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2-[^{18}F]Fluoroethyl tosylate – a versatile tool for building ^{18}F-based radiotracers for positron emission tomography

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Abstract

Positron emission tomography (PET) is a modern *in vivo* imaging technique and an important diagnostic modality for clinical and pre-clinical research. The incorporation of a radionuclide like fluorine-18 into a target molecule to form PET radiopharmaceuticals is a repeated challenge for radiochemists. ^{18}F-Fluoroethylation is a well acknowledged method for ^{18}F-radiolabeling and 2-[^{18}F]fluoroethyltosylate ([^{18}F]FETs) is a preferred reagent because of its high reactivity to phenolic, thiophenolic, carboxylic and amide functions. The review will highlight the role of [^{18}F]FETs in PET-chemistry, and summarize its applicability in radiotracer design. The radiolabeling conditions and pros and cons of direct and indirect radiolabeling as well the aspects of reactivity of [^{18}F]FETs compared with other [^{18}F]fluoroalkylating reagents will be discussed comprehensively.

Introduction

Positron emission tomography (PET) is a modern *in vivo* imaging technique and has become for clinical and pre-clinical research an important diagnostic modality. By incorporation of a radionuclide like fluorine-18 ($t_{1/2}$=109.8 min, β^+=97%) into a target molecule PET radiopharmaceuticals are formed, enabling the non-invasive visualization of biochemical processes and the imaging and quantification of pathological situations *in vivo.* The most prominent example, 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), a radiopharmaceutical for glucose metabolism is for more than 25 years widely used as diagnostic tool for neuropsychiatric[1,2] and cardiovascular[2,3] disorders and the working horse for the diagnosis, monitoring and staging of the cancerous disease.[4]

Radiochemists have developed a number of methods of [^{18}F]radiofluorination via electrophilic and nucleophilic pathways for the incorporation of fluorine-18 into molecules of interest. Due to easy handling the direct nucleophilic substitution using [^{18}F]fluoride has been established as the preferred approach yet. However, the weak nucleophilic character of the fluoride ion often displays a major

hindrance. Aprotic conditions, highly reactive leaving groups, and in case of aromatic nucleophilic substitution an electron deficient aromatic system are basic requirements for successful incorporation of [^{18}F]fluoride into the organic molecule.[5] As alternative further labeling strategies have been developed, making use of so-called prosthetic groups by first attaching the radionuclide to a small bifunctional reactive unit and subsequent coupling of them to the target compound. An overview on prosthetic groups for ^{18}F-radiolabeling comprising activated esters, aldehydes and amines as well as halogenides, azide- and alkyne based small molecules can be found in a number of excellent reviews.[6-8] Beyond this, [^{18}F]fluoroalkylating agents like [^{18}F]fluoromethyl bromide, 2-[^{18}F]fluoroethyl bromide ([^{18}F]FEBr), 2-[^{18}F]fluoroethyl triflate or 2-[^{18}F]fluoroethyl tosylate ([^{18}F]FETs) or even [^{18}F]fluorocyclobutyl tosylate[9] have gained increased interest because they are providing a high reactivity to phenolic, thiophenolic, carboxylic and amide functions.[10] In general, the fluoroethyl group holds similar sterical properties like an ethyl and methyl group and is expected to maintain the pharmacological properties of the lead compound. Compared to the methyl the fluoroethyl moiety is more lipophilic; so the shift in lipophilicity by introduction of a fluoroethyl moiety (ΔlogP = 0.70) is stronger than by using methyl substituent (ΔlogP = 0.54).[11] However the increased lipophilicity originating from the fluoroethyl group may be beneficial for addressing specific targets or organs e.g. the brain. From radiochemists point of view, the [^{18}F]fluoroethyl group may act as a surrogate for a [^{11}C]methyl radiolabel because it can be coupled to the same functional groups. Precursors already available for methylation with [^{11}C]methyl iodide may be used without modification for [^{18}F]fluoroethylation. This is advantageous for the transfer of a promising ^{11}C-labeled radiotracer into its ^{18}F-labeled counterpart by exploitation of the longer half-life of fluorine-18 for extended PET investigations. Besides that [^{18}F]fluoroethylation can be performed with unprotected compounds and the reaction conditions are less restricting in comparison to direct nucleophilic substitution.

[^{18}F]FETs is one of the mostly used reagents for [^{18}F]fluoroethylation due to its beneficial properties: it is easy to prepare, possesses a low volatility, is almost stable and shows high reactivity in correspondence with some chemo-selectivity. On the other hand some disadvantages are known: [^{18}F]FETs may be sensible to distinct solvents, bases and high temperatures. and [^{18}F]FETs may react unselectively e.g. if multiple hydroxyl and amino groups are present in the target molecule and often it shows an inadequate chemical purity. To circumvent the latter drawback, intermediate purification steps of [^{18}F]FETs have been developed comprising solid phase extraction (SPE) as well as HPLC-based procedures, partly remote-controlled, which in consequence afford the implementation of a two-pot radiolabeling procedure.[12,13]

This review will summarize the role of [^{18}F]FETs as labeling block in PET radiochemistry, its usage in the synthesis of radiopharmaceuticals with clinical relevance like [^{18}F]fluoroethylcholine ([^{18}F]FECh)

or [^{18}F]fluoroethyltyrosine ([^{18}F]FET), but also its broad application in the radiolabeling of a multitude of radiotracers in pre-clinical research. The radiolabeling conditions of [^{18}F]FETs, aspects of reactivity towards functional groups, will be reviewed for a number of *O*- and *N*-[^{18}F]fluoroethylations. The pro and cons of direct ([^{18}F]fluoride based) and indirect ([^{18}F]FETs based) labeling approach will be discussed as well as different purification methods of [^{18}F]FETs in accordance with the radiochemical yield.

