salt remaining in a solution did have a negative effect on the reaction. Since alternative reaction conditions had been given by Årstad *et al.* and had already proven successful in the synthesis of **11**, it was not tried to optimize method A and instead method B was applied. [81] Synthesis using method B proceeded without problems and gave products **16** and **17** in quantitative yields. All reagents were stirred under argon at room temperature in until

TLC confirmed completion of the reaction, whereupon the reaction was quenched and the solvent was evaporated *in vacuo*.

The raw product was crystallized from methanol at least twice, if it was supposed to be used as labeling precursor, in order to ensure highest purity. While minor contaminations of by-products usually do not impact organic reactions on macroscopic scale, even the smallest amounts of other compounds can cause the formation of by products during radiolabeling reactions. Therefore purification by crystallization from a suitable solvent or *flash*-column chromatography, if crystallization is not possible, is normally applied. After crystallization the naphthyl precursor **16** was obtained in high purity (> 99 % by HPLC) but in yields of about 43 %, while the phenyl precursor **17** was obtained in yields of approximately 25 % in similar high purity (> 99 % by HPLC). The loss in yield is high and thus should be avoided if the product is used in any other reaction than a labeling reaction.

3.1.2.3 Synthesis of *N*-(2-hydroxybenzyl)-isoindoline-1-imine (19)



18 **10** **19**

Scheme 3.7: Synthesis of *N*-(2-hydroxyethoxybenzyl)-isoindoline-1-imine (**19**)

The synthesis of the cyclic amidine precursor **19** was performed as described by Nguyen *et al.* (cf. Scheme 3.7). [82]

2-Hydroxybenzylamine (**10**) was dissolved in ethanol and equally dissolved 2-(bromomethyl)-benzonitrile (**18**) was added to the solution which was then heated to reflux. Control with TLC showed a good conversion rate and after 3 hours the reaction was stopped by removal of solvent and the solid residue purified by crystallization. The product was obtained as light yellow needles in very good yields (75 %) and high purity (> 98 % by HPLC).

3.1.2.4 Synthesis of *N*-(2-toslyoxyethoxybenzyl)cinnamamidine (27)

The labeling precursor **27** for a direct labeling reaction by substitution with [^{18}F]fluoride had to be synthesized in 4 steps starting from cinnamylimidoester **7** and salicylaldehyd **24** (see Scheme 3.8). While generally reaction conditions for production of amidines from imidoesters need to be water-free, a method was used that tolerates small amounts of water and will give the amidines in good yield. [94, 95]

The imidoester was transferred into the amidine by stirring with triethylamine and ammonium chloride in ethanol. The reaction proceeded as planned and gave the desired product **23**, which was obtained as colorless needles in low yields (17 %).

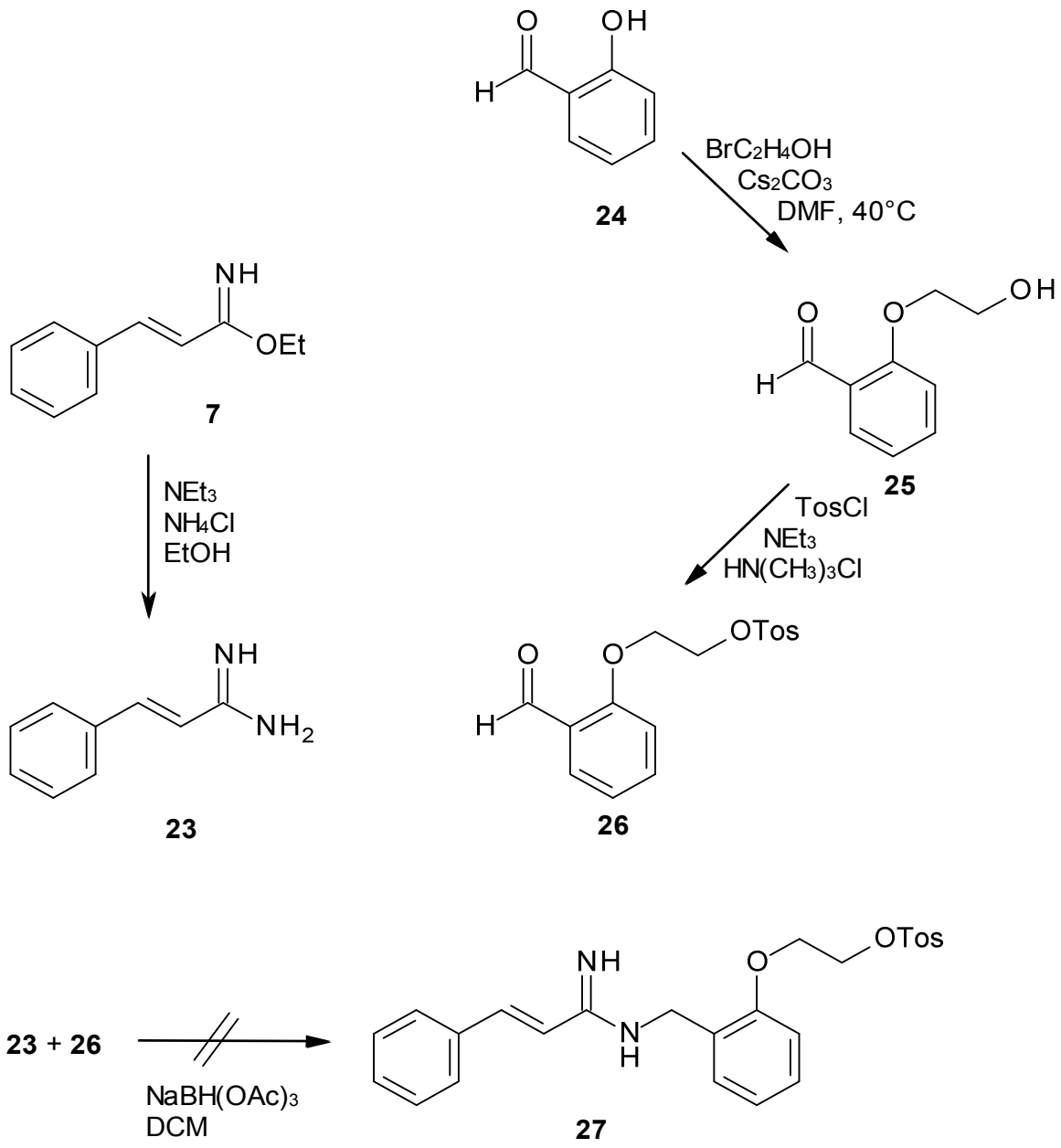
The tosylated salicylaldehyd **26** was synthesized similarly to the fluoroethoxy compound by first coupling 2-bromoethanol with the salicylaldehyd **24** in DMF using cesium carbonate as base. After column chromatography the product **25** was obtained in quantitative yields as brown oil.

Tosylation of the alcohol was performed in a quickly proceeding reaction with tosyl chloride, trimethylammonium chloride and triethylamine in dichloromethane (DCM). After 1 hour of reaction time the control TLC showed no remaining reagent. The tosylated compound **26** was obtained as light yellowish to brownish crystals in good yield (75 %).

Before synthesis of the final amidine compound **27** it was examined if a coupling by reductive amination would be possible, and if so, which conditions would be mild enough to allow the use of a tosylated compound. A publication from 1901 showed that the reaction of amidines to Schiff bases is generally possible under mild conditions. [96] The reaction was subsequently planned following these conditions but no product was found even after prolonged reaction times. As an alternative the reduction of the Schiff base to the desired product **27** was conducted according to methods given by Abdel-Magit *et al.*. [97]

Sodium triacetoxyborohydride, a very mild reducing agent, was utilized, which is easily employable, since the substance is not sensitive to air and moisture.

Synthesis of *N*-(2-tosyloxyethoxy)cinnamamidine (**27**) was attempted by coupling of the amidine **23** and the aldehyde **26** in DCM under mild heating in the presence of sodium triacetoxyborohydride in order to reduce the produced imine. The reaction time was 7 days and a product was obtained as a colorless solid. Mass spectrometry proved, however that the product was not the desired precursor.



Scheme 3.8: Method of synthesis of the precursor **27** for a direct labeling reaction

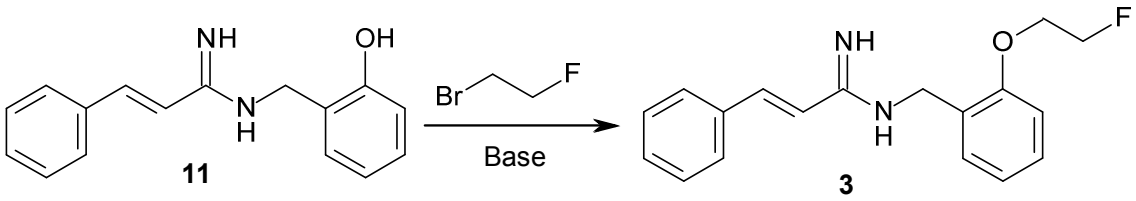
Therefore, it was tried to couple the amidine and aldehyd as described by Kunckell *et al.*. [96] When after a day of reaction time no conversion was noticed, equilibrium of the reaction was shifted by adding 4Å molsieve and the reaction was stirred for another 8 hours. Again no reaction product could be found by TLC and one equivalent of formic acid was added to catalyze the reaction. After another 16 hours the reaction was stopped since no product could be found. It was concluded that **27** cannot be produced in the way described in Scheme 3.8. This is different from the findings of Kunckell *et al.*, who reported a fast reaction between benzylamidine and salicylaldehyde. [96]

3.1.3 Synthesis of the authentic fluoro-compounds

It is necessary to synthesize the target molecules **3** - **6** containing a stable fluorine atom. Besides employing those as standards for establishing a chromatographic system, they sometimes are also used as carrier. This is of great importance since molecules under n.c.a. conditions often show different characteristics than expected and adding carrier to the solution containing the labeled molecule may help verifying the identity of the product. The authentic standard compound is further used for biological evaluations in order to establish systems for further *in vitro* and *in vivo* studies.

While all the precursor molecules could be prepared by methods described in the literature, no paper has been published to date containing molecules of this type that are fluoroethyl-substituted at the hydroxyl group. Therefore standard reaction conditions were applied that had succeeded at the introduction of the desired functional group in other molecules containing an arylic hydroxyl group.

3.1.3.1 Direct methods



Scheme 3.9: General method of synthesis of 2-fluorethoxybenzylcinnamamidine **3**

Generally, reactions of functionalized alkanes with arylic hydroxyl functions are simple methods of introducing a wide range of functional groups. Often the presence of a hydroxyl and an amine function leads to disubstituted products. In this molecule it was expected that the amidine group would slightly deactivate the amine and the higher acidity of the hydroxyl group would lead to a single product. Therefore standard reaction conditions for the introduction of 1-bromo-2-fluoroethane (BFE) into an aromatic molecule were chosen (cf. Scheme 3.9). While following this approach, a range of different reaction conditions was tested, which are listed in Table 3.1.

A reaction at room temperature using potassium carbonate as base and acetone as solvent was employed (cf. Table 3.1, entry 1). These conditions are generally considered favorable, since normally no or very few by-products are observed and

the carbonate can be removed by filtering after the reaction is completed, thus simplifying workup considerably. The reaction was monitored by TLC and when after 24 hours no product could be found in the reaction mixture, the temperature was increased and additional equivalent of 1-bromo-2-fluoroethane was added. After 2 days of stirring under reflux no reagent was left as confirmed by TLC and the reaction was stopped. After removing the solvent the obtained solid was analyzed with mass spectroscopy, but the desired product could not be found.

Table 3.1: Reaction conditions for the synthesis of 2-hydroxybenzylcinnamamidine

entry	Base	Additions	Solvent	Temperature	Yield
1	K_2CO_3	-	Acetone	25 °C	-
2	K_2CO_3	NaI	Acetone	25 °C	-
3	K_2CO_3	-	Acetone	60 °C	-
4	K_2CO_3	NaI	Acetone	60 °C	~ 10%
5	NEt_3	-	DMF	100 °C	-
6	NaOMe	-	Methanol	70 °C	-
7	NaOMe	NaI	DMF	70 °C	Traces
8	NaH	-	THF	70 °C	-
9	NaH	-	THF	0 °C- 70 °C	-
10	NaH	-	DMF	0 °C – 70 °C	Traces
11	NaH	-	MeCN	85 °C	-
12	KOH	-	DMSO	25 °C	-
13	KOH	-	EtOH	100 °C	50 %[a]
14	Na_2CO_3	NaI	4-Methyl-2-pentanone	100 °C	-
15	K_2CO_3	NaI	4-Methyl-2-pentanone	100 °C	-
16	Cs_2CO_3	NBu_4Br	MeCN	25 °C	-
17	K_2CO_3	-	DMF	120 °C	30 %[b]
18	K_2CO_3	KI	DMF	120 °C	70 %[b]

[a] Product could not be separated since decomposition occurred during purification

[b] Product contained both the *O*- and the *N*-alkyl compound, as shown by NMR. These isomers could not be separated

In a second attempt (entry 2) a small amount of sodium iodide (NaI) was added to the solution to enable the in-situ formation of 1-iodo-2-fluoroethane, which is a better reactant. [98] While reaction at room temperature again gave no product, at 60 °C about 10% of product could be found in the solution as confirmed by mass spectroscopy (entry 4).

Since higher temperatures were expected to lead to both the *N*-alkylated and the *O*-alkylated product, it was decided to try reaction conditions at moderate temperatures using stronger bases. A stronger base should make deprotonation of the phenole more likely, which would reduce the possibility of generation of two products. It was expected that under the mild conditions the possible by-product, if at all, would only be produced in small amounts and that a separation of the two products would be possible, if necessary.

An alternative to the use of carbonate as base was the comparably mild base triethylamine, which was utilized in DMF at 100 °C (entry 5). When control with TLC showed no unreacted compounds in the reaction solution after 24 hours the reaction was stopped and the product extracted with DCM, but only no product was found.

As stronger base sodium methanolate was used directly with the BFE present in the solution. It was also tried to produce the sodium salt of the amidine first and add BFE after change of the solvent (entry 6 and 7). The reaction with the amidine in methanol at 70 °C did not give any product and while the sodium salt seemed to form readily, a reaction in DMF at 70 °C in presence of NaI did not give more than traces of product as determined by mass spectroscopy.

Since the use of weak and medium bases seemed unsuccessful, the strong base sodium hydride was then utilized with THF, MeCN or DMF as solvent and at temperatures between 0 °C and 70 °C (entries 8 to 11). Reactions in THF and MeCN did not prove successful and a reaction in DMF gave only traces of the desired product. The starting compound could also not be found in the solution, and since mass spectroscopy did show a number of by-products with masses lower than that of the amidine, it was concluded that decomposition of the compound had taken place.

In order to avoid decomposition a weaker base had obviously to be applied and potassium hydroxide was chosen (entry 12 and 13). As solvent DMSO and ethanol were employed and while the simple and direct reaction of the amidine in DMSO with NaI and BFE did not yield any product, formation of the potassium salt of the amidine and subsequent reaction in ethanol showed success, as about 50% product was found in the reaction solution (analyzed by mass spectroscopy). The product decomposed during the aqueous workup and could not be isolated, which pointed towards this method of reaction also not being favorably.

