# 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.