Radiosynthesis, reactivity and purification

The first radiosynthesis of [^{18}F]FETs and its methyl- and propyl analogs was published in 1987 by the group of Stöcklin who described the reaction of the corresponding bis-tosylated, -mesylated and -brominated alkanes with [^{18}F]fluoride, Kryptofix K_{222} and K_2CO_3 in acetonitrile.[14] By this straightforward approach [^{18}F]FETs was obtained in 77-82% radiochemical yield (RCY) and this reaction is more or less still used without major modifications up to now. (Figure 1) Notably, dibromo-alkane precursors gave lower yields of [^{18}F]fluoroalkylated products than their bis-tosylated and bis-mesylated counterparts. The corresponding [^{18}F]fluoromethylated compounds could be obtained in only 1% RCY by the same method. Furthermore, the impact of the solvent and reaction time for the synthesis of [^{18}F]FETs was evaluated. Acetonitrile providing 82% RCY was superior to dichloromethane and acetone providing 52% and 28% RCY, respectively. A reaction time of 10-15 min was found to be optimal with a precursor concentration of ~0.015 mmol/mL.

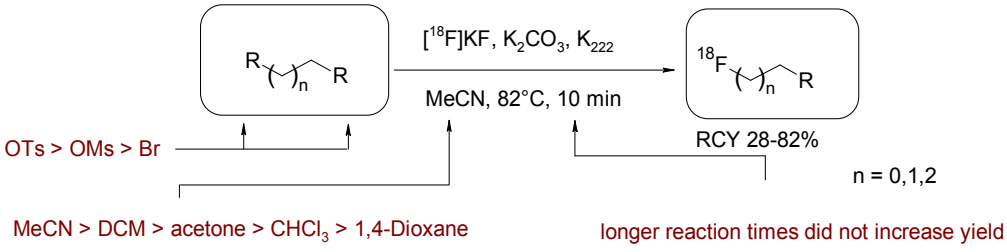

Fig. 1. First [^{18}F]FETs synthesis: influence of leaving group, solvent, chain lenght.[14]

Later on, Stöcklin et al. optimized the [^{18}F]fluoroalkylation on simple H-acidic compounds with respect to leaving groups, reaction times, substrate concentration and basicity.[15] Interestingly, the yield of the [^{18}F]fluoroethylation of phenol in refluxing tetrahydrofuran or 1,4-dioxane (76-79%) was significantly higher than in acetonitrile (50%). In a comparison of [^{18}F]FETs with 2-[^{18}F]fluoroethyl

bromide and 2-[^{18}F]fluoroethyl methanesulfonate in the reaction with phenol, [^{18}F]FETs gave the highest RCY (51%) on [^{18}F]fluoroethyl phenolate.[15]

In some cases the reactivity of [^{18}F]fluoroethylating reagents may be limited. Rösch et al. reported that reacting the weak nucleophilic amino group of *p*-anisidine with 2-[^{18}F]fluoroethyl bromide or [^{18}F]FETs using sodium hydride or lithium diisopropylamide (LDA) as base, respectively, resulted in a low RCY of 15%.[16] However, the authors found that the RCY was increased to 80% by adding 22-143 µmol of NaI, KI or LiI to the labeling mixture containing [^{18}F]FEBr or [^{18}F]FETs. (Figure 2) By application of this alkali iodides in the [^{18}F]fluoroethylation the isolated radiochemical yield of clinical relevant radiopharmaceuticals, namely [^{18}F]fluoroethyl-4-piperidyl benzilate and [^{18}F]fluoroethylcholine could almost be doubled. This does most probably originate from the in situ formation of 2-[^{18}F]fluoroethyl iodide which is the stronger fluoroethylating agent.[16] From the series of alkali iodides examined LiI was the most potent one and the more polar solvents DMF and DMSO were superior to acetonitrile.

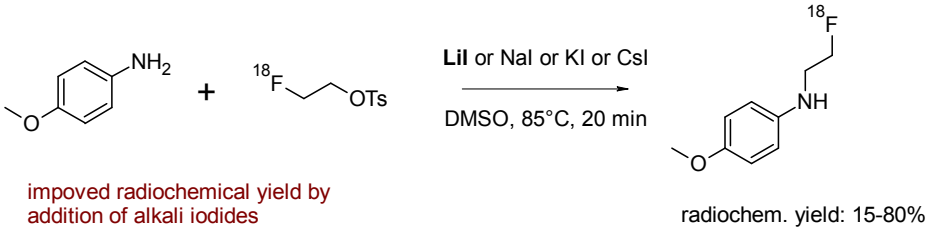

Fig 2. [^{18}F]Fluoroethylation of model compounds.[16]

In recent years, microwave dielectric heating has brought a high impact on organic chemistry and radiochemistry.[17] Accordingly, Lu et al. reported how to improve the RCY in the reaction of [^{18}F]FETs with model compounds having carboxylic, secondary amine and phenolic functionalities.[18] Under microwave enhanced conditions (acetonitrile, 2-10 min, 300 W) the RCY was increased by ~20% compared to conventional heating. (Figure 3) Notably, under microwave conditions [^{18}F]FETs reacts with DL-pipecolinic acid, having both a carboxylic and an amine function, exclusively with the amino group.

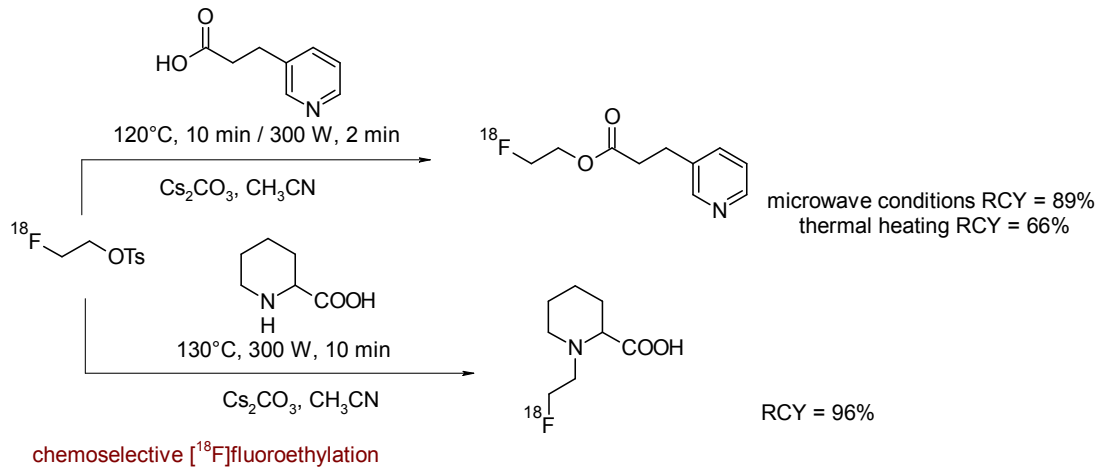

Fig. 3. [^{18}F]Fluorethylation of model compounds under microwave enhanced conditions.[18]