Up to this point only the use of potassium carbonate and potassium hydroxide had given good results, which lead back to the use of carbonates as bases (entries 14 to

18). As an alternative to potassium carbonate, sodium and cesium carbonate were applied. Since reactions in acetone gave no product even after days of reaction, an alternative solvent that could be used at higher temperatures was employed. Both potassium and sodium carbonate were used with 4-methyl-2-pentanon at 100 °C, but again no product could be found. To be able to further rise the temperature DMF was used as a solvent at 120 °C. Under these conditions the reaction gave 30 % of the fluoroalkylated product which was cleaned by column chromatography and analyzed with mass spectroscopy and NMR (entry 17).

The product still contained by-products that could not be removed with normal chromatographic methods. In an attempt to lower the rate of by-products reaction conditions were slightly altered by adding sodium iodide to the reaction, which gave very good yields (entry 18). The product was first cleaned by *flash*-chromatography and when this proved insufficient, by semi-preparative HPLC. The product was obtained in very small yields and analyzed using mass spectroscopy and NMR. While mass showed a clean product the ^{19}F-NMR showed small residues of another fluorine compound that could not be removed chromatographically. Since HPLC was > 99 % pure, it seems reasonable to conclude that the other compound found is the *N*-fluoroethyl isomer. This pointed towards both isomers having very similar properties, thus making chromatographic purification very difficult. The direct approach showed little prospect of success and no further experiments were done.

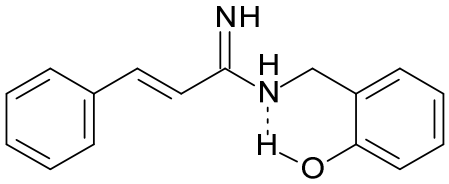
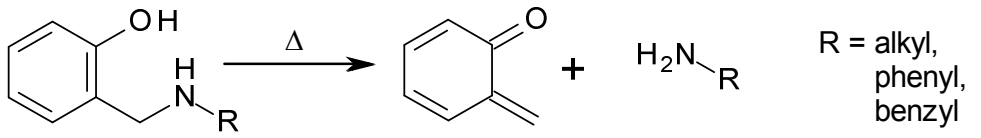


Figure 3.3: Potential deactivation of the hydroxyl group through formation of a hydrogen bond

Generally reaction yields suggest that the hydroxyl group is not very reactive when positioned in ortho-position to the benzylamine. Reactions that were undertaken using the amine as a reagent showed low yields even under reaction conditions that are known to lead to good or very good results with other compounds. Normally the addition of a fluoroalkane to a phenole is a simple reaction that has been applied for many different reagents, and especially in radiochemistry is a standard reaction.

A possible reason for the reaction problems may be the formation of hydrogen bonds between the amine and the hydroxyl group, leading to formation of a stable ring, which could cause a deactivation of the oxygen atom (see Figure 3.3). At the same time it would normally be expected that the reaction takes place without hindrance, since deprotonation of the oxygen should be even facilitated.

With all reactions using temperatures above 60 °C a high fraction of by-products could be found, but most of the time they were the only compounds produced. It is known that when heated 2-hydroxybenzylamine compounds decompose under formation of ortho-quinone methide (cf. Scheme 3.10).[99] The use of hydroxybenzylamines as protection groups for amines has been suggested and been successfully applied in literature.[100] However, temperatures at which such decompositions normally occur are at about 200 °C. Therefore decomposition was not expected to take place in the course of this work, but given the unfavorable results of several reactions, the possibility has to be considered. The amount of by-products found in the reactions was generally high and it could be discussed whether some kind of decomposition may have taken place.



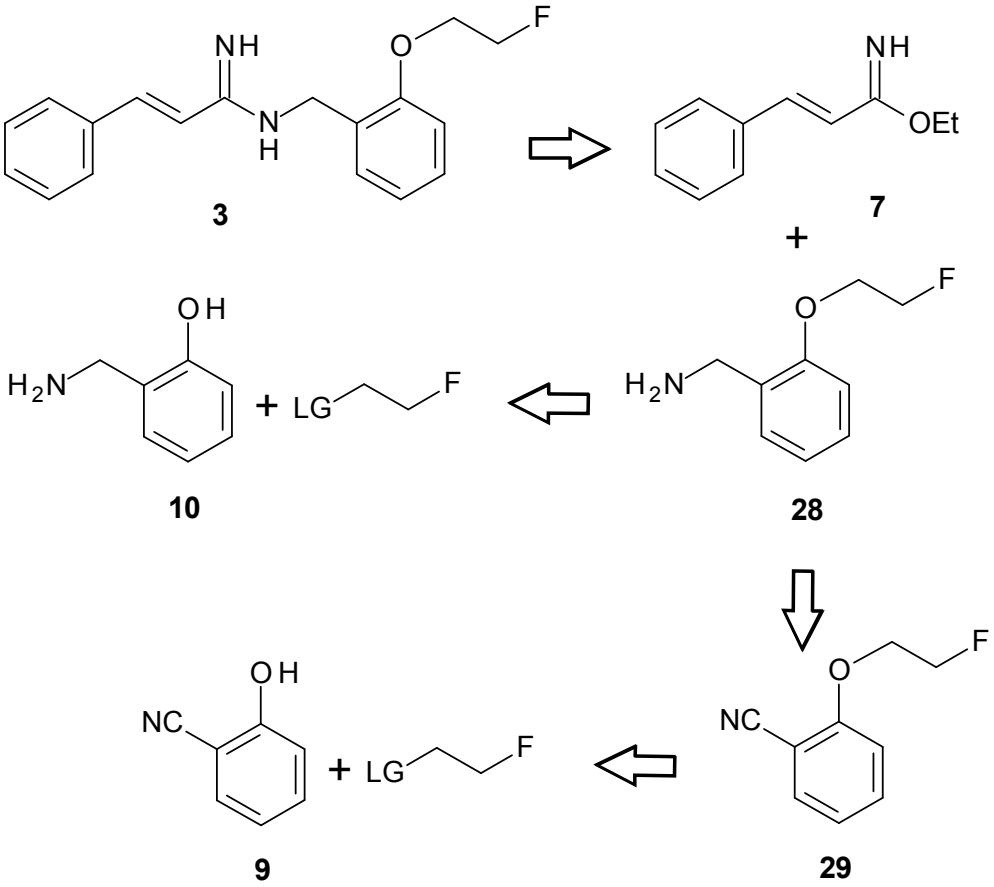
Scheme 3.10: Thermal decomposition of 2-hydroxybenzylamines

Since ortho-quinone methide is a very reactive compound it would react with other molecules present in the reaction solution almost instantly. This would account for the wide range of different by-products found under strongly basic conditions. Analysis of the by-products with mass spectroscopy, however, did not point towards this, since the masses found could not be allotted to potential multimers of ortho-quinone methide.

3.1.3.2 Indirect methods

An alternative to the reaction of 1-bromo-2-fluoroethane with 2-hydroxybenzyl cinnamamidine was the protection of the amidine. Attempts were made to protect the amidine with a *N-tert*-butoxycarbonyl (Boc) group. Introduction of this protecting group into the molecule by reacting Boc-anhydride in triethylamine and dimethylformamide with the precursor molecule was not successful and since the

protection of amidines, even though described in literature, is difficult, it was decided to avoid the introduction of a protective group.



Scheme 3.11: Possible retrosyntheses of 2-fluorethoxybenzylcinnamamidine **3** (LG = leaving group)

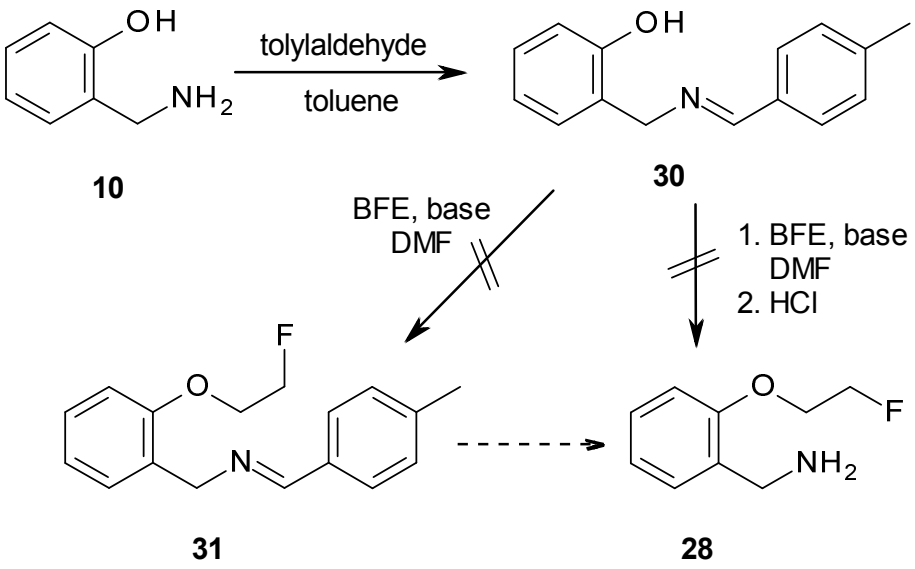
Attempts to introduce BFE directly into the cinnamamidine **11** had not given the expected results and a different approach had to be taken. Retrosynthesis indicated that an introduction of the fluoroethyl group might be done directly on the 2-hydroxybenzylamine (**10**) (see Scheme 3.11). A possible method of synthesis was reaction of BFE with the 2-hydroxybenzylamine **10** followed by coupling to the imidoester **7**. An alternative to that approach would be the alkylation directly at the benzonitrile **9** and following reduction to the amine. The conditions under which reduction of the nitrile to the amine takes place could lead to partial decomposition of the product and made a reaction at the amine **10** more promising. This was subsequently planned.

Upon first attempts under simple reaction conditions with potassium carbonate (acetone at RT) did not give any product, more rigorous reaction conditions using

refluxing DMF and different bases (carbonates, methanolates, sodium hydride) were applied, however even under those conditions the unprotected compound **10** gave no product at all.

Since previous experience had shown that the formation of both possible alkylation products might be expected under harsh reaction conditions, the better alternative to use of stronger bases was a protection of the amine. The protected product would give only a single product since the nitrogen would not be available for reaction. At the same time it was estimated that functionalization of the amine would reduce the strength of the presumed hydrogen bond in the molecule enough to enable a reaction easily to take place (cf. 3.1.3.1 for further discussion).

The simplest way to protect the amine **10** was the transformation into a Schiff base (see Scheme 3.12). The reduced basicity of the imine should inhibit the formation of an intramolecular hydrogen bond and therefore enable better reaction conditions for the alkylation.



Scheme 3.12: Planned methods of synthesis of 2-fluorethoxybenzylamin (**28**)

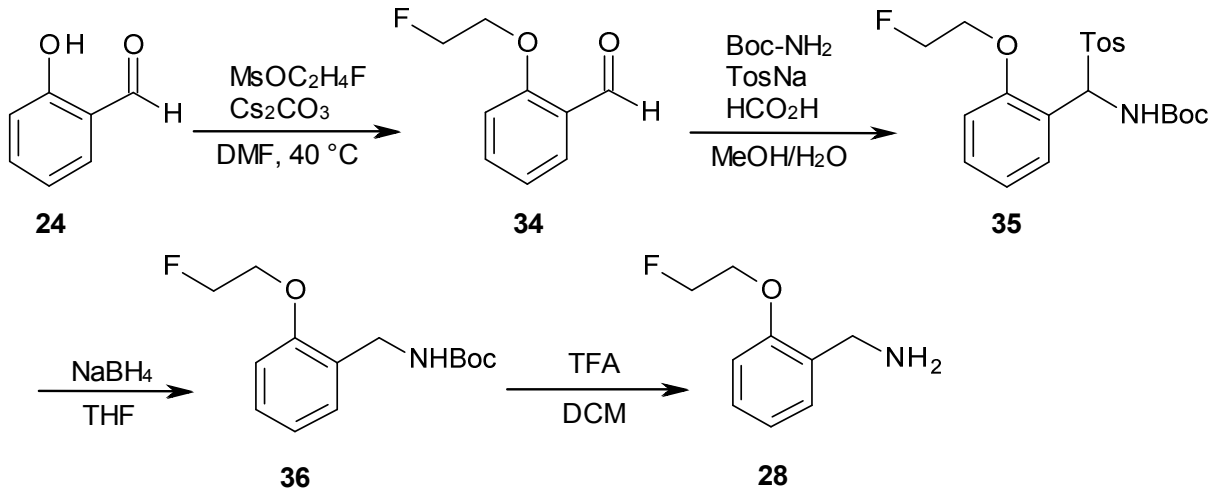
The imine **30** was produced by reacting 2-hydroxybenzylamine **10** with p-tolyl aldehyde in toluene. The desired product was readily obtained in good yields (43 % to 77 %) after 3 hours of reaction time.

The plan was to introduce the fluoroalkyl group into compound **30** and then to deprotect the amine **31** without intermediate purification to give **28**. For this reaction the imine **30** was reacted in DMF with potassium or caesium carbonate as base.

After the reaction the product was extracted, the solvent evaporated and the residue dissolved in ethyl acetate. After deprotection with HCl, no product could be found.

Since the reaction did not proceed as expected, the reaction pathway was changed attempting to separate and analyze the alkylated product before further reactions. However, it was found, that the desired fluoroalkylated imine **31** was not formed as expected. Reaction conditions were varied by addition of potassium or sodium iodide, but still the desired product **31** could not be found.

Formation of both the lithium and potassium salt of **30** was done, but a reaction with BFE using different carbonates in DMF did not yield any product. The fact that the imine **30** did not yield any product contributed to the above mentioned hypothesis that the formation of a hydrogen bond does deactivate the molecule, even though intramolecular formation of a true salt is not expected in this molecule. The fact that product **31** could not be found in mass spectrometry and TLC, even after reactions under harsh reaction conditions, even though no reagent was present anymore, implies that other problems than simply hindered reaction conditions occur. It is possible that the alkylated product **31** is formed during the reaction, but decomposes during workup. Different methods of obtaining the product **31** were attempted but were also unsuccessful.



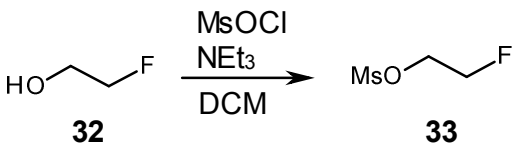
Scheme 3.13: Pathway of synthesis for 2-fluoroethoxybenzylamine **28**

Generally the reaction conditions described in Scheme 3.12 should have given the desired product **28**, as the reaction has been described on a similar 4-hydroxy compound in the literature good yields were obtained. [101]

In order to remove the potential problem of the intermolecular hydrogen bond it was decided to change the synthesis precursor and salicylaldehyde was used as starting compound (cf. Scheme 3.13). Reaction conditions were similar to those given by Bernacka *et al.*. [102]

Due to changes in regulations 1-bromo-2-fluoroethane is not available commercially anymore. An alternative was 2-fluoroethylmesylate **33**, which was synthesized from 2-fluoroethanol (**32**) and methanesulfonyl chloride in a quick and easy reaction that gave good yields of 82 % to 88 % (see Scheme 3.14).

