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Abstract

Labeling of physiologically relevant molecules with no carrier added (n.c.a.) [^{18}F]fluoride is performed with the aim of an application of these “radiotracers” in positron emission tomography. In order to find ^{18}F-labeled radioligands of the AMPA receptor on the basis of the urea structure, the first task of this work was to develop a synthesis of 4-[^{18}F]fluorophenyl urea derivatives.

A direct labeling of the electron rich, i.e. not activated, 4-fluorophenyl ureas by an S_NAr-reaction is not possible. Thus, radiosyntheses involving three or four step procedures were developed. 4-[^{18}F]Fluoroaniline was produced in the first two steps, whose synthesis has already been described several times in the literature. All those concepts were combined to give an optimum method regarding efficiency and duration. The reaction of para-dinitrobenzene with n.c.a. [^{18}F]fluoride, activated by the potassium-Kryptofix-complex, in DMSO at 120°C leads to 4-[^{18}F]fluoronitrobenzene. For the subsequent reduction of the nitro-group, palladium (black) and phosphoric acid in methanol under reflux conditions proved to be the best system to yield 4-[^{18}F]fluoroaniline. The time of synthesis until this step was 30 min with a radiochemical yield of at least 80%.

Subsequent transformation to the 4-[^{18}F]fluorophenyl ureas was performed via carbamate-4-nitrophenylesters, whereas strategies involving isocyanate compounds failed. In general, reactions of carbamate-4-nitrophenylesters with an amine yielded the corresponding urea. Hence, two different ways for the radiosynthesis are possible. One is to produce the carbamate in a reaction of [^{18}F]fluoroaniline with 4-nitrophenyl chloroformate, followed by addition of an amine of choice. To avoid one radiosynthetic step, the [^{18}F]fluoroaniline can alternatively be reacted directly with the 4-nitrophenyl carbamate of the amine.

The yields of the [^{18}F]fluorophenylcarbamate-4-nitrophenylester from [^{18}F]fluoroaniline are nearby quantitative. Subsequent reaction with n-propyl amine, cyclo-hexyl amine, benzyl amine or aniline leads to the corresponding ureas with an overall radiochemical yield of more than 70%. The shorter radiosyntheses with [^{18}F]fluoroaniline and phenylcarbamate, benzylcarbamate or cyclo-hexylcarbamate gave generally a lower RCY in the range of 50 to 70%. With the n-propylcarbamate nearly no conversion to the urea is detectable.

A direct application of this general method to preparation of n.c.a. [^{18}F]fluorophenyl ureas for the synthesis of two putative AMPA-receptor ligands of the quinoxalinedione-family (QX) failed. Even the synthesis of the stable reference substances was not successful. Therefore a change to less complex quinoxalinediones appeared necessary, and those labeling approaches led to success. Dinitro- and cyano-nitro-QX are established antagonists of the AMPA-receptor. The aromatic rings in these molecules are activated by the nitro- or cyano-group, respectively, for the S_NAr-reaction with [^{18}F]fluoride, whereas one nitro-group might function as leaving group. The relevant stable analogues fluoro-nitro-QX (FNQX) and fluoro-cyano-QX (FCQX) were synthesized as reference substances. The radiosynthesis of [^{18}F]FNQX was performed with DNQX as precursor and Kryptofix-activation in DMSO at 180°C and a reaction time of 20 min. With the low RCY of 5 – 10% and a molar activity of 1.5 GBq/µmol there was enough labeled product for first pharmacological experiments. ^{18}F-labeling of FCQX was only possible by an ^{18}F-for-^{19}F isotopic exchange reaction. Therefore, the [^{18}F]FNQX contains carrier, and the RCY with about 5% is again very low.

For the general evaluation of the affinity of FNQX and FCQX the stable reference substances were utilized in competition studies with tritiated AMPA in vitro. Their affinities in form of the inhibition constants K_i were determined with 1.4 µmol and 3.9 µmol, respectively, which are clearly lower than those of CNQX (0.5 µmol) or AMPA (0.06 µmol). Nevertheless, the general mode of structur-activity-relationship in QX-derivatives could be affirmed by these studies.

Blocking experiments with rat brain slices showed that the binding of [^{3}H]AMPA or [^{18}F]FNQX can be neither completely blocked by an excess of stable FNQX nor by an excess of stable FCQX. This is an evidence for a large fraction of unspecific binding of the ligand in brain tissue. Finally, the extremely low brain uptake of [^{18}F]FNQX, which was determined in ex vivo examinations, led to the conclusion that these substances are not suited for radiopharmaceutical application of in vivo imaging.

Zusammenfassung

Die Markierung physiologisch relevanter Moleküle mit trägerarmem (n.c.a.) [^{18}F]Fluorid erfolgt im Hinblick auf eine mögliche Anwendung dieser „Radiotracer“ als Diagnostika in der Positronen-Emissions-Tomographie. Um neue, ^{18}F-markierte Radioliganden mit Harnstoffstruktur für den AMPA-Rezeptor zu erhalten, wurde in dieser Arbeit zunächst eine Synthese von 4-[^{18}F]Fluorphenylharnstoffen entwickelt.

Eine direkte Markierung von 4-Fluorphenylharnstoffen über eine S_NAr-Reaktion an diesen elektronenreichen und daher nukleophil nicht aktivierten Aromaten ist nicht möglich. Deshalb wurden Radiosynthesen in drei bzw. vier Schritten entwickelt. In den ersten beiden Stufen wurde 4-[^{18}F]Fluoranilin hergestellt, dessen Synthese bereits mehrfach beschrieben wurde. Die vorteilhaften Aspekte all jener Synthesekonzepte wurden in Bezug auf weitere Optimierung von Ausbeute und Synthesezeit berücksichtigt. Von para-Dinitrobenzol gelangte man nach Umsetzung mit n.c.a. [^{18}F]Fluorid, aktiviert durch einen Kalium-Kryptofix-Komplex, in DMSO bei 120°C nach 3 min zum 4-[^{18}F]Fluornitrobenzol. Die nachfolgende Reduktion der Nitro-Funktion mit Palladium (schwarz) und Phosphoriger Säure in Methanol unter Rückflussbedingungen erwies sich als beste Methode, um trägerarmes 4-[^{18}F]Fluoranilin zu erhalten. Die Synthesezeit bis zu diesem Schritt betrug 30 min bei einer radiochemischen Ausbeute von über 80%.

Für die Weiterreaktion zu Harnstoffen erwies sich der Weg über aktivierte Carbamatester geeignet, nachdem Methoden über Isocyanate nicht zum Erfolg führten. Nach Umsetzung eines Carbamat-4-nitrophenylester mit einem Amin erhielt man den entsprechend substituierten Harnstoff. Bei der Radiosynthese konnte nun alternativ das [^{18}F]Fluoranilin mit Chlorameisensäure-4-nitrophenylester zum Carbamat und weiter mit einem gewünschten Amin umgesetzt werden, oder es wurde mit einem Carbamat-4-nitrophenylester eines beliebigen Amins reagieren lassen.

Es zeigte sich, dass der [^{18}F]Fluorphenylcarbamat-nitrophenylester in nahezu quantitativer Ausbeute erhalten wird. Seine Umsetzung mit n-Propylamin, cyclo-Hexylamin, Benzylamin oder Anilin führte mit radiochemischen Gesamtausbeuten von über 70% zu den entsprechenden trägerarmen [^{18}F]Fluorphenylharnstoffen. Um bei der Radiosynthese Schritte, d. h. Zeit und Aufwand einzusparen, wurde von denselben vier Aminen auch der jeweilige Carbamat-4-nitrophenylester hergestellt und dieser dann direkt mit n.c.a. [^{18}F]Fluoranilin zur Reaktion gebracht. Hierbei waren die Ausbeuten beim Phenylcarbamat, Benzylcarbamat und cyclo-Hexylcarbamat mit 50 bis 70% generell geringer, während beim n-Propylcarbamat kaum ein Umsatz erkennbar war.

Die Übertragung dieser allgemeinen Radiosynthese von [^{18}F]Fluorphenylharnstoffen auf die Darstellung zweier potentieller AMPA-Rezeptorliganden aus der Familie der Quinoxalindione (QX) schlug fehl. Schon die Synthese der stabilen Referenzsubstanzen gelang nicht.

Versuche zur Radiosynthese weniger komplexer Quinoxalindione führten jedoch zum Erfolg. Dinitro- und Cyano-nitro-QX sind etablierte Antagonisten des AMPA-Rezeptors. Durch die Nitro- bzw. die Cyano-Gruppe ist der aromatische Ring für eine S_NAr-Reaktion mit [^{18}F]Fluorid aktiviert, wobei eine Nitro-Gruppe selbst als gute Abgangsgruppe dienen kann. Als Referenzsubstanzen konnten die entsprechenden Fluoranaloge Fluor-nitro-QX (FNQX) und Fluor-cyano-QX (FCQX) hergestellt werden. Die Radiosynthese von [^{18}F]FNQX erfolgte mit DNQX als Vorläufer unter Kryptofix-Aktivierung in DMSO bei 180°C innerhalb von 20 min. Die radiochemische Ausbeute mit 5 - 10% bei einer molaren Aktivität von 1,5 GBq/µmol war gering aber ausreichend für erste pharmakologische Untersuchungen. Eine ^{18}F-Markierung von FCQX gelang nur über einen ^{18}F-für-^{19}F Austausch mit ebenfalls niedrigen Ausbeuten.

Zur pharmakologischen Evaluierung wurden nicht radioaktives FNQX und FCQX in Kompetitionsstudien mit tritiiertem AMPA in vitro eingesetzt, um ihre Inhibitionskonstante K_i zu bestimmen. Es zeigt sich, dass ihre Affinität zum AMPA-Rezeptor (1,4 µM bzw. 3,9 µM) im Vergleich zu CNQX (0,5 µM) oder AMPA (0,06 µM) deutlich niedriger war. Die Werte ordnen sich dabei gut in die Struktur-Wirkungs-Beziehung des allgemeinen Substitutionsschemas der QX ein. In Blockierungsexperimenten an Rattenhirnschnitten zeigte sich, dass die Bindung von [^{3}H]AMPA oder [^{18}F]FNQX weder durch einen Überschuss an FNQX noch an FCQX vollständig verhindert wird. Dies spricht für einen hohen Anteil unspezifischer Bindung der Liganden im Hirngewebe. Ex vivo Untersuchungen mit [^{18}F]FNQX an Ratten ergaben, dass die Hirnaufnahme des Radiotracers extrem gering ist. Diese Ergebnisse schließen eine Nutzung der Substanzen als Radiopharmaka für die in vivo Bildgebung aus.

Contents

1 Introduction

1.1 Radiopharmaceuticals

The broad use of radioactive nuclides in medicine can generally be divided into two fields. On one hand, there are therapeutic, radioactive pharmaceuticals, with the purpose to destroy malignant tissues endogenously. On the other hand, there exist several diagnostic methods using the tracer principle and the penetrating properties of γ-rays.

This work deals with the synthesis and evaluation of putative ligands for the AMPA receptor system as diagnostic radiopharmaceuticals. The radioactive label fluorine-18 is a positron emitting nuclide, most suitable for in vivo positron emission tomography (PET) studies.

1.1.1 Therapeutic and diagnostic radiopharmaceuticals

In most cases radiation resulting from a nuclear decay causes more or less damage or destruction of biological tissues. This might be used for medical treatment, if one can deposit a radioactive nuclide in malignant tissue. The goal is to localize the destruction in order not to affect healthy tissues.

For the placement of the radionuclide several alternatives are suitable. Direct implantation of a capsulated probe of radioactive material is done with heavy metal nuclides. This so called brachytherapy requires a physical invasion and is only useful for large tumors[1]. Since the radioactivity is located there, metastases are not reached at all.

Another localized application of a radioisotope is that of yttrium-90. It is injected as solution of [^{90}Y]YCl into the capsule of infectious, aching joints and stays there. The curative effect of radioactivity in this case is palliative, as neurons are destroyed.

Some chemical elements accumulate in a defined chemical form directly in the tissue of interest. The iodide anion, for example, accumulates in the thyroid. Therefore [^{131}I]iodide is a very common therapeutic agent against thyroid cancer[2].

The most versatile method for deposition of radioisotopes in selected tissues is the use of labeled pharmaceuticals as vehicle for the radioactivity. A designed compound, carrying a radioactive atom, is administered to the organism. This compound, or metabolic products of it, should have high affinity to the target tissue. This affinity might result from incorporation into special cells, for example by trapping mechanisms. Integration of the pharmaceutical into DNA or RNA can be examples for trapping. Outside the cell the pharmaceutical could bind to proteins on the cell surface[3].

The most important requirement of all these mechanisms is, that the radioactivity is localized in malignant tissue for its whole lifetime. It may not affect other parts of the body. The lifetime of therapeutic radionuclides should be in the range of few days. This is the compromise between the radiation dose, given to the healthy tissue, effect on malignant tissue and controllability of the substance in the body.

Suitable decay modes for therapeutic uses are Auger electron emission, beta minus and alpha decay. The emitted corpuscles differ in their spatial range and their potency. The low energy Auger electrons might only destroy the cell nucleus, if the nuclide is there. Electrons from the beta minus decay, which have much higher energies, destroy tissue in a sphere of up to 100 cells. Alpha particles with their high mass and energy deposition reach only neighboring cells.

The boron neutron capture method does not use radiopharmaceuticals, but rather a nuclear reaction that is generated in situ[4]. A pharmaceutical prodrug, that contains many boron atoms, is administered and has to accumulate in the target tissue. Afterwards, the patient is irradiated with slow neutrons. From the $^{10}B(n,\alpha)^{7}Li$ reaction result an α-particle and a ^{7}Li nucleus, which destroy the surrounding tissue, as they are in a highly excited state and bear a large amount of recoil energy.

While destruction is the purpose of therapeutic radionuclides or irradiation therapy, diagnostically used isotopes should not harm the body at all. Gamma rays of adequate low energy penetrate the body, while their energy deposition is minimal. This kind of radiation is found in nearly all decay modes, but only some nuclides are suitable for SPECT or PET diagnostics as described below and in the following chapters. Gamma rays of defined energy should be the only or at least the major part of the occurring radiation. Otherwise, there is too much noise in the detection process and the body is needlessly strained. For minimization of the dose given to

the patient the half life of the radionuclide should ideally not be longer than the diagnosis lasts. This is in the timescale of minutes to several hours.

If a suitable nuclide and an adequate vehicle are found, the diagnostic radiopharmaceuticals enable functional examinations of physiological processes. This is done by correlation of the distribution of the radioactivity in an organism and the metabolic fate of the labeled molecule.

1.1.2 SPECT and PET as diagnostic methods

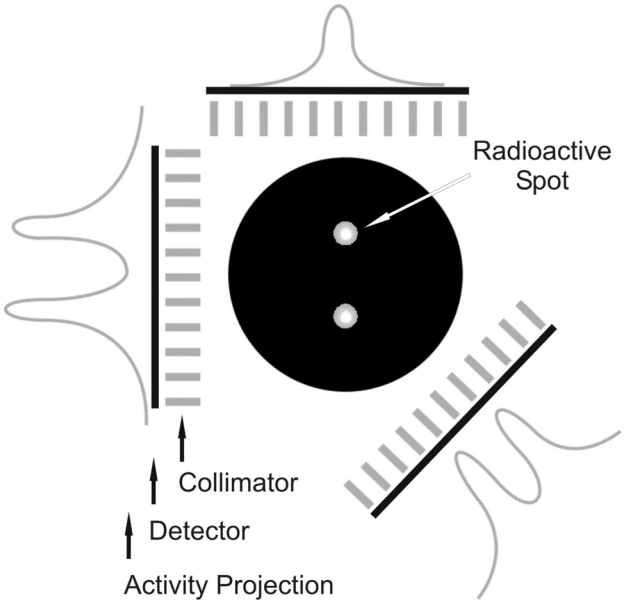
Single Photon Emission Computer Tomography – SPECT

This tomographic method uses tracers which are labeled with a radionuclide, that ideally emits only one detectable gamma ray of defined energy. Every other radiation should be negligible. This is the case for some nuclei that decay by the electron capture process such as iodine-123 or thallium-201. The energies of their main gamma ray of 159 keV or 167 keV, respectively, are high enough to penetrate the body and close to the detection maximum for common Na(Tl)I crystal γ-detectors. Molecules labeled with iodine-123 or $[^{123}I]I^-$ and $[^{201}Tl]Tl^+$ themselves find used as tracers for this purpose.

Besides these and several other nuclides the isomeric transition of technetium-99m to technetium-99g (E_γ = 141 keV; $T_{1/2}$ = 6.1 h) plays an outstanding role in nuclear medicine. The decay of ^{99}Mo with a physical abundance of 87% and a half life of 66 h leads to ^{99m}Tc. These properties are very well suited to make use of the generator technique. ^{99}Mo, that is produced by neutron induced fission of uranium in a reactor and carefully isolated from other fission-products, is fixed on a lead shielded Al_2O_3-column in the chemical form of MoO_4^-. This column-generator might be distributed over longer distances due to the half life of ^{99}Mo. The ^{99m}Tc generated in the form of $[^{99}Tc]TcO_4^-$ can be eluted from this column with an aqueous saline solution while $[^{99}Mo]MoO_4^-$ stays fixed. This solution is a radiopharmaceutical itself for bone scintigraphy or blood flow measurement. Otherwise the pertechnetate anion can be transformed into several different chemical forms. By appropriate complexation of the metal core, a tracer for special applications can be prepared. Those syntheses take place in ready-made kit-systems, where the radioactive

solution of TcO_4^- is added into an ampule which contains all chemicals needed. After a defined time stirring the radiopharmaceutical is ready for administration[5].

In general, the SPECT tomogram is recorded following a strict medical protocol. The camera consists of three or five collimated detector blocks placed around the patient's body as shown in Scheme 1 [6]. The detectors are moved in a circular path around the object until they together cover a field of view of 360°.



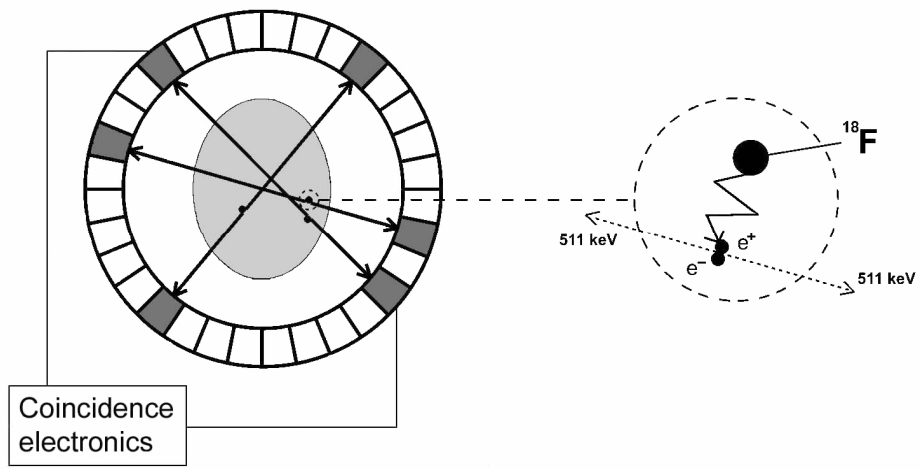
Scheme 1: Schematic setup of a SPECT system; Detectors and collimators rotate 120° around the body [modified from 6]

By using collimators, every signal of a detector can be allocated to a decay on a line perpendicular to the detector plane. This is one geometric information that is needed for the three-dimensional reconstruction of activity distribution. The other information is gained from mathematical algorithms, in which the frequency of signals is combined with the geometric parameters of the measurement. The tomographic images, that results from this procedure, show the planar distribution of the radionuclide in the body.

Positron Emission Tomography – PET

The PET method is more sophisticated than SPECT but also more limited due to its premises. A PET camera consists of several closed rings of many radiodetectors placed around a patient as shown in Scheme 2. The β^+-decay itself cannot be detected, but the resulting positron is the corresponding antimatter to an electron. After loosing its energy from the decay by being scattered in matter and then colliding at an adequately low energy with an electron, both annihilate to two γ-quanta, each with an energy of 511 keV. Another annihilation mode with three photons is possible but very rare[29]. The 511 keV γ-quanta are emitted antipodal and simultaneously. They penetrate the body and might be detected in two separate detectors of the ring. A connected computer system then assigns a decay event on a direct line between the segments to these two signals, if they occur in coincidence. This is one major advantage of PET against SPECT. Noise from incidental radiation is minimized. The spatial resolution of the images is much higher, since it is not physically limited by the collimator geometry, but by the distance the positron needs to become thermalized. This is only few millimeters for suitable positron emitters.

The camera has not to be moved, because the ring of detectors is closed, and the field of view already comprises 360°. The reconstruction of the tomographic images is also done with mathematic algorithms depending on the abundance of registered events.



Scheme 2: Setup for a PET measurement;
The zoomed part in the right illustrates an annihilation event [from 19]

The biggest advantage, however, is that besides the graphical information of the radionuclide distribution, its amount can be quantified. For this purpose an additional measurement is necessary. In a so-called transition measurement with a focused γ-ray beam of known intensity penetrates the body by external irradiation. The source is moved on a circular path at the inside of the detector ring. The detectors on the opposite site of the ring detect a signal, that correlates with the loss of intensity of the beam in the body in the given projection. In summa, upon the β^+-annihilation both γ–quanta travel the same distance and therefore their intensity is reduced by the same factor as the single beam. With the known attenuation of this external γ-ray, the measured intensity of the annihilation-quanta can be corrected and thus quantified.

To gain quantitative medicinal relevant information from the distribution of the radioisotope in the body, the temporal and local change of the radiotracer has to be combined with a suitable bio-mathematical compartment model describing the pharmacokinetic behavior of the pharmaceutical[15].

1.1.3 The tracer principle

In 1911 Georg de Hevesy and Fritz Paneth first tried to separate the so called radium-D from lead. They did not know at that time, that this was the lead isotope Pb-210. When they had realized this fact, they started to use the chemical identical behavior of isotopes for labeling of stable probes with radioactive additives[7]. They gave trace amounts with defined activity of the radioactive lead isotope to a macroscopic mass of lead and mixed it carefully. When they took a small amount of the mixture and measured its activity they could calculate which part of the starting activity this was. If they knew how much lead was in the starting probe, they know how much lead is in the small amount of the mixture. If they determined the amount of lead in the part of the mixture, they could calculate how much lead was in the starting probe. Thus radioactivity measurement replaced weighting. This “dilution analysis” is applicable in many different ways. Solubility, velocity of diffusion processes and a lot other things can be determined.

After several “inorganic” applications Hevesy made the next step and administered radioactive minerals to plants in 1923 [8]. This was the first tracer experiment with living organisms[8,9]. Hevesy was the first who used radioactive material to visualize

metabolism. He first gave small amounts of labeled lead, using thorium-B (Pb-212) as indicator, to a plant. Afterwards the incorporated activity could even be displaced with an excess of stable lead, which was given to the living organism. The method was limited to natural occurring isotopes until Curie and Joliot found the way to produce artificial radioactivity[10]. Fermi invented a procedure to produce phosphor-32[11]. This is an isotope of an element with a high abundance in all organisms. Using this biological element, physiological sequences could be traced without disturbing the organism, as the used tracers were part of the natural process. Finally the invention of the cyclotron by Lawrence made a lot of radioisotopes at a high level of activity available[12].