In 2005 the group of Pike reported the synthesis of modified 2-[^{18}F]fluoroethyl arylsulfonates bearing a less electron-rich aryl group offering enhanced reactivity.[19] A series of novel 2-[^{18}F]fluorethylated benzenesulfonyl- ([^{18}F]FEBs), 4-bromobenzenesulfonyl- ([^{18}F]FEBrBs), 4-nitrobenzenesulfonyl- ([^{18}F]FENs) and 3,4-dibromobenzenesulfonyl-derivatives ([^{18}F]FEBr$_2$Bs) were synthesized and compared to [^{18}F]FETs in terms of handling and reactivity. (Figure 4) The novel 2-[^{18}F]fluoroethyl arylsulfonates were obtained in yields of 47-53%, suggesting that the substitution pattern on the phenyl ring has minimal effects on the yields of [^{18}F]fluorine incorporation. However, in the subsequent [^{18}F]fluoroethylation reaction with the target molecule 2β-carboxymethoxy-3β-(4-chlorophenyl)nortropane (CNT), the [^{18}F]FEBr$_2$Bs provided a considerably higher RCY (87%) than [^{18}F]FETs (64%). A similar tendency was observed with two other model compounds, an amyloid probe and *p*-nitrophenol where [^{18}F]fluoroethylation with [^{18}F]FEBr$_2$Bs also provided higher RCY compared to [^{18}F]FETs. According to these results and the easy synthesis of [^{18}F]FEBr$_2$Bs, this reagent may be considered as a powerful alternative to [^{18}F]FETs.

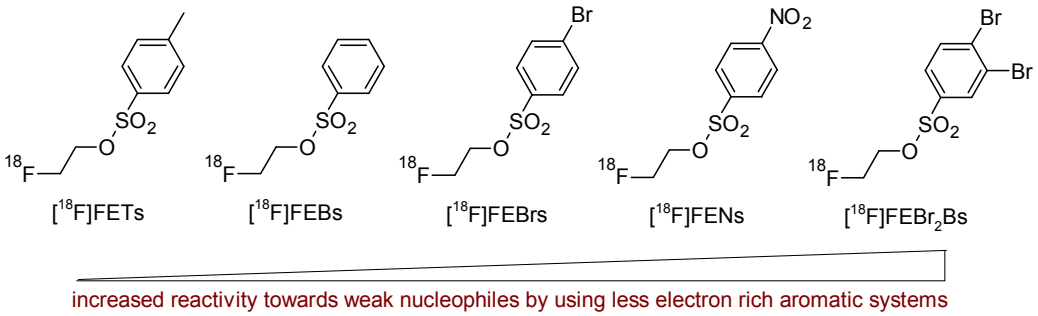

Fig 4. Graduation of reactivity of [^{18}F]fluoroethylating reagents.[19]

In a recent study, the comparison of the reactivity of [^{18}F]FETs, [^{18}F]FENs and [^{18}F]FEBr$_2$Bs for the [^{18}F]fluoroethylation of an azadipeptide nitrile based inhibitor of cathepsin revealed that [^{18}F]FENs provided higher RCY (74 %) compared to [^{18}F]FETs and [^{18}F]FEBr$_2$Bs.[20] (Figure 5)

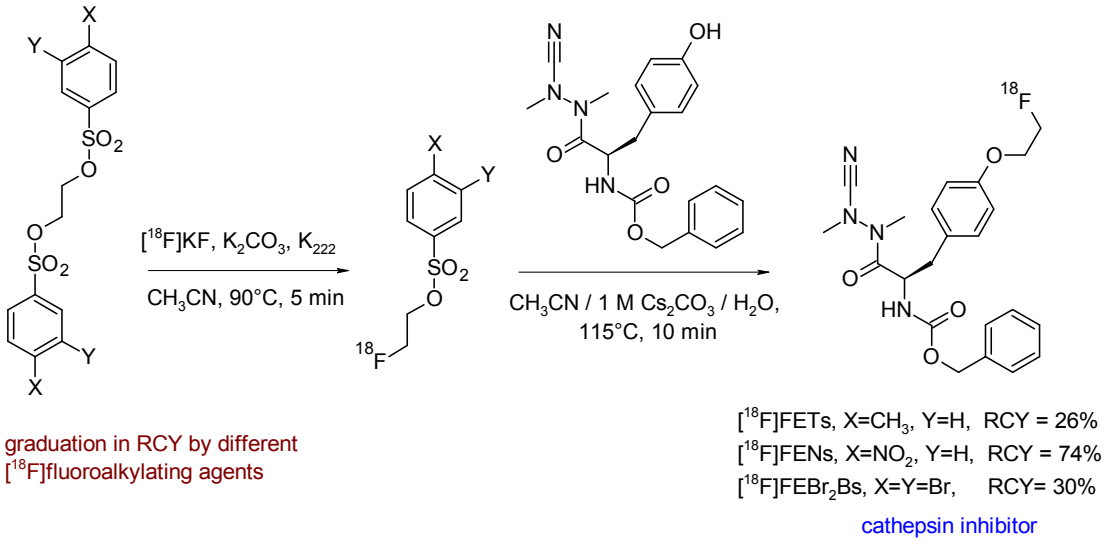

Fig 5. [^{18}F]Fluoroethylation of a cathepsin inhibitor.[20]

A new approach for the synthesis of [^{18}F]FETs was presented by Lemaire et al. using a technique circumventing the time consuming and often by loss of radioactivity characterized azeotropic drying step of the [^{18}F]fluoride/K_{222}/K_2CO_3 complex.[21] It is characterized by the elution of [^{18}F]fluoride from the anion exchange cartridge (SAX) with acetonitrile containing water (6300 ppm) and the strong organic base tert-butyl-tetramethyl-guanidine (BTMG). The direct reaction of this mixture with 1,2-ethylene glycol-bis-ditosyltate provided [^{18}F]FETs in 83% RCY and 85% purity. This innovative technique with short reaction times of ~5 min and the use of small solvent volumes of 0.5-1.0 mL offers advantages for the development of micro reactors or microfluidic based devices.