The mesylate was then reacted with salicylaldehyde **24** and caesium carbonate at 40 °C in DMF (cf. Scheme 3.13). [103] The resulting 2-fluoroethoxybenzylaldehyde **34** was obtained in yields between 57 % and 73 %. The fact that the reaction did proceed without problems under mild conditions seems to support the theory that an intramolecular interaction takes place in the benzylamine. Only harsh reaction conditions gave comparable results for the synthesis of **3** starting from **11** (cf. Table 3.1), and no product had been found after a reaction on **10**. At the same time this hypothesis cannot be proven satisfactorily, since examples for similar problems could not be found in literature as encountered in this work.



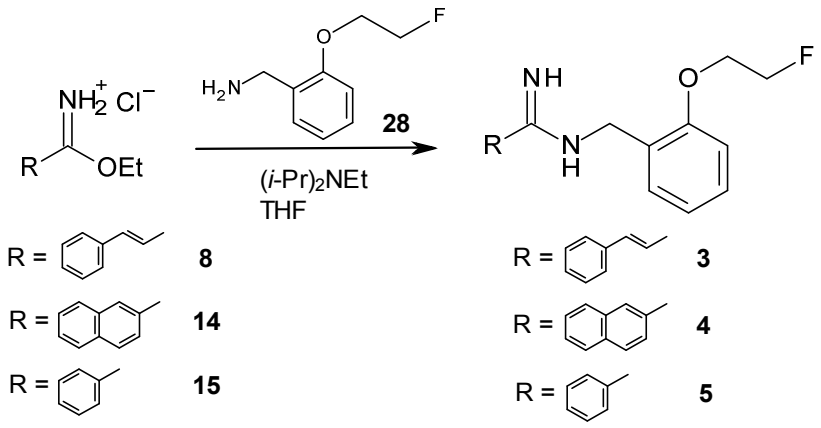
Scheme 3.14: Synthesis of 2-fluoroethylmesylate (**33**)

Product **34** was purified by *flash*-column chromatography with ethyl acetate/hexan 3/7 (v/v) as eluent and then employed in the next step.

Introduction of the nitrogen into molecule **34** was conducted by a reaction of the aldehyde with *tert*-butylcarbamate (cf. Scheme 3.13). The reaction was employed, using water and methanol as solvents, with a simple reductive amination taking place. The amination is slightly changed in comparison to normal reaction routes by adding sodium toluene to the reaction. The sulfonate prevents the formation of an imine by addition to the carbon atom in the last reaction step giving the tosylated and Boc-protected amine **35** as a light yellow to colorless solid in good yields of 54 % to 79 %. The solid precipitated during the reaction and was obtained by filtering off the solvents.

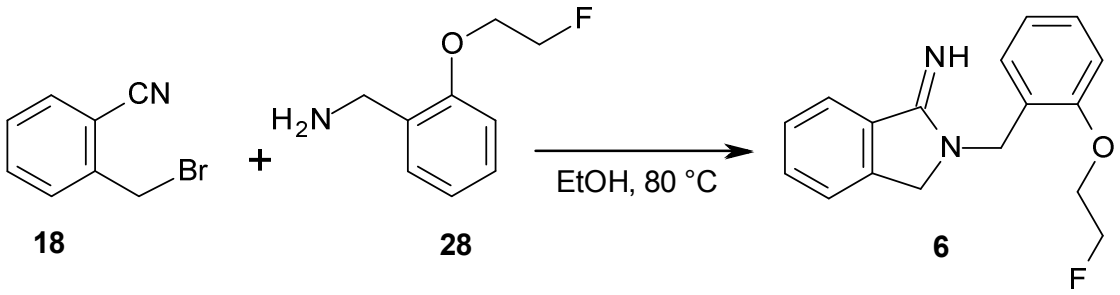
Removal of the tosyl group from **35** was achieved by a reaction with sodium borohydride in THF at room temperature. In this reaction the hydride acts both as base and reducing agent, first inducing an elimination reaction that removes the tosylate and then reducing the resulting Boc-protected imine to the Boc-protected amine. The reaction proceeded easily with good yields between 87 % and 94 % and after crystallization from ethyl acetate product **36** was obtained as colorless needles.

Deprotection of the amine **36** to yield **28** was done under standard conditions with DCM and trifluoroacetic acid (TFA) in a 1/1 mixture (v/v) at room temperature. The product **28** was purified by *flash*-column chromatography with ethyl acetate and dipropylamine (8/2, v/v). Purification was difficult since column chromatography did not remove all traces of by-products and dipropylamine from the eluent could also not be completely removed after the column chromatography. Product **28** was therefore reacted in the next step without further purification.



Scheme 3.15: Synthesis of the authentic standard compounds **3**, **4** and **5**

Coupling between the 2-fluoroethoxybenzylamine (**28**) and the respective imidoester was achieved in THF using Hünig's base (cf. Scheme 3.15). The reaction proceeded at room temperature with moderate yields. The resulting product was purified by repeated column chromatography and in the case of cinnamamidine (**3**) by crystallization from ethanol and water. The two compounds **4** and **5** obtained by this way could not be crystallized from any of the classic solvents and were thus only obtained as viscous oils.



Scheme 3.16: Synthesis of the authentic standard compound **6**

The cyclic compound **6** was obtained after reacting the benzylamine with 2-(bromomethyl)-benzonitrile (**18**) in ethanol (see Scheme 3.16). After removal of solvent and crystallization the product was obtained as a white solid.

3.2 Radiosynthesis of fluorine-18 labeled compounds

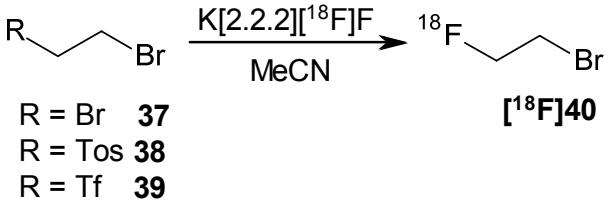
Labeling of organic molecules with fluorine can be achieved under several different conditions. Radiofluorine can be produced in both electrophilic and nucleophilic form and labeling reactions are possible directly on the precursor molecule or by synthesizing a synthon that is then reacted with the precursor (cf. chapter 1.6).

For the lead compound a labeling reaction with [^{11}C]methyl triflate has been described by Thominiaux *et al.*. [80] Since a direct labeling reaction with [^{18}F]fluoride could not be performed on the molecule due to missing activating groups, an indirect reaction pathway was planned. As shown in the introduction the utilization of a labeled synthon can be helpful with the synthesis of labeled molecules that cannot be obtained in a direct labeling reaction. In the past molecules that had previously been labeled successfully with a [^{11}C]methyl group, often have been labeled with a [^{18}F]fluoroalkane. The [^{18}F]fluoromethyl group would normally be the first choice, but its handling is difficult and a fluoromethylene group bound to a heteroatom is often not stable. The [^{18}F]fluoroethyl group is often employed instead. At the same time this group is promising, since electronic, steric and thus binding properties of the molecule will only marginally be changed compared to the [^{11}C]methyl group.

For this reaction most of the time one of the two reagents is used: either 2-[^{18}F]fluoroethyltosylate (FET) or 1-bromo-2-[^{18}F]fluoroethane (BFE). For each reagent, new methods of radiosynthesis and especially work-up have been published during the last 10 years and a lot of advancement has been made towards simpler and better reaction conditions since the first publications highlighting these new labeling synthons. [45, 46] While Block *et al.* and other groups following them used a

HPLC separation of the labeled synthon, publications during the last few years gave examples for new methods successfully using solid phase exchange cartridges (SPE cartridges), that were also applied in this work. [104, 105]

3.2.1 Production of 1-bromo-2-[^{18}F]fluoroethane



Scheme 3.17: Labeling reaction of 1,2-dibromoethane with [^{18}F]KF/Kryptofix 2.2.2

1-Bromo-2-fluoroethane (**40**) is normally produced from three precursors: 1,2-dibromoethane (**37**), 2-bromoethyl tosylate (**38**) or 2-bromoethyl triflate (**39**) (cf. Scheme 3.17). While the latter is not as widely used, it has been described in the literature for preparation of [^{18}F]BFE **[^{18}F]40**. [106] Another precursor that has been developed but not been widely introduced is 2-bromoethyl tosylate (**38**). [107] While the two sulfonic acid containing precursors promise to give better yields in the primary labeling reaction, 1,2-dibromoethane (**37**) is commercially available and easier to handle and therefore used for routine production in this work. The reaction conditions for the labeling reaction have been described in literature and were adjusted to our needs. [49, 104]

As suggested by Block *et al.* the polar aprotic acetonitrile was employed as solvent for the primary labeling reaction. [45] This solvent gives best results for almost all labeling reactions and due to its lower boiling point and high volatility compared to DMF or DMSO is generally the best choice for labeling of alkyl derivatives, since it can be removed from the reaction easily, if necessary.

Separation of the desired product from unreacted [^{18}F]fluoride was done using a LiChrolut EN® cartridge as first published by Comagic *et al.*. [49] The cartridge was preconditioned with methanol and water and the reaction mixture was slowly passed through it. The amount of reactivity fixated on the column corresponded to the amount of total product and varied largely between 25 % to 60 % radiochemical yield.

These large differences can be attributed to several possible reasons. If the cartridge was conditioned right before use yields generally were lower, while conditioning the cartridge up to 2 hours before the reaction was started showed better results.

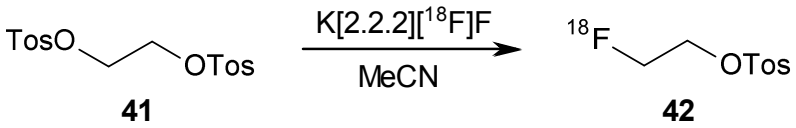
The product was eluted from the cartridge through a Waters AluminaB SPE cartridge to remove traces of water. Since the secondary labeling reaction was performed in either DMF or DMSO, the cartridge was conditioned with the respective solvent and the product eluted with 4 mL of DMF or DMSO heated to 70 °C. The amount of product obtained this way lay between 8 % and 45 % (decay corrected).

Radiochemical yields lowered significantly in 2010 though the method of synthesis was not altered. Contamination of chemicals, solvents or other materials could be ruled out. Therefore the [^{18}F]fluoride for synthesis was obtained from another cyclotron for a few days, to exclude a potential problem with the production of fluorine-18 at the Baby cyclotron. Surprisingly considerably better radiochemical yields were gained using the different fluoride. After verification of problems by other group members production at the Baby cyclotron was changed and radiochemical yields rose.

After elution from the SPE cartridge the product was obtained radiochemically clean and no radioactive by-products were found as tested by HPLC. The radioactive product showed to peaks in HPLC that corresponded to the authentic standard for BFE, which also showed two peaks. This is probably due to the low pH of the HPLC eluent. The labeled [^{18}F]BFE **[^{18}F]40** was used without further purification in the final labeling reaction.

The complete reaction time was between 16 to 19 minutes from the moment of addition of the dibromoethane (**37**) to the fluoride complex until finishing the measurement of radioactivity in the collected fractions. Since fast reaction times are an absolute necessity for good yields with high specific activity, the use of SPE in comparison to the more time consuming HPLC separation proved crucial. With this way of synthesis the next step in the labeling reaction could be started within 20 minutes from the start of the primary labeling reaction.

3.2.2 Production of 2-[^{18}F]fluoroethyl tosylate



41 **42**

Scheme 3.18: Labeling reaction on ethylenglycole-1,2-ditosylate (**41**) with [^{18}F]KF/Kryptofix 2.2.2

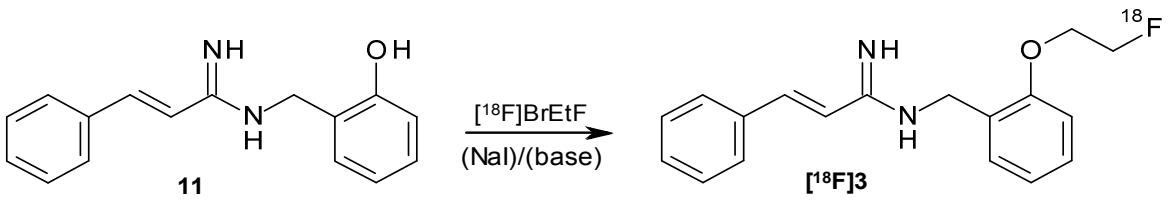
An alternative to dihalogenethane as precursor is ethylenglycole-1,2-ditosylate (**41**), which has been researched during the last 20 years. Just like 1,2-dibromoethane both ends of the alkane are functionalized. Since concentration of [^{18}F]fluoride in the solution is very small compared to the concentration of the precursor, it is almost statistically impossible that a molecule disubstituted with fluorine-18 would be formed. As shown by Block *et al.* the labeling of the 1,2-bis(tosyloxy)ethane **41** with [^{18}F]fluoride proceeds with better yields than that of the respective halogen precursors and gives the product 2-[^{18}F]fluoroethyl tosylate (FET, **42**) with a yield of about 82 %. [46]

While in the late 1980s, with the beginning research on [^{18}F]fluoroalkane prosthetic groups, the labeling precursor **41** had to be synthesized, it is now commercially available and can be used without further purification. However reaction times for the labeling reaction are longer than with 1,2-dibromoethane and until recently the product had to be separated from the precursor by HPLC separation, which caused longer times of synthesis. In 2007 a solid phase extraction of 2-[^{18}F]fluoroethyl tosylate was published by Honer *et al.*. [105] This method utilizes a tC18 cartridge and an AluminaN cartridge to separate the product from by-products and the labeling precursor.

The labeling reaction (see Scheme 3.18) proceeds similar to the synthesis of 1-bromo-2-[^{18}F]fluoroethane, acetonitrile acts a solvent and the dissolved precursor is added to the dried [^{18}F]fluoride. After the reaction the solution is dissolved with water and the product fixated on a Waters tC18 cartridge that is then rinsed with water. About 70 % of total radioactivity was found on the cartridge and could be eluted with tempered acetonitrile or DMF through an AluminaN cartridge. Radiochemical yield (RCY) was a bit higher than with the halogen precursor and lay at about 39 % ± 9 % (n = 2).

Reaction time from start of synthesis to measurement of radioactivity in the collected fractions was 25 minutes, which is significantly higher than for the synthesis with the dibromo precursor as described in chapter 3.2.1.

3.2.3 Synthesis of *N*-(2-[^{18}F]fluoroethoxybenzyl)-cinnamamidine



Scheme 3.19: Labeling reaction as planned for the cinnamamidine compound **[^{18}F]3**

Introduction of the prosthetic group into the secondary precursor was planned following examples in several publications. [45, 49, 104] As shown by many groups, phenol can be labeled with BFE or FET in good yields. [45] Since nitrogen is also a potential site for labeling, depending on its basicity, it was considered if protection of the amidine group was necessary. After trials to protect the group were unsuccessful (cf chapter 3.1.2.2), labeling was tried without protection of the amidine, based on the consideration that the phenolic hydrogen is more acidic then the amidine hydrogen atoms and the oxygen will be deprotonated more easily than the amidine when a base is present. The pk_a of the phenolic group in 2-hydroxybenzylamine is roughly 8, while that of different amidines ranges between 12 and 30, with substituted amidines generally having a higher pK_a due to their mesomeric stabilization. [108, 109]

As first implemented for radiolabeling reactions by Zhang *et al.*, sodium iodide was added to the reaction mixture in order to enable the *in situ* formation of 1-iodo-2-[^{18}F]fluoroethane that will be a better reactant than the bromo compound. [98] In a publication from 2003 Bauman *et al.* have published their results of a study on different iodides and reaction conditions. [104] Best results were found with lithium iodide followed closely by sodium iodide, while using MeCN, DMF and DMSO as solvents. It was therefore decided to use NaI of high purity and DMF as solvent for first experiments, which had worked well for macroscopic reactions and had given good results for the Baumann group (cf. Scheme 3.19).