1.1.4 Requirements for PET pharmaceuticals

Today the cyclotron is the major source for positron emitters. However, the combination of the tracer principle and the use of the geometric characteristics of the annihilation γ-quanta in vivo took about fifty years until Mike Phelps et al. invented the PET-camera in 1975 [13]. For this application of tracers labeled with positron emitters together with PET, special requirements have to be fulfilled for the radioisotope and the resulting radiopharmaceutical.

Most of the used tracers are labeled molecules. The chemistry for the synthesis has to be easy to handle and should ideally not last longer than one half life of the nuclide. Typically the labeled molecules are artificial tracers. Therefore large amounts of the substance would disturb the physiology of the organism or even be toxic. The minimization of material is one of the most important challenges for syntheses. Subnanomolar quantities of radioactive nuclei – some 100 MBq – create enough signals to construct a tomographic image. Isotopic dilution with stable nuclei (carrier) has to be as little as possible. If there is no carrier added (n.c.a.) one can assume, that the organism is not disturbed at all[14].

For radiotracers of the central nervous system the blood brain barrier (BBB) is another major hindrance for application. The BBB protects the brain especially against polar (ionic) substances (with toxic properties) from the regular blood circuit.

If the bioorganic behavior and metabolic stability of the radiotracer is known, the quantified distribution of radioactivity in the body can be correlated with functions or

dysfunctions of the organism. Therefore, a mathematical compartment model is drafted[15]. It describes the relation of the rate constants on the tracer's metabolic pathway. The time-dependent change of radioactivity in defined regions of the tissue of interest is registered and the non-metabolized fraction of the administered radiotracer in blood are monitored[16]. With this data the rate constants can be calculated. The general limitation of this procedure is, that models with more than three compartments are too complex for exact calculations. Therefore, metabolic pathways with less than four compartments allow to quantify bioorganic processes. However, this feature is by now outstanding for non-invasive tomographic methods.

Quantified SPECT measurements and functional MRI studies are under development but not as established as PET at the moment.

1.2 Production of PET-nuclides and synthesis therewith, especially ^{18}F

1.2.1 PET-nuclides

The β^+-decay of neutron-deficient radionuclides is competing with the electron capture decay mode. In both cases a proton transforms into a neutron:

$$\beta^+\text{-Decay:} \quad p \longrightarrow n + e^+ + \nu_e$$

$$\text{Electron capture:} \quad p + e^- \longrightarrow n$$

Neutron deficient isotopes can generally not be produced in neutron sources (e. g.: reactor, spallation source etc.) or from fission products, because these procedures lead to neutron rich isotopes. Mainly, β^+-emitters are produced by irradiation of stable nuclides with charged particles in a cyclotron. This facility is useful for large medical institutions with an on-site production laboratory for radiopharmaceuticals or for a company which produces on demand and distributes the radiopharmaceuticals to medical facilities[17].

According to the requirements for PET pharmaceuticals there are only a few radionuclides, which are suited for application. Some of them are listed in Table 1

together with their important physical properties and most common production routes.

The multitude of parameters, like half life, β^+-energy and chemical behavior, entails very special applications for most of the nuclides. ^{82}Rb for example is available from a ^{82}Sr-generator system of long $T_{1/2}$ which can be distributed over large distances. Its short half life of $T_{1/2}$ = 13 min in contrast demands fast handling and prompt application. As a alkali metal it is used as blood flow tracer in cardiology in form of the cation.

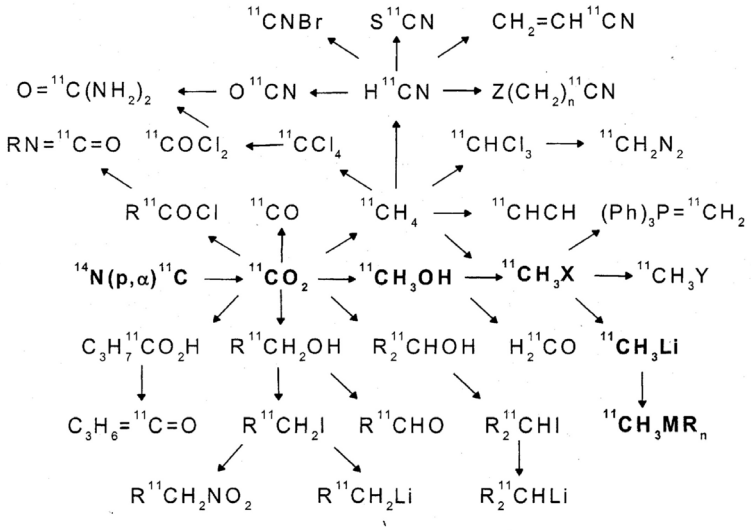
Table 1: Important PET-nuclides with their nuclear data and common production routes[18]

Nuclide	Half life	Decay Modes	Maximum β^+-Energy [MeV]	Production Route
^{11}C	20.4 min	β^+ (99.8%); EC (0.2%)	0.96	^{14}N(p,α)^{11}C
^{13}N	10.0 min	β^+ (100%)	1.19	^{12}C(d,n)^{13}N
^{15}O	2.0 min	β^+ (99.9%); EC (0.1%)	1.72	^{14}N(d,n)^{15}O
^{18}F	109.7 min	β^+ (97%); EC (3%)	0.64	^{18}O(p,n)^{18}F
^{73}Se	7.1 h	β^+ (65%); EC (35%)	1.30	^{75}As(p,3n)^{73}Se
^{75}Br	98.0 min	β^+ (75.5%); EC (24.5%)	1.74	^{76}Se(p,2n)^{75}Br
^{82}Rb	1.3 min	β^+ (96%); EC (4%)	3.35	generator ^{82}Sr ($T_{1/2}$ = 25 d)
^{120}I	1.4 h	β^+ (64%); EC (36%)	4.10	^{122}Te(p,3n)^{120}I
^{124}I	4.2 d	β^+ (25%); EC (75%)	2.14	^{124}Te(p,n)^{124}I

^{73}Se has a relatively long half-life, which allows chemical syntheses and shipment, but besides the positron emission also one third of disturbing EC decay occurs. Furthermore the daughter nuclide ^{73}As is not stable, but has a half life of 80.3 d. Several radiosyntheses with radioselenium are developed. However, the applicability of corresponding tracers has not been demonstrated so far[19,20].

Syntheses with positron emitting bromine and iodine isotopes have been performed since the beginning of PET chemistry[21] but are also in the scope of actual research. Especially ^{124}I and ^{120}I seem to be interesting due to their chemical versatility and physical half-life, even though that positron decay represents only one fourth or two third, respectively, of their radiation[22].

Besides the chemical and physical properties of radionuclides their physiological behavior and acceptance is most important. From the group of commonly occurring elements in organisms ^{15}O, ^{13}N and ^{11}C represent suitable PET-nuclides. Their half-life is generally short, making an on-site cyclotron facility necessary. Complex chemical syntheses with ^{15}O and ^{13}N are not reasonable, because most of the produced activity is lost during the process. The tracers used are $[^{13}N]NH_3$, $[^{15}O]O_2$, $[^{15}O]H_2O$ or $[^{15}O]C_4H_9OH$ mostly for perfusion measurement[18].



Scheme 3: "Zoo" of small molecules for $[^{11}C]$carbon labeling [modified from 23]

Carbon-11 with a half life of 20.4 min offers the great possibility of an authentic labeling of biomolecules. For that reason, great efforts are taken to develop fast chemical syntheses with $[^{11}C]CO_2$, which is already formed in the target. A whole "zoo" of small molecules[23], which is shown in Scheme 3, is available to incorporate ^{11}C in following steps into a biomolecule. The most convenient and widely spread method is to produce $[^{11}C]$MeI or $[^{11}C]$MeTos. With those a "precursor" molecule is methylated afterwards[24]. This simple and fast procedure is sufficient, therefore many

biomolecules or pharmaceuticals comprise an ester or amide function and are not even changed by being labeled.

This fact opens three directions of research for authentically labeled molecules:

- If the functional mechanisms of an organism are unknown, the behavior and metabolism of the radiotracer can clarify pathways.
- If all functions of the organism and the tracer's metabolism are known, a PET examination is useful as a diagnostic tool to visualize dysfunction.
- Drug development can be facilitated, because the pharmacodynamics and –kinetics of new substances can easily be monitored in vivo.

Fluorine-18, as the most important PET nuclide, is only occasionally an authentic label. Mostly, ^{18}F-compounds are analogue tracers, whose biochemistry cannot be described by the compartment models of the original compounds.

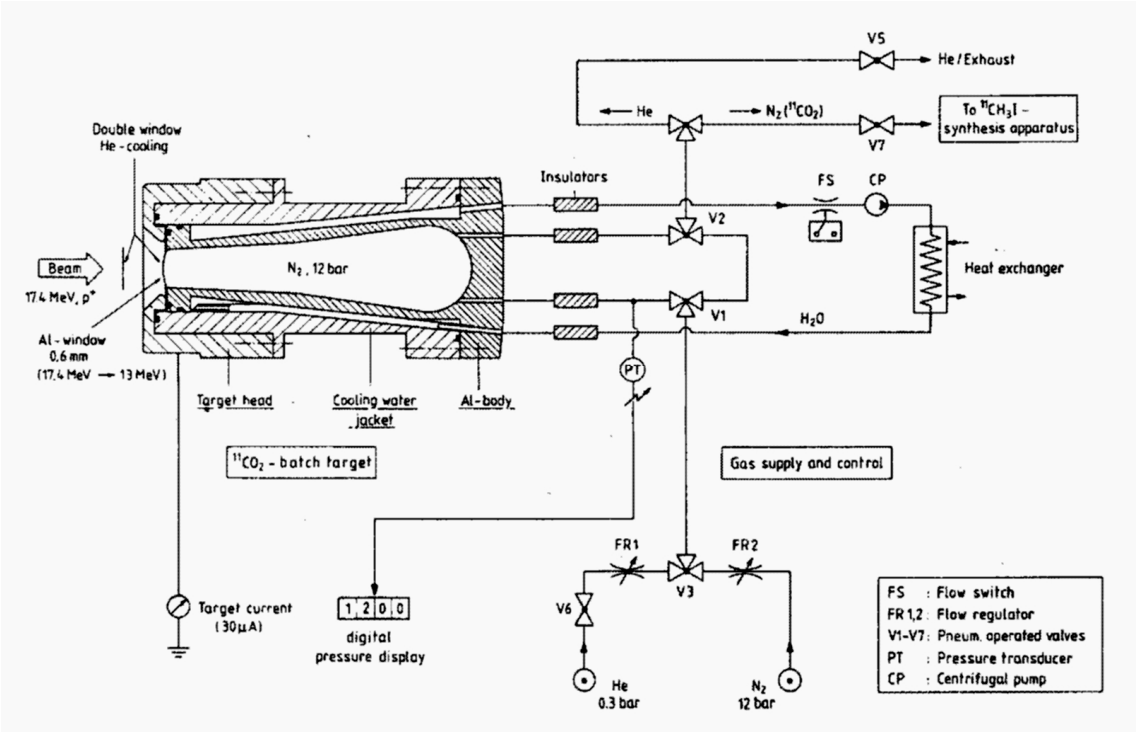
The importance of ^{18}F arises from several factors. The half life of 109.7 min is very well suited for complex syntheses, longer PET studies or a broad distribution to medical facilities after a short synthesis. The maximum β^+-energy of 640 keV is one of the lowest of all positron-emitters. This leads to the best resolution of images with PET.

1.2.2 Cyclotron production of radionuclides

Production of radionuclides with a cyclotron includes the irradiation of a target with accelerated charged particles like protons, deuterons, $^3He^{2+}$ or α-particles. Fast separation of the produced isotopes in a defined chemical form, without contamination by coproducts or target material, is essential.

Solid targets, as they are used for the production of ^{73}Se (irradiation of Cu_3As or AlAs) for example, offer a high density of material. The probe is only about one cm in diameter and just several mm thick. Cooling of the irradiated pill or plate can be performed easily, but the separation of the product takes some effort. For ^{73}Se it is done with a multistep thermochromatography at several hundred degrees Celsius in a stream of oxygen[19,25].

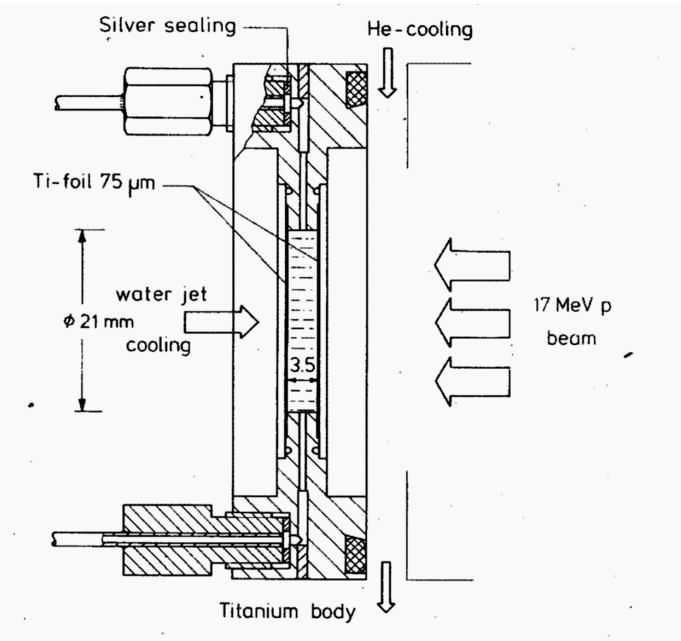
In a gas target the molecules are of course much less closely packed, which leads to larger dimensions. The bulb volume of about 200 cm^3 of a nitrogen target (Scheme 4) is shaped like this, because the focused proton beam is spread by scattering in the target. The carbon atoms formed by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction are in a highly excited state and bear a lot of recoil energy. These “hot atoms” react with traces of oxygen to finally form $[^{11}C]CO_2$. These molecules might be separated easily from the carrier gas nitrogen by freezing out the $[^{11}C]CO_2$ in a cooling trap.



Scheme 4: Gas target and control scheme for $[^{11}C]CO_2$ production [from 18]

Also for the production of fluorine-18 there is an established gas target available. The $^{20}Ne(d,\alpha)^{18}F$ reaction is useful, since the natural abundance of ^{20}Ne is over 90% and the target material has to be pure, but not isotopically enriched. Again the radioisotopes are generated in a highly excited state, with much recoil energy and react with random partners. The produced amounts in the subnanomolar range do not allow to form $^{18}F_2$ for statistically reasons. The only way to prevent chemical absorption on the target wall is to add $^{19}F_2$ carrier gas in concentrations of 0.1 to 0.2%. After the nuclear reaction carrier-added (c.a.) $[^{18}F]F_2$ is formed, which can be used for electrophilic labeling reactions.

For production of n.c.a. fluorine-18 another nuclear reaction is used. Here, the $^{18}O(p,n)^{18}F$ in isotopically high enriched water is the method of choice[26]. Water is a dense material, which can be handled easily. The target volume is about one to two cm^3 in a stainless steel plate with an aluminum or titanium window (Scheme 5).



Scheme 5: [^{18}O]Water target for production of [^{18}F]F$^-$ [from 18]

The produced ^{18}F is dissolved as $[^{18}F]F^-$ in the enriched water. Separation of the radionuclide is performed by retrieving the fluoride on a carbonate form ion exchange resin[27] or electrochemical, anodic absorption[28]. In both cases the enriched water can be recycled easily. After recovery, it has to be purified and might be used for several further irradiations. Isotopic dilution by natural water is the limiting factor for its devaluation.

Theoretically, this process leads to carrier free (c.f.) $[^{18}F]F^-$. Nevertheless, as fluorine is present nearly everywhere in the environment and especially in the technical world, isotopic dilution of the ^{18}F with natural occurring ^{19}F cannot be prevented. Thus, it should be minimized by avoiding materials containing fluorine. These are of course perfluorinated polymers in valves and tubing, but also several solvents and reactants in chemical reactions. Since the theoretically calculated possible molar activity is never reached for a naturally occurring element, one has to speak of no carrier added conditions.

1.2.3 Chemistry under no carrier added conditions

The most typical example for first order reaction kinetics is the radioactive decay. The general equation in this case is[29]:

$$N_t = N_0 \cdot e^{-\lambda \cdot t}$$

N: Number of particles

λ: Decay constant

Activity is defined as decays per time (dimension: 1 s^{-1} = 1 Bq):

$$A_t = \frac{dN_t}{dt}$$

Following this definition the number of particles for a concrete activity is given as:

$$N = \frac{A}{\lambda}$$

For fluorine-18: $\lambda = 1.05 \cdot 10^{-4}\ s^{-1}$

Average production at a small cyclotron: A = 55 GBq

$$N_{^{18}F} = 5.79 \cdot 10^{14} \quad (9.62 \cdot 10^{-10}\,mol)$$

As outlined in chapter 1.2.2 this number is valid for carrier free conditions, which are never reached for fluorine-18. However, the dilution with stable fluorine-19 leads to amounts of atoms in the nano-molar range, where one has to speak of no carrier added conditions. Thus it appears, that chemical reactions with the resulting concentrations of n.c.a. fluorine-18 follow special kinetics. The precursor molecules

(P) are present in an enormous excess. Their concentration should approximately not decrease due to reactions with the radioisotope (R*) to yield the labeled compound (L*).

This issue can be described with kinetics of pseudo first order[30]:

$$P + R^* \longrightarrow L^*$$

$$-\frac{d[L^*]}{dt} = k' \cdot [R^*] \quad \Leftrightarrow \quad k' = k \cdot [P]$$

Using these premises rate constants can be determined quite simple with radioactive labels. In order to take into account the radioactive decay of the nuclide the radiochemical yield (RCY) has to be used, which is corrected for the decay. The absolute yield – measured as activity at a defined time t - of reaction is projected to t = 0 and then divided by the starting activity.

In general, the quality of a radiolabeling reaction can be evaluated by the following major criteria:

- Duration of the process in relation to the half-life of the used radionuclide.
- Simplicity of the handling and number of steps with radioactive material.
- Chemical and radiochemical purity of the product.
- Molar activity (Bq/mol) of the product.

1.2.4 Electrophilic labeling with fluorine-18

The gas target for ^{18}F-production leads to c.a. [^{18}F]F_2 as discussed above. This starting material offers the possibility of electrophilic reactions as they are known from organic chemistry.

Addition of [^{18}F]F_2 to a double bond and subsequent hydrolyses was an important application as it was the first way to yield [^{18}F]fluorodeoxyglucose[31]. Besides this important aliphatic compound, an aromatic electrophilic substitution reaction leads to the often used 6-[^{18}F]fluoro-DOPA (see chapter 1.2.6 for details).

Both examples show the usefulness of elemental fluorine gas as a labeling reagent, since aliphatic and electron rich aromatic precursors can be labeled easily. The limitation is, that the formation of isomers cannot be excluded. To minimize the formation of isomers three mechanisms are used.

One is to generate a more mild fluorination agent from F_2. One of these agents is [^{18}F]AcOF, what is formed in a column filled with AcOK and AcOH. The isomeric selectivity is about twice as high as with fluorine gas for addition reaction to double bonds[32]. For substitution reactions on arenes this effect is much less pronounced[33].

The second mechanism is to tune the reaction conditions as there are acidity of solvents, temperature and time. Some remarkable effects can be found, individual for each reaction[34, 35].

Finally, the synthesis of a precursor with a designated leaving group is reasonable. Here, fluoro-demetallation of stannyl or mercury molecules is the method of choice. Destannylation is preferred in order to prevent possible contamination of the product with mercury[34].

All these radiosyntheses are performed with carrier added [^{18}F]F_2. The highest RCY, that can theoretically be obtained, is 50% because only ^{18}F-^{19}F or ^{19}F-^{19}F molecules are present. If there is a ^{18}F atom in the fluorine molecule, it is only built into the product with the statistical abundance of 50%. The other half of activity is lost as the “nucleophilic part” of the reaction.

Against these general constraints stands the synthetic potential of electrophilic labeling. That is why several methods are under development to produce an electrophilic labeling agent from n.c.a. [^{18}F]fluoride.

An already used alternative is to form [^{18}F]MeF from n.c.a. [^{18}F]F^- and MeI. The purified product is mixed with an minimum of carrier $^{19}F_2$ in Ne gas (150 nmol – 1.5 µmol). The gaseous mixture is atomized by electric discharge (20 – 35 kV, 400 µA, 10 s). Recombination of the components leads to [^{18}F]F_2 with a medium amount of added carrier and molar activities of about 55 GBq/µmol can be reached[36].

1.2.5 Nucleophilic labeling with fluorine-18

Fluorine-18 has become the radionuclide, that is most often used for routine diagnosis with PET, because many radiopharmaceuticals are readily available by nucleophilic labeling reactions under no carrier added conditions. The preparation und use of those compounds have been summarized in many recent reviews[34,37,38,39,40, 55].

Nucleophilic labeling is generally done with n.c.a. [^{18}F]fluoride, which is formed in an ^{18}O-enriched water target. After recovering of [^{18}O]H_2O it is solved in pure water. However, the aqueous environment is not suitable for these reactions. The anion is protonated or at least deactivated by a solvent cage. That is why the solvent for the reaction has to be aprotic. Most common solvents are acetonitrile, DMF or DMSO.

The change of solvent is done by an azeotropic distillation with acetonitrile after addition of a base, which prevents formation of HF in the radioactive solution. The formed azeotropic mixture with about 85% of water and 15% of acetonitrile leaves dried [^{18}F]F^- behind. The fluoride is dissolved again, now in the solvent of choice. Drying can also be performed in the electrochemical cell as described above[28].

For good yields in nucleophilic substitution reactions, another factor has to be considered. A counter cation can foreclose or promote chemical reaction. Fluoride forms a strong bond with a proton or small cations. Therefore they are completely useless. Also the potassium cation alone would be to small and hard. However, special aminopolyethers form voluminous complexes with the cation. The ionic partner for the fluoride anion is now much softer and its reactivity is increased dramatically. Kryptofix 2.2.2. is the aminopolyether which is generally used as “cryptand” for potassium[41, 42, 43]. The higher homologues of the alkali metals in some cases do not need to be complexed, since the ionic bond is not as strong. Another alternative for weak ionic bonds is the use of tetra-alkylammonium cations, which are generally used as phase transfer catalysts.

For all systems, carbonate as basic compound shows the best results. Sometimes buffering with oxalate is necessary, if the precursor is not stable under extremely basic conditions[44, 45].

Thus, the underlying chemical reaction type for labeling with n.c.a. [^{18}F]fluoride is nucleophilic substitution. For aliphatic compounds in aprotic dipolar solvents it proceeds by the S_N2 mechanism. The fluoride-anion approaches the carbon from the opposite of the leaving group. Thus, the substitution leads to a stereochemical inversion at an asymmetrically substituted atom.