As another example for miniaturization Pascalini et al. reported the production of [^{18}F]FETs in a microfluidic system, providing the [^{18}F]fluoroethylating agent in 67% RCY within 90 s reaction time.[22] As the [^{18}F]fluoride drying procedure was performed in macro scale and apart from the microfluidic system, the authors were able to divide the dry [^{18}F]fluoride/K_{222}/K_2CO_3 complex into several batches and to perform a number of radiolabeling reactions from a single stock solution. This protocol may be useful to obtain individual doses of the labeling reagent permitting the dose-on-demand production of radiopharmaceuticals.

One major challenge in the synthesis and application of [^{18}F]FETs is the chemical purity because the 1,2-ethylene glycol-bis-tosylate precursor, if not removed from the [^{18}F]fluoroalkylating mixture, will act as a competitive reagent to the targeted functional groups resulting in tosyl-ethylated, hydroxyl-ethylated or cross-linked by-products. (Figure 6) The first two often show a similar chromatographic behavior like the [^{18}F]fluoroethylated main product, making its purification by semi-preparative HPLC difficult and finally resulting in products with a similar binding behavior but preventing specific binding of the radiotracer.

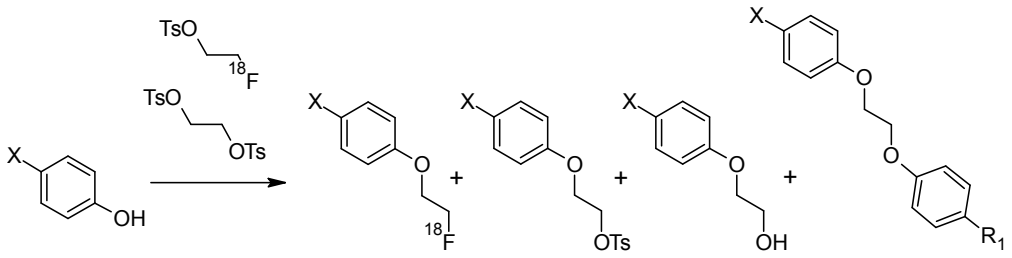

Fig 6. Expectable by-products in radiolabeling with [^{18}F]FETs

To circumvent this disadvantage, a minimum of 1,2-ethylene glycol-bis-tosylate precursor (1-2 mg) may be used as precursor but this often results in decreased RCY of [^{18}F]FETs. Another and more sophisticated alternative, is the implementation of a purification step for [^{18}F]FETs either by solid phase extraction or semi-preparative HPLC. Both approaches afford enhanced technical expense, e.g. a two pot radiolabeling device, additional valves and at least two HPLC steps. However, these expanded efforts are justified by an enhanced purity and reactivity of [^{18}F]FETs resulting in higher RCY and improved specific activity of the final radiotracer.

The first purification of [^{18}F]FETs was reported in 2000 by the group of Wester for the radiosynthesis and biological evaluation of 6-*O*-(2-[^{18}F]fluoroethyl)-6-*O*-desmethyldiprenorphine ([^{18}F]FDPN), a ligand for the opioid receptor system.[23] (Figure 7) In brief, [^{18}F]FETs was purified by RP18 based HPLC. After fixing on a C18 cartridge and drying with a nitrogen stream [^{18}F]FETs was finally eluted with acetonitrile to react with the corresponding hydroxyl precursor. In this manner, [^{18}F]FETs was obtained as intermediate in 65% RCY. The two-step radiosynthesis provided [^{18}F]FDPN within 100 min with 22% overall decay corrected RCY and a specific activity of 37 GBq/µmol at end of synthesis (EOS). The [^{18}F]fluoroethylation was reproduced later by Mueller et al. under almost identical conditions providing [^{18}F]FDPN in 19% RCY.[24]

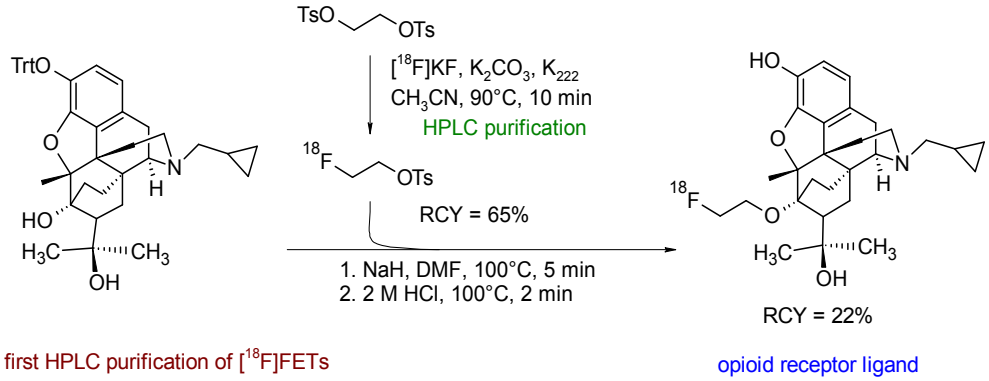

Fig 7. Radiosynthesis of [^{18}F]FDPN.[23]

In two recent papers Schoultz et al. published the full radiosynthesis of the opioid receptor ligand [^{18}F]FDNP together with a partial agonist [^{18}F]fluoroethyl-buprenorphine ([^{18}F]FBPN) and the agonist [^{18}F]fluoroethyl-phenethyl-orvinol ([^{18}F]FBEO).[12, 25] The method is based on an innovative automated production and purification of [^{18}F]FETs where the excess of 1,2-ethylene glycol-bis-tosylate precursor is removed by precipitation and subsequent filtration and the [^{18}F]FETs is purified by gradient elution from the SPE cartridges. After mobilization of [^{18}F]FETs with DMF it was reacted with the FDNP precursor and NaH.[12] This new two-pot two-cartridges based system avoids HPLC purification of [^{18}F]FETs and provides [^{18}F]FDNP within 100 min in 25 % overall RCY and high chemical and radiochemical purity. The other ^{18}F-fluoroethylated orvinol-based PET tracers [^{18}F]FBPN and [^{18}F]FBEO were obtained by the same method in similar RCY from their *O*-desmethyl-precursors demonstrating the reliability of the method.