Synthesis using NaI in DMF at 80 °C showed a radioactive product in HPLC that was about 50 % of total activity. This was a surprise, since without the addition of a base

no formation of product was expected. It seemed possible that due to high basicity of the amidine the phenol was deprotonated even without addition of base. TLC (DCM/MeOH 9/1 + 0.1 % NEt_3) showed a product, though with lower results. The product could not be clearly identified, since R_f-values were very close to those of BFE.

Improved conditions for TLC were developed and utilized for the radio TLC (ethyl acetate/hexane/diethylamine 8/1/1, v/v/v). It proved that for the fluoroalkylated cinnamamidine a sufficient separation of precursor and standard was only possible by using very alkaline eluent conditions, which made it necessary to add a large amount of amine to the eluent.

Using the new TLC conditions and performing radioactive synthesis again, it was expected to find a product with an R_f value identical to that of the authentic standard. Again HPLC showed 40 % to 50 % (decay corrected) of a product with retention times identical to those of the authentic standard compound. But TLC did not give a product that with a R_f value similar to that of the standard compound, showing only unreacted BFE (R_f 0.6 to 0.7) and a product that had a R_f of 0. It was assumed that this spot resulted from an ion that had formed during the reaction or due to the very basic eluent conditions. Different methods were used to free the ion and get a better R_f value.

For monitoring reaction conditions aliquots of 20 µL were taken after 2, 4, 6, 9, 12, 15, 20 and 30 minutes reaction time, added to an Eppendorf vial containing 30 µL of DCM. In order to determine if a variation of dissolving method would have an impact, various solvents were tested. Acetonitrile, methanol, chloroform and acetone were used as solvents but did not show any change in comparison to DCM.

Another option was to add acid to the aliquot, for which tests were performed with trifluoro acetic acid, concentrated hydrochloric acid and glacial acetic acid. A small amount of acid was added to the solvent and after addition of the sample from the reaction briefly shaken to mix all phases and then utilized for TLC. TLC still showed one radioactive spot that could be identified as BFE and another that showed a R_f value of 0.

As an alternative to Si-60 plates for TLC, others coated with RP-18 material (MeCN/H_2O 35/65 + 0.1 % TFA) or aluminium oxide (AlOx, EE/Hex/DEA 8/1/1) were tested. With these conditions a spot remained at the starting point and only [^{18}F]BFE could be identified, similar to the previous tests. To verify if the product that remained

at the starting point was the desired target compound, some milligrams of the authentic tracer were dissolved in DCM, 10 µL of that solution was added to the aliquot and a TLC was developed. Even though the authentic standard showed good retention with an R_f value of about 0.25 the radioactive product remained at the starting point. It could therefore be determined that this product was not the desired compound.

At the same time HPLC analysis still showed a radioactive product with high yields that was identical to the standard compound in HPLC. Strikingly, a substantial non-radioactive peak could be seen in the UV spectrum of the HPLC diagram, even though no carrier had been added. Inquiries about possible low specific activities after production showed that during 2010 for the whole time frame during which radioactive labeling had been performed, specific activity had been worse than before and the [^{18}F]fluoride produced had not been as low on carrier as before.

This had no impact on routine production, but greatly impacted smaller scale work as done in the frame of the thesis. While production of [^{18}F]BFE gave results that were clearly inferior to those found in the past, reactions also showed a wider range of product with results varying greatly between each day. To prove that the problems encountered with labeling were connected to the [^{18}F]fluoride produced at the Baby cyclotron, [^{18}F]fluoride was obtained from a GE PET-Trace cyclotron. Tests with this product showed the expected outcome with no radioactive spots but BFE on the plates. Therefore it can be assumed that the product that was found in previous measurements was a contamination of the produced [^{18}F]fluoride from the Baby cyclotron.

Correspondingly the radiochemical yields of the production of [^{18}F]BFE rose significantly (cf. chapter 3.2.1). The method of production of [^{18}F]fluoride at the Baby cyclotron was subsequently changed and after that change no product could be found anymore at the starting point on TLC plates. This proved that the radioactive product found so far was not the desired product but a contamination, at the same time neither the desired product nor any other radioactive product that would account for the peak found in HPLC could be found in TLC measurements.

It was concluded that the desired product *N*-(2-[^{18}F]fluoroethoxybenzyl)-cinnamamidine **[^{18}F]3** was not produced at all. Since strikingly HPLC showed a product that had the same retention times as the authentic sample, it was necessary to conclusively prove or disprove that this product was the desired compound.

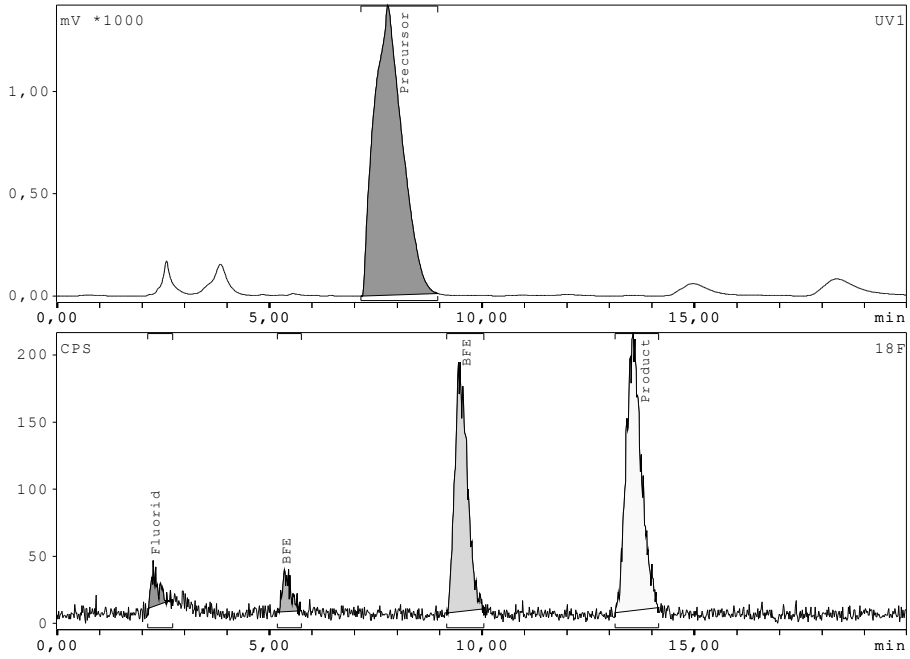


Figure 3.4: HPLC diagram of a radiolabeling with [^{18}F]BFE
(Kromasil C-18, MeCN/H_2O 35/65 + 0.1%TFA, 1 mL/min, 285 nm)

HPLC conditions until this point were a Kromasil C-18 column, with MeCN/H_2O 35/65 + 0.1 % TFA as eluent mixture at 1 mL/min flow rate at 285 nm UV detection. For this system a radioactive product with a retention time of 13.57 min was found, while times of retention for [^{18}F]BFE (see Figure 3.4) were 5.37 min and 9.47 min. Those peaks are found both with inactive BFE and freshly labeled [^{18}F]BFE and can definitely be assigned to this compound. An explanation for the occurrence of two peaks could be an elimination reaction on the alkane removing HBr from the molecule leaving fluoroethene, though this cannot be proven since the authentic standard compound was not available.

A co-injection of the separated radioactive product and the authentic standard was performed in order to verify the identity of a peak which, at approximately 13 minutes, was surmised to be the desired product.

For this purpose a product fraction of 200 µL was injected directly from the reaction into HPLC and the desired product peak was cut after analysis. The collected fraction was mixed with 50 µL of a solution of the authentic standard in acetonitrile (1 mg/mL) and 200 µL of this solution was injected into the HPLC. After measurement 20 µL of the standard solution were injected and measured. It could be proven that the difference between retention times of the sole standard and that of the sole radioactive product were the same as when co-injected into HPLC (see Figure 3.5).

At the same time the standard compound showed retention times lower than the labeled compound, which is impossible, since the UV detector is situated before the radioactivity detector and the UV peak must have a lower retention time than the radioactive peak. Since the product found in HPLC could not be the desired compound, but retention times of the standard and the by-product were nearly the same, the HPLC system was deemed unsuitable for further analysis.

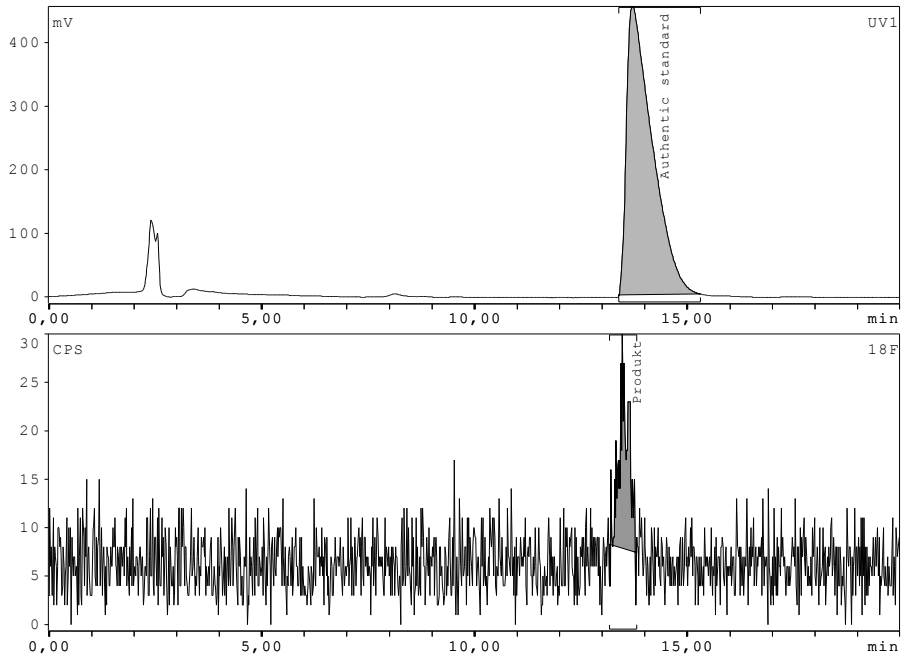


Figure 3.5: HPLC diagram of a co-injection of authentic sample and separated active product (Kromasil C-18, MeCN/H_2O 35/65 + 0.1%TFA, 1 mL/min, 285 nm)

To unravel the conflicting results from HPLC and TLC an alternative HPLC system was established. As an alternative to a Kromasil C-18 column a Phenomenex Luna PFP was chosen. Eluent conditions used for analysis were identical to those for the Kromasil system (MeCN/H_2O 35/65 + 0.1%TFA, 1 mL/min, 285 nm) and retention times of the radioactive compounds were comparable. Like the Kromasil system the Luna PFP system showed three radioactive peaks that could be assigned to BFE and to one unknown product. In order to identify the unknown product retention times for the authentic standard were determined. For measurements where the standard was injected into HPLC alone it gave retention times that were close to those of the radioactive compound. To confirm this finding, a co-injection was performed and as described earlier a mixture of both the authentic sample and the radioactive product was injected into HPLC. Both substances showed distinctly different retention times,

with the authentic standard having a higher retention time than the radioactive product. Thus it could be proven that the radioactive product found was not the desired compound (cf. Figure 3.6).

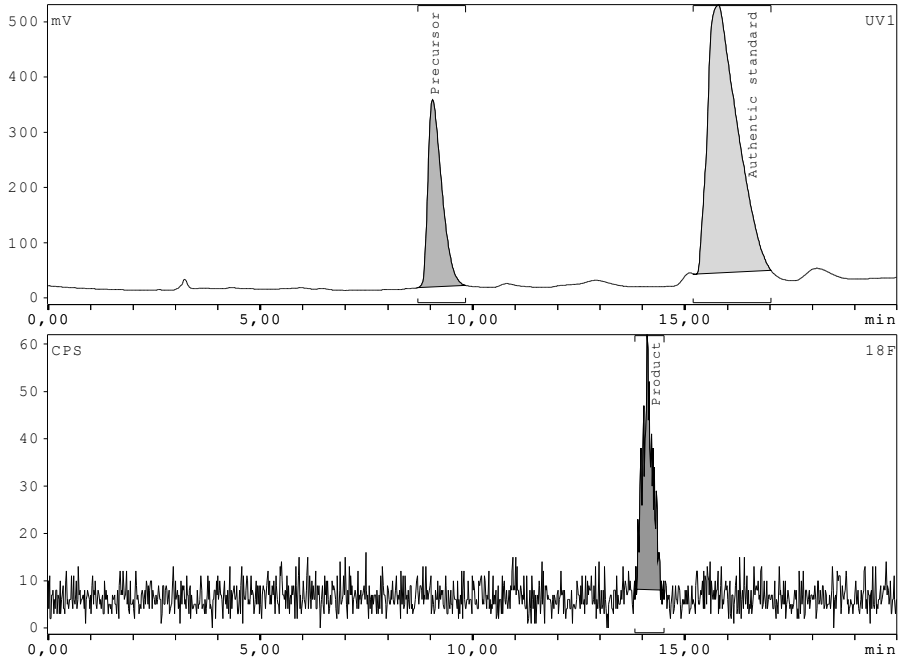


Figure 3.6: Co-injection of the separated product and the authentic standard on a Luna PFP column
(Luna PFP, MeCN/H_2O 35/65 + 0.1%TFA, 1 mL/min, 285 nm)

A reaction that would account for the product found and give similar yields would be the formation of the *N*-alkylated product. In order to confirm formation of this compound in the labeling reaction, a synthesis of the *N*-fluoroethyl compound in a direct reaction, which should give good yields, if the reaction is favored against the O-alkylation, was attempted. At this point the available supply of BFE had been exhausted and fluoroethylmesylate was utilized instead. Due to the findings in radioactive analysis and the theory that the N-alkylated product was formed, it was expected that a reaction would occur primarily at the amine nitrogen atom under these reaction conditions.

The reaction was conducted in DMF at 120 °C under addition of caesium carbonate and sodium iodide. It was stopped by the addition of water, once TLC showed no remaining reagent in the reaction solution. Analysis of the purified product was a surprise, since instead of the expected compound only the authentic *O*-alkylated standard compound was obtained in good yields. No *N*-alkylated product was found as proven by 2D-NMR analysis that showed the *O*-substituted amidine. This was

surprising since all trials at introducing the fluoroethoxy function were unsuccessful during organic synthesis of the authentic standard compounds which had led to the conclusion that the direct introduction was not possible.

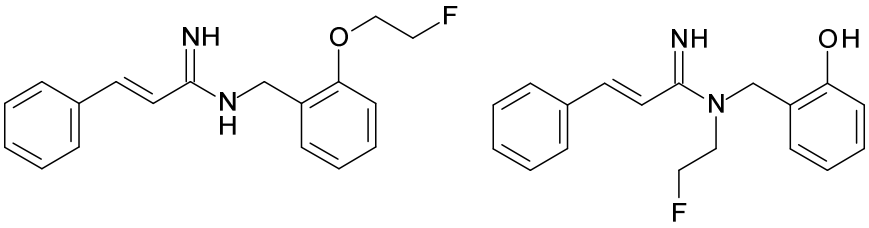


Figure 3.7: Structure of the *O*-alkylated (left) and the *N*-alkylated (right) product

This finding suggests that the phenol is the only target of an alkylation reaction and hints towards this compound also being the product in a possible labeling reaction. Since the product actually formed in the n.c.a. radiolabeling is not the desired compound, it must be concluded that the product formed in the labeling reaction is neither the O-alkylated nor the N-alkylated compound but an unknown by-product.