Aliphatic labeling can be performed at moderate temperature in acetonitrile. Leaving groups are halogens or mesylate, tosylate and triflate esters. This diversity of possibilities most often and mild reaction conditions often allow a direct labeling. A precursor is to be synthesized with a leaving and if necessary protection group. After the labeling step and deprotection the radiotracer is purified by fast radiochromatogrphic methods and then applicable.

For compounds where fluorine should be attached to an aromatic ring, the reaction is much more difficult. In general, the aromatic nucleophilic substitution S_NAr requires an activated compound. These are electron deficient aromatic rings with an electron withdrawing substituent in ortho- or para-position[46]. Strongly activating are the benzaldehyde, cyano or nitro group. Good leaving groups are again halogens, the nitro group or a trimethylammonium cation[47]. In contrast to aliphatic compounds, in arenes the ^{19}F is the best leaving group beside the trimethylammonium cation. However, the use of fluorine is limited, since the product would contain carrier in form of the precursor after an isotopic exchange reaction.

More, non-activating or even de-activating substitutes at the aromatic ring lower the yield of the labeling reaction, which takes generally place at high temperatures above 120°C. That is why direct labeling of complex aromatic compounds is often difficult. The aimed pharmaceuticals are not stable under those labeling conditions or do not bear activating substituents.

A loophole in this situation could be the use of microwaves. In a microwave the reaction energy is transferred directly to the molecules without raising the solvent's temperature. Sensitive functions in a complex molecule might not be affected. Microwaves are applied already in several labeling reactions[48].

A change of concept is the utilization of the iodonium chemistry. Two electron rich aromatic rings are bridged over an iodine atom. The iodine is oxidized to give an iodonium salt[49]. The offered nucleophilic n.c.a. [^{18}F]F^- binds to one of the aromatic rings. This direct labeling of electron rich aromatics is under development[50, 51].

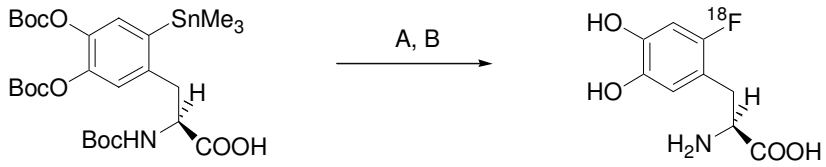
If direct labeling fails, built-up multi-step reactions have to be utilized. The most common strategy is to label a short alkyl chain at one end with ^{18}F in a first step. The other end functions as a linker. This linker could be a leaving group for another S_N2 reaction[52, 53] or a protected alcohol or amine for condensation reactions in the second step[54]. The fluoroalkylation often leads to analogue tracers, which should not extremely change their biochemical behavior or loose anything of their functionality compared to the original molecule.

Aromatic built-up reactions consist of several steps. Starting from simple, labeled compounds like fluoronitrobenzene, fluorobenzaldehyde, fluorohalobenzene or fluorobenzonitrile a manifold of second step reaction is possible. Direct linkage to a suitable precursor would be preferable. But the half-life of ^{18}F allows to perform more extended chemical reactions. So called synthons like [^{18}F]fluoroaniline or [^{18}F]fluorophenol represent electron rich arenes that can be prepared in less than an hour and are important parts in complex synthetic strategies for labeling.

Multistep reactions are very intricate for automation. That is the main hindrance for many potential tracers to be widely utilized in medicine. Application demands starting activities of up to 55 GBq, what implies shielding and automated processes.

1.2.6 Examples of ^{18}F-labeled tracers with application in PET studies

The following examples should give a brief insight into the chemical feasibility of fluorine-18 labeling and the diversity of applications of PET-tracers, which are established as diagnostic tools or under clinical evaluation of their potence[55].

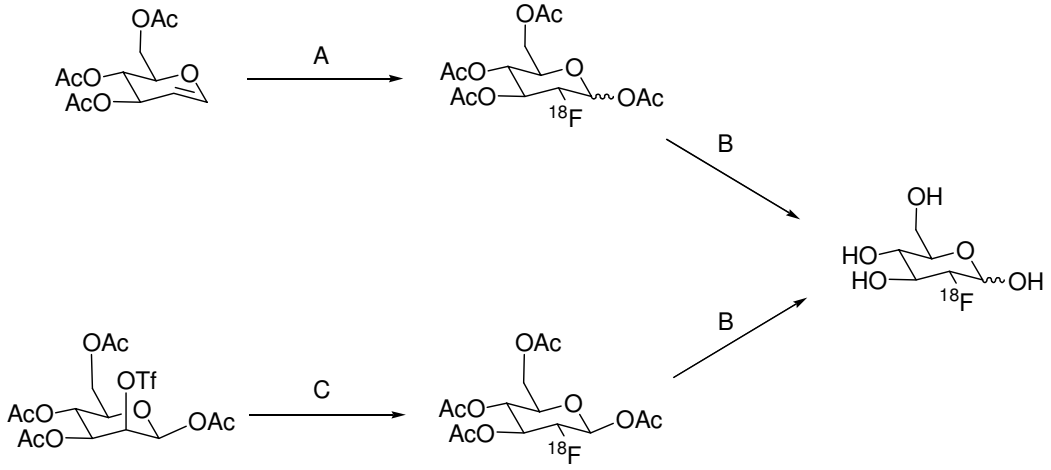


Scheme 6: Electrophilic radiosynthesis of 6-[^{18}F]fluoro-L-DOPA [57, 58]
(A) [^{18}F]F_2, $CFCl_3$, RT, 10 min; (B) HBr 48%, 130°C, 10 min

The first molecule discussed is 6-[^{18}F]fluoro-L-DOPA. DOPA itself is a precursor of dopamine, the neurotransmitter of the dopaminergic neurons in the central nervous

system (CNS). 6-[^{18}F]fluoro-L-DOPA follows nearly the whole metabolic pathway of DOPA, both in the blood and in the brain. Due to this fact there exists no simple compartment model for quantification of the metabolism of the radiotracer. Nevertheless, the integrity of the presynaptic innervation of the dopaminergic system can be studied qualitatively by a PET examination with 6-[^{18}F]fluoro-DOPA. The most important applications are in the diagnoses of Parkinson's Disease[56].

Since the aromatic ring in DOPA is not activated for the S_NAr-reaction with n.c.a. [^{18}F]F$^-$ the simplest way of synthesis is the electrophilic demetallation with c.a. [^{18}F]F_2, as depicted in Scheme 6 [57]. This synthetic approach is established and automated[58]. Even as the RCY is not very high and the product comprises carrier, it is sufficient for application, whereas it is not toxic. The still little amount of artificial carrier 6-fluoro-DOPA is tolerated by the organism given the huge excess of endogenous DOPA.



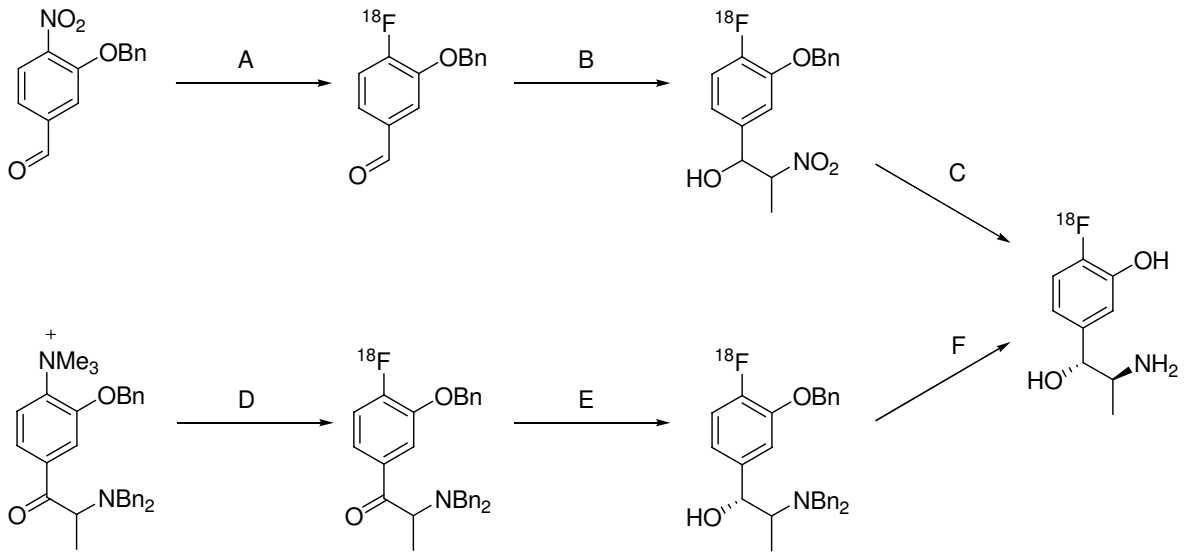
Scheme 7: Electrophilic and nucleophilic radiosyntheses of 4-[^{18}F]FDG [59, 60]

(A) [^{18}F]AcOF, $CFCl_3$, RT, 10 min;
(B) HCl 1 M, 130°C, 15 min; RCY: via (A) ca. 20%, via (C) ca. 60%;
(C) [K⊂2.2.2][^{18}F]F, MeCN, reflux, 5 min

Another example for an applied radiotracer comprising carrier was c.a. 2-deoxy-2-[^{18}F]fluoro-D-deoxyglucose ([^{18}F]FDG). The excess of the authentic substance glucose present in the body against the analogue tracer is immense, and the amount of carrier causes no negative effects in the organism. [^{18}F]FDG, like 6-[^{18}F]fluoro-DOPA, was first prepared by an electrophilic synthesis. The addition reaction of [^{18}F]AcOF with 3,4,6-tri-O-acetyl-D-glucal is depicted in Scheme 7 [59]. However, the

RCY of [^{18}F]FDG is not satisfactory and the stereoselectivity of the adduct is poor. With a synthesis of a mannose-triflate precursor and a labeling methodology with n.c.a. [^{18}F]F$^-$, invented in 1985 [42, 43], the change to a nucleophilic synthesis became possible[60]. This synthesis, consisting of a labeling and one deprotection step, was soon automated, and [^{18}F]FDG became available in high batch yields and purity[27]. Due to the half life of fluorine-18 and the short time of synthesis, a distribution of the product over longer distance is possible.

The importance of [^{18}F]FDG results, apart from is availability, from its advantageous pharmacological behavior. Fluoro-deoxyglucose, like 6-fluoro-DOPA, is also a false substrate for the organism. It is transported into cells and phosphorylated like glucose. However, it is not tolerated for the next metabolic step and cannot leave the cell in the phosphorylated form. This trapping mechanism leads to a three compartmental model for quantification of glucose-transport into cells[61]. PET images with [^{18}F]FDG show the glucose demand in every part of the body. The number of different applications mainly in oncology is tremendous. That is why this tracer is called the “working horse of PET”.



Scheme 8: Built up and direct nucleophilic radiosyntheses of 4-[^{18}F]fluorometaraminol [62, 63, 64]

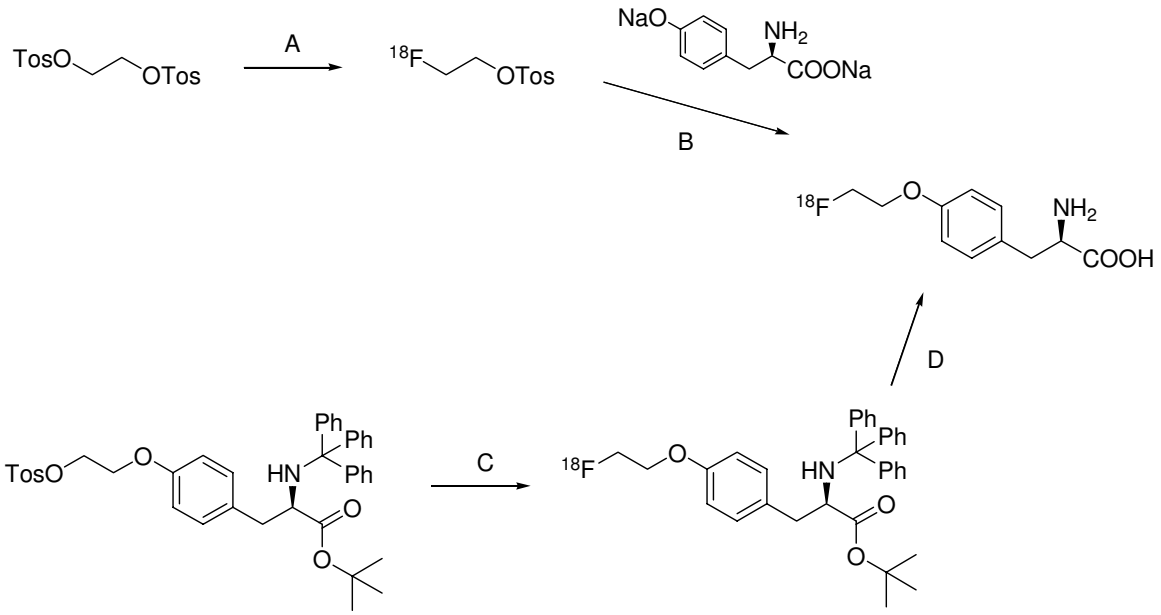
(A) [K⊂2.2.2][^{18}F]F, DMSO, 130°C, 15 min; (B) $EtNO_2$, $NaOH_{aq}$, MeOH, 0°C, 5 min;
(C) Pd/ C, $[NH_4][HCOO]$, MeOH, 130°C, 20 min, chiral HPLC;
(D) [K⊂2.2.2][^{18}F]F, DMSO, 140°C, 5 min; (E) BH_3*THF, 80°C, 10 min;
(F) Pd (black), $[NH_4][HCOO]$, MeOH, 80°C, 10 min, chiral HPLC

No other PET-compound is as versatile in its applications as [^{18}F]FDG. However, for special diagnostic purposes [^{18}F]fluorine labeling syntheses of particular radiotracers are developed, as it is illustrated for 4-[^{18}F]fluorometaraminol in Scheme 8. This potential tracer for monitoring the myocardial innervation is not accessible by a direct nucleophilic S_NAr-reaction with n.c.a. [^{18}F]F$^-$. Therefore, two labeling concepts were developed.

The first one is a built up synthesis where the activated 3-benzyloxy-4-nitro-benzaldehyde is reacted with n.c.a. [^{18}F]F$^-$. Subsequently the propyl-sidechain is built up by addition of the nitro ethyl moiety. Reduction of the nitro group and debenzylation lead in one step to all stereoisomers of 4-[^{18}F]fluorometaraminol[62]. The alternative method for preparation of this radiotracer is to react n.c.a. [^{18}F]F$^-$ with a precursor that already comprises the aliphatic sidechain. The aromatic ring is activated by a carbonyl group. The amino and phenol groups are protected with the benzyl group. After the labeling step the carbonyl function is stereoselectively reduced to yield the hydroxy moiety. Subsequent debenzylation again leads to a stereoisomeric mixture of 4-[^{18}F]fluoromeraraminol[63,64]. Both variations make further enantioselective HPLC-purification steps necessary. At first the two favored diastereomeres are isolated from the mixture, and from these the pharmacological active enantiomere 1'R,2'S-4-[^{18}F]fluorometaraminol is separated.

Both procedures are extremely demanding for automation of syntheses, because they consist of several synthetic steps and HPLC purifications. This complexity is a major hindrance for a wider application of this tracer in PET studies.

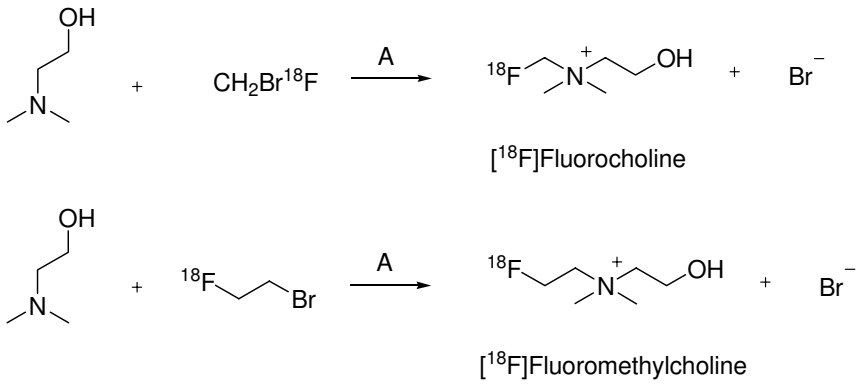
An example for improvement of a radiosynthesis by reducing its complexity is the formation of [^{18}F]fluoroethyltyrosine ([^{18}F]FET). The originally presented method started with the synthesis and purification of 1-[^{18}F]fluoro-2-tosyloxyethane in a first step. Consecutively, reaction with the disodiumsalt of tyrosin yields [^{18}F]FET (Scheme 9)[65].



Scheme 9: Radiosyntheses of [^{18}F]FET by direct nucleophilic or [^{18}F]fluoroalkylation [65, 66]

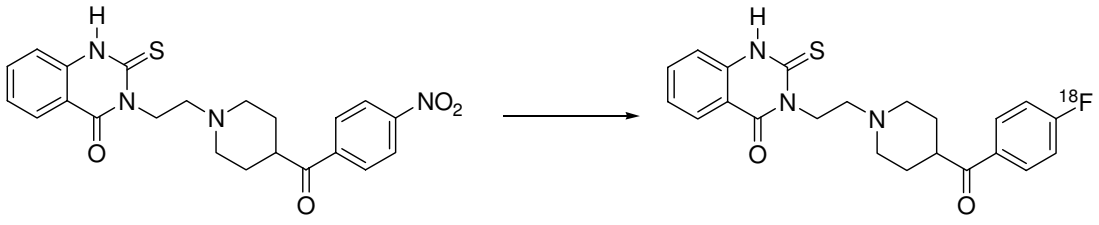
(A) [K⊂2.2.2][^{18}F]F, MeCN, 90°C, 10 min, HPLC separation;
(B) DMSO, 90°C, 10 min
(C) [N(n-Bu)$_4$][^{18}F]F, MeCN, 85°C, 10 min;
(D) TFA/1,2-dichloroethane, 70°C, 7 min;

Whereas a one-pot-synthesis of the radiotracer became possible when the protected and tosylated O-ethyltyrosine as labeling precursor was synthesized. Its reaction with n.c.a. [^{18}F]F$^-$ and subsequent deprotection can be performed in one reactor. Purification and formulation are carried out by solid phase extraction and HPLC. This simplified method makes n.c.a. [^{18}F]FET available in high radiochemical yields (> 50%) and high batch-yields of radioactivity of up to 20 GBq[66]. It is a radiotracer for special amino acid transporters into cells. Therefore it shows the high demand for amino acids of tumor cells, especially in several types of brain cancer. Sometimes its application is advantageous compared to [^{18}F]FDG, because [^{18}F]FET does not accumulate in inflammatory tissues but only in the tumor[67]. In contrast, [^{18}F]FDG, which reflects the glucose transport into cells, accumulates in every tissue with an increased energy demand.



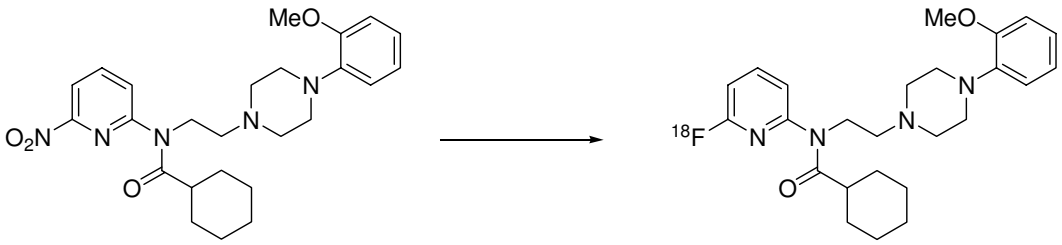
Scheme 10: Radiosynthesis of [^{18}F]fluorocholin and [^{18}F]fluoroethylcholine [68, 69]
(A) Dimethylethanolamine 0.1 M, acetone, 100°C, 10 min

Besides amino acid tumor markers like [^{18}F]FET, there also exist analogue tracers of other substance classes for oncologic applications. Two examples are [^{18}F]fluorocholine and [^{18}F]fluoromethylcholine. As shown in Scheme 10, they are synthesized by [^{18}F]fluoroalkylation of 2-hydroxyethyl-dimethylamine to yield the corresponding choline derivatives[68,69]. Both tracers accumulate in prostate carcinomas and metastases. Furthermore they show a biodistribution, that is very similar to the authentically labeled [^{11}C]choline, apart from their rapid renal excretion. This fact demonstrates, that even conspicuous changes in small molecules do not necessarily change their pharmacological behavior in vivo as dramatically. Thus, there might be several options for the choice of the most suitable radiotracer and labeling feasibilities.



Scheme 11: Radiosynthesis of [^{18}F]altanserin [70]
[K⊂2.2.2][^{18}F]F, DMSO, microwave irradiation

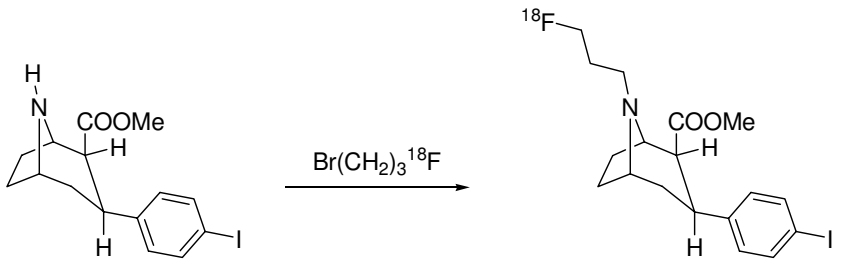
Some molecules are even not chemically changed by being labeled with ^{18}F. This is the case for altanserin, which already contains a 4-fluorophenyl moiety. Furthermore, its aromatic ring is activated for the S_NAr-reaction by a carbonyl function in para-position. Thus, nitro-altanserin can directly be used as precursor for labeling with n.c.a. $[^{18}F]F^-$ (Scheme 11). Due to this simple procedure $[^{18}F]$altanserin is prepared with high molar activity, what is very favorable for the application as radioligand of the 5-HT_{2A} serotonin receptor[70]. This receptor occurs in very low concentrations in the CNS and larger amounts of carrier would block the binding sites for the radiotracer and possibly cause pharmacological effects. Thus, only a small ratio of the radioactivity could reflect the distribution of the receptor.



Scheme 12: Radiosynthesis of 6-[^{18}F]fluoro-WAY-100635 [72]
[K⊂2.2.2][^{18}F]F, DMSO, 145°C, 3-10 min

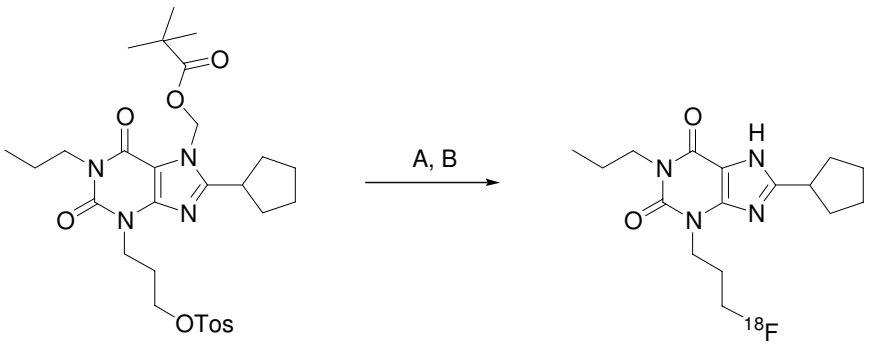
A radioligand for another subtype of the serotonergic receptor, i.e. 5-HT_{1A}, is 6-[^{18}F]fluoro-WAY-100635 [71]. It is an analogue of WAY-100635 and an example for labeling of a pyridine ring. This heteroarene is generally activated in ortho and meta position to the nitrogen atom for an S_NAr-reaction with $[^{18}F]F^-$ [72]. Therefore the radiosynthesis is a one step procedure, as illustrated in Scheme 12.