With the aim to translate ^{11}C-labeled radiopharmaceuticals into their respective [^{18}F]fluoroethylated analogs, Wadsak and Mitterhauser investigated several methods for the purification of [^{18}F]FEBr and [^{18}F]FETs with view on the implementation in commercially available synthesizers.[13] In this study the reactivity of [^{18}F]FETs purified by SPE and HPLC-based methods was compared with that of [^{18}F]FEBr and the results were transferred to the synthesis of five radiotracers of clinical interest. Several SPE materials, like Silica SepPak®, C18plus SepPak® and AluminaN® have been evaluated for the purification of [^{18}F]FETs. Finally, it was demonstrated that e.g. for the synthesis of 2-[^{18}F]fluoroethyl-(R)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate ([^{18}F]FETO) HPLC-purified [^{18}F]FETs provided the best results regarding RCY and chemical and radiochemical purity. Additional [^{18}F]FETs was synthesized in 55% RCY which was substantially higher than for distilled [^{18}F]FEBr (34%). However, this benefit is accompanied by an enhanced need for hardware components like additional HPLC and SPE units. However for highest demands on chemical purity owed to the low receptor densities of the target HPLC-purified [^{18}F]FETs is the best choice.

[^{18}F]FETs is a radiolabeling agent widely used to form a broad variety of radiotracers as will be demonstrated in the following sections. In the majority of cases the resulting radiolabeled probe was comprehensively investigated regarding its in vivo behavior displayed in parameters as biodistribution, stability and metabolism. However to the best of our knowledge no studies were made concerning the radiopharmacology of [^{18}F]FETs istself so that it's properties in vivo have still to be explored.

O-[^{18}F]fluoroethylation

O-[^{18}F]fluoroethyl-phenols

The phenol group is a recurrent functionality in a variety of biomolecules, and is ideally suitable for the introduction of radionuclides such as ^{11}C as [^{11}C]methyl via [^{11}C]CH_3I or ^{18}F as [^{18}F]fluoroethyl via [^{18}F]FETs. The following examples may demonstrate the successful application of [^{18}F]FETs in the syntheses of a multitude of radiotracers by *O*-[^{18}F]fluoroethylation.

Radiolabeled amino acids have raised clinical interest in neurology and oncology because they are biological building blocks for the synthesis of neurotransmitters and proteins. Especially for diagnosis of human brain tumors, radiolabeled amino acids are advantageous since they are accumulating in tumor tissue because of upregulated transporters and higher protein synthesis.[26, 27]

A number of radiolabeled amino acids are used for that purpose, as [^{11}C]L-methionine, 2-[^{18}F]fluoro-L-tyrosine, 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine ([^{18}F]FDOPA) or 6-[^{18}F]fluoro-L-3-methoxy-4-hydroxyphenylalanine ([^{18}F]OMFD). After its introduction *O*-(2-[^{18}F]fluoroethyl)tyrosine ([^{18}F]FET) has become a very useful PET tracer since it shows high brain uptake, has a sufficient metabolic stability, is not involved in protein synthesis, its accumulation in tumors is solely based on amino acid transporters, and is of prognostic relevance.

The first radiosynthesis of [^{18}F]FET was published in 1999 by Wester et al. by direct ^{18}F-alkylation of tyrosine with [^{18}F]FETs.[28] (Figure 8) The di-sodium salts of unprotectedted L-tyrosine or D-tyrosine were reacted with HPLC-purified [^{18}F]FETs in DMSO at 90°C and the corresponding [^{18}F]FET was provided within 60 min in 40% overall RCY.

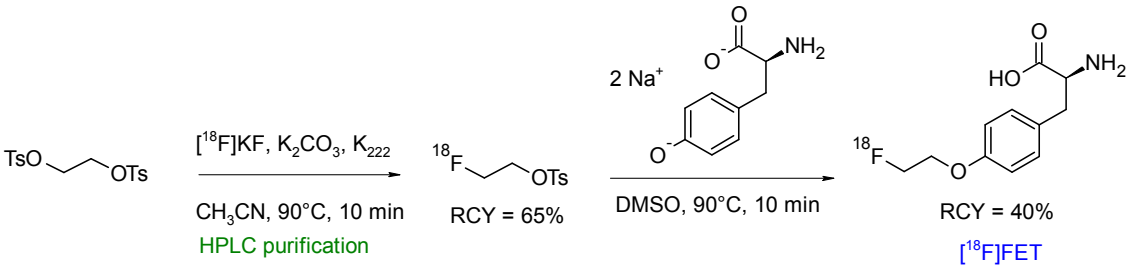

Fig 8. First radiosynthesis of *O*-(2-[^{18}F]fluoroethyl)tyrosine.[28]

In a separate study, Tsukada et al. has synthesized a series of radiolabeled unnatural amino acids, including D-[^{18}F]FET to compare the properties with the L-isomer.[29] Radiosynthesis of D-[^{18}F]FET was performed by heating SPE-purified [^{18}F]FETs with D-tyrosine in DMSO for 15 min and subsequent HPLC purification; no RCY was provided.

The first automated synthesis of [^{18}F]FET using a commercial synthesizer was reported in 2003 by Tang et al.[30] In this case, the [^{18}F]fluoroalkylating agent [^{18}F]FETs was synthesized starting from [^{18}F]fluoride that was previously fixed on a quaternary 4-(4-methylpiperidinyl)-pyridinium functionalized anion exchange resin (SAX). The reaction was performed directly on the resin by passing 1,2-ethylene glycol-bis-tosylate in acetonitrile through the heated cartridge. The [^{18}F]FETs was eluted from the resin and added to a mixture of L-tyrosine and NaOH. After evaporation of the solvent, DMSO was added and the mixture was heated to form [^{18}F]FET. This protocol avoids any HPLC-purification step and provided [^{18}F]FET within 52 min in 8-10% overall RCY.