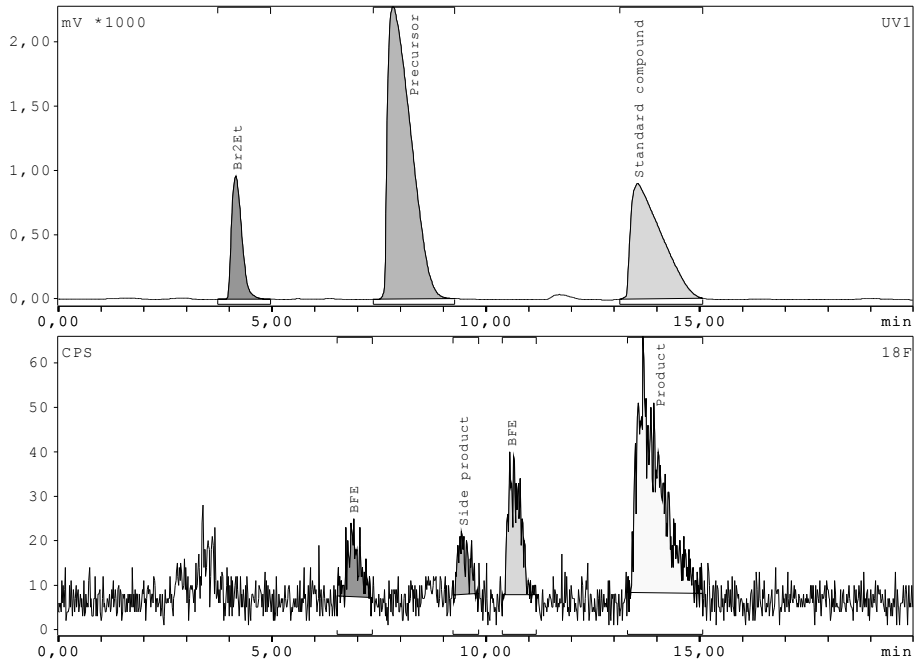


Figure 3.8: Co-injection of the radioactive product and the authentic standard of the labeling reaction in DMF at 80 °C using NaOH as base
(Luna PFP, MeCN/H_2O 35/65 + 0.1%TFA, 1 mL/min, 285 nm)

Since the synthesis of **[^{18}F]3** should generally be possible, other reaction conditions were explored. As originally planned the use of bases was tested in further detail. Adding one equivalent Cs_2CO_3 or five equivalents 5N NaOH did give a product that

showed retention times identical to the authentic standard compound and also gave a product on TLC that had the same R_f-values as the authentic standard (cf. Figure 3.8).

Co-injection showed that even though retention times of the authentic standard and the radiolabeled product were slightly different, adding carrier to the radiolabeled product showed that retention times were the same. The form of the peaks of both the product and the authentic standard compound in Figure 3.8, which show the same tailing on the UV and the radioactive scale, is striking.

If base and sodium iodide were added to the reaction, HPLC analysis gave the same pattern of products as with only NaI, while TLC did not show any product. This strongly indicates that the peak found in HPLC does not belong to any reaction product but is a side product from a reaction of the NaI on the BFE or the precursor.

Therefore the addition of NaI to the reaction was stopped and the reaction optimized in reference to solvent and temperature.

As potential solvents DMF and DMSO were chosen, since they promised the best results. MeCN was not tested due to low solubility of the precursor and the restrictions of low temperatures applicable.

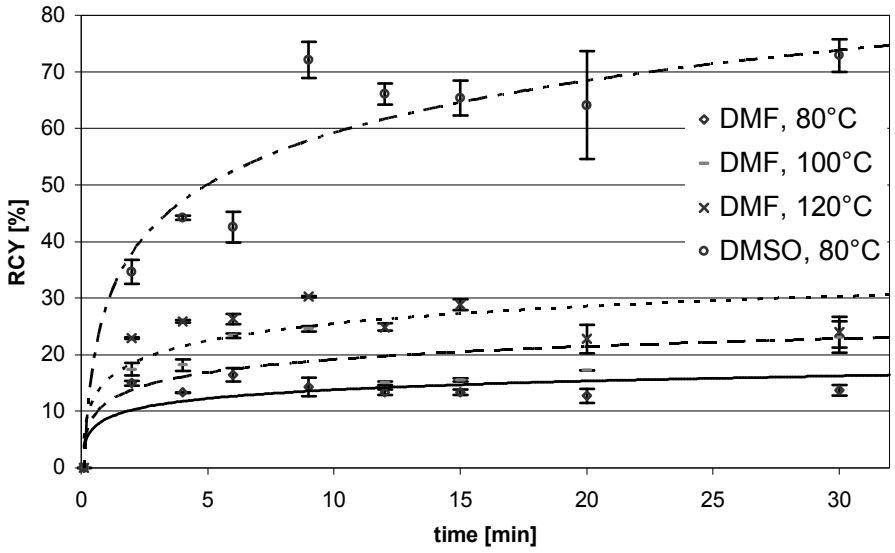


Figure 3.9: Optimization of the labeling reaction of **[^{18}F]3** (n = 1)

The first tests with DMF gave very good results, showing 49% RCY after 30 minutes. Trials at reproducing these results failed and for further trials radiochemical yields generally were about 10 %. Raising the temperature up to 100 °C and 120 °C did not give much better results, staying at about 25 % to 30 % (cf. Figure 3.9). Time

restrictions did not allow repeating those tests and therefore these results need to be considered as preliminary tests that should be expanded in a later optimization work.

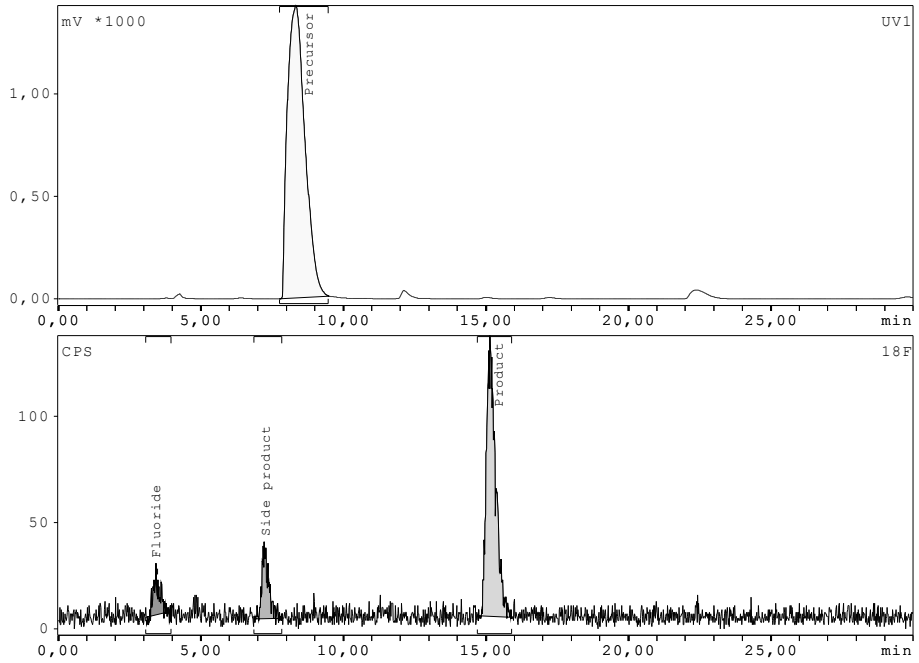


Figure 3.10: HPLC diagram of the labeling reaction of **[^{18}F]3** in DMSO at 80°C (Luna PFP, MeCN/H_2O 35/65 + 0.1%TFA, 1 mL/min, 285 nm)

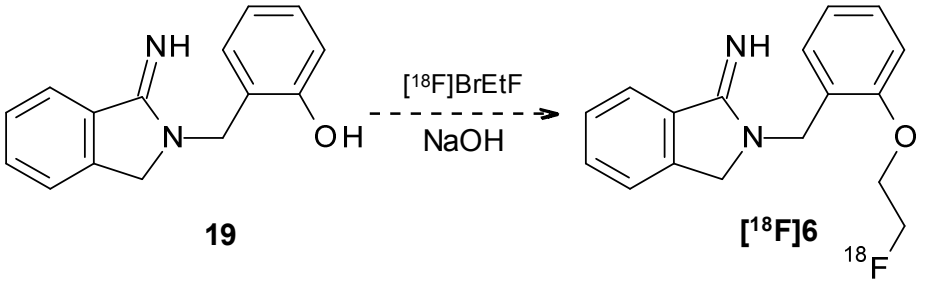
A single test with DMSO as solvent gave excellent results, giving 78 % RCY after 30 minutes, not showing any leftover BFE and only about 14 % of a by-product and 8 % unreacted fluoride (cf. Figure 3.10). These conditions therefore seem to be nearly perfect and encourage further investigation and optimization for preparative routine-labeling.

During the course of this work it could be proven that the labeling of **[^{18}F]**3 is possible using the methods applied and that reaction yields are very good, as long as the conditions are right. At the same time the bad reproducibility of the labeling reaction needs to be looked into further and its causes should be found and eliminated.

3.2.4 Synthesis of 2-(2-[^{18}F]fluoroethoxybenzyl)-isoindoline-1-imine

After the [^{18}F]fluoroalkyl-labeling of the cinnamamidine **[^{18}F]3** had proven successful, it was tried to label the cyclic precursor **19** with [^{18}F]BFE (cf. Scheme 3.20). Reaction conditions were chosen similar for those of the production of **[^{18}F]3**. Aliquots for TLC were diluted with 50 µL of dicholoromethane. Aliquots for HPLC analysis were taken after 15 and 30 minutes and diluted with HPLC eluent. 20 µL of this solution were injected into HPLC. The conditions for HPLC were similar to those used for the

cinnamamidine standard with exception of the eluent, which was MeCN/H_2O 25/75 + 0.1%TFA and the wavelength for UV absorption, which was set to 200 nm.



19 **[^{18}F]6**

Scheme 3.20: Labeling reaction of the cyclic amidine **[^{18}F]6**

TLC plates, which were developed with an eluent mixture of ethyl acetate and propylamine in an 8/2 ratio, did not show a product and HPLC did not show any radioactive peak that could be identified as the desired product **[^{18}F]6** (cf. Figure 3.11).

It seems that reaction conditions working for the labeling of **[^{18}F]3** are not easily transferable to the labeling of the cyclic compound **[^{18}F]6**. More tests should be performed to generate an optimized labeling reaction for this compound for performing *in vitro* tests to verify the potential usefulness of the tracer.

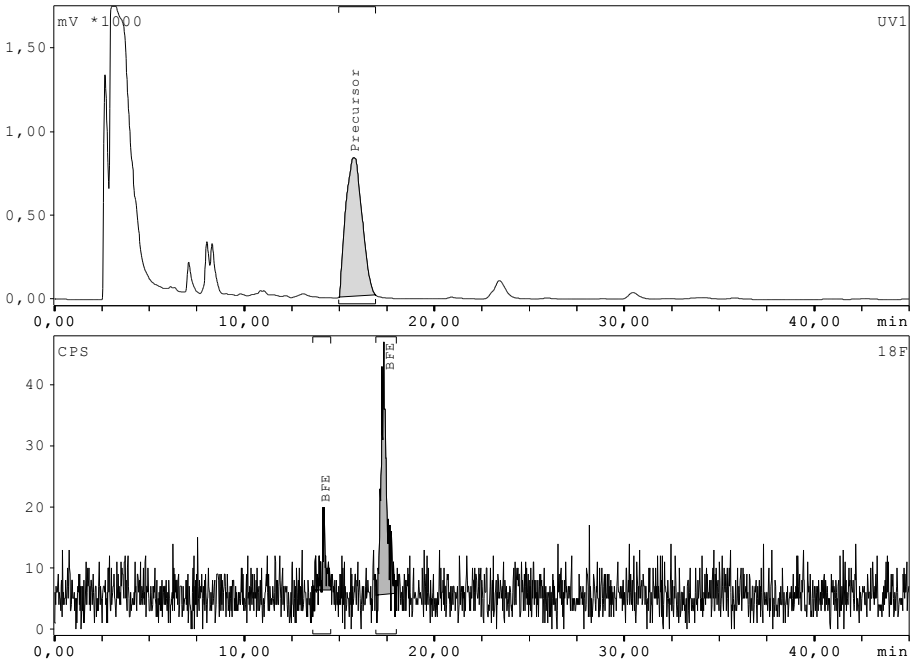


Figure 3.11: HPLC diagram of the labeling reaction on **19**
Luna PFP column (Luna PFP, MeCN/H_2O 25/75 + 0.1%TFA, 1 mL/min, 200 nm)

4 Experimental section

4.1 General

All experiments were conducted using chemicals obtained from one of the following companies: Aldrich (Germany), Fluka (Switzerland), VWR (Germany), Acros Organics (Belgium) or Merck (Germany). They were utilized without further cleaning and were of p.a. quality.

For analytic thin layer chromatography (TLC), silica (SiO_2) coated plates from Macherey & Nagel (Germany) were utilized. They were visualized under a UV lamp with the wave length of 254 nm. For column chromatographic separation, silica gel 60 (0.063-0.200 mm) from Merck was used. Eluent mixtures are given as volume proportion. Melting points were determined using a BÜCHI (B-540) melting point apparatus and are uncorrected.

Mass spectra were measured on a Thermoquest Automass Multi III mass spectrometer. Fourier transform mass spectrometry (FTMS) spectra for determination of the identity of the previously unknown authentic standard compounds were measured at the BIOSPEC Kompetenzzentrum für organische Massenspektrometrie at the Forschungszentrum Jülich.

For NMR spectra were recorded using a Bruker DPX Avance 200, chemical shifts (δ) are given in ppm and the internal standard was tetramethylsilane (TMS).

For determination of radiochemical yields radio-TLC and radio-HPLC was utilized. Aliquots were taken from the reaction vessel and analysed using the non active standard as relation. Please refer to page 76 for detailed information.

The eluent systems used for TLC to determine the R_f-values of the compounds and for (*flash*-)column chromatography are given in Table 4.1.

Table 4.1: Eluent Systems used for TLC and column chromatography

Eluent	Proportions (v/v)	Name
Ethyl acetate/n-hexane+ 0.1% NH_3	1/1	system 1
Ethyl acetate/dipropylamine	8/2	system 2
Ethyl acetate/n-hexane/DEA	8/1/1	system 3
Ethyl acetate/n-hexane/DEA	4/4/2	system 4
Ethyl acetate/n-hexane/DEA	4/5/1	system 5

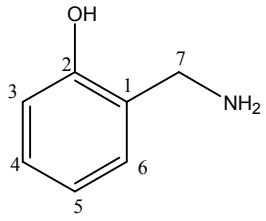
4.2 Synthesis of standard compounds and labeling precursors

4.2.1 2-Hydroxybenzylamine

In a three neck flask, equipped with reflux condenser, dropping funnel and septum, 4.935 g (41.47 mmol) of 2-hydroxybenzonitrile are dissolved in 50 mL of THF under argon atmosphere. Slowly 90 mL (90 mmol) of 1M borane in THF solution is added and after addition has finished, the mixture is refluxed for 3 hours. After cooling to room temperature (RT) 150 mL of 3M hydrochloric acid are added and the mixture is once again heated to reflux for 30 minutes and then cooled to RT. The reaction mixture is extracted with 2 x 150 mL of DCM, then set to alkaline with 250 mL of 2M NaOH and once again extracted with 2 x 150 mL DCM.