The last three examples of ^{18}F-labeled radiotracers are also ligands for neuronal receptors or for transporters of neurotransmitters in the brain. All these compounds are highly specific for their targeted protein. The syntheses make use of the S_N-mechanism with n.c.a. $[^{18}F]F^-$ on aliphatic molecules.



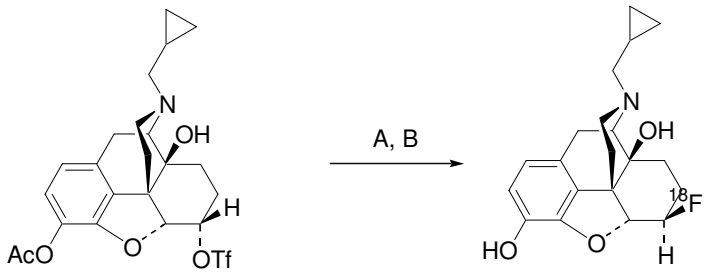
Scheme 13: Radiosynthesis of [^{18}F]FP-β-CIT by [^{18}F]fluoroalkylation [73]
DMSO, 130°C, 25 min

3-[^{18}F]Fluoropropylation of 2β-carbomethoxy-3β-(4-iodophenyl)nortropane (β-CIT) leads to [^{18}F]FP-β-CIT (Scheme 13). This compound is an extremely potent radioligand for the dopamine reuptake transporter. It is known, that disorders of the presynaptic dopaminergic system are closely linked with Parkinson's disease. [^{18}F]FP-β-CIT might become a standard diagnostic tool for this illness[73].



Scheme 14: Radiosynthesis of [^{18}F]CPFPX [74]
(A) [K⊂2.2.2][^{18}F]F, DMSO, 85°C, 10 min; (B) $NaOH_{aq}$ 2 M, RT, 3 min

The reaction of 8-cyclopentyl-3-(3-tosyloxy-propyl)-7-pivaloyloxymethyl-1-propyl-xanthine with n.c.a. [^{18}F]F$^-$ and subsequent deprotection yields the corresponding xanthine derivative [^{18}F]CPFPX as shown in Scheme 14 [74]. This compound, with high specific affinity to the A_1 adenosine receptor subtype, was recently applied for PET studies concerning the behavior of receptor binding in brains after sleep deprivation[75].



Scheme 15: Radiosynthesis of [^{18}F]cyclofoxy [79, 80]
(A) $[N(Me)_4][^{18}F]F$, MeCN, 100 °C, 15 min; (B) $NH_{3\ aq}$ 28%, 55 °C, 3 min

Finally, 6-deoxy-6-β-[^{18}F]fluoronaltrexone ([^{18}F]cyclofoxy) is an antagonist of opiate μ and κ receptors. The main application with PET is for the diagnosis of epilepsy[76], but also Alzheimer's disease and heroine addiction are clinically examined therewith[77, 78]. The radiosynthesis again is performed in two steps. The first is the labeling step with a triflate precursor[79] and n.c.a. [^{18}F]F^-, and in a second step the protection group is removed[80] as shown in Scheme 15.

In general all these example show, that routine production routes of radiotracers labeled with fluorine-18 mostly consist of one or two synthetic steps and chromatographic purification of the product. More complex procedures do not lend themselves for automation. Thus, ready availability is the major limitation for the application of putative new radiotracers. This is why less complicate and fast labeling methods are mandatory. Besides this technical restriction the general pharmacological premises as outlined in chapter 1.1.4 have of course to be fulfilled by any radiotracer.

1.3 Glutaminergic receptor system

1.3.1 Anatomical distribution of glutaminergic neurons

Glutamic acid is the principal excitatory neurotransmitter in the central nervous system and plays a significant role in regulating neuronal activity via various glutamate receptors[81]. This fact implies that those receptors are ubiquitously spread over the whole nervous system. Dysfunctions and diseases associated with the glutaminergic system generally seem to arise from an overstimulation of the receptors[82]. Glutamic acid appears to be neurotoxic in high concentrations. This relation between overstimulation and toxic concentrations leads to the term “excitotoxicity”. Antagonists of the receptors could reveal a “neuroprotective” effect, by decreasing glutamate concentration[83, 84].

As the receptors are present nearly everywhere in the brain, many different pathologic conditions are connected to glutamate receptors. Here are some diseases exemplary mentioned, where glutamate receptors are or might be involved.

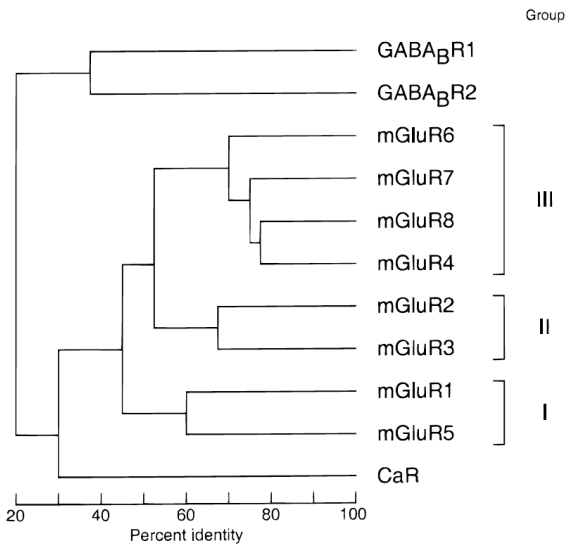
After a stroke or brain injury, the neuronal activity in the affected region is strongly stimulated. Perhaps this stimulation causes excitotoxicity[85]. In brains of patients with Huntington’s, Parkinson’s and Alzheimer’s disease[86,87,88] disintegration of neuronal tissues in specific regions was proven. Although the mechanisms of these destructions are not exactly known, there are evidences that glutaminergic neurons are involved. In patients with amyotrophic lateral sclerosis an elevation of the glutamic acid level in plasma and cerebrospinal fluid was found[89]. The anticonvulsive effect of receptor antagonists might help in the treatment of epilepsy[90]. Finally symptoms of schizophrenia, relief from pain and anxiety[91,92,93] are medical issues connected to the glutaminergic receptor system.

Those complex coherences have to be clarified and radiopharmaceuticals could arise from new radioligands for the glutaminergic system.

1.3.2 Subtypes of the glutamate receptor system

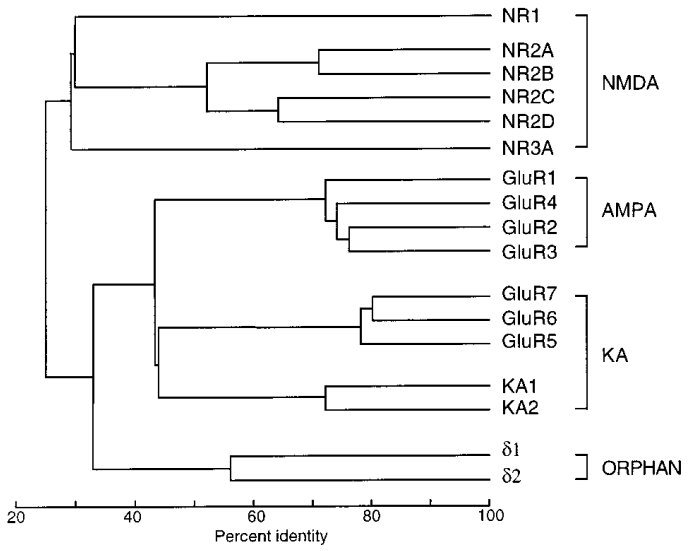
The complexity of symptoms and diseases is even multiplied by the variety of different glutaminergic receptors. At least 28 different subtypes are identified. In general those can be divided into two groups. There are metabotropic or ionotropic receptors.

The metabotropic glutamate receptors (mGluRs) belong to the superfamily of G-protein coupled receptors[82]. The subtypes mGluR 1 to 8 are divided into three families. Furthermore two type B receptors which show selective binding to GABA (γ-amino-n-butyric acid) are also metabotropic glutamate receptors as it is depicted in Scheme 16.



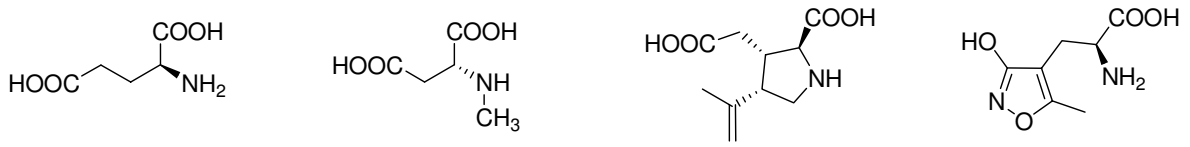
Scheme 16: Subtypes of the metabotropic glutamate receptors and their genetic identity [from 82]

For this class of neurons exists one radioligand, that is applicable with PET. Recently, a ^{11}C-labeled radiotracer was developed and is now under evaluation[94]. [^{11}C]ABP688 is a noncompetitive antagonist of the mGluR5 subtype.



Scheme 17: Subtypes of the ionotropic glutamate receptors and their genetic identity [from 82]

The ionotropic receptor subclasses are named after the substances that bind selectively to them in vitro. These substances are NMDA (N-methyl-D-aspartic acid), KA (kainic acid) and AMPA ((RS)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazoly)propionic acid).



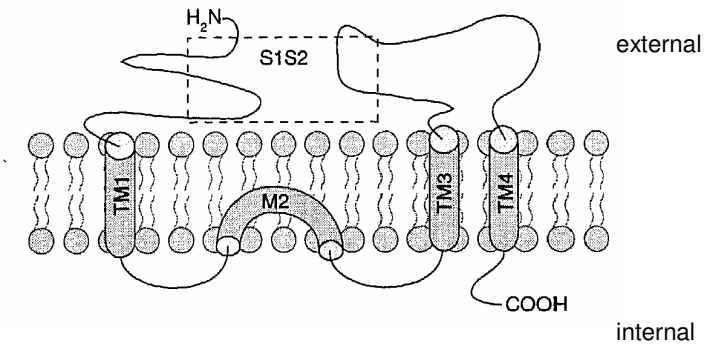
S-Glutamic Acid NMDA S-Kainic Acid S-AMPA

Scheme 18: Ligands naming the ionotropic glutamate receptors

A manifold of more or less specific agonists and antagonists was designed for these different groups. Most of them are amino acids. Amino acid analogues comprise at least an acidic proton in an amino function and a neighboring keto-group.

1.3.3 AMPA receptors

The AMPA receptors, as important ionotropic receptors, are mainly located in the postsynapse[95]. An illustration of the pore is given in Scheme 19. It is composed of four domains M1 – M4 in the membrane. M1, M3 and M4 are transmembrane-spanning domains, where M3 and M4 build the ion channel. M2 is embedded in the membrane as a re-entry loop, connecting M1 and M3. Glutamate is bond extra-cellular in two discontinuous domains S1 and S2. This is also the binding site for most agonists or antagonists. The large extra-cellular domain ends in the N-terminus, while the C-terminus is in the cell at the end of a smaller domain[96].

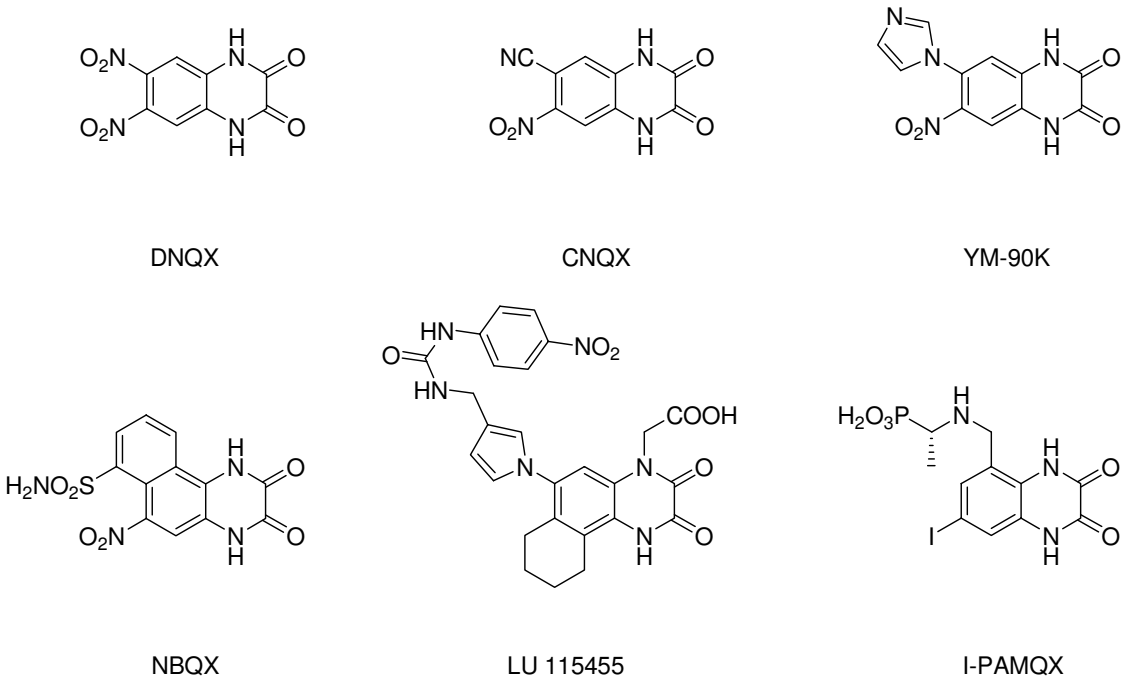


Scheme 19: Schematic illustration of an ionotropic glutamate receptor pore [from 96]

Glutamate binding opens the channel pore and leads to an influx of Na^+ and Ca^{2+} into the postsynapse. Simultaneously, the sensitivity of the synapse is decreased to prevent excitotoxicity.

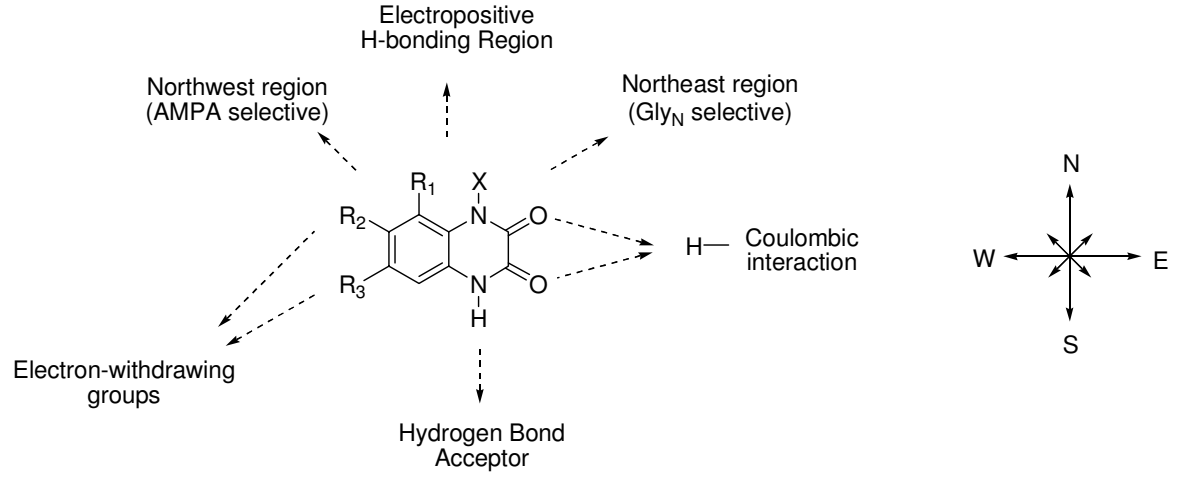
1.3.4 Quinoxalinediones as antagonists of the AMPA receptor

The quinoxaline-2,3-diones form the single most important class of compounds, which act as amino acid analogs and AMPA antagonists[81]. Their development was forced to find orally available drugs with neuroprotective properties[96].



Scheme 20: Examples of ligands of the glutamate receptors from the QX-family

Some simple derivatives like those in Scheme 20 show very good affinity to the AMPA receptor. However, minor changes in the substitution pattern lead to unselective or much less potent antagonists. The studies about the relation of substitutes and selectivity was refined and is shown in Scheme 21.



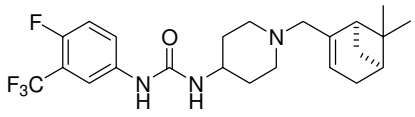
Scheme 21: Relation of substitutes and selectivity of ligands from the QX-family
[modified from 81]

An example for a QX-derivative that binds to the NMDA receptor is the radioactive labeled (D)-7-[^{123}I]Iodo-N-(1-phosphonoethyl)-5-aminomethylquinoxaline-2,3-dione ([^{123}I]I-PAMQX, Scheme 20)[97].

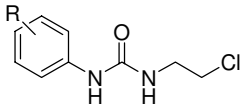
Although CNQX, DNQX[98, 99] or YM-90K[100] are used as standard competitors for in vitro studies evaluating new compounds, so far only the tritiated molecules are known. More and different radioactive labeled analogues and derivatives could feature new applications for this class of compounds. Several strategies might be used for labeling especially with fluorine-18. The simple structures like CNQX and DNQX comprise both activating and leaving groups for direct nucleophilic labeling. Some of the promising, more complex molecules as LU 115455 (Scheme 20), of which the in vivo activity was determined[101], enclose substituted phenyl urea units where a build up labeling synthesis might be developed.

1.4 Ureas in pharmaceutical chemistry

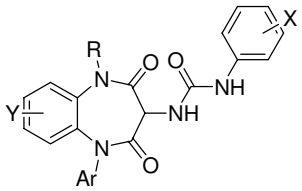
Besides several AMPA antagonists, there is a manifold of urea derivatives in pharmaceutical chemistry. They are found as a structural element in a large variety of biologically active molecules[102]. Especially para substituted phenyl ureas (e.g. with halogen) are quite often found like the examples in Scheme 22 [103,104,105,106].



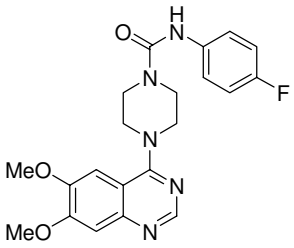
CXCR3 receptor antagonist



Antineoplastic agents



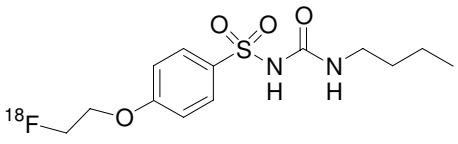
Cholecystokinin antagonists



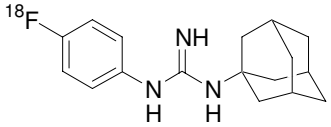
Platelet-derived growth factor
receptor phosphorylation inhibitor

Scheme 22: Examples of ureas used as pharmaceutical compounds [from 100 – 103]

Until now, there is no example where these structures are used for the preparation of n.c.a. ^{18}F-labeled analogues or original tracers. On the other hand, there are several urea derivatives described, which are labeled via ^{18}F-fluoroalkylation or ^{18}F-fluoroacylation indicating the principle interest in this class of compounds[107]. Also, a 4-[^{18}F]fluorophenylguanidine was prepared as an example of a related substance class (Scheme 23)[108].



β-Cell imaging agent



σ-Receptor ligand

Scheme 23: Examples of ^{18}F-labeled ureas and related compounds, prepared with the purpose of radiotracers [from 107, 108]

2 Aims and scope

The outstanding role of the radionuclide fluorine-18 for radiopharmaceutical applications in positron-emission-tomography is demonstrated by many examples. For the glutaminergic receptor system, however, which is one principal excitatory neurotransmission pathway in the central nervous system, there are only few suitable tracers known.

Thus, the aim of this work was the implementation of the potent radiolabel fluorine-18 into ligands for the glutaminergic receptor system with a focus on the AMPA receptor, which is an ionotropic receptor, located in the postsynapse. The antagonist α-**A**mino-3-hydroxy-5-**M**ethyl-3-isoxazol**P**ropionic **A**cid is an in vitro ligand with high affinity and specificity for the thus termed AMPA binding site.

In classical medicinal chemistry several classes of substances with affinity to the AMPA receptor in vitro were identified, e. g. derivatives of the quinoxalinediones (QX). Focusing on the QX-family is the second specification of aims for this work.

Due to the fact that the QX derivative “LU 115455” is a very potent antagonist of the AMPA receptor, this complex structure was the first compound, that should be labeled. The most promising part for ^{18}F-labeling is the para-nitrophenyl urea moiety. In several other examples the substitution of a nitro-group by a fluorine atom did not lead to a significant change of pharmacological properties of the basic molecule.

Until now there is no radiosynthesis of no carrier added (n.c.a.) [^{18}F]fluorophenyl ureas described. The n.c.a. strategy is important for an application of radioligand studies in the central nervous system with PET, because the total amount of molecules has to be in the subnanomolar range, in order not to disturb the equilibrium processes of the organism.

Labeling of an aromatic compound like the phenyl urea with n.c.a. [^{18}F]fluoride presumes an aromatic ring with an appropriate leaving group that is activated for the S_NAr reaction. The urea moiety is not an activating group. It can be assumed, that direct labeling by a fluorine-for-nitro substitution without further activation would fail.

Therefore, a built-up synthetic strategy should be developed. The amine of the QX-part of the desired ligand was provided by ABBOTT pharmaceutical company.

According to the literature, it is combined with an isocyanate to yield the urea derivative. This procedure exemplifies a possible access to radiofluorinated phenyl ureas.

First of all, the n.c.a. radiolabeling of the accordingly needed fluorophenyl isocyanate should be tried. Alternatively, [^{18}F]fluorophenyl ureas should be synthesized by built-up syntheses starting from [^{18}F]fluoroaniline and phosgene analogues, e. g. diphosgene, triphosgene or carbonyl diimide azol (CDI), which are well known substitute reagents for the highly toxic, gaseous compound.

Besides the synthesis of ureas via isocyanates, other routes lend themselves for their preparation. In general, a substitution reaction of carbamate nitrophenyl esters with amines, where the nitrophenol moiety of the activated ester serves as leaving group, leads to high yields of ureas with disparate substitutes. Using [^{18}F]fluoroaniline as amino compound, or its transformation into the carbamate ester should yield [^{18}F]fluorophenyl ureas corresponding to the chemical properties of the product. Consequently, an elaborated strategy for radiolabeling of fluorophenyl ureas would facilitate several new syntheses of putative ligands of the AMPA receptor.