A further automated, high yielding radiosynthesis of [^{18}F]FET was presented by Müller et al. using a modified synthesizer designed for ^{11}C-radiolabeling chemistry consisting of two reaction vessels.[31] [^{18}F]FETs was formed in the first reactor by conversion of 1,2-ethylene glycol-bis-tosylate with the dried [^{18}F]KF/K_{222}/K_2CO_3 complex. After addition of water the [^{18}F]FETs was collected by SPE and eluted with hot DMSO into the second reactor containing the L-tyrosine disodium salt. The formed [^{18}F]FET was purified by SAX and SCX-based SPE systems and provided the radiotracer in typically 41% RCY with a chemical and radiochemical purity that meets the current Pharmacopeia requirements. Nowadays, the preferred synthesis of [^{18}F]FET is based on direct nucleophilic substitution of a protected, 4-tosyl-ethoxy-tyrosine with [^{18}F]fluoride yielding the radiotracer in about 60% RCY[32] however, the above mentioned automatic [^{18}F]FETs based radiolabeling method may serve as a very useful radiolabeling protocol for further [^{18}F]fluoroethylated radiotracers.

A convenient remote controlled radiosynthesis of a new tryptophan analog L-5-(2-[^{18}F]fluoroethoxy)-tryptophan (5-[^{18}F]FETP) by [^{18}F]fluoroethylation of 5-hydroxy-L-tryptophan disodium salt was reported in 2010.[33] (Figure 9) The radiolabeling took place in a two-pot synthesizer, where in the first

reactor [^{18}F]FETs was formed in 45-60% RCY and transferred into the second vessel for [^{18}F]fluoroethylation in DMSO. (Figure 12) After purification of 5-[^{18}F]FETP by silica-and RP18-based SPE the radiolabeled amino acid was isolated within 65 min in 12-16% overall RCY.

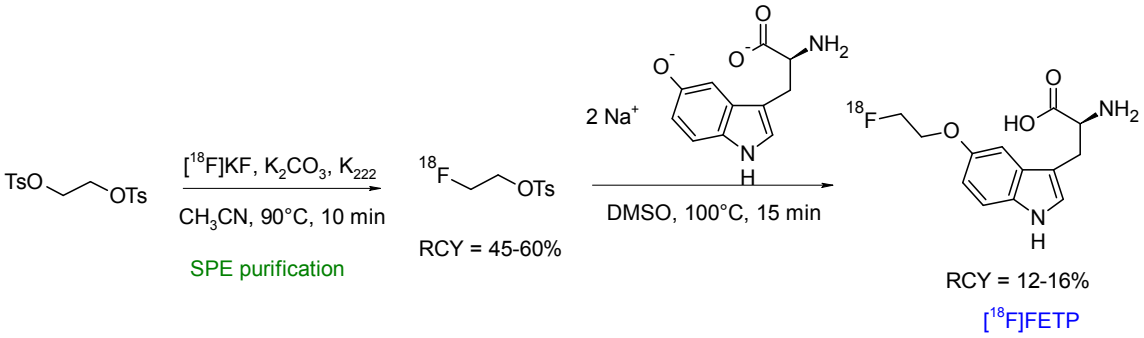

Fig 9. Radiosynthesis of L-5-(2-[^{18}F]fluoroethoxy)-tryptophan.[33]

4-Phenylpiperazines have attracted radiopharmaceutical interest since derivatives as FAUC346 have proved to be highly selective and affine dopamine D_3 receptor antagonists. Aiming at the development of dopamine D_3 radioligands Hocke et al. synthesized ^{18}F-labeled compounds derived from FAUC346 with the methoxy function being replaced by a fluoroethoxy bioisostere.[34] (Figure 10) *O*-[^{18}F]fluoroethylation with [^{18}F]FETs was the method of choice to obtain four different radioligands for the D_3 receptor. HPLC-purified [^{18}F]FETs was reacted with the phenol precursor and a base in DMF at 120°C; under these conditions 24-65% RCY were observed. Interestingly, the best RCY was achieved by using $N(Bu)_4OH$ as base whereas with NaH and $NaOCH_3$ no radiotracers were formed.

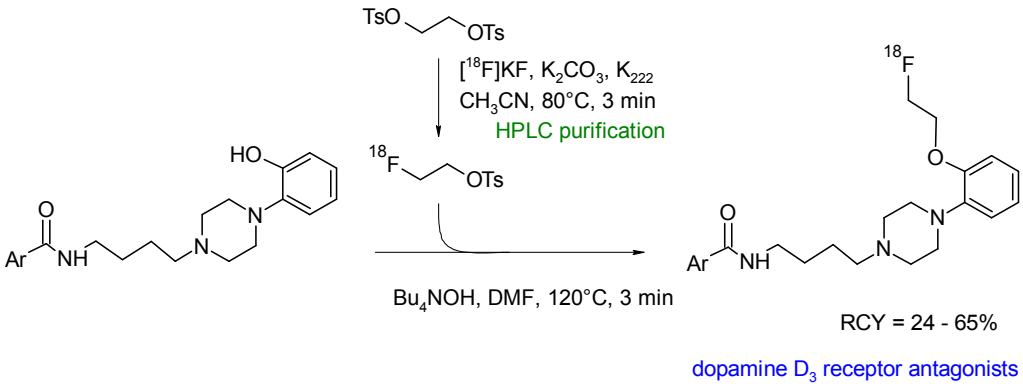

Fig 10. Synthesis of *O*-[^{18}F]fluoroethylated 4-phenylpiperazine.[34]

Another series of radiolabeled dopamine $D_{2/3}$ receptor agonists, [^{18}F]fluoroalkylated derivatives of 2-aminomethylchromane, has been presented by van Wieringen et al.[35] (Figure 11) In this study, [^{18}F]FETs as well as 4-[^{18}F]fluorobutyl tosylate were synthesized from the corresponding bis-tosylated alkyl precursors and the intermediates were purified by semi-preparative HPLC. The *O*-[^{18}F]fluoroethylation was performed by addition of the methoxymethyl-protected phenolic precursor, sodium hydride, and DMF followed by a deprotection step. The protective group was required to prevent [^{18}F]fluoroethylation at the second hydroxyl group of the target molecule. The overall RCY of this three-step radiosynthesis containing two HPLC runs was up to 11%. Notably, the reaction with 4-[^{18}F]fluorobutyl tosylate provided a better RCY up to 22%.