The organic phases are combined and dried over Na_2SO_4 and the solvent is evaporated. The reaction yields 4.34 g (85 %) of crude product that is crystallized from methanol to give the pure product as colorless crystals.

Yield:	3.52 g (28.6 mmol, 69 %)
Melting point	128.3 °C
MS (m/z)	124
R_f-Value (System 3)	0.43
^{1}H-NMR (d_6-DMSO)	δ [ppm] = 7.06 (m, 2**H**, H-4/6), 6.75 (m, 2**H**, H-3/5), 3.78 (s, 2**H**, H-7)



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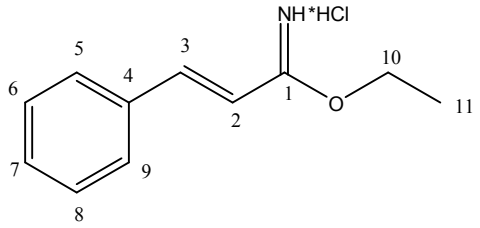
4.2.2 General procedure for the synthesis of imidate hydrochlorides

In a three neck flask, equipped with a gas inlet, a septum and a glass stopper, 50 to 80 mmol of nitrile was diluted in 150 to 250 mL of absolute ethanol under argon. The reaction mixture was cooled to 0 °C and HCl was bubbled through for 4 hours, keeping the temperature between 0 °C and 5 °C. The reaction was left stirring at room temperature for another 18 to 72 hours, during which the product formed as white precipitate. The solvent was removed under vacuum and the remaining solid taken up with diethyl ether. After filtering off, the product was dried under vacuum giving the clean imidate salt.

4.2.2.1 Ethyl cinnamimidate hydrochloride

Cinnamylnitril (10 mL, 80 mmol) was dissolved in 250 mL absolute ethanol and stirred under HCl atmosphere for 18 hours. The reaction was stopped when TLC showed no starting compound remaining in the reaction mixture.

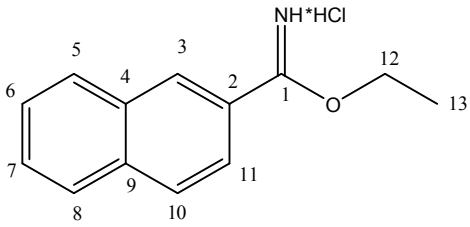


7

Yield:	15.25 g (72.3 mmol, 91 %)	
Melting point	128.8 °C	
MS (m/z)	176	
R_f-Value (System 1)	0.62	
^{1}H-NMR (d_6-DMSO)	δ [ppm] =	11.92 (bs, 1**H**, N-H), 7.92 (d, 1**H**, H-3), 6.65 (m, 2**H**, H-5/9), 7.43 (m, 3**H**, H-6/7/8), 7.22 (d, 1**H**, H-2), 4.58 (m, 2**H**, H-10), 1.39 (t, 3**H**, H-11)

4.2.2.2 Ethyl 2-naphthylimidate hydrochloride

7.659 g (50 mmol) of naphthylnitril were dissolved in 150 mL absolute ethanol and stirred under HCl atmosphere for 72 hours until no starting compound remained in the reaction mixture.



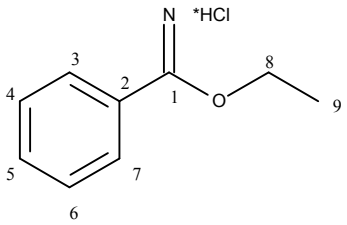
14

Yield:	10.29 g (43.7 mmol, 87 %)	
Melting point	n/a	
MS (m/z)	176	
R_f-Value (System 1)	0.75	
^{1}H-NMR (d_6-DMSO)	δ [ppm] =	12.06 (bs, 1**H**, N-H), 8.91 (s, 1**H**, H-11), 8.12 (m, 4**H**, H-5/6/7/8), 7.75 (m, 2**H**, H-3/10), 4.75 (q, 2**H**, H-12),1.45 (t, 3**H**, H-13)

4.2.2.3 Ethyl phenylimidate hydrochloride

Benzonitril (5.1 mL, 50 mmol) was dissolved in 150 mL absolute ethanol and stirred under HCl atmosphere for 72 hours. The reaction was stopped when TLC showed no starting compound remaining in the reaction mixture.

Yield:	8.85 g (47.8 mmol, 96 %)
Melting point	120.5 °C
MS (m/z)	150
R_f-Value (System 1)	0.77



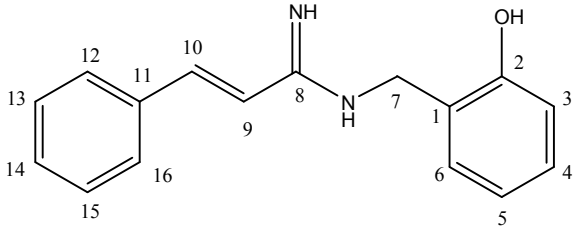
15

^{1}H-NMR (d_6-DMSO)	δ [ppm] =	8.20 (d, 2**H**, H-3/7), 7.73 (m, 3**H**, H-4/5/6), 4.75 (q, 2**H**, H-8),1.45 (t, 3**H**, H-9)

4.2.3 *N*-(2-Hydroxybenzyl)cinnamamidine

In a 50 mL beaker 1.69 g (8 mmol) of imidoester **7** are dissolved in 20 mL DMF. 1.2 mL (8 mmol) triethylamine is added and the resulting mixture briefly stirred. The resulting solution is filtered off into a 50 mL flask and another 1.2 mL (8 mmol) triethylamine and 20 mL DMF are added, and the flask is then flooded with argon. Finally 985.2 mg (8 mmol) of 2-hydroxybenzylamine are added. The mixture is stirred at room temperature for 4 hours and then evaporated to dryness. The resulting solid, which is crystallized from methanol, yields light yellow needles.

Yield:	1.5 g (6.1 mmol, 77 %)
Melting point	202.7 °C
MS (m/z)	253
R_f-Value (System 3)	0.36



11

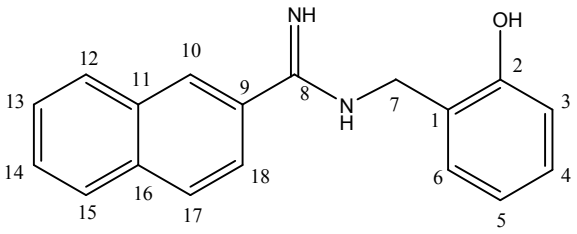
^{1}H-NMR (d_6-DMSO)	δ [ppm] =	7.75 (m, 5**H**, H-12/13/14/15/16), 7.33 (s, 1**H**, H-10), 7.10 (m, 2**H**, H-4/6), 6.75 (m, 2**H**, H-3/5), 6.59 (s, 1**H**, H-9), 4.28 (s, 2**H**, H-7)
$\{^1$H$\}^{13}$C-NMR (d_6-DMSO)	δ [ppm] =	162.08 (C-8), 159.26 (C-2), 136.55 (C-10), 135.91 (C-11), 131.44 (C-13/15), 130.19 (C-6), 129.86 (C-4), 129.47 (C-14), 128.08 (C-12/16), 126.77 (C-1), 122.81 (C-5), 118.65 (C-3), 118.41 (C-9), 44.24 (C-7)

4.2.4 General procedure for the synthesis of *N*-(2-hydroxybenzyl)-naphthyl amidine and *N*-(2-hydroxybenzyl)phenyl amidine

In a 10 mL one neck flask 370 mg (3 mmmol) of 2-hydroxybenzyl amine **10** are dissolved in 10 mL absolute methanol and 0.13 g (2.4 mMol) NaOMe are added. After the solids have dissolved 2 mmol of the corresponding imidoester are added. The reaction is stirred at room temperature under argon overnight (18h) and then quenched with a mixture of 1 mL of concentrated hydrochloric acid in 9 mL methanol. After removing the solvents in vaccuo the resulting solid is crystallized from methanol and yields colorless crystals.

4.2.4.1 *N*-(2-Hydroxybenzyl) naphthyl amidine

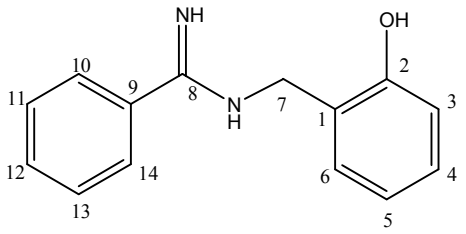
Yield:	235 mg (0.85 mmol, 43 %)
Melting point	170.0 °C
MS (m/z)	277
R_f-Value (System 4)	0.42
^{1}H-NMR (d_4-MeOH)	δ [ppm] = 8.11 (m, 3**H**, H-13/14/18), 7.78 (m, 4**H**, H-10/12/15/17), 7.27 (m, 4**H**, H-3/4/5/6), 4.69 (d, 2**H**, H-7)



16

4.2.4.2 *N*-(2-Hydroxybenzyl) phenyl amidine

Yield:	113 mg (0.5 mmol, 25 %)
MS (m/z)	227
R_f-Value (System 4)	0.50
^{1}H-NMR (d_4-MeOH)	δ [ppm] = 7.68 (m, 5**H**, H-10/11/12/13/14), 7.22 (m, 4**H**, H-3/4/5/6), 4.63 (d, 2**H**, H-7)



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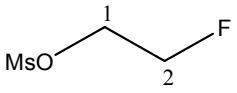
4.2.5 2-Fluoroethylmesylate

25 g (0.39 mol) 2-fluoroethanol are dissolved in DCM and 65 mL (0.47 mol) triethylamine are added. The resulting mixture is cooled to 0 °C and then 33.3 mL (0.43 mol) methansulfonylchloride are slowly added to the solution. The cooling is stopped and the reaction stirred one hour at room temperature. After adding 100 mL

DCM the solution is extracted with 150 mL water followed by extraction with the same amount of 10 % hydrochloric acid, followed by extraction with 150 mL of saturated $NaHCO_3$ and then 150 mL of saturated NaCl. After drying over $NaSO_4$ the solvent is removed in vaccuo without heating and the resulting oil kept under argon at 7 °C.

Yield: 48.75 g
(0.34 mol, 88 %)



33

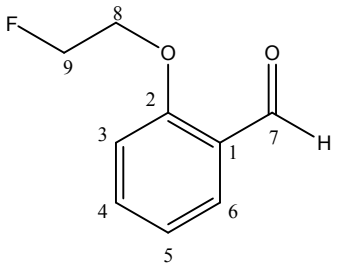
MS (m/z) n/a
R_f-Value n/a

^{1}H-NMR ($CDCl_3$) δ [ppm] = 4.82 (t, 1**H**, H-1), 4.55 (m, 2**H**, H-2), 4.42 (t, 1**H**, H-1), 3.10 (s, 3**H**, Ms-CH_3)

4.2.6 2-Fluoroethoxysalicylaldehyde

In a 250 mL flask 16.2 mL (0.16 mol) salicylaldehyde and 63.2 g (0.19 mol) caesium carbonate are dissolved in 200 mL DMF. 19.3 g (0.14 mol) of 2-fluoroethanmesylate are slowly added and the resulting mixture is heated to 40 °C and stirred at that temperature for 1 hour. After that time the heating is removed and the reaction stirred at room temperature for 23 hours. The mixture is then poured onto 1000 mL of ice water and extracted with 3 x 150 mL DCM. Drying over $NaSO_4$ and evaporation of the solvent gives 28.8 g of the crude product as brown oil. For purification of the product column chromatography (system 1) was employed. After removal of the solvent the product is obtained as light yellow oil.

Yield: 12.9 g
(0.77 mol, 57 %)



34

MS (m/z) 169

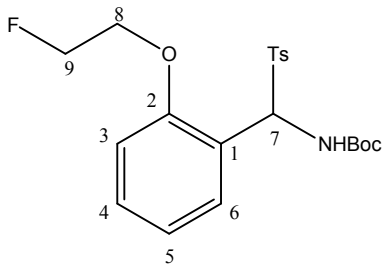
R_f-Value n/a

^{1}H-NMR ($CDCl_3$) δ [ppm] = 10.44 (s, 1**H**, H-7), 7.85 (d, 1**H**, H-2), 7.54 (t, 1**H**, H-4), 7.07 (m, 2**H**, H-3/5), 4.95 (m, 1**H**, H-9), 4.72 (m, 1**H**, H-9), 4.43 (m, 1**H**, H-8), 4.29 (m, 1**H**, H-8)

^{19}F-NMR ($CDCl_3$) δ [ppm] = -223.92

4.2.7 2-Fluoroethoxy-α-tosyl-*N*-boc-benzylamine

In a 250 mL one neck flask 5 g (29.73 mmol) 2-fluoroethoxysalicylaldehyde **34** together with 3.44g (29.73 mmol) t-butyl carbamate and 5.22 g (29.73 mmol) sodium p-toluenesulfinate are dissolved in a mixture of 60 mL water, 30 mL methanol and 7.5 mL formic acid. After stirring for 15 minutes all reagents have dissolved and the reaction mixture is left standing without stirring for 24 hours. The product is obtained as colorless precipitate and is separated by filtering off the solvent. Drying over P_2O_5 in an exsiccator gives the product in excellent yield.



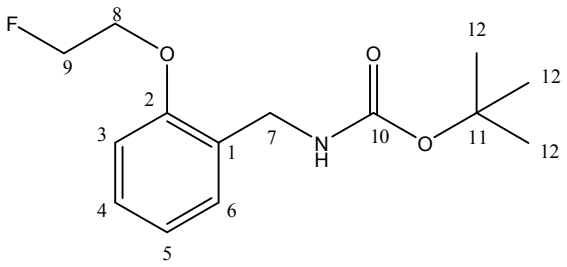
35

Yield:	9.91 g (23.4 mmol, 79 %)	
Melting point	248.0 °C	
MS (m/z)	267 (Schiff base)	
R_f-Value (System 5)	0.73	
^{1}H-NMR ($CDCl_3$)	δ [ppm] =	7.79 (d, 2**H**, Ts-H), 7.35 (m, 4**H**, 2 x Ts-H and H-4/6), 7.06 (m, 1**H**, H-5), 6.93 (d, 1**H**, H-3), 6.34 (m, 1**H**, H-7), 4.91 (m, 1**H**, H-9), 4.65 (m, 1**H**, H-9), 4.30 (m, 1**H**, H-8), 4.17 (m, 1**H**, H-8), 2.44 (s, 3**H**, Ts-CH_3), 1.32 (s, 9**H**, Boc-CH_3)
^{19}F-NMR ($CDCl_3$)	δ [ppm] =	-224.03

4.2.8 2-Fluoroethoxy-*N*-boc-benzylamine

In a 250 mL one neck flask 3.18 g (84 mmol) sodium borohydride are dissolved in 70 mL THF and slowly over 15 minutes 17.734 g (42 mmol) 2-fluoroethoxy-α-tosyl-*N*-boc-benzylamine **35** are added. The reaction mixture is then stirred for 2 hours at room temperature. Subsequently it is cooled to 5 °C and 30 mL saturated ammonium chloride solution is added carefully to quench the reaction. After separating the organic phase the water phase is extracted with 3 x 30mL DMC. The organic fractions are combined and dried over Na_2SO_4 before the solvent is removed *in vacuo*. After crystallization from ethanol/water the product is obtained as colorless solid.