Derived from cyano-nitro-QX (CNQX), a urea is conceivable, where the nitro group is substituted by the [^{18}F]fluorophenyl moiety. However, in order to study the biodistribution of QX-derivatives, which is not totally clarified until now - especially the extent of the brain uptake - the established antagonists dinitro-QX (DNQX) or CNQX should be radiofluorinated. Both molecules comprise an activating and a leaving group for the S_NAr reaction with n.c.a. [^{18}F]fluoride.

With regard to an application, all radiosyntheses demand a synthesis of the stable fluorinated compounds. Several concepts for this are described in the literature, which had to be adopted or adapted as necessary.

The non-radioactive substances had to be used as chromatographic standards for identification of the labeling products and for planned competition studies in vitro. The competition studies would give a preliminary information of the ligand's potency. Therefore, the inhibition constants K_i were to be determined in displacement experiments with a suitable tritiated ligand like [^{3}H]AMPA or [^{3}H]CNQX.

3 Results and Discussion

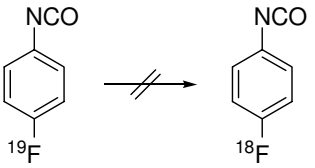
As described in chapter 1.3.4 quinoxalinedione derivatives form the single most important substance class, which act as antagonists for the AMPA receptor. Therefore it is obvious to search in this class of compounds for molecules, which can be labeled with n.c.a. ^{18}F and utilized as radiotracers for the AMPA system.

In the literature several promising studies with complex phenyl urea derivatives of the QX family are described, why this was the substance class chosen here. Because the ^{18}F-labeling of phenyl urea moieties has not been described until now, a general strategy for radiosyntheses has to be developed before an application to a concrete radiotracer is possible.

3.1 Radiosynthesis of [^{18}F]fluorophenyl ureas

There are many possibilities to prepare urea derivatives on a laboratory scale. The most simple way is to combine an isocyanate compound with an amine[109,110]. In the case of fluorophenyl ureas this method works well. Fluorophenyl isocyanate is commercially available and stable under standard conditions. Its further reaction with different amines leads to the corresponding products. A transfer of this methodology to radiolabeling reactions is not quite simple on a no carrier added scale; while a n.c.a. synthesis is advantageous with regard to a later application of products in vivo, as explained in chapter 1.1.4.

For preliminary tests of the proof of principle, isotopic exchange reactions are first to be performed. But the S_NAr-reaction on 4-fluorophenyl isocyanate with n.c.a. [^{18}F]fluoride failed under several conditions tested. Experiments were performed in DMF and DMSO at temperatures of 140°C, 160°C and even 180°C. No radioactive products, however, could be observed. Whereas fluorine is one of the best leaving groups in this kind of reaction, further efforts with isocyanates are not reasonable. The isocyanate group, with a Hammett constant of +0.28 [111], is obviously not activating the aromatic ring enough in order to enable a nucleophilic substitution.

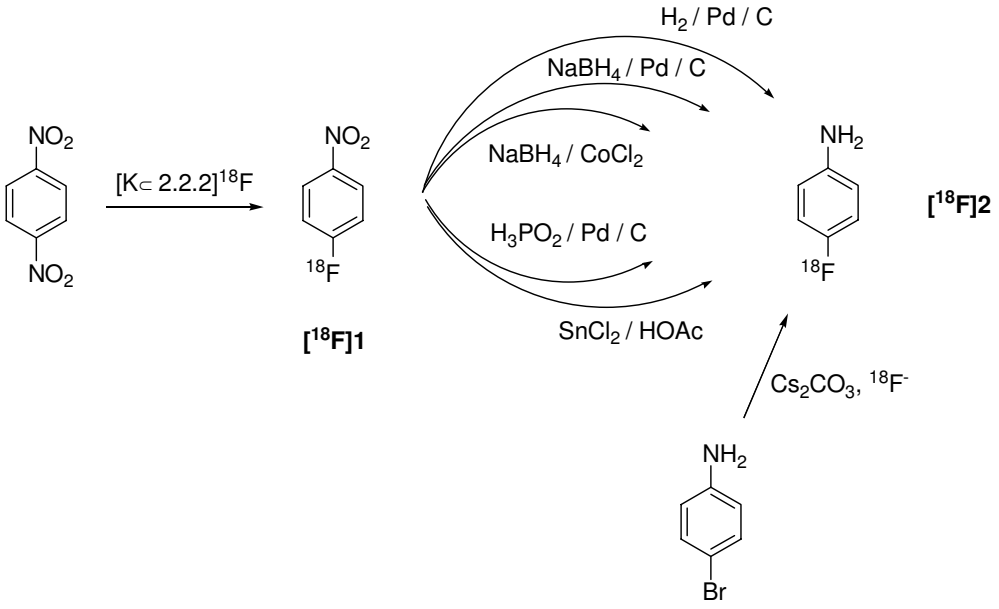


Scheme 24: Direct labeling of fluorophenyl isocyanate by isotopic-exchange failed

Alternatives for this direct synthetic approach for [^{18}F]fluorophenylisocyanates are multistep syntheses via 4-[^{18}F]fluoroaniline as intermediate. Generally, organic isocyanates can be made from aniline via a reaction with phosgene or a substitute for this highly toxic gas. This pathway, however, demands the n.c.a. preparation of [^{18}F]fluoroaniline.

3.1.1 Radiosynthesis of n.c.a. 4-[^{18}F]fluoroaniline

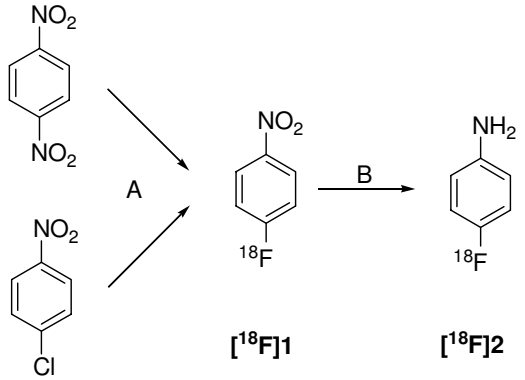
The preparation of n.c.a. 4-[^{18}F]fluoroaniline (**[^{18}F]2**) was earlier described using several different routes as outlined in Scheme 25.



Scheme 25: Known preparation routes for n.c.a. [^{18}F]fluoroaniline

The direct labeling of bromoaniline leads to a RCY of only 8% due to the missing activation for an S_NAr reaction[112]. The other procedures are two-step radiosyntheses. They have in common that the intermediate para-[^{18}F]fluoronitrobenzene is prepared in a first step. Subsequent reduction of the nitro-group leads to [^{18}F]fluoroaniline. Each of these syntheses, however, is somehow limited. The use of gaseous hydrogen gives a pure product without contamination of inorganic salts, but the handling of gaseous, extremely flammable substances and radioactivity is very demanding for safety reasons[113]. The reduction with sodium borohydride and Pd on charcoal[108,114,115] or $CoCl_2$[116], respectively, in general gives good results. Also hypophosphoric acid[117,118] and tin dichloride[114] were successfully applied. Nevertheless, the fate of the inorganic salts has to be monitored, as some of them are toxic. All syntheses with charcoal have the disadvantage that the suspensions are hardly to handle with syringes or tubes, as they are plugged nearly immediately. Also n.c.a. intermediates and final ^{18}F-products are easily absorbed by the charcoal.

That is why efforts were taken in order to modify the methods described above in several aspects. According to Scheme 26 the radiosynthesis starts with dried potassium [^{18}F]fluoride. Potassium is complexed with Kryptofix 2.2.2 to activate the nucleophilic properties of [^{18}F]F^-. The preparation of this potassium-complex solution is a standard procedure in labeling syntheses with n.c.a. [^{18}F]F^- as described in chapter 1.2.5. The radiosynthesis is performed in DMSO.



Scheme 26: Preparation of para-[^{18}F]fluoroaniline;
(A) [K⊂2.2.2][^{18}F]F, K_2CO_3, DMSO, 120°C, 3 min;
(B) Pd black, H_3PO_3, MeOH, reflux, 15 min

Two precursors for the preparation of para-[^{18}F]fluoronitrobenzene (**[^{18}F]1**) were tested. 1,4-Dinitrobenzene and 4-chloronitrobenzene lead to very high radiochemical yields after nucleophilic exchange labeling with [^{18}F]fluoride at 120°C for three minutes. The radiochemical yield of **[^{18}F]1** alluded to the dried [^{18}F]fluoride is constantly at least 85% after cartridge absorption. The purification is performed on a Waters C18+ cartridge in a simple procedure. The reaction mixture is diluted with water and passed through the cartridge.

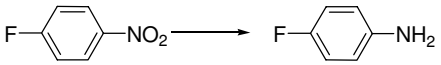
Elution of the product is done with methanol, which is the solvent for the subsequent reduction of the nitro group. This reduction is the crucial step in the synthesis of n.c.a. [^{18}F]fluoroaniline. As outlined in Scheme 25 there already exist several modifications in the literature. In order to find the most effective method, seven alternatives were examined on an equimolar and n.c.a. scale – see Table 2 for details. From those, the combination of phosphorous acid or hydrazine, respectively, and palladium black in methanol at 80°C proved to be the best on the n.c.a. level. The reaction is quantitative within 15 min. Hydrazine is as effective but it is toxic and its degree of decomposition is elusive. This problem does not occur with phosphoric acid which is not toxic but soluble in water, as all the other products are. They are removed in the next reaction and purification steps.

For the following purification step a cartridge filled with a Merck Lichrolut EN phase is utilized. Fixation of **[^{18}F]2** is only achieved under alkaline conditions. Under the acidic conditions of reduction the ammonium salt is formed and the RP cartridge does not retain the cation. Dilution of the reduction mixture with 1 M sodium hydroxide solution assures that the electronic neutral 4-[^{18}F]fluoroaniline is present in the solution.

The catalyst Pd black is in general preferable against Pd on charcoal. Its coarse particles do not plug cannulas and are filtered off easily during cartridge purification. Furthermore, no product is absorbed, as it is the case with Pd on charcoal.

For subsequent reactions the cartridge charged with **[^{18}F]2** is eluted with the solvent of choice. Referring to the dried [^{18}F]fluoride the radiochemical yield of **[^{18}F]2** is 84 ± 5 % within 30 min of preparation, what means a major improvement compared to the procedures described in the literature.

Table 2: Methods tested for the reduction of 4-fluoronitrobenzene to 4-fluoraniline

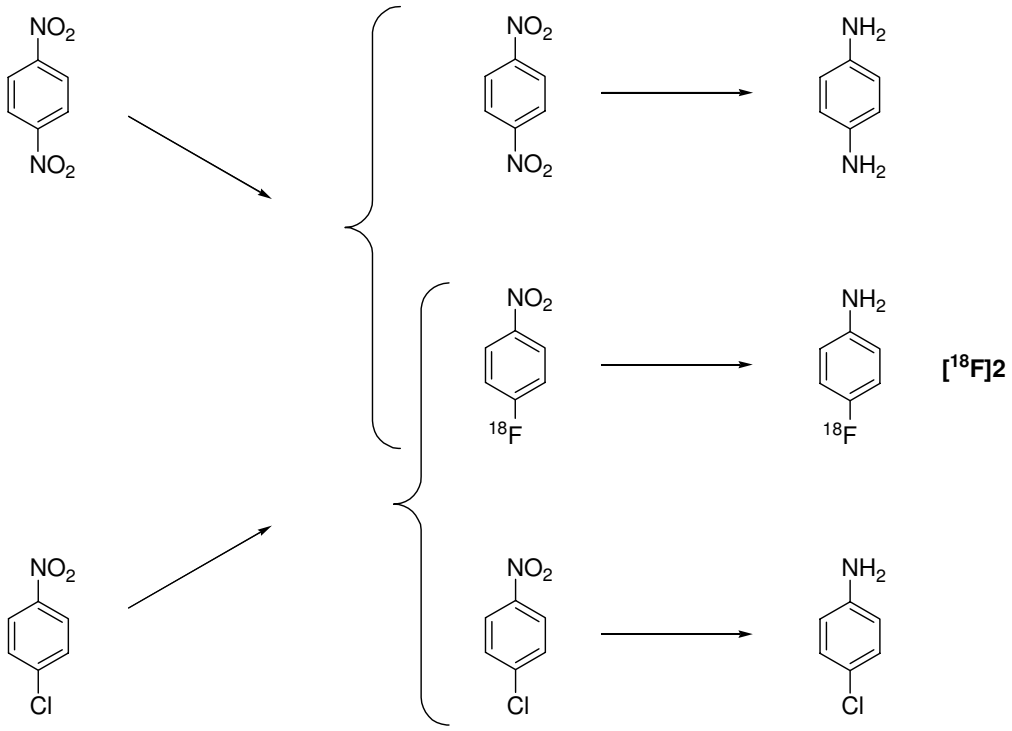


equimolar scale		n.c.a. scale	
In/ acetic acid	not quantitative	Pd/ $[NH_4][HCOO]$	not quantitative
Pd/ BER*	no conversion	$NaBH_4$	no conversion
Pd/ hydrazine	not quantitative	Pd/ hydrazine	complete conversion
$SnCl_2$	not quantitative	$SnCl_2$	not quantitative
		NiAc/ $NaBH_4$	no conversion
		Pd/ H_3PO_3	complete conversion

*BER: borohydride, polymer supported

Both precursors 1,4-dinitrobenzene and 4-chloronitrobenzene show similar results until this step. Nevertheless, the first molecule seems to be more suitable for further reactions. Reduction of excess 1,4-dinitrobenzene yields 1,4-diaminobenzene, which shows a completely different chemical and chromatographic behavior than 4-fluoroaniline. In contrast, 4-chloroaniline is formed from the excess of the other precursor as shown in Scheme 27.

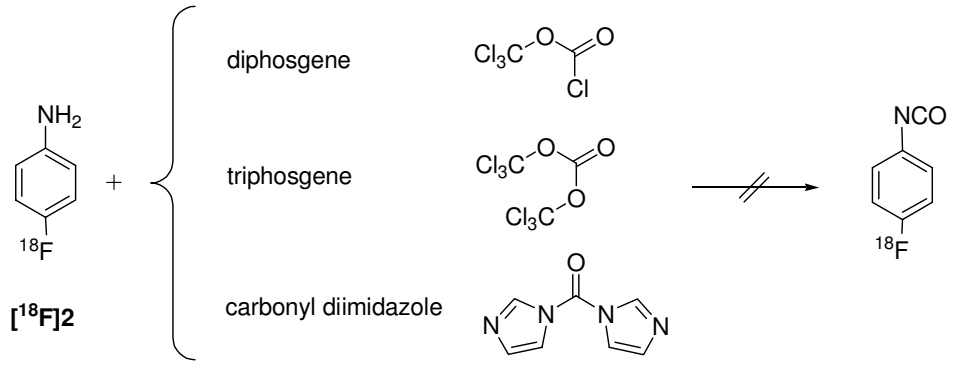
Since it is not easy to separate 4-chloroaniline completely from **[^{18}F]2** via HPLC or other quantitative methods, it may lead to analogous chloroarenes in subsequent reactions and thus to a non-isotopic dilution rendering a reduction of the apparent molar activity of the ^{18}F-labeled product.



Scheme 27: Byproducts of [^{18}F]fluoroaniline after labeling and reduction using different precursors

3.1.2 Approaches to the preparation of n.c.a. 4-[^{18}F]fluorophenyl isocyanate

In order to avoid the synthesis of the isocyanate using phosgene, three alternatives were tested. Diphosgene and triphosgene[119,120,121,122] are colorless solids and stable under ambient conditions. One molecule diphosgene releases two, triphosgene three equivalents of phosgene in situ. Further, carbonyl diimidazole is a direct substitute for phosgene, where the imidazole moieties replace the chlorine atoms[104,123].



Scheme 28: Attempts of n.c.a. 4-[^{18}F]fluorophenyl isocyanate preparation;
performed in chlorinated or etheric solvents at temperatures from -68°C to 60°C

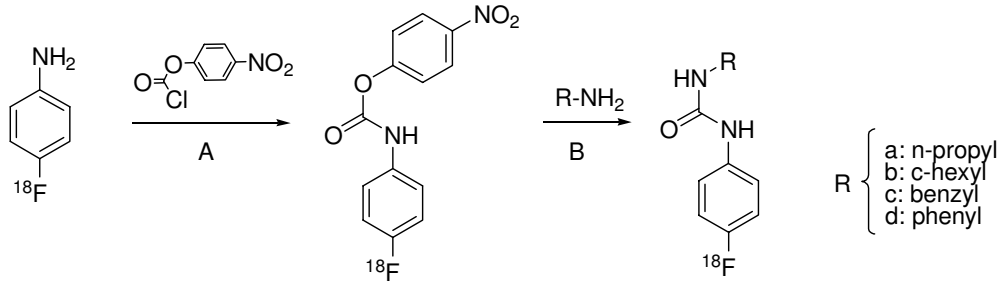
These three substances were used in several experiments for reaction with n.c.a. 4-[^{18}F]fluoroaniline as depicted in Scheme 28, but no conversion of the free amino group could be observed. Reactions were performed in THF, diethyl ether, acetonitrile and chloroform. The temperatures were varied from – 68°C to 0°C, room temperature and even slightly elevated temperatures. Several substances like proton sponge, pyridine, triethyl amine or Hünigs base were tested as assistant base. Also drying of the solution by passing the cartridge eluat through sodium sulphate showed no success.

If the formation of an isocyanate would mechanistically proceed as an intramolecular reaction of a carbamate intermediate, this failure is hard to explain. The results obtained may point to a multistep reaction, which would not be possible for statistical reasons at the n.c.a. level.

3.1.3 N.c.a. 4-[^{18}F]fluorophenyl carbamate 4-nitrophenylester ([^{18}F]3e) and 4-[^{18}F]fluorophenyl urea derivatives ([^{18}F]4a - 4d)

Carbamate nitrophenylesters were therefore tested as alternative intermediates. Reactions of amines with 4-nitrophenyl chloroformate or bis-4-nitrophenyl carbonate lead to carbamate 4-nitrophenylesters[124,125]. These substances are also stable under standard conditions and easy to separate. Further reaction with another amine gives disparately substituted ureas. This strategy is well known in organic chemistry and appeared also attractive for the radiosynthesis. Therefore the n.c.a. **[^{18}F]2** is eluted

from the Merck EN-cartridge, and the solution is subsequently dried by passing it through sodium sulphate and kieselguhr powder. After addition of 4-nitrophenyl chloroformate the solution is stirred at room temperature. HPL- and thin layer chromatograms indicate the conversion to the desired carbamate 4-nitrophenylester (**[^{18}F]3e**).



[^{18}F]2 **[^{18}F]3e** **[^{18}F]4a-4d**

Scheme 29: Preparation of 4-[^{18}F]fluorophenylcarbamate nitrophenyl ester and N-4-[^{18}F]fluorophenyl-N'-substituted ureas
(A) THF, Na_2SO_4-cartridge; (B) THF, Δ

For conversion to the desired urea, an amino compound of choice is added to the carbamate. After stirring at slightly elevated temperatures the urea derivative is obtained and can be separated by a further cartridge purification step. The conversion takes place under alkaline conditions. The basicity of aliphatic amines is high enough, so that addition in excess is sufficient. With less basic aromatic amines, however, an assistant base such as a tertiary amine, for example ethyl diisopropyl amine, is necessary. The yields for labeled fluorophenyl ureas are listed in Table 3. They are generally high, but the whole syntheses, as described, comprises four steps.

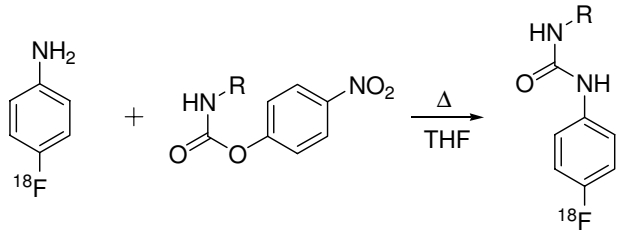
One of the last two steps in the radiosynthesis can be avoided, if it is possible to form the carbamate 4-nitrophenylester of the non radioactively labeled part of the urea. In fact, adding n.c.a. 4-[^{18}F]fluoroaniline to a carbamate (**3a-3d**) while stirring the mixture at 30 – 40 °C, yielded the corresponding urea derivative. Raised temperatures accelerate the conversion. This reaction concept is simpler than the four step procedure, but it does not work equally effective with all tested amines, respectively carbamates, as shown in Table 4.

Table 3: RCY of labeled fluorophenyl urea derivatives formed via [^{18}F]fluorophenylcarbamate nitrophenyl esters

Amine	Urea	RCY (referring to dry [K⊂2.2.2] [^{18}F]F)
n-propyl amine	N-4-[^{18}F]fluorophenyl-N'-*n*-propyl urea (**[^{18}F]4a**)	76 %
c-hexyl amine	N-4-[^{18}F]fluorophenyl-N'-*c*-hexyl urea (**[^{18}F]4b**)	73 %
benzyl amine	N-4-[^{18}F]fluorophenyl-N'-benzyl urea (**[^{18}F]4c**)	82 %
aniline*	N-4-[^{18}F]fluorophenyl-N'-phenyl urea (**[^{18}F]4d**)	73 %

temperature for coupling reaction: 30 – 40 °C
*addition of ethyldiisopropyl amine (ca. 50 µL)

The 4-[^{18}F]fluorophenyl-carbamate-4-nitrophenylester (**[^{18}F]3e**) is more reactive than some aliphatic compounds, which becomes clear by comparison of the results shown in Tables 3 and 4. Reactions with **[^{18}F]3e** constantly result in yields > 70% for all four amines. Whereas RCY of reactions of **[^{18}F]2** with the carbamate-4-nitrophenylesters are generally lower, and with n-propyl-carbamate-4-nitrophenyl-ester nearly no conversion can be determined.



[^{18}F]2 **[^{18}F]3a-d** **[^{18}F]4a-d**

Scheme 30: Preparation of N-4-[^{18}F]fluorophenyl-N'-substituted ureas via carbamate 4-nitrophenylesters; a-d, see Scheme 29

Table 4: RCY of labeled fluorophenyl urea derivatives via various carbamate nitrophenyl esters; ureas fixed on Merck EN cartridge

Carbamate	Urea	RCY*
n-propyl carbamate 4-nitrophenyl ester (**3a**)	N-4-[^{18}F]fluorophenyl-N'-*n*-propyl urea (**[^{18}F]4a**)	< 5 %
c-hexyl carbamate 4-nitrophenyl ester (**3b**)	N-4-[^{18}F]fluorophenyl-N'-*c*-hexyl urea (**[^{18}F]4b**)	57 ± 6 %
benzyl carbamate 4-nitrophenyl ester (**3c**)	N-4-[^{18}F]fluorophenyl-N'-benzyl urea (**[^{18}F]4c**)	62 ± 5 %
phenyl carbamate 4-nitrophenyl ester (**3d**)	N-4-[^{18}F]fluorophenyl-N'-phenyl urea (**[^{18}F]4d**)	74 ± 7 %

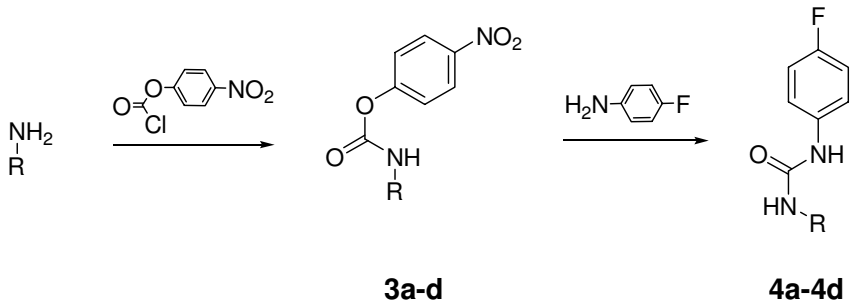
temperature of coupling reaction: 30 – 40 °C; * referring to dry [K⊂2.2.2] [^{18}F]F

The choice of the right combination of carbamate and amine is obviously depending on the structure and possibility of synthesis of the desired labeling product.