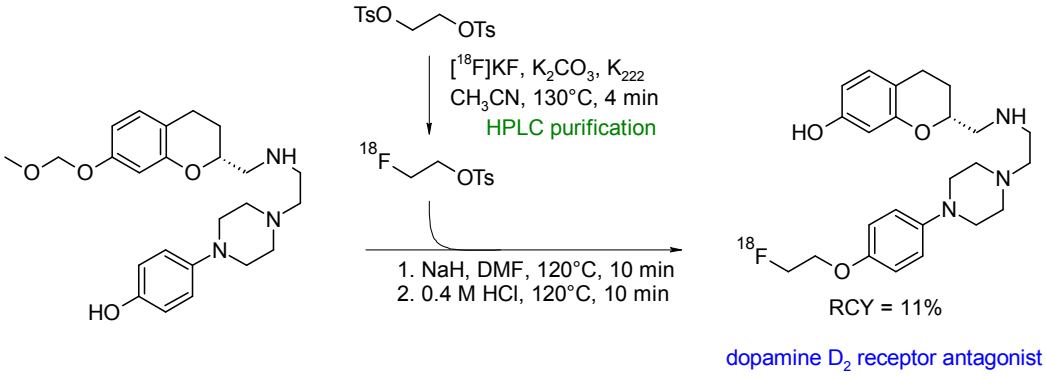

Fig 11. Synthesis of a *O*-[^{18}F]fluoroethylated 2-aminomethylchromane.[35]

To provide potential PET imaging probes for the dopamine D_4 receptor, Tietze et al. developed [^{18}F]fluoroethylated 5-cyano-indole derivatives and reported optimized radiolabeling conditions with [^{18}F]FETs.[36] [^{18}F]FETs was synthesized within 3 min reaction time at 90°C, purified by gradient reversed-phase HPLC, fixed on a SPE unit and eluted with dry DMF into a reaction vessel containing the hydroxyl precursor in DMSO and a base. (Figure 12) Surprisingly, the optimized reaction conditions for the *ortho*-fluoroethylation could not be transferred to the *para*-derivative. As an alternative for the reagent system DMSO/NaOMe, which was efficient for the *ortho*- but failed for the *para*-hydroxyl group, the mixture DMF/N(Bu)$_4$OH finally lead to an effective *para*-[^{18}F]fluoroethylation. The RCY of the *O*-[^{18}F]fluoroethylation step was 81% for the 2-hydroxy phenyl and 47% for the 4-hydroxy phenyl precursor. As a part of these investigations the authors compared the solvents DMF and DMSO and pointed out that the use of DMSO resulted in an accelerated product formation; 80% in DMSO versus 43% in DMF under identical reaction conditions. By optimizing the reaction temperature it turned out that at 140°C a significant drop of the RCY was

observed due to decomposition of [^{18}F]FETs and oxidation to 2-[^{18}F]fluoroacetaldehyde according to Kornblum conditions (see Figure 70).

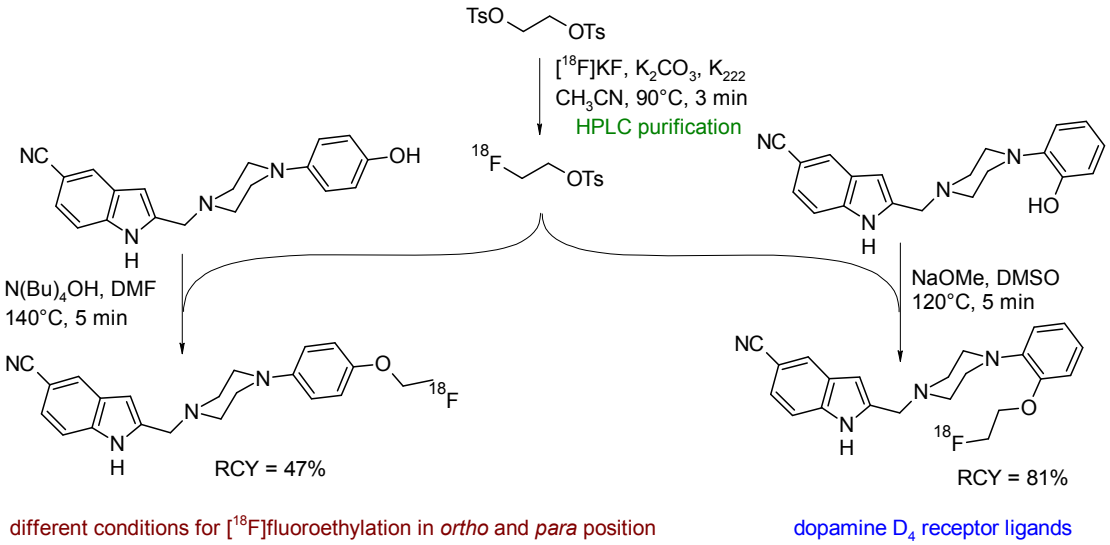

Fig 12. Synthesis of *O*-[^{18}F]fluoroethylated 5-cyano-indoles.[36]

A further radioligand targeting the dopamine D_4 receptor was prepared by *O*-[^{18}F]fluoroethylation of a dimethoxy-substituted benzyl-piperazine derivative.[37] Employing HPLC-purified [^{18}F]FETs the hydroxyl precursor was reacted in DMF and $N(Bu)_4OH$ for 3 min to provide [^{18}F]fluoroethylated product in 92% RCY. The desired radiotracer was isolated after semi-preparative HPLC purification in high purity. (Figure 13)