Yield: 10.68 g (40 mmol, 94 %)



Melting point 145.2 °C

MS (m/z) 270

R_f-Value (System 5) 0.83

36

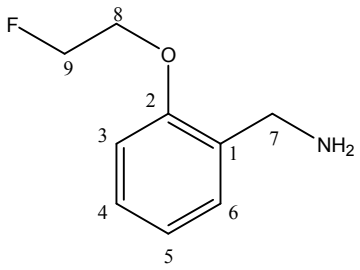
^{1}H-NMR ($CDCl_3$) δ [ppm] = 7.26 (m, 2**H**, H-5/6), 6.94 (m, 2**H**, H-3/4), 4.90 (m, 1**H**, H-9), 4.66 (m, 1**H**, H-9), 4.35 (m, 3**H**, H-7/8), 4.19 (m, 1**H**, H-8), 1.47 (s, 9**H**, H-12)

^{19}F-NMR ($CDCl_3$) δ [ppm] = -223.92

4.2.9 2-Fluoroethoxybenzylamine

Into a one neck flask 10.68 g (40 mmol) of 2-fluoroethoxy-*N*-boc-benzylamine **36** are diluted in 20 mL DCM and 20 mL TFA are added. The mixture is stirred at room temperature for 30 minutes and then reduced to dryness. The resulting solid is diluted in 20 mL DCM and adjusted to pH 14 with 5M NaOH. The solution is then washed with each 20 mL water, $NaHCO_3$ and saturated NaCl solution. After drying over Na_2SO_4 the crude product is obtained in quantitative yield. It is purified by column chromatography using system 5 producing the title compound as light yellow oil.

Yield: 2.9 g (17 mmol, 43 %)



MS (m/z) 170

R_f-Value (System 5) 0.75

28

^{1}H-NMR ($CDCl_3$) δ [ppm] = 7.34 (m, 2**H**, H-5/6), 6.99 (m, 2**H**, H-3/4), 4.93 (m, 3**H**, H-7/9), 4.70 (m, 1**H**, H-9), 4.37 (m, 1**H**, H-8), 4.23 (m, 1**H**, H-8)

^{19}F-NMR ($CDCl_3$) δ [ppm] = -223.72

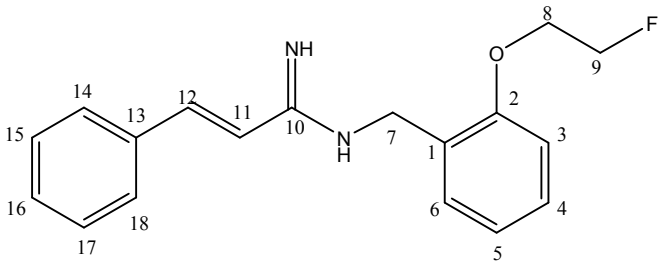
4.2.10 General procedure for the synthesis of 2-fluoroethoxybenzyl amidines

In a beaker 17 to 20 mmol of the imidoester are dissolved in 30 mL THF and 3.5 mL (20 mmol) *N*,*N*-diisopropylethylamine (Hünig's base) are added. When a precipitate has formed the solution is filtered into a one neck flask. Then 3.4 g (20 mmol) 2-fluoroethoxybenzylamine dissolved in 20 mL THF are added to the solution and it is stirred under argon atmosphere at room temperature for 4 hours.

The reaction mixture is poured onto 50 mL water and then neutralized using acetic acid. The product is then extracted using 3 x 50 mL DCM. After drying over Na_2SO_4 the solvent is evaporated *in vacuo* giving the crude product which is purified by individual means for each compound.

4.2.10.1 *N*-(2-Fluoroethoxybenzyl)cinnamamidine

The crude product is recrystallized from ethanol/water with a few drops of acetic acid producing colorless crystals.



3

Yield:	199 mg (0.67 mmol, 12 %)	
Melting point	163.0 °C	
FTMS (m/z)	299.1554	
R_f-Value (System 3)	0.56	
^{1}H-NMR (d_6-DMSO)	δ [ppm] =	7.78 (d, 1**H**, H-12), 7.66 (m, 2**H**, H-14/18), 7.47 (m, 5**H**, H-4/6/15/16/17), 7.09 (m, 2**H**, H-3/5), 6.77 (d, 1**H**, H-11), 4.93 (t, 1**H**, H-9), 4.70 (t, 1**H**, H-9), 4.65 (s, 2**H**, H-7), 4.44 (t, 1**H**, H-8), 4.29 (t, 1**H**, H-8)
$\{^1H\}^{13}$C-NMR (d_6-DMSO)	δ [ppm] =	160.68 (C-10), 156.66 (C-2), 143.20 (C-12), 133.71 (C-13), 130.83 (C-15/17), 130.08 (C-6/16), 129.92 (C-4), 128.87 (C-1), 127.97 (C-14/18), 121.80 (C-5), 114.82 (C-3), 111.71 (C-11), 83.28 (C-9), 67.63 (C-8), 42.00 (C-7)
^{19}F-NMR (d_6-DMSO)	δ [ppm] =	-224.93

4.2.10.2 *N*-(2-Fluoroethoxybenzyl)naphthyl amidine

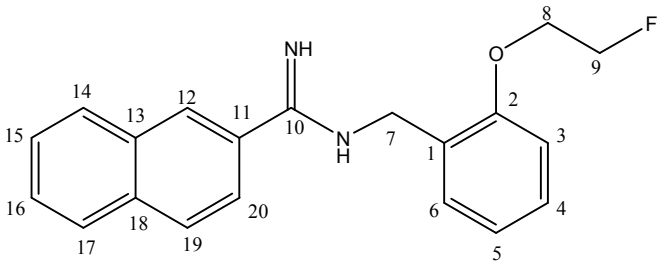
The reaction is started using 4.1 g (17 mmol) naphthylimidoester with the amount of the other reagents indicated in the general description.

The crude product is purified by column chromatography using system 2 followed by a second column chromatography using system 3. This produces the product as light beige oil.

Yield: 1.243 g (3.9 mmol, 20 %)

FTMS (m/z) 323.1554

R_f-Value (System 4) 0.74



4

^{1}H-NMR (d_3-MeCN) δ [ppm] = 8.23 (s, 1**H**, H-20), 7.96 (m, 4**H**, H-14/15/16/17), 7.57 (m, 3**H**, H-4/12/19), 7.28 (t, 1**H**, H-6), 7.02 (t, 2**H**, H-3/5), 4.93 (t, 1**H**, H-9), 4.70 (t, 1**H**, H-9), 4.54 (s, 2**H**, H-7), 4.38 (t, 1**H**, H-8), 4.24 (t, 1**H**, H-8)

{^{1}H}^{13}C-NMR (d_3-MeCN) δ [ppm] = 159.82 (C-10), 156.21 (C-2), 134.80 (C-18), 133.96 (C-13), 132.86 (C-11), 129.06 (C-19), 128.77 (C-1), 128.52 (C-6), 127.90 (C-4), 127.59 (C-14/17), 129.93 (C-16), 126.55 (C-15), 125.61 (C-12), 124.36 (C-20), 121.03 (C-5), 111.87 (C-3), 82.55 (C-9), 67.78 (C-8), 42.63 (C-7)

^{19}F-NMR (d_3-MeCN) δ [ppm] = -223.84

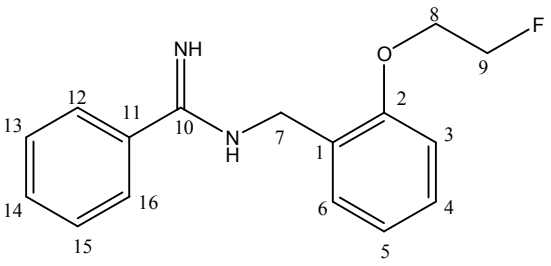
4.2.10.3 *N*-(2-Fluoroethoxybenzyl)phenyl amidine

The crude product is purified by column chromatography (system 2) followed by a second column chromatography using system 5. This produces the product as light beige oil.

Yield: 227 mg (0.8 mmol, 4 %)

FTMS (m/z) 273.1397

R_f-Value (System 4) 0.70



5

^{1}H-NMR (d_6-DMSO)	δ [ppm] =	7.73 (m, 2**H**, H-12/16), 7.45 (m, 4**H**, H-6/13/14/15), 7.29 (t, 1**H**, H-4), 7.01 (t, 2**H**, H-3/5), 4.93 (t, 1**H**, H-9), 4.69 (t, 1**H**, H-9), 4.49 (s, 2**H**, H-7), 4.38 (t, 1**H**, H-8), 4.23 (t, 1**H**, H-8)
{^{1}H}^{13}C-NMR (d_6-DMSO)	δ [ppm] =	160.25 (C-0, 156.20 (C-2), 137.58 (C-11), 129.78 (C-14), 128.93 (C-6), 128.72 (C-1), 128.37 (C-13/15), 127.88 (C-4), 126.32 (C-12/16), 121.00 (C-5), 111.85 (C-3), 82.53 (C-9), 67.77 (C-8), 42.24 (C-7)
^{19}F-NMR (d_6-DMSO)	δ [ppm] =	-223.96

4.2.11 2-(2-Hydroxybenzyl)-isoindoline-1-imine

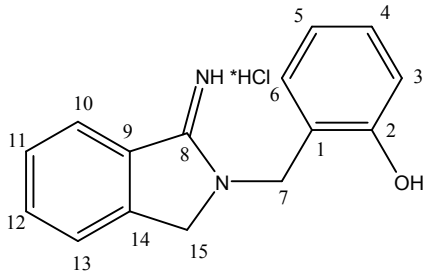
308 mg (2.5 mmol) 2-hydroxybenzylamine are dissolved in 20 mL ethanol, 431 mg (2.2 mmol) 2-(bromomethyl)-benzonitrile are added and the mixture is then stirred for 3 hours under reflux. After cooling the solvent is removed *in vacuo*, the resulting solid is washed with diethyl ether and then crystallized from methanol to produce the title compound as light yellow needles.

Yield: 130 mg (0.55 mmol, 25 %)

Melting point 51.4 °C

MS (m/z) 239

R_f-Value (System 4) 0.61



19

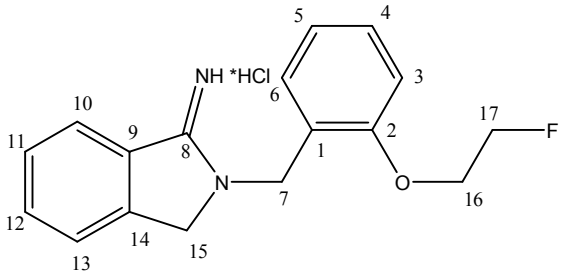
^{1}H-NMR (d_4-MeOH)	δ [ppm] =	9.85 (bs, 2**H**, -NH/-OH), 8.29 (d, 1**H**, H-12), 7.72 (m, 3**H**, H-10/11/13), 7.24 (m, 2**H**, H-4/6), 6.90 (m, 2**H**, H-3/5), 4.98 (s, 2**H**, H-15), 4.69 (s, 2**H**, H-7)
{^{1}H}^{13}C-NMR (d_4-MeOH)	δ [ppm] =	161.90 (C-8), 156.52 (C-2), 142.89 (C-14), 134.19 (C-12), 130.76 (C-6), 130.72 (C-13), 129.48 (C-4), 129.26 (C-9), 124.50 (C-10), 124.29 (C-10), 121.14 (C-1), 120.16 (C-5), 116.28 (C-3), 56.36 (C-15), 45.53 (C-7)

4.2.12 2-(2-Fluoroethoxybenzyl)-isoindoline-1-imine

3.4 g (20 mmol) 2-hydroxybenzylamine are dissolved in 50 mL ethanol, 3.9 g (23 mmol) 2-(bromomethyl)-benzonitrile are added and the mixture is then heated to reflux until no starting compound remains. After 3 days the reaction is stopped by

letting it cool and removing the solvent *in vacuo*. The remaining solid is then washed with diethyl ether and recrystallized from ethanol/water to produce the title compound as colorless crystals.

Yield:	686 mg (2.4 mmol, 12 %)
Melting point	231.1 °C
FTMS (m/z)	285.1397
R_f-Value (System 4)	0.49



6

^{1}H-NMR (d_6-DMSO)	δ [ppm] =	8.34 (d, 1**H**, H-14), 7.68 (m, 3**H**, H-12/13/15), 7.40 (m, 2**H**, H-4/6), 7.07 (m, 2**H**, H-3/5), 5.08 (s, 2**H**, H-17), 4.87 (t, 1**H**, H-9), 4.71 (s, 2**H**, H-7), 4.63 (t, 1**H**, H-9), 4.36 (t, 1**H**, H-8), 4.22 (t, 1**H**, H-8)
{^{1}H}^{13}C-NMR (d_6-DMSO)	δ [ppm] =	161.50 (C-10), 156.73 (C-2), 142.44 (C-16), 133.75 (C-14) 130.70 (C-6), 130.54 (C-15), 129.00 (C-4), 128.88 (C-11), 124.05 (C-13), 123.99 (C-12), 122.65 (C-1) 121.51 (C-5), 112.67 (C-3), 82.55 (C-9), 67.97 (C-8), 56.90 (C-17), 45.27 (C-7),
^{19}F-NMR (d_6-DMSO)	δ [ppm] =	-222.00

4.3 Radiosyntheses

4.3.1 Radioanalysis

Radiochemical yields of desired products and by-products formed during the reaction are analyzed using radio-TLC. TLC plates (Polygram SIL G/UV254 40x80 mm) were obtained from Macherey-Nagel (Germany) and the following eluent systems were used. A Packard Instruments Instant Imager™ was used for measuring the distribution of radioactivity on the TLC plates.

Table 4.2: Eluent systems used for radio-TLC

Compound	Eluent	Proportions (v/v)
N-(2-[^{18}F]Fluoroethoxybenzyl)-cinnamamidine **[^{18}F]3**	Ethyl acetate/n-hexane+ DEA	8/1/1
2-(2-[^{18}F]Fluoroethoxybenzyl)-isoindoline-1-imine **[^{18}F]6**	Ethyl acetate/dipropylamine	8/2

Analytic HPLC was used for determining specific activity and radiochemical yields. The HPLC system used consisted of a Knauer WellChrom Mini-Star K500 pump, a Rheodyne 7125 injection valve, a Merck/Hitachi L4000 UV/Vis photometer and a Na(Tl)-detector connected to a photomultiplier and amplifier (EG&G Ortec, 925 Scint Amplifier and Bias Supply) for determination of radioactivity. A analytical Kromasil 100-5 C18 (250 x 4,6 mm) column (obtained from CS-Chromatographie Service GmbH, Germany) was used with this system. The analysis was performed under isocratic conditions with a flow of 1 mL/min using one of the eluent systems given in Table 4.3. For analysis of the reaction progress samples of 20 µL were taken directly from the reaction vial and injected into the HPLC without purification.