3.1.4 Synthesis of carbamate 4-nitrophenylesters and corresponding 4-fluorophenyl urea derivatives as chromatographic standards

Syntheses of carbamate 4-nitrophenylesters are well described for many different substances[125]. Nevertheless, some modifications are applied in this work to optimize the results for the individual molecules. Four types of substances were examined.

Benzyl, cyclohexyl and *n*-propyl amine represent different primary aliphatic amines. The reaction of those with 4-nitrophenyl chloroformate takes place in methylene chloride at room temperature and is completed within several hours. As an assistant base ethyl diisopropyl amine is added in slight excess. This base is better suited than triethyl amine, because the formed ammonium salt does not precipitate. The reaction mixture is washed with hydrochloric acid and the precipitated product (**3a - 3c**) is recristallised from iso-propyl alcohol. The yields with all three aliphatic compounds are high and products are pure after a simple purification procedure.



3a-d **4a-4d**

Scheme 31: Preparation of carbamate 4-nitrophenylesters and 4-fluorophenyl ureas;
a-d. see Scheme 29

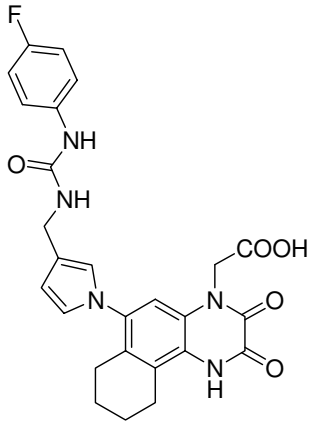
In contrast, the conversion of aniline derivatives is not as simple as of aliphatic ones. The rapidly formed carbamate is not stable under alkaline conditions. After a short time, the only product with 4-nitrophenyl chloroformate and an excess of organic bases is bisphenyl urea. Therefore Hünig base, ethyl diisopropyl amine, was only used in equimolar amounts. Cooling of the reaction flask is necessary. For purification, the solution is washed with hydrochloric acid. Further washing, if necessary, is then done by suspending the solid product in diethyl ether.

The four carbamate compounds **3a** - **3d** described above are further reacted in methylene chloride with para-fluoroaniline to give the corresponding urea derivatives. This simple procedure works out very well, except for the *n*-propyl compound (**3a**) where nearly no reaction can be observed. This finding corresponds to the results of the radiosyntheses where **[^{18}F]2** does not react with **3a**.

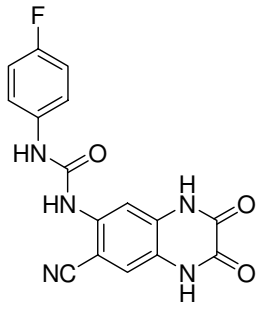
Reaction of *n*-propyl amine with 4-fluorophenyl carbamate nitrophenylester (**3e**), on the other hand, leads to the desired urea with good yields (91%). The 4-fluorophenyl carbamate nitrophenylester was prepared like the phenyl compound described above.

3.1.5 Application of the developed labeling synthesis to putative AMPA receptor ligands of the QX-family

As the pathway for radiolabeling of fluorophenyl ureas was developed for application on derivatives of the QX-family, two compounds, which are shown in Scheme 32, were chosen as putative ligands for the AMPA receptor.



(5)



(6)

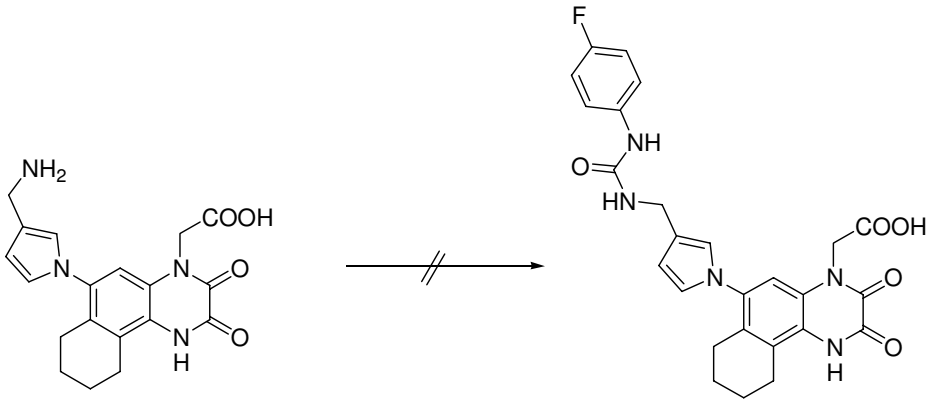
Scheme 32: Fluorophenyl ureas of the QX substance class as putative AMPA antagonists

Compound **5** is an analogue of the already mentioned LU 115455, where the para-nitro-group in the phenyl moiety is substituted by a fluorine-atom. This change could possibly have minor influence on the biological behavior of the analogue molecule. LU 115455 itself exhibits a great binding potency to the AMPA-receptor. The corresponding methylene amine of the pyrolyl-QX-part of the molecule was provided by Abbott GmbH & Co KG. The chromatographic reference should be synthesized analogously to literature methods. Unfortunately, all attempts failed.

The solubility of the amine is extremely low. Reactions in DMF or DMSO at temperatures above 120°C with fluorophenyl isocyanate gave no result. NMR data of separated products appeared to be those of the educts.

The poor reactivity is also affirmed by radiosyntheses. The prepared [^{18}F]fluorophenyl carbamate nitrophenylester (**[^{18}F]3e**) is added to the amine. No

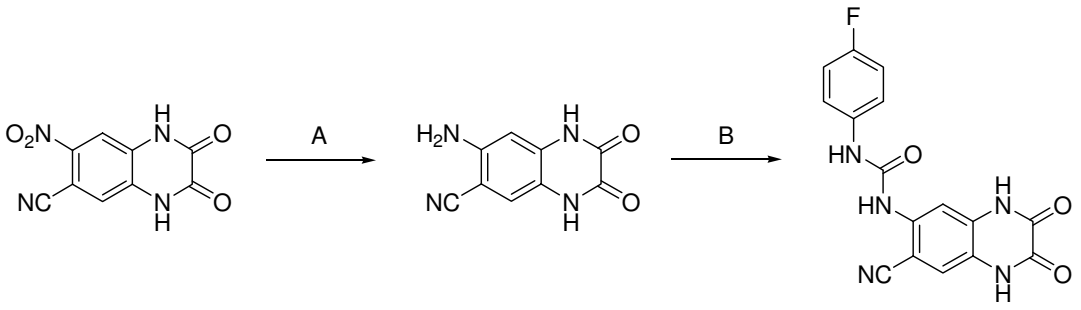
conversion to a radioactive product could be observed. Tests included addition of base, increasing temperatures and different solvents.



(5)

Scheme 33: Syntheses of the fluoro analogue of LU 115455 failed

The second aspired urea is derived from CNQX, whose synthesis is described below in chapter 3.2.1. Reduction of its nitro group leads to amino-cyano-QX (ACQX), which in turn should be transformed into the fluorophenyl urea **6** as depicted in Scheme 34.



CNQX **ACQX** **(6)**

Scheme 34: Syntheses of the stable fluorophenyl-CQX-urea
(A) Pd / C, H_2N-NH_2, MeOH, reflux; (B) F-C_6H_4-NCO, DMF, 110°C

Reduction of CNQX is preferably done with hydrazine and Pd on charcoal in methanol, therefore products of the reduction agent are gaseous and volatile[126]. Purification of ACQX is complicated. Inorganic salts as impurities of CNQX cannot be

excluded. The solubility of the amine in methanol is low, and it precipitates during filtration of the charcoal. Dissolving ACQX in DMF is an alternative, but the solvent cannot be evaporated easily. Precipitation as the corresponding hydro chloride by addition of hydrochloric acid gave no result. Fixation of the product on a cation exchange resin is possible, but desorbing it afterwards is not simple.

HPLC, NMR and mass spectroscopy of the crude reaction mixture show, that ACQX is produced. Only minor organic impurities can be determined so that purification was not performed and the coupling reaction to the fluorophenyl urea was attempted with the crude reaction product.

In order to obtain the desired urea ACQX is stirred with fluorophenyl isocyanate in DMF at 110°C. Further isocyanate is added several times. After cooling the mixture to room temperature, water is added to the solution. The precipitate is filtered off and analyzed via NMR in d_6-DMSO. The spectrum shows evidence for the conversion of the amine to a urea. However, this result cannot be confirmed by mass spectrometry, since the product is almost not soluble in suitable solvents. All detected signals are related to impurities and products of the added fluorophenyl isocyanate. HPL-chromatograms also are ambiguous. An attempt of application of the synthesis route via fluorophenyl carbamate nitrophenyl esters also leads to unsatisfying results. All spectroscopic data is ambivalent.

The use of the ACQX in a radioactive labeling experiment should clarify things. The n.c.a. [^{18}F]fluorophenyl carbamate nitrophenyl ester (**[^{18}F]3e**) is again prepared like described above. Addition of the ACQX results in no success. According to HPLC several products are formed in minor amounts, but they cannot be assigned to concrete molecules.

In general the quinoxalinediones with affinity to the AMPA-receptor bear at least four functional groups, which complicate organic syntheses. The non-aromatic ring is formed by two carbamate functions. Their behavior and chemical conformation at different pH values cannot be determined exactly. Keto-enol-tautomerism can occur, and reaction of at least one of the nitrogen atoms with reactants cannot be excluded. The solubility of the two chosen QX-amines is generally low in organic solvents, but crystalline precipitates are not formed. This makes purification often difficult or impossible.

While the radiosyntheses of a manifold of [^{18}F]fluorophenyl urea derivatives works without any problems, the preparation of the two QX ureas under examination failed. The choice of target molecules for pharmacological use has to be reconsidered. Whereas these complex structures are not easy to process, some more simple QX molecules labeled with [^{18}F]fluorine could possibly answer pharmaceutical questions.

3.2 ^{18}F-labeling of basic quinoxalinediones

As described in chapter 1.3.4 the basic quinoxalinediones DNQX and CNQX are very well established antagonists for in vitro competition studies of the AMPA-receptor system. And even if their affinity to the receptor is only in the µM range n.c.a. ^{18}F-labeled tracers also might be useful for in vitro application. Perhaps the introduction of a fluorine atom into these molecules even improves their binding affinity.

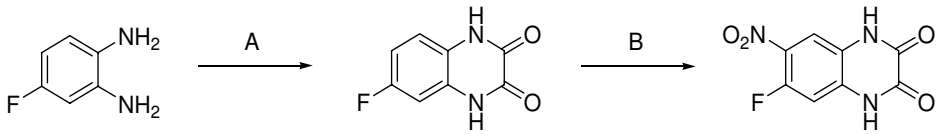
Both molecules are twice substituted in the “western” region. The nitro and the cyano groups, respectively, are electron withdrawing functions and therefore activating S_NAr reactions. The nitro group is also a very good leaving group. Hence possibilities for direct labeling with n.c.a. [^{18}F]fluoride are given. The resulting molecules are 6-fluoro-7-nitroquinoxaline-2,3(1H,4H)-dione (FNQX) and 7-fluoro-2,3-dioxo-1,2,3,4-tetrahy-droquinoxaline-6-carbonitrile (FCQX).

DNQX and CNQX are purchased. For the radiosynthesis of [^{18}F]FNQX only the commercial DNQX was used as precursor. Whereas additional CNQX and all chromatography standards are synthesized. Most of these molecules have been described before as cited in the text. Synthesis procedures are adopted or adapted as necessary.

3.2.1 Syntheses of CNQX, FNQX and FCQX

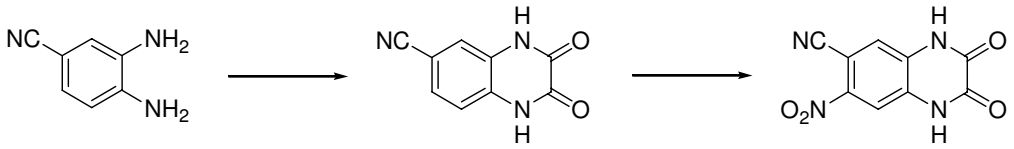
Stable FNQX, which is used as chromatographic standard and for competitive studies with tritiated AMPA, is synthesized in a two step procedure starting from 3,4-diaminofluorobenzene according to Scheme 35. Cyclisation with oxalic acid and

nitration in sulfuric acid leads to the desired compound. This synthesis follows a concept which was used by Ohmori et al. for several QX derivatives[127].



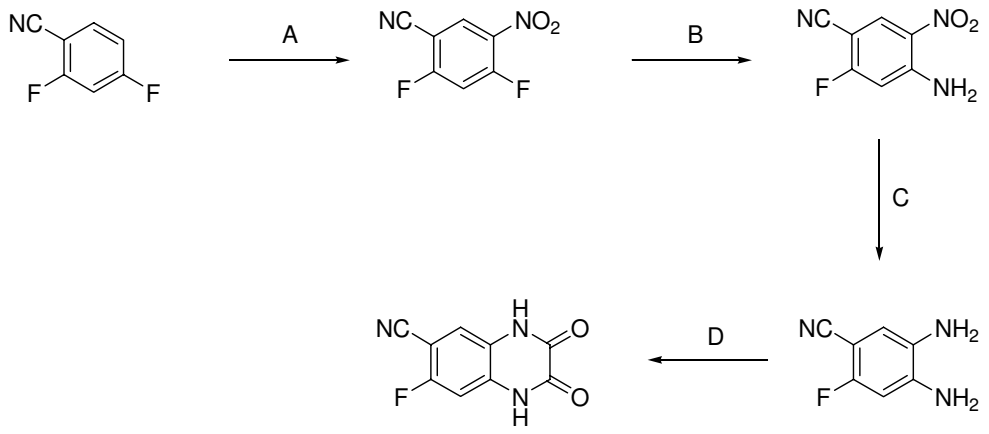
Scheme 35: Preparation of FNQX
(A) Oxalic acid, 4 N HCl, 110°C; (B) KNO_3, H_2SO_4 (96%), 0°C – room temperature

This strategy is also applicable for the preparation of CNQX as is illustrated in Scheme 36. The molecule is built up from 3,4-diaminobenzonitrile. The resulting CNQX was prepared as labeling precursor, because the purchased substance is very expensive.



Scheme 36: Preparation of FCQX
(A) Oxalic acid, 4 N HCl, 110°C; (B) KNO_3, H_2SO_4 (96%), 0°C – room temperature

Preparation of stable FCQX, also necessary as chromatographic standard and for competition studies, is done in the four step synthesis depicted in Scheme 37. The first three steps are described in a patent and have been optimized[128]. Nitration of the commercially available 2,4-difluorobenzonitrile in with potassium nitrate concentrated sulfuric acid follows a standard procedure[129]. The subsequent S_NAr reaction with ammonia is performed at room temperature and yields pure 2-fluoro-4-amino-5-nitrobenzonitrile without any byproducts. This is interesting, because the enormous influence of the activating groups is revealed as no isomers are formed. Its reduction is performed with Pd on charcoal and phosphoric acid as reducing agent. This is not according to the described method, but the use of gaseous hydrogen and autoclaving are evaded, while the yield is comparably good. Cyclisation of 4,5-diamino-2-fluorobenzonitrile, again with oxalic acid in hydrochloric acid, finally leads to FCQX.

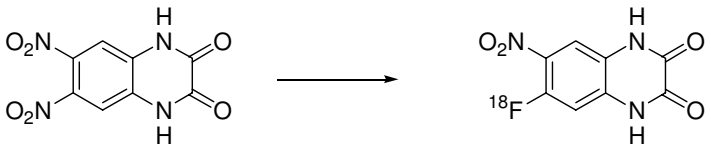


Scheme 37: Preparation of FCQX

(A) KNO_3, H_2SO_4 (96%), 0°C – room temperature;
(B) NH_3 (conc., aq.), EtOH, room temperature; (C) Pd / C, H_3PO_3, MeOH, reflux;
(D) Oxalic acid, 4 N HCl, 110°C;

3.2.2 Radiosyntheses of [^{18}F]FNQX and [^{18}F]FCQX

In order to obtain radiofluorinated FNQX and FCQX nucleophilic substitution reactions with n.c.a. [^{18}F]fluoride were performed on DNQX and CNQX. This works for DNQX at 180°C in DMSO with the potassium-Kryptofix-complex, which activates the n.c.a. [^{18}F]F^- for nucleophilic reactions. Radiochemical yields, however, are not very good. After HPLC purification and cartridge formulation they are between 5% and 10% only, with a molar activity of 1.5 GBq/µmol.

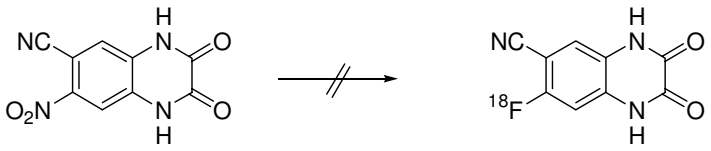


Scheme 38: Preparation of n.c.a. [^{18}F]FNQX; [K⊂2.2.2][^{18}F]F, K_2CO_3, DMSO, 180°C, 20 min

In contrast, direct labeling of CNQX was not achieved. Whether in DMF nor in DMSO at the highest reachable temperatures in these solvents [^{18}F]FNQX is formed with the Kryptofix system, which is applied in standard procedure. Tests also included the change of the cation to tetrabutyl ammonium, which is an alternative for

the potassium-Kryptofix-complex. Also both, synthesized and purchased CNQX were used as precursor.

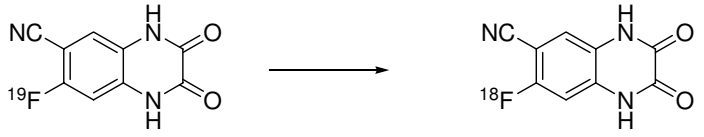
Even though the cyano group as an electron withdrawing group is activating the aromatic ring for the S_NAr-reaction, like the nitro-groups in DNQX, no conversion can be determined. Obviously, the carbamat functions reduce this effect as they are deactivating moieties for nucleophilic substitution reactions. The lesser activation by a cyano group than by a nitro group, however, is crucial while the two nitro groups in DNQX provide just enough activation for the reaction with $[^{18}F]F^-$ to proceed, while activating and functioning as a leaving group.



Scheme 39: Preparation of n.c.a. [^{18}F]FCQX from CNQX failed

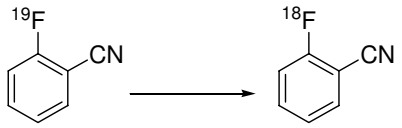
However, a loophole for synthesis can be the use of fluorine as leaving group. For S_NAr reactions fluorine is a better leaving group than the nitro-moiety, and this effect has to be used for FCQX to compensate the reduced activation. Since stable FCQX is the precursor and cannot be separated from the radiofluorinated compound, the [^{18}F]FCQX contains carrier. Thus this carrier added product is limited to in vitro applications, but could be sufficient for preliminary pharmacological studies.

The reaction conditions used are analogous to that for the production of [^{18}F]FNQX with DNQX as precursor, and the yield was accordingly low with only up to 5%. The molar activity was not determined, because results of in vitro experiments were not promising and thus further experiments with c.a. [^{18}F]FCQX seam to be inappropriate.



Scheme 40: Preparation of n.c.a. [^{18}F]FNQX; [K⊂2.2.2][^{18}F]F, K_2CO_3, DMSO, 180 °C, 20 min

However, the general proof, that the labeling system works, can be given by a control reaction with 2-fluoro-benzonitrile. For this simple molecule, also activated by only one cyano group, the isotopic exchange can be performed at 120°C only in the standard Kryptofix system. The yield is nearby quantitative alluded to the dried [^{18}F]fluoride.



Scheme 41: Preparation of c.a. [^{18}F]fluoro benzonitrile as proof of principle;
[K⊂2.2.2][^{18}F]F, K_2CO_3, DMSO, 120°C, 5 min

3.3 Pharmacological evaluation of FNQX, [^{18}F]FNQX and FCQX

N.c.a. [^{18}F]FNQX and the stable compounds FNQX and FCQX are available for pharmacological evaluation, which are performed by the radiopharmacology group of the Institute of Nuclear Chemistry, FZJ. The results are mentioned in this work, because the radiotracers are synthesized for this purpose and the values found are basic facts for the modeling of new ligands in an iterative approach. The interpretation of the results is a dialogue between pharmacologist and chemist.

Tritiated AMPA is the radioligand of choice for AMPA receptor binding studies, which are performed as competition studies. In basic experiments most AMPA-binding-sites were measured in hippocampus, followed by cortex and striatum. Low but measurable binding sites were detected in cerebellum. In autoradiographic competition assays unlabeled AMPA was the most potent competing ligand, followed by CNQX, FNQX and FCQX as shown in Table 5. At concentrations of 10 µM, AMPA and CNQX blocked [^{3}H]AMPA binding to 95%, FNQX to 85% and FCQX to only 70%.

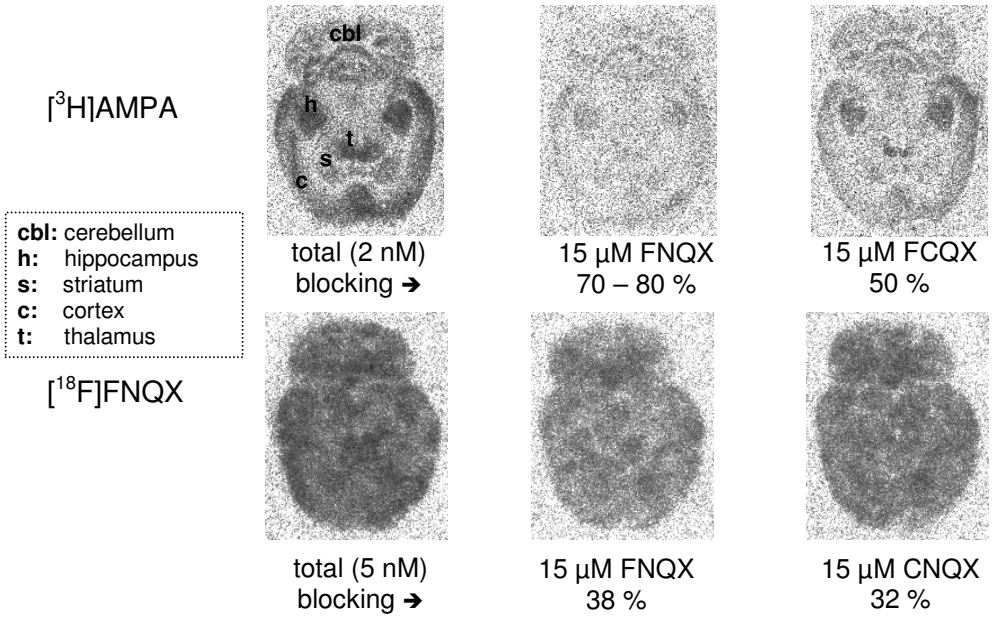
Table 5: K_i-values of the AMPA-ligands versus [^{3}H]AMPA using autoradiography of rat brain sections; data given as µM

Compound	Hippocampus	Cortex	Striatum	Cerebellum
FNQX	1.1 ± 0.2	1.7 ± 0.3	1.3 ± 0.2	1.5 ± 0.3
FCQX	2.2 ± 0.4	2.1 ± 0.7	3.9 ± 1.0	7.5 ± 0.7
CNQX	0.7 ± 0.1	0.30 ± 0.08	0.30 ± 0.09	0.7 ± 0.2
AMPA	0.080 ± 0.005	0.040 ± 0.006	0.07 ± 0.02	0.04 ± 0.01

The K_i-values are means of 2 to 4 experiments, each with 2 or 3 different rat brains. The [^{3}H]AMPA concentrations ranged from 2.3 nM to 6.7 nM.

The autoradiograms with [^{18}F]FNQX revealed a blurred binding distribution in horizontal rat brain slices as shown in Scheme 42. At 50 nM detailed brain structures are recognizable. But this image includes a high fraction of nonspecific binding, because with FNQX at a concentration of 15 µM only 38% and at 15 µM CNQX not more than 32% of [^{18}F]FNQX binding could be blocked.

Basic ex vivo experiments are perfomed with [^{18}F]FNQX on female NMRI mice. The radiotracer is applicated by tail vein injection and the animals are sacrified. Subsequently, the distribution of the radioactivity in several organs is measured. These tests show that [^{18}F]FNQX penetrates the blood-brain-barrier and accumulates in brain to an extent of 0.028% ID/g 10 min after injection and decreased to 0.016% ID/g after 60 min. Due to this very low brain uptake useful autoradiographic ex vivo images could not be obtained. Therefore, further experiments with c.a. [^{18}F]FCQX seam to be inappropriate.

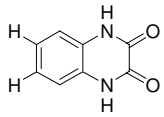
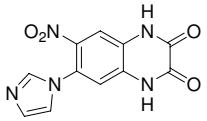
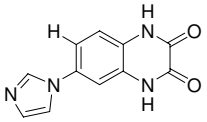
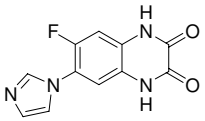
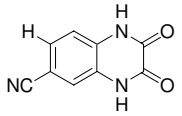
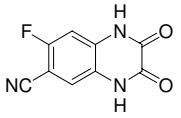
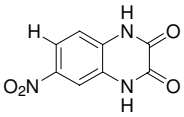
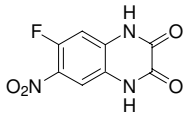


Scheme 42: Autoradiographs of in vitro experiments on horizontal rat brain slices;
% values indicate the blocking achieved of [^{3}H]AMPA or [^{18}F]FNQX, respectively, by addition of an excess (15µM) of FNQX, FCQX or CNQX

For in vitro investigations the tritiated quinoxalinedione derivatives CNQX and NBQX had earlier been used[130, 131]. In order to obtain a ligand for PET studies in vivo, the quinoxalinediones FNQX and FCQX with the potential possibility for a fluorine-18 label were synthesized as described above. The autoradiograms of the assays with [^{3}H]AMPA represented the typical AMPA receptor binding distribution[132]. As unlabeled competitor in these assays FNQX exhibits an about twofold higher affinity than FCQX in hippocampus, as this is the region with the highest density of AMPA receptors, but proved to be considerably less affine than CNQX. In this region AMPA was still about 10fold more affine than CNQX confirming several recent results[133,134,135]. However, FNQX has been labeled with fluorine-18 and showed a high nonspecific binding and diffuse binding distribution in in vitro autoradiography. Preliminary in vivo tests resulted also in a rather low brain uptake. So far this fact was postulated for AMPA antagnoists of the QX-family[136,137]. Only once the low brain uptake could be monitored by ex vivo experiments with a radiotracer for the NMDA-receptor by Ametamey et al.[97]. In conclusion, [^{18}F]FNQX and [^{18}F]FCQX appear to be rather unsuitable as ligands for AMPA receptor studies.

However, the pharmacological data of the ligands can be integrated in the general concept of SAR for quinoxalinediones. According to the values in Table 6, a comparison of the K_i-values of the new QX-derivatives FCQX and FNQX to the monosubstituted cyano- and nitro-QX shows negligible small differences (FCQX: 5.0 µM to CQX: 3.9 µM; FNQX: 2.0 µM to NQX: 1.4 µM). Compared to derivatives with two electron withdrawing groups like CNQX (0.27 µM) and DNQX (0.20 µM) their loss of affinity is > 10fold.

Table 6: Comparison of different QX derivatives and their K_i-values;
data from J. Ohmori et al.[127], if not otherwise indicated

Substance	K_i-values [µM]	Substance	K_i-values [µM]
	24% (100 µM)*	 YM 90K	0.08
	1.6		14% (1.0 µM)*
 CQX	5.0	 FCQX	3.9#
 NQX	2.0	 FNQX	1.4#

* percent value shows resulting inhibition of binding; applied substance concent. in brackets

\# Data from this work as mean values from Table 5

In compounds, where the affinity to the AMPA receptor results from sterical effects, like for the imidazolyl-moiety the influence of a neighboring atom is extreme. YM 90K (imidazolyl-nitro-QX: 0.08 µM) is one of the most potent ligands. Imidazolyl-QX with a K_i-value of 1.6 µM exhibits a 20fold lower affinity. Finally, for imidazolyl-fluoro-QX no K_i-value can be determined. This comparison corroborates the thesis, that an

electron withdrawing group in the southwest region and a voluminous moiety in the northwest region of QX-molecules are essential for good AMPA-receptor affinity.

In summary two new ligands for the AMPA-receptor system FNQX and FCQX could be synthesized and labeled with fluorine-18. Even though that they proved not suitable for in vivo application some insight into SAR of QX-derivatives could be gained.

4 Experimental

4.1 Chemicals

All chemicals are purchased from Merck (Germany, KMF (Germany), Riedel-de Häen (Germany), Fluka (Switzerland) or Aldrich (Germany) in p. a. quality, if not otherwise mentioned in the text.

4.2 (Radio-)HPLC

The HPLC-system used for identification of compounds consists of a Sykam S1000 pump (constant flow 1 mL/ min) and a Phenomenex Bondclone (10 µ C18, 300 x 3.8 mm) column. Detection was done by a Knauer UV detector K2500 at 254 nm and an EG & G Ortec NaI radiodetector with an ACE Mate Amplifier and BIAS supply.

Semi-preparative separation was done with a Merck Hitachi L6000 pump (constant flow 5 mL/ min) and a µ-Bondapack (C18, 300 x 8 mm) column. Detection was done by a Knauer UV detector K2501 at 254 nm and an EG & G Ortec NaI radiodetector with an ACE Mate Amplifier and BIAS supply.

Data acquisition and interpretation was performed via a Raytest Gina box and the corresponding Gina Star software.

4.3 Thin layer chromatography

Thin layer chromatography was performed on TLC aluminium roll Silica gel 60 F_{254} from Merck. It was developed with diethyl ether/ hexane (volume ratio 1:1) plus 20 µL acetic acid per 5 ml eluent or as otherwise mentioned in the text. Spots were visualized under a Desaga MinUVIS lamp at 254 nm or by heating in a hot air stream after spraying with a solution of 0.45 g ninhydrin in 150 mL ethanol and 3 mL acetic acid. Radio-TLC plates were measured on a Raytest Mini Gita system equipped with a closed β-counting tube and interpreted with the corresponding software.

4.4 NMR spectroscopy

^{1}H-, ^{13}C- and ^{19}F-NMR spectra were recorded on a Bruker Avance 200 spectrometer by Dr. M. Holschbach or Mr. W. Wutz with samples dissolved in solvents mentioned in the text. All shifts are given in δ ppm using the signals of the appropriate solvent as a reference.

4.5 Melting points

All melting points were measured in a BÜCHI Melting Point B-540 apparatus and data is corrected.

4.6 Organic syntheses

4.6.1 General method for preparation of alkyl-carbamate nitrophenylesters (3a - 3c) [modified from 125]

In a 100-mL flask 10 mmol of 4-nitrophenyl chloroformate are dissolved in 5 mL dry methylene chloride. 10 mmoL of ethyl diisopropyl amine and 10 mmol of the corresponding alkylamine are dissolved in 5 mL of dry dichloromethane and added dropwise. The solution is stirred for 4 hours.

The solution is washed with 1 M hydrochloric acid three times. The organic layer is dried over sodium sulphate and evaporated to dryness after filtration. Recrystallization of the residue from iso-propyl alcohol leads to the desired alkyl carbamate 4-nitrophenylester.

n-Propyl carbamate 4-nitrophenylester (3a)

^{1}H-NMR (DMSO-d6): (2H, d, 8.27 ppm Ar-H), (1H, t, 8.06 ppm N-H), (2H, d, 7.41 ppm Ar-H), (2H, q, 3.07 ppm C-H_2), (2H, q, 1.51 ppm C-H_2), (3H, t, 0.91 ppm C-H_3)

m.p.: 99.8 °C

MS (ESI): (m+H)/z = 225.2

Yield: 1.90 g (8.44 mmol; 84 %)

cyclo-Hexyl carbamate 4-nitrophenylester (3b)

^{1}H-NMR (DMSO-d6): (1H, s, 8.34 ppm N-H), (2H, m, 7.40 ppm Ar-H), (2H, m, 7.09 ppm Ar-H), (1H, d, 6.03 ppm C-H), (10H, b, 1.83 – 1.03 ppm C-H_2)

m.p.: 135.8 °C [Lit.: 144-145 °C]

MS (ESI): (m+H)/z = 265.1

Yield: 2.32 g (8.75 mmol; 88 %) [Lit.: 78 %]

Benzyl carbamate 4-nitrophenylester (3c)

^{1}H-NMR (DMSO-d6): (1H, t, 8.62 ppm N-H), (2H, m, 8.27 ppm Ar-H), (7H, m, 7.34 ppm Ar-H), (2H, d, 4.36 ppm C-H_2)

m.p.: 131.0 °C

MS (ESI): (m+H)/z = 295.1

Yield: 2.80 g (9.5 mmol; 95 %)

4.6.2 General method for preparation of aryl-carbamate nitrophenylesters (3d - 3e) [modified from 125]

In a 100-mL flask 10 mmol of 4-nitrophenyl chloroformate are dissolved in 5 mL of dry methylene chloride. The flask is cooled in a water bath. 10 mmol of ethyl diisopropyl amine and 10 mmol of the selected aniline derivative are dissolved in 5 mL of dry dichloromethane and added dropwise. This solution is stirred for 3 hours and afterwards washed three times with 1 M hydrochloric acid. The organic layer is dried over sodium sulphate and evaporated to dryness after filtration. The residue is the analytically pure aryl carbamate 4-nitrophenylester.

Phenyl carbamate 4-nitrophenylester (3d)

^{1}H-NMR ($CDCl_3$): (1H, s, 10.46 ppm N-H), (2H, d, 8.32 ppm Ar-H), (4H, m, 7.55 ppm Ar-H), (1H, t, 7.12 ppm Ar-H)

m.p.: 148.6 °C [Lit.: 147-150 °C]

MS (ESI): (m+H)/z = 259.3

Yield: 1.72 g (6.66 mmol; 67 %) [Lit.: 44 %]

Fluorophenyl carbamate 4-nitrophenylester (3e)

^{1}H-NMR ($CDCl_3$): (2H, d, 8.32 ppm Ar-H), (4H, m, 7.47 ppm Ar-H), (2H, m, 7.11 ppm Ar-H), (1H, b, 7.01 ppm N-H)

m.p.: 157.1 °C

MS (ESI): (m+H)/z = 277.2

Yield: 2.29 g (8.30 mmol; 83 %)

4.6.3 General method for preparation of fluorophenyl urea derivatives (4a - 4d) [modified from 125]

The corresponding alkyl or aryl carbamate nitrophenyl ester (5 mmol) is dissolved in 5 ml of dry THF. After addition of 5 mmol 4-fluoroaniline (or *n*-propylamine) and 5 mmol of ethyl diisopropyl amine the mixture is stirred for several hours at room temperature. The solvent is evaporated under reduced pressure and the residue dissolved in ethyl acetate.

The solution is washed three times with 1 M sodium hydroxide solution and 1 M hydrochloric acid. The organic layer is dried over sodium sulphate and evaporated to dryness after filtration.

N-Fluorophenyl-N'-n-propyl urea (4a)

Preparation only via fluorophenyl carbamate nitrophenyl ester and *n*-propyl amine.

^{1}H-NMR (DMSO-d6): (1H, s, 8.19 ppm N-H), (2H, m, 7.30 ppm Ar-H), (2H, m, 6.88 ppm Ar-H), (1H, b, 5.90 ppm N-H), (2H, q, 3.07 ppm C-H_2), (2H, m, 1.46 ppm C-H_2), (3H, t, 0.88 ppm C-H_3)

m.p.: 155.0 °C

MS (ESI): (m+H)/z = 197.1

Yield: 0.90 g (4.57 mmol; 91 %)

N-Fluorophenyl-N'-cyclohexyl urea (4b)

^{1}H-NMR (DMSO-d6): (1H, s, 8.31 ppm N-H), (2H, m, 7.38 ppm Ar-H), (2H, t, 7.05 ppm Ar-H), (1H, d, 6.03 ppm N-H), (1H, b, 3.4 ppm C-H), (5H, b, 1.83 – 1.54 ppm C-H_2), (5H, b, 1.42 – 1.08 ppm C-H_2)

m.p.: 104.4 °C

MS (ESI): (m+H)/z = 237.2

Yield: 0.65 g (2.74 mmol; 55 %)

N-Fluorophenyl-N'-benzyl urea (4c)

^{1}H-NMR (DMSO-d6): (1H, s, 8.62 ppm N-H), (7H, m, 7.36 ppm Ar-H), (2H, t, 7.08 ppm Ar-H), (1H, t, 6.62 ppm N-H), (2H, d, 4.31 ppm C-H_2)

m.p.: 179.0 °C

MS (ESI): (m+H)/z = 245.1

Yield: 1.10 g (4.49 mmol; 90 %)

N-Fluorophenyl-N'-phenyl urea (4d)

^{1}H-NMR (DMSO-d6): (1H, d, 8.66 ppm N-H), (4H, m, 7.48 ppm Ar-H), (2H, t, 7.30 ppm Ar-H), (2H, t, 7.13 ppm Ar-H), (1H, t, 6.97 ppm Ar-H)

m.p.: 235.0 °C

MS (ESI): (m+H)/z = 231.2

Yield: 0.96 g (4.15 mmol; 83 %)

4.6.4 6-Fluoroquinoxaline-2,3(1H,4H)-dione [modified from 127]

In a 100-mL flask 7.93 mmol of 4-fluoro-1,2-phenylendiamine are dissolved in 20 mL of 4 N hydrochloric acid. After addition of 15.77 mmol oxalic acid, the mixture is heated for 4 h in an oil bath at 110°C. The solution is poured on ice and the precipitate separated and washed with water

Yield: 1.35 g (7.48 mmol; 94 %) [Lit.: 94 %]

4.6.5 6-Fluoro-7-nitroquinoxaline-2,3(1H,4H)-dione (FNQX) [modified from 127]

7.49 mmol of 6-Fluoroquinoxaline-2,3(1H,4H)-dione are dissolved in 10 mL of concentrated sulfuric acid and cooled in an ice bath. At this low temperature 8.24 mmol of potassium nitrate are added slowly in portions and the mixture is stirred for 30 min. After the cooling bath is removed it is stirred for another 150 min. The precipitate is separated and washed with water.

^{1}H-NMR (DMSO-d6): (1H, d, 7.07 ppm Ar-H), (1H, d, 7.85 ppm Ar-H), (1H, s, 12.11 ppm N-H), (1H, s, 12.39 ppm N-H)

[Lit: ^{1}H-NMR (DMSO-d6): (1H, d, 7.06 ppm Ar-H), (1H, d, 7.84 ppm Ar-H), (1H, s, 12.09 ppm N-H), (1H, s, 12.36 ppm N-H)]

^{18}F-NMR (DMSO-d6): (1F, s, 124.01 ppm)

Anal. Calcd for $C_8H_4FN_3O_4$: C, 42.68; H, 1.79; F, 8.44; N, 18.66

Found: C, 42.7 ± 0.2; H, 1.92 ± 0.02; N, 18.7

Yield: 1.01 g (4.49 mmol; 60 %) [Lit.: 54.6 %]

4.6.6 2,3-Dioxo-1,2,3,4-tetrahydroquinoxaline-6-carbonitrile [modified from 127]

In a 50-mL flask 3.76 mmol of 3,4-diamino benzonitrile are dissolved in 15 mL of 4 N hydrochloric acid. After addition of 7.52 mmol oxalic acid, the mixture is heated for 4 h in an oil bath at 110°C. The solution is poured on ice and the precipitate separated and washed with water.

^{1}H-NMR (DMSO-d6): (3H, m, 7.2 – 7.7 ppm Ar-H), (1H, s, 12.11 ppm N-H), (1H, s, 12.18 ppm N-H)

MS (ESI): (m+H)/z = 188.0

Yield: 0.65 g (3.45 mmol; 97.7 %)

4.6.7 7-Nitro-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-carbonitrile (CNQX) [modified from 98]

3.74 mmol of 2,3-Dioxo-1,2,3,4-tetrahydroquinoxaline-6-carbonitrile are dissolved in 12 mL of concentrated sulfuric acid and cooled in an ice bath. At this low temperature 6.00 mmol of potassium nitrate are added slowly, in portions and the mixture is stirred for 30 min. After the cooling bath is removed it is stirred for another 150 min. The precipitate is filtered off and washed with water.

^{1}H-NMR (DMSO-d6): (1H, s, 7.57 ppm Ar-H), (1H, s, 8.05 ppm Ar-H), (1H, s, 12.47 ppm N-H), (1H, s, 12.51 ppm N-H)

[Lit.: ^{1}H-NMR (DMSO-d6): (7.7 ppm Ar-H), (8.2 ppm Ar-H) and downfield of TMS]

MS (ESI): (m+H)/z = 225.2

Yield: 0.36 g (1.55 mmol; 41 %) [Lit.: 75 %]

4.6.8 2,4-Difluoro-5-nitrobenzonitrile [from 128]

In a 50-mL flask 7.20 mmol of 2,4-difluoro benzonitrile are dissolved in 12 mL of concentrated sulfuric acid and cooled in an ice bath. After addition of 14.40 mmol of potassium nitrate in small portion, the mixture is stirred for 30 min. Afterwards the cooling bath is removed, and the mixture is stirred at room temperature for another 45 min. The suspension is poured on ice and the precipitate separated and washed with water.

^{1}H-NMR (DMSO-d6): (1H, q, 8.79 ppm Ar-H), (1H, q, 8.82 ppm Ar-H)

[Lit.: ^{1}H-NMR (DMSO-d6): (1H, q, 8.093 ppm Ar-H), (1H, q, 8.982 ppm Ar-H)]

Yield: 1.0 g (5.43 mmol; 75 %) [Lit.: 38 %]

4.6.9 4-Amino-2-fluoro-5-nitrobenzonitrile [from 128]

In a 100-mL flask 5.43 mmol of 2,4-Difluoro-5-nitrobenzonitrile are dissolved in 10 mL of ethanol. An aqueous ammonia solution is added slowly (ca. 40 mL). The yellow product is filtered off, washed with ammonia solution and water and dried in an exsiccator at reduced pressure.

^{1}H-NMR (DMSO-d6): (1H, d, 6.82 ppm Ar-H), (2H, b, 8.10 ppm N-H), (1H, d, 8.49 ppm Ar-H)

[Lit.: ^{1}H-NMR (DMSO-d6): (1H, d, 6.85 ppm Ar-H), (2H, b, 8.22 ppm N-H), (1H, d, 8.57 ppm Ar-H)]

Yield: 0.71 g (3.92 mmol; 72 %) [Lit.: 87 %]

4.6.10 4,5-Diamino-2-fluorobenzonitrile [modified from 128]

A suspension of 1,38 mmol 4-amino-2-fluoro-5-nitrobenzonitrile, 2 g of phosphorous acid and about 100 mg of Pd on activated carbon (10 wt. %) in 15 mL of methanol are refluxed in an oil bath at 80°C for 4 h. The carbon is filtered of. Addition of aqueous ammonia solution to the filtrate leads to precipitation of the product as a light yellow substance.

^{1}H-NMR (DMSO-d6): (2H, b, 4.74 ppm N-H), (2H, b, 5.90 ppm N-H), (1H, d, 6.43 ppm Ar-H), (1H, d, 6.68 ppm Ar-H)

[Lit.: ^{1}H-NMR (DMSO-d6): (2H, b, 4.7 ppm N-H), (2H, b, 5.863 ppm N-H), (1H, d, 6.375 ppm Ar-H), (1H, d, 6.56 ppm Ar-H)]

MS (ESI): (m+H)/z = 152.1

Yield: 0.13 g (0.86 mmol; 62 %) [Lit.: 100 %]

4.6.11 7-Fluoro-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-carbonitrile (FCQX)

In a 50-mL flask 2.34 mmol of 4,5-diamino-2-fluorobenzonitrile are dissolved in 10 mL of 4 N hydrochloric acid. After addition of 5.00 mmol oxalic acid, the mixture is heated for 4 h in an oil bath at 110°C. The solution is poured on ice and the precipitate filtered off and washed with water.

^{1}H-NMR (DMSO-d6): (1H, d, 7.08 ppm Ar-H), (1H, d, 7.41 ppm Ar-H), (1H, s, 12.11 ppm N-H), (1H, s, 12.32 ppm N-H)

MS (ESI): (m+H)/z = 206.6

Anal. Calcd. for $C_9H_4FN_3O_2$: C, 52.69; H, 1.97; N, 20.48

Found: C, 52.4 ± 0.1; H, 2.08 ± 0.02; N, 20.6 ± 0.3

Yield: 0.20 g (0.94 mmol; 41.8 %)

4.7 Radiochemical syntheses

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

N.c.a. [^{18}F]fluoride is produced via the $^{18}O(p,n)^{18}F$ nuclear reaction by bombardment of an isotopically enriched [^{18}O]water target with a 17 MeV proton beam at the BC 1710 cyclotron (JSW), as described previously[18].