Table 4.3: Eluent conditions used for radio-HPLC

Compound	Eluent	Proportions (v/v)
N-(2-[^{18}F]Fluoroethoxybenzyl)-cinnamamidine **[^{18}F]3**	Acetonitrile/water + 0,1% TFA	35/65
2-(2-[^{18}F]Fluoroethoxybenzyl)-isoindoline-1-imine **[^{18}F]6**	Acetonitrile/water + 0,1% TFA	25/75

An alternative system was a analytical Phenomenex Luna PFP 100-3 (250 x 4,6 mm) column (obtained from Phenomenex, Germany). Here the analysis was performed under isocratic conditions with a flow of 1 mL/min using one of the eluent systems given in Table 4.3. Samples of 50 µL were taken and dissolved in the same amount of HPLC eluent before injection of 20µL of the prepared solution into the HPLC. For determination of radiochemical yields (RCY) peak activity was compared to total activity measured during the chromatographic analysis and is given in relation to this total activity.

4.3.2 Production of n.c.a. [^{18}F]fluoride

Production of n.c.a. [^{18}F]fluoride is done routinely at the institute for nuclear chemistry (INM-5) at the Forschungszentrum Jülich utilizing the “Baby cyclotron” BC 1710 (JSW) or the GE PETtrace. A ^{18}O-enriched water target is irradiated by a proton beam of 17 MeV generating fluorine-18 through the ^{18}O(p,n)^{18}F reaction. Separation of the product is achieved via electrostatic adsorption using a Sigradur®-Anode and

reelution of the product into 5-fold distilled water, after removal of the enriched target water. [20]

4.3.3 Preparation of the [^{18}F]fluoride-Kryptofix complex.

The labeling reaction with [^{18}F]fluoride is conducted using a dry 2.5 mL wheaton reactor (glass vial) made from borosilicate glass. The vial is closed with a silicone septum and a twist off cap, ensuring isochoric conditions, and then flushed with argon. For drying of the aqueous [^{18}F]fluoride solution the reaction vial is equipped with connections to the argon supply and vacuum pump.

Typically about 150 MBq [^{18}F]fluoride solution (20 to 150 µL depending on the production time) are mixed with 13 µL (13 µmol) 1 M K_2CO_3 and 10 mg (26.56 µmol) Kryptofix® in 1mL acetonitrile and given into the vial. The vial is then placed into an oil bath set to 80°C and pressure in the vial is reduced to 800 mbar under a constant argon stream. After evaporation of the solvent another 1 mL actonitrile is added and the mixture is again reduced to dryness. Depending on the amount of water present before the azeotropic drying, this step is repeated up to three times. After this process total vacuum (~0.01 mbar) is applied to the vial for about 3-5 minutes and the vial again flushed with argon until atmospheric pressure is reached.

4.3.4 Nucleophilic substitution on 1,2-dibromoethane for production of 1-bromo-2-[^{18}F]fluoroethane as prosthetic group

The vial containing the dried fluoride-Kryptofix complex is heated in a second oil bath with a temperature of 70 °C and 2.3 µL (5 mg, 26.62 µmol) 1,2-dibromoethan dissolved in 1 mL acetonitrile are added to the [^{18}F]fluoride. The reaction is left running for 3 minutes and then stopped by diluting the product with 19 mL of water.

The mixture is then passed through a LiChrolut® EN cartridge which has been conditioned using 5 mL of methanol followed by 5 mL of water. The cartridge is dried by a stream of argon through it for about 1 minute. The desired product is then eluted from the cartridge through an AluminaB cartridge (conditioned with 5 mL DMF or DMSO) using 4 mL hot (70 °C) DMF or DMSO.

The solvent is collected in four fractions of 1.4 mL, 1.0 mL and then two of 0.8 mL. Fraction 2 almost exclusively contains the product. Radiochemical yields of 1-bromo-2-[^{18}F]fluoroethane are 34 % ± 4 %. This product can be used for further reactions without any further purification.

4.3.5 Nucleophilic substitution on ethylenglycole-1,2-ditosylate for production of 2-[^{18}F]fluoroethyl tosylate as prosthetic group

After drying of the [^{18}F]fluoride and generation of the Kryptofix complex, the vial is then heated in a second oil bath with a temperature of 70 °C and 4.5 mg (12 µmol) ethylenglycole-1,2-ditosylate dissolved in 1 mL acetonitrile are added to the [^{18}F]fluoride. The reaction is stirred for 10 minutes and then stopped by diluting the reaction mixture with 9 mL of water.

The mixture is then passed through a Waters tC18 cartridge which was conditioned using 5 mL of ethanol followed by 5 mL of water. The cartridge is purged with 5 mL of water and dried by purging argon through it for about 1 minute. The desired product is then eluted from the cartridge through an AluminaN cartridge (conditioned with 5 mL DMF) using 4 mL hot (70 °C) DMF.

The solvent is collected in three fractions of one time 2.0 mL and two times 1.0 mL. Fraction 2 almost contains most of the product. Radiochemical yields of [^{18}F]fluoroethyl tosylate are 39 % ± 9 %. This product can be used for further reactions without any further purification.

4.3.6 General method of synthesis of 2-[^{18}F]fluoroethoxybenzyl amidines

In a dry glass vial flushed with argon 25 µmol precursor, dissolved in 0.5 mL DMF or DMSO, is mixed with 2.4 µL 5M NaOH. 0.5 mL (~ 5 MBq) of the prosthetic group dissolved in the reaction solvent are added and the reaction allowed to run for 30 minutes. During this time 50 µL aliquots are taken from the reaction after 2, 4, 6, 9, 12, 15, 20 and 30 minutes and dissolved in 50 µL DCM or methanol. These are analyzed with radio-TLC and radio-HPLC.

5 Summary

Radiosynthesis of a receptor ligand selective for the NMDA receptor subtype NR2B is of great interest for *in vivo* studies, since the NMDA receptor is surmised to be involved in the etiology of diseases like Alzheimer's or Parkinson's. Availability of a selective radioligand for that receptor subtype would enable further exploration of the origin of these diseases and those of others that are supposedly connected to malfunctions of the NMDA receptor.

In the course of this work the synthesis of the authentic standards and labeling precursors for four possible radiotracers was performed. The compounds that were to be synthesized were chosen following a lead structure known from literature. While radiolabeling with a [^{11}C]methyl group had previously been performed on the lead structure, in the scope of this work radiolabeling with a [^{18}F]fluoroethyl group was planned. The synthesis of a ^{18}F-analogue would be of great interest, as fluorine-18 has a significantly longer half-life than carbon-11. Thus, while still offering no-carrier-added conditions, the use of fluorine-18 enables longer study times.

The compounds to be synthesized were *N*-(2-[^{18}F]fluoroethoxybenzyl)-cinnamamidine (**[^{18}F]3**), *N*-(2-[^{18}F]fluoroethoxybenzyl)-naphthyl amidine (**[^{18}F]4**), *N*-(2-[^{18}F]fluoroethoxybenzyl)-phenyl amidine (**[^{18}F]5**) and 2-(2-[^{18}F]fluoroethoxy-benzyl)-isoindoline-1-imine (**[^{18}F]6**). Since the desired target compounds were not accessible in a direct labeling reaction, an indirect synthesis by ^{18}F-fluoroalkylation using 1-bromo-2-[^{18}F]fluoroethane was planned and executed.

Synthesis of the necessary labeling precursors *N*-(2-hydroxybenzyl)-cinnamamidine (**11**), *N*-(2-hydroxybenzyl)-naphthyl amidine (**16**), *N*-(2-hydroxy-benzyl)-phenyl amidine (**17**) and the cyclic precursor 2-(2-hydroxybenzyl)-isoindoline-1-imine (**19**) was performed in a two step synthesis starting from 2-hydroxybenzylamine and the respective imidoester for the non-cyclic compounds and 2-hydroxybenzylamine and 2-(bromomethyl)-benzonitrile for the cyclic compound.

Problems with the synthesis of 2-hydroxybenzylamine were overcome after changing the reaction procedure from a reduction employing hydrogen and palladium on active charcoal to one utilizing borane in THF. After reduction of 2-hydroxybenzonitrile with this reagent, the desired product 2-hydroxybenzylamine was obtained within three

hours of reaction time. Crystallization from methanol gave the clean product in good yields (69 %).

The respective imidoesters were obtained from their nitriles in a Pinner synthesis. Reaction of the nitrile in absolute ethanol und hydrogen chloride atmosphere was successful and gave excellent yields (87 % - 96 %) after short to medium reaction times (18 h - 72 h).

The cinnamyl precursor **11** was obtained after a coupling reaction between the amine and the imidoester in DMF using triethylamine as base. The phenyl and naphthyl compounds were obtained after a reaction in methanol using sodium methanolate as base. All precursors could be purified by crystallization from methanol and were suitably pure for use in a labeling reaction. Yields for the cinnamyl precursor were satisfying with 77 % while those for the phenyl and naphthyl compound were significantly lower with 25 % and 43 % respectively.

The cyclic standard compound was synthesized directly from 2-(bromomethyl)-benzonitrile and 2-hydroxybenzylamine by a ring closing reaction in refluxing ethanol. Like the other precursors crystallization from methanol gave the pure compound that could be employed for the labeling reaction without further purification. Like the phenyl and naphthyl precursor yields of the pure compound were low with 25 %.

In order to verify identity of the labeled product in chromatographic methods the stable authentic fluoro-compounds were synthesized as chromatographic standards.

Attempts of the syntheses of the authentic standards *N*-(2-fluoroethoxybenzyl)-cinnamamidine (**3**), *N*-(2-fluoroethoxybenzyl)-naphthyl amidine (**4**), *N*-(2-fluoroethoxy-benzyl)-phenyl amidine (**5**) and 2-(2-fluoroethoxybenzyl)-isoindoline-1-imine (**6**) were first performed on the labeling precursors, using 1-bromo-2-fluoroethane and different bases. Those experiments were unsuccessful and the application of different solvents did not enhance yields. Finally, even after the use of strong bases had not led to any success, reaction conditions were found that gave the desired product. The reaction using potassium carbonate and potassium iodide in DMF at 120 °C gave good yields of about 70 % of the desired product. Analysis of the product using NMR showed that a fluorine containing by-product had been produced that could not be removed from the target compound by any means. It was concluded that alkylation of the precursor had taken place not only at the oxygen

atom but also at one of the nitrogen atoms of the amidine. It was therefore necessary to find a different method of synthesis for the desired product.

In order to avoid potential alkylation on the nitrogen atom a reaction pathway was planned that started from salicylaldehyde. Introduction of the fluoroethoxy group into the salicylaldehyde was facile using mild reaction conditions of 40 °C in DMF and cesium carbonate as base. Transformation of the aldehyde into an amine was achieved in a three step synthesis. Introduction of the nitrogen atom into the molecule was achieved by reaction with *tert*-butylcarbamate and sodium toluene sulfonate. The desired product 2-fluoroethoxy-α-tosyl-*N*-boc-benzylamine was obtained in good yields of 79 % as a colorless solid and used in the next step without further purification. A reduction with sodium borohydride in THF was performed to remove the tosyl group and 2-fluoroethoxy-*N*-boc-benzylamine was obtained in an excellent yield of 94 %.

Deprotection of the amine was achieved by stirring the tosyl compound with a 1/1 mixture of DCM and TFA for 30 minutes at room temperature. After purification by *flash*-column chromatography the desired product 2-fluoroethoxybenzylamine was obtained in yields of 43 %. Coupling of the 2-fluoroethoxybenzylamine with the respective imidoester, or in the case of the cyclic compound 2-(bromomethyl)-benzonitrile, gave the desired product which was then purified by *flash*-column chromatography and in the case of the cinnamyl and the cyclic compound by crystallization from ethanol and water. Yields for all compounds were low (between 4 % to 10 %) but the substances were > 99 % pure as confirmed by HPLC and mass spectrometry.

Once the authentic standard compounds were available, radiolabeling could be conducted. For the ^{18}F-fluoroalkylation 1-bromo-2-[^{18}F]fluoroethane was synthesized starting from 1,2-dibromoethane. The desired labeled prosthetic group was obtained in radiochemical yields of 34 %± 4 %. An alternative route of synthesis for a prosthetic group was also employed using ethylenglycole-1,2-ditosylate, leading to radiochemical yields of 39 % ± 9 % of 2-[^{18}F]fluoroethyl tosylate.

The labeling reaction was performed with the cinnamyl precursor **11** using either DMF or DMSO as solvent at different temperatures. The n.c.a. ^{18}F-fluoroalkylation with addition of sodium iodide gave a product that showed the same retention time as the authentic standard, but could not be verified using thin layer chromatography.

Co-injection of the labeled product and the authentic standard made it doubtful that the desired product had been obtained. Indeed changing HPLC conditions by utilizing a different column (Luna PFP 100-3, instead of the previously used Kromasil 100-5 C18) showed that the product was not the desired labeled compound.

The addition of five equivalents of sodium hydroxide to the reaction without presence of sodium iodide eventually showed a product different from that one so far obtained. Co-injection of *N*-(2-[^{18}F]fluoroethoxybenzyl)-cinnamamidine (**3**) and the authentic standard compound showed the same retention time for both compounds. It was thus proven that the desired product was formed and subsequent experiments to optimize the reaction were performed.

Both DMF and DMSO were compared as reaction solvent in preliminary tests and showed the formation of a product. While yields were generally lower than 30 % even after 30 minutes of reaction time using DMF at different temperatures (80 °C, 100 °C and 120 °C), DMSO gave much better yields of about 78 % at 80 °C after 30 minutes.

In analogy, labeling of the cyclic precursor 2-(2-hydroxybenzyl)-isoindoline-1-imine (**19**) was performed using DMSO and sodium hydroxide at 80 °C. However, no product could be found and the labeling reaction was unsuccessful.

Further experiments to establish a labeling procedure for *N*-(2-[^{18}F]fluoroethoxy-benzyl)-naphthyl amidine (**[^{18}F]4**), *N*-(2-[^{18}F]fluoroethoxybenzyl)-phenyl amidine (**[^{18}F]5**) and 2-(2-[^{18}F]fluoroethoxybenzyl)-isoindoline-1-imine (**[^{18}F]6**) are therefore still necessary. However, *N*-(2-[^{18}F]Fluoroethoxybenzyl)-cinnamamidine (**[^{18}F]3**) has been successfully synthesized and is now available for *in vitro* and *in vivo* tests to determine its suitability as radioligand for the *in vivo* monitoring of the NMDA receptor.

6 List of abbreviations

ACN	acetonitrile
BFE	1-bromo-2-fluoroethane
Bq	Becquerel
c.a.	carrier added
c.f.	carrier free
Ci	Curie (1 Ci = $3.7 \cdot 10^{10}$ MBq)
CNS	central nervous system
d	doublet (NMR), days (half-life)
DCM	dichloromethane
DEA	diethylamine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DPA	dipropylamine
FDG	2-fluoro-2-deoxy-D-glucose
HPLC	high performance liquid chromatography
m	multiplet (NMR)
m.p.	melting point
n.c.a.	no-carrier-added
NMDA	*N*-methyl-D-aspartate
NMR	nuclear magnetic resonance
q	quartet (NMR)
PET	positron emission tomography
RCY	radiochemical yield
RT	room temperature
s	singulet
t	triplett (NMR)
TLC	thin layer chromatography
TMS	tetramethylsilane

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