4.7.2 Drying of n.c.a. [^{18}F]fluoride

The aqueous [^{18}F]fluoride solution (10 - 50 µL, e.g. 64.2 MBq) is added to 20 mg of Kryptofix 2.2.2. and 26 µl of a 1 M potassium carbonate solution. The mixture is diluted with 0.9 mL of dry acetonitrile (< 0.003 % H_2O) and transferred via syringe to a 5 mL conical vial (Reactivial) closed with a silicon septum. The solvent is evaporated under a stream of argon at 80°C and 750 mbar. The azeotropic drying is repeated with 1 mL acetonitrile, followed by evaporation of the dry Reactivial for 5 min to 40 - 50 mbar.

4.7.3 Radiosynthesis of n.c.a. 4-[^{18}F]fluoroaniline ([^{18}F]1)

The dry cryptate complex (example of reference activity: 52.9 MBq) and approx. 4 mg 1,4-dinitrobenzene are solubilised in 0.2 mL of dry DMSO. The stirred solution is heated in an oil bath at 120°C for 3 min.

Afterwards the reaction mixture is diluted with 10 mL of water and passed through a Waters C18+ cartridge (500 mg, conditioned with 10 mL of ethanol and 10 mL of water).

The 1,4-[^{18}F]fluoronitrobenzene formed (**[^{18}F]1**, 45.3 MBq, RCY: 88 %) is eluted with 2 mL of methanol into a 25 mL two necked flask. After addition of approx. 10 mg palladium black and approx. 100 mg phosphorous acid the flask is closed with a plug and a septum. A cannula in the septum is needed for pressure equalization, while the flask is heated under stirring in an oil bath at 60°C for 15 min. The reaction mixture is

diluted with 20 mL of a 1 M sodium hydroxide solution and passed through a Merck EN cartridge (500 mg, conditioned with 10 mL of ethanol and 10 mL of a 1 M sodium hydroxide solution). After a synthesis time of 30 min 34.9 MBq (RCY: 82 %) of n.c.a. 4-[^{18}F]fluoroaniline (**[^{18}F]2**) are fixed on the cartridge. No radioactive by-products can be determined by radio HPL- or thin layer chromatography.

4.7.4 Radiosynthesis of n.c.a. 4-[^{18}F]fluorophenyl ureas ([^{18}F]4a - 4d)

[^{18}F]2 is eluted from the cartridge (cf. above) with 2 mL of dry THF and the solution passed through a Merck Lichrolut cartridge filled with a mixture of kieselguhr powder and anhydrous sodium sulphate (volume ratio 1:1, wetted with 2 mL THF) for drying.

*Synthesis via n.c.a. 4-[^{18}F]fluorophenyl carbamate 4-nitrophenylester (**[^{18}F]3e**)*

About 30 mg of 4-nitrophenyl chloroformate are added to the THF solution containing n.c.a. 4-[^{18}F]fluoroaniline (**[^{18}F]2**) in a Reactivial. The mixture is stirred at room temperature for 15 min. Afterwards 40 µL of the corresponding amine are added. In the case of aniline, addition of an assistance base, e. g. 30 µL Hünigs base, is necessary. After another 15 min of stirring at slightly raised temperature (30 – 40 °C) in an oil bath HPL- and thin layer chromatography show the desired ureas **[^{18}F]4a - 4d** as the only radioactive products. Fixing the ureas on a Merck EN cartridge is done after dilution of the reaction mixture with 20 mL of a 1 M sodium hydroxide solution (cartridge conditioned with 10 mL of ethanol and 10 mL of a 1 M sodium hydroxide solution). The radiochemical yields are listed in Table 3 (cf. chapter 3.1.3).

*Synthesis of ureas **[^{18}F]4a - 4d** via n.c.a. 4-[^{18}F]fluoroaniline and carbamate 4-nitrophenylesters (**3a - 3d**)*

About 30 mg of the corresponding carbamate-4-nitrophenylester **3a - 3d** are added to the THF elution containing n.c.a. 4-[^{18}F]fluoroaniline in a Reactivial. The mixture is stirred at slightly raised temperature for 15 min. After this time, HPL- and thin layer chromatography show the desired ureas **[^{18}F]4b - 4d** as the major or exclusive products. Nearly no conversion is observed with the *n*-propyl derivative **[^{18}F]4a**. The remaining radioactivity signals could be identified in all cases as 4-[^{18}F]fluoroaniline.

Fixing the ureas on a Merck EN cartridge is done after dilution of the reaction mixture with 20 mL of a 1 M sodium hydroxide solution (cartridge conditioned with 10

mL of ethanol and 10 mL of a 1 M sodium hydroxide solution). The radiochemical yields are listed in Table 4 (cf. chapter 3.1.3).

4.7.5 N.c.a. 6-[^{18}F]Fluoro-7-nitroquinoxaline-2,3(1H,4H)-dione (n.c.a. [^{18}F]FNQX)

The dry cryptate complex (70.7 MBq, reference activity) and ca. 3 mg DNQX are solubilised in 0.15 mL of dry DMSO. The stirred solution is heated in an oil bath at 180°C for 20 min.

Afterwards the vial is cooled to room temperature with dry ice. The crude mixture is diluted with 0.4 mL of HPLC eluent and applicated to the semipreparative HPLC-system (MeCN (20) : H_2O (80) + HOAc (10 mL / L). The product fraction is collected in about 15 mL of eluent. The solution is passed through a Merck EN cartridge (500 mg, conditioned with 10 mL of ethanol and 10 mL of water).

The [^{18}F]FNQX (4.8 MBq, RCY: 10%) is eluted with 1 mL of ethanol. An aliquot of the ethanol solution is applicated to the analytic HPLC system for quality control and determination of the molar activity (1.5 GBq / µmol).

4.7.6 C.a. 7-[^{18}F]Fluoro-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-carbonitrile (c.a. [^{18}F]FCQX)

The dry cryptate complex (55.2 MBq, reference activity) and ca. 8 mg DNQX are solubilised in 0.15 mL of dry DMSO. The stirred solution is heated in an oil bath at 180°C for 20 min.

Afterwards the vial is cooled to room temperature with dry ice. The crude mixture is diluted with 10 mL of water and passed through a Merck EN cartridge (500 mg, conditioned with 10 mL of ethanol and 10 mL of water). The [^{18}F]FCQX (2.4 MBq, RCY: 5%) is eluted with 1 mL of ethanol.

4.7.7 Determination of the molar activity

For determination of the molar activity of labeling products the amount of carrier has to be measured. This is achieved by quantification of the UV-signal in HPL-chromatograms of the stable compounds. For each measurement an aliquot of 10 µL of a prepared solution of FNQX or FCQX in the eluent was applied to the HPLC system. The corresponding UV-signal was integrated and the values are shown in Diagram 1 and 2. The dilution series result from two basis solutions for each substance. For every value an uncertainty of 15% is assumed based on errors of weighting, handling and UV-absorption-detection. The formula in the diagram can be used for calculation of an unknown amount of substance in a probe, applied to the HPLC. This procedure is utilized to determine the amount of carrier in an aliquot of the radioactive product solution. With the known activity of the aliquot the molar activity is the quotient of activity of the aliquot and the amount of carrier.

FNQX

Initial weight 1: 5.27 mg
Initial weight 2: 5.79 mg
Eluent: [H_2O (80%) : MeCN (20%)] + HOAc (10 mL/ L)

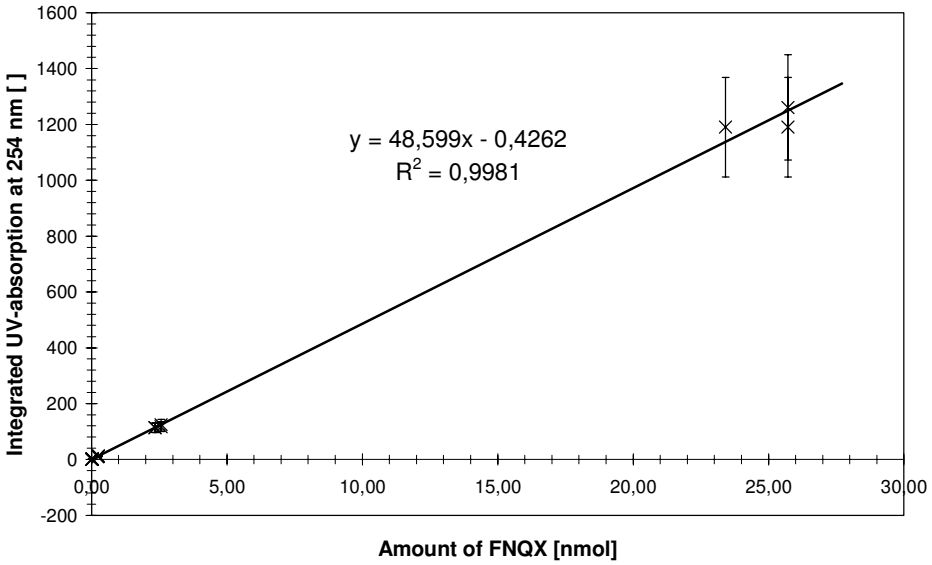


Diagram 1: Determination of the amount of FNQX in HPL-chromatograms

FCQX

Initial weight 1: 0.38 mg
Initial weight 2: 0.35 mg
Eluent: [H_2O (90%) : MeCN (10%)] + HOAc (10 mL/ L)

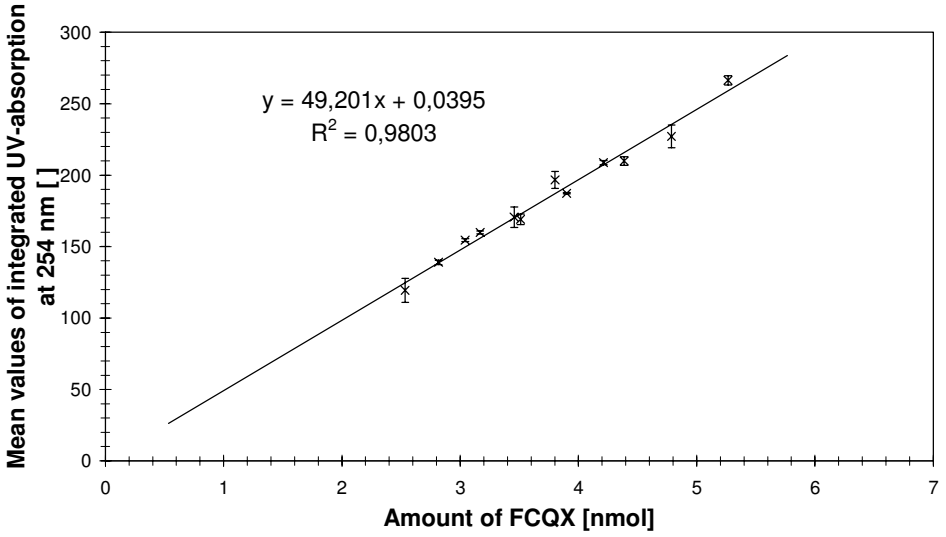


Diagram 2: Determination of the amount of FCQX in HPL-chromatograms

4.8 Pharmacological evaluation

Pharmacological evaluation was performed by the radiopharmacology group of the Institute of Nuclear Chemistry, namely Mrs. Dr. W. Sihver, Mrs. A. Schulze and Mr. W. Wutz. The experimental details are mentioned here for the sake of completeness.

4.8.1 In vitro autoradiography

For autoradiography, frozen brains of Wistar rats were cut horizontal at -18°C into 20 µm thick sections (Leica AG Microsystems, Germany), mounted onto gelatin-coated object glasses (LO-Laboroptik GmbH) and stored at -80°C.

For the assay, the brain sections were thawed and dried at room temperature. After preincubation for 15 min at 4°C in 50 mM Tris-HCl containing 0.5 mM $CaCl_2$, the

cryosections were incubated with additionally 50 mM KSCN and 4.3 ± 1.0 nM [^{3}H]AMPA (spec. act. 45.5 Ci/mmol) or two different concentrations (5 and 50 nM) of [^{18}F]FNQX (spec. act. 42.1 Ci/mmol) for 45 min at 4°C. For competition assays with [^{3}H]AMPA adjacent sections were incubated in the presence of different concentrations of unlabeled CNQX, APMA (both Sigma-Aldrich), FNQX or FCQX, respectively. In order to block the [^{18}F]FNQX binding, adjacent sections were incubated with 15 µM unlabeled FNQX or FCQX. After treatment the sections were washed twice for 15 sec in Tris-HCl at 4°C, dipped in deionized water, dried and in the case of [^{3}H]AMPA exposed for 5 days or for 2 h with [^{18}F]FNQX to phosphor image plates (Fuji). These were scanned with a laser phosphor imager (BAS 5000, Fuji) utilizing software from the vendor (Version 3.14, Raytest, Germany). Data from regions of interest, such as hippocampus, cortex, striatum and cerebellum, were obtained by the image analyzer program AIDA (Version 3.52, Raytest, Germany) and further processed in Excel and a curve fitting program GraphPad Prism.

4.8.2 Ex vivo autoradiography

In order to investigate if [^{18}F]FNQX passes the blood-brain barrier, female NMRI mice weighting 34 ± 2 g received 3 µCi [^{18}F]FNQX by tail vein injection. The animals were sacrificed 10 min and 60 min after tracer injection, the brains removed, weighted and the radioactivity measured in well a well-counter.

(Institutional approval of animal studies: 50.203.2-KFA 12/02).

5 Summary

Glutamic acid is the principal excitatory neurotransmitter in the central nervous system and plays a significant role regulating neuronal activity via various glutamate receptors. The AMPA receptor as part of this neuronal family was in the scope of research of this work.

Putative ligands labeled with fluorine-18 should provide an insight in the relationship of antagonist binding in vitro and visualization of disorders in vivo. The method of imaging with such radiolabeled tracers is positron emission tomography, with several advantages towards other nuclear medicinal applications.

Derivatives of quinoxalinedione (QX) were chosen as chemical lead. Especially complex phenyl urea derivatives show promising results in several studies performed in vitro and in vivo. Affinities are high and stability is given under physiological conditions. The nitro group in a known nitrophenyl urea (LU 115455) was to be substituted by a fluorine atom. A second tracer was derived from cyano-nitro-QX. In this case a fluorophenyl urea moiety displaces the nitro group.

The radiosynthesis of [^{18}F]fluorophenyl ureas was not described before. Besides AMPA antagonists there is a manifold of urea derivatives in pharmaceutical chemistry. Therefore these structures appeared generally interesting as class of compounds.

Attempts to yield these ureas on a no carrier added scale were hampered by various obstacles. For the most common route, a reaction of an isocyanate with an amine, the fluorophenyl isocyanate had to be labeled. Direct experiments with isotopic exchange reactions with n.c.a. [^{18}F]fluoride on 4-[^{19}F]fluorophenyl isocyanate failed. The isocyanate group is obviously not activating the aromatic ring enough, in order to enable a nucleophilic substitution.

An alternative multistep synthesis comprises the formation of para-[^{18}F]fluoroaniline, subsequent conversion to an isocyanate and finally reaction with a corresponding amine. The production of n.c.a. [^{18}F]fluoroaniline refined and optimized known procedures. The product is made in two steps from cheap dinitrobenzol as precursor within 30 min with a radiochemical yield of over 80%. Reduction with Pd black and phosphoric acid in methanol is easily done. Cartridge purification leads to a radiochemical pure product.

The next step towards ureas is more demanding. Isocyanates are made of phosgene or a substitute reagent. None of the tested reagents, however, reacted

with n.c.a. [^{18}F]fluoroaniline. Diphosgene, triphosgene and CDI are the most common reagents in this field, but their application yielded no result.

As an alternative route, the formation of carbamate nitrophenyl esters was chosen. The activated ester is substituted in a second step with an amine of choice. These syntheses are well described for many different substances. Nevertheless, some modifications are applied to optimize the results for the individual molecules. Four types of substances were examined. The resulting n.c.a. N-[^{18}F]fluorophenyl ureas are substituted at the N'-nitrogen with the n-propyl, c-hexyl, benzyl or phenyl moiety. Either the carbamate nitrophenyl ester of the unlabeled amine or of the [^{18}F]fluoroaniline is formed. The choice of concept depends on the individual molecule and synthetic feasibility. Both ways proved possible for the formation of labeled ureas.

Application of the method for syntheses of the designated AMPA-ligands failed due to the chemical limitation of the chosen QX-derivatives. Solubility of the corresponding amines is generally low. Purification is very complicate. Furthermore behavior of their reaction at different pH values is not predictable.

Before more efforts were taken, labeling of compounds with reduced complexity should clarify basic pharmacological questions. The new tracers [^{18}F]FNQX and [^{18}F]FCQX are derived from DNQX and CNQX. Those last two substances are standard antagonists for in vitro competition studies with new putative AMPA ligands. In those studies the tritiated molecules are used while ^{18}F-labeled tracers might be alternatives.

The chromatographic standards FNQX and FCQX were synthesized in a two step and a four step procedure, respectively. They are adapted from described synthetic routes and optimized in several aspects. The general concept is to react a substituted ortho-diamino benzene compound with oxalic acid in hydrochloric acid. A double condensation reaction leads to a substituted quinoxalinedione. In CNQX and FNQX the nitro group is inserted by a subsequent nitration step.

Stable FNQX and FCQX were used in competition studies with [^{3}H]AMPA in vitro. The precursor CNQX was synthesized for reasons of costs. Radiosyntheses were performed with DNQX or CNQX and [^{18}F]fluoride, which was activated by the cationic potassium-Kryptofix complex. This worked out for DNQX with low yields of [^{18}F]FNQX after HPLC purification and cartridge formulation of 5% to 10% with a molar activity of 1.5 GBq/µmol. The results were sufficient for basic in vitro and ex vivo tests.

Labeling experiments with CNQX failed, so that an alternative was necessary. The effect of the better leaving group fluorine had to be used to yield [^{18}F]FCQX, and a fluorine-18 for fluorine-19 exchange reaction appeared necessary. Reaction

conditions are analoguous to that for the production of [^{18}F]FNQX. The yield of [^{18}F]FCQX was correspondingly low with < 5%.

In autoradiographic competition assays, which were performed by the radiopharmacology group of the INC, AMPA was the most potent ligand, followed by CNQX, FNQX and FNQX. Complete blocking of the binding of [^{3}H]AMPA by FNQX or FCQX was not observed. [^{18}F]FNQX showed high nonspecific binding in vitro autoradiography and a diffuse binding distribution. Preliminary ex vivo tests resulted in rather low brain uptake, what so far was just postulated for antagonists from the QX-family and once shown for a related NMDA ligand.

Nevertheless, comparison of the K_i-values of the fluorinated compounds with several other compounds was interesting. In FCQX and FNQX the fluorine atom is in the ortho position to the strong electron withdrawing cyano or nitro group, respectively. Compared to the mono-substituted cyano- or nitro-QX, respectively, the difference of K_i-values is negligibly small (FCQX: 5.0 µM to CQX: 3.9 µM; FNQX: 2.0 µM to NQX: 1.4 µM). Compared to derivatives with two electron withdrawing group like CNQX (0.27 µM) and DNQX (0,20 µM) the loss of affinity is a factor > 10.

In compounds, where the affinity to the AMPA receptor results from sterical effects, like for the imidazolyl-moiety the influence of a neighboring atom is extreme. YM 90K (imidazolyl-nitro-QX: 0.08 µM) is one of the most potent ligands. Imidazolyl-QX with a K_i-value of 1.6 µM exhibits a 20fold lower affinity. Finally, for imidazolyl-fluoro-QX no K_i-value can be determined. This comparison corroborates the thesis, that an electron withdrawing group in the southwest region and a voluminous moiety in the northwest region of QX-molecules are essential for good AMPA-receptor affinity.

In summary, two new ligands for the AMPA-receptor system could be synthesized and labeled with fluorine-18. Even though that they are not suited for standard application some insight into SAR of QX-derivatives could be gained.

Furthermore, the search for labeling concepts of more complex molecules led to a synthesis route for preparation of n.c.a. [^{18}F]fluorophenyl ureas and an optimum access to n.c.a. [^{18}F]fluoroaniline. They represent important moieties in pharmacologically relevant molecules and versatile intermediates in radiosyntheses, respectively.

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Abbreviations

Abbreviation	Meaning	Abbreviation	Meaning
°C	degree Celsius	CNQX	cyano-nitro-quinoxalinedione
µA	micro Ampère	CNS	central nervous system
$^{10}B(n,\alpha)^{7}Li$	nuclear reaction: target nuclide (projectile,leaving particle) resulting nuclide	CPFPX	8-cyclopentyl-3-(3-fluoro-propyl)-1-propyl-xanthine
5-HT2A	subtype of the seretonergic receptor system	CQX	cyano-quinoxalinedione
A	activity [Bq or Ci]	d	doublet (NMR)
A1	subtype of the adenosine receptor system	d	day (unit)
Ac	acetyl-moiety	DMF	N,N-dimethylformamide
ACQX	amino-cyano-quinoxalinedione	DMSO	methyl sulfoxide
AMPA	(RS)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazoly)propionic acid	DNA	deoxyribonucleic acid
aq	aqueous solution	DNQX	dinitro-quinoxalinedione
b	broad (NMR)	DOPA	3,4-dihydroxyphenylalanine
β^+	positron from radioactive decay	EC	electron capture
BBB	blood-brain-barrier	Et	ethylene-moiety
BER	borohydride, polymer supported	FCQX	fluoro-cyano-quinoxalinedione
Bn	benzyl-moiety	FDG	2-fluoro-2-deoxyglucose
Bq	Becquerel [37 GBq = 1 Ci]	FET	2-fluoroethyl-tyrosine
Bu	butyl-moiety	FNQX	fluoro-nitro-quinoxalinedione
c.a.	carrier-added	FP-β-CIT	N-3'-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane
c.f.	carrier-free	g	gram
CDI	carbonyl diimidazol	GABA	γ-amino-n-butyric acid
Ci	Curie [37 GBq = 1 Ci]	HPLC	high performance liquid chromatography

Abbreviation	Meaning	Abbreviation	Meaning
ID	injected dose	NQX	nitro-quinoxalinedione
I-PAMQX	(D)-7-iodo-N-(1-phosphonoethyl)-5-aminomethylquinoxaline-2,3-dione	PET	positron emission tomography
K⊂2.2.2	potassium-Kryptofix-(2.2.2)-complex	Ph	phenyl-moiety
KA	kainic acid	ppm	parts per million
keV	kilo electron Volt	QX	quinoxalinedione
K_i	inhibition constant	RCY	radiochemical yield
kV	kilo Volt	RNA	Ribonucleic acid
m	multiplett (NMR)	RT	room temperature
m	molar mass (MS)	s	singlet (NMR)
Me	methyl moiety	s	second
mGluR	metabotropic glutamate receptor	SAR	structure-activity-relationship
min	minute	S_NAr	nucleophilic substitution on arenes
MRI	magnetic resonance imaging	SPECT	single photon emission computer tomography
MS	mass spectrometry	t	triplett
N	number of molecules	$T_{1/2}$	half-life
n.c.a.	no carrier added	TFA	trifluoroacetic acid
Na(Tl)I	sodium idodide doted with thalium	THF	tetrahydrofurane
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-quinoxalinedione	tosyl	p-toluenesulfonate
NMDA	N-methyl-D-aspartic acid	triflate	trifluoromethanesulfonate
NMR	nuclear magnetic resonance spectroscopy	z	number of charges (MS)

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