Prante et al. published a series of piperazine-based radioligands with high affinity towards the dopamine D_4 receptor, including 2- and 3-substituted pyrazolo[1,5-*a*]pyridines.[38] Two compounds were selected for [^{18}F]fluoroethylation. For that purpose, the corresponding hydroxyl precursors were allowed to react with [^{18}F]FETs in DMF and NaOMe. (Figure 13) After 3 min and 5 min, respectively, at 120°C 87-92% of the [^{18}F]fluoroethylated products were detected. The overall RCY of the dopamine D_4 receptor ligands after purification and formulation for *in vivo* experiments was 12-13% within a total synthesis time of 120 min. In this case and in opposite to former findings[36], no difference in reactivity towards [^{18}F]FETs between the 4-hydroxyl- and the 2-hydroxyl-phenyl derivative was observed. Interestingly, the authors synthesized in a parallel approach one of the radioligands by a direct kryptate-assisted nucleophilic ^{18}F-fluorination using a tosylate precursor and standard conditions. By the direct method, the rate of ^{18}F-incorporation was 26% but after workup, purification and formulation the radioligand was obtained in less than 10% overall RCY. Both

approaches give more or less a similar RCY. This is not always the case as will be shown later on in a separate chapter of this review comparing [^{18}F]fluorethylation with [^{18}F]FETs with direct [^{18}F]fluoride substitution to generate the same radiotracer.

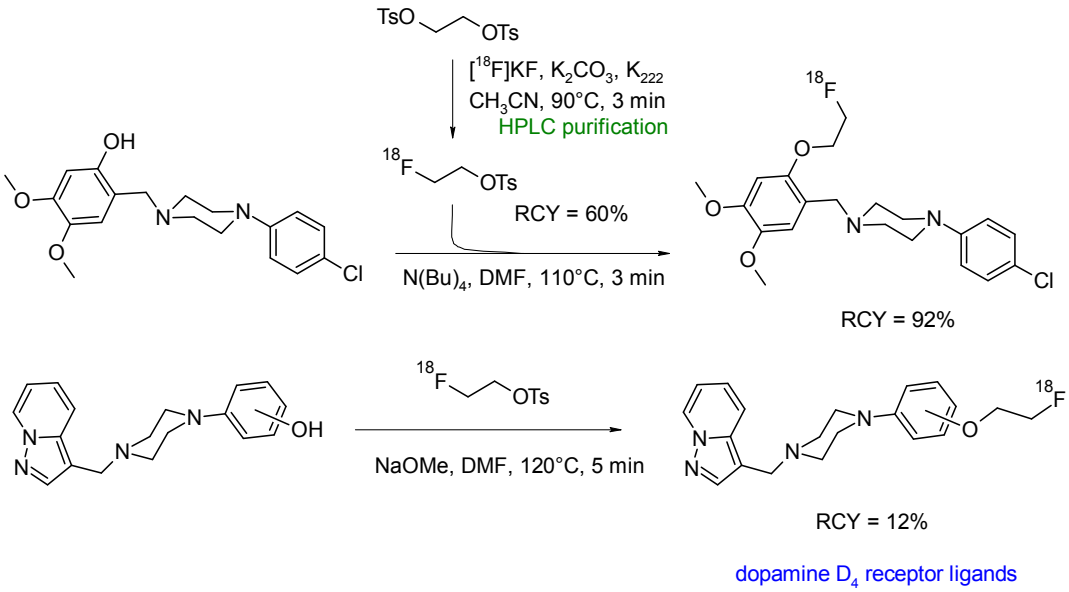

Fig 13. Synthesis of *O*-[^{18}F]fluoroethylated piperazines.[37, 38]

The compound class of imidazobenzodiazepines is known to be antagonists for the γ-aminobutyric acid ($GABA_A$) receptor. A prominent example is flumazenil which has been radiolabeled as well with carbon-11 and fluorine-18.[39] With the aim to find alternative routes for radiolabeling Jackson et al. synthesized a [^{18}F]fluoroethylated derivative which was obtained by the reaction of a hydroxyl precursor with [^{18}F]FETs (Figure 14).[39] The overall RCY for the three-step synthesis of [^{18}F]fluoroethylated flumazenil was 18-20%.

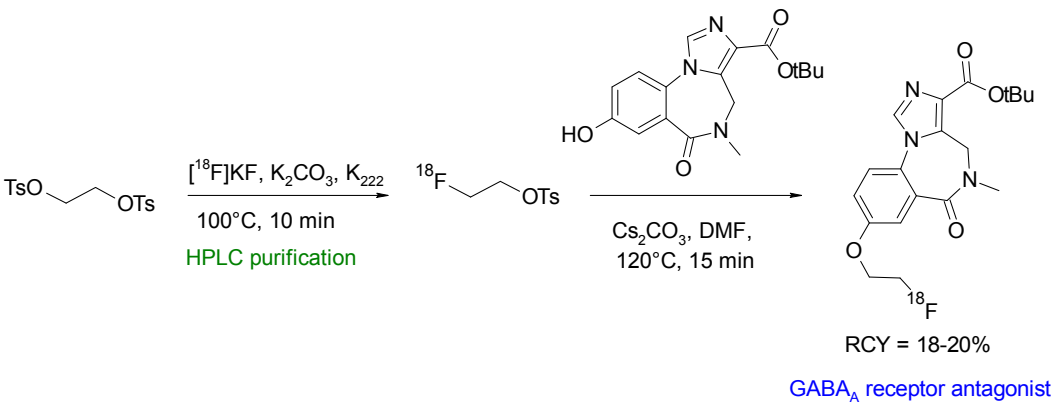

Fig 14. Radiosynthesis of *O*-[^{18}F]fluoroethylated flumazenil.[39]

Since serotonin (5-hydroxytryptamine, 5-HT), is involved in important brain functions; radiolabeled serotonin receptor antagonists are highly desired compounds for the investigation of human brain disorders. Herth et al. reported in a series of piperazine-substituted azatricyclo-dec-8-ene-3,5-diones targeting the serotonin 5-HT$_{1A}$ receptor one radioligand, synthesized via [^{18}F]fluoroethylation with [^{18}F]FETs.[40] HPLC-purified [^{18}F]FETs was reacted for 20 min at 120°C with the corresponding hydroxyl precursor. (Figure 15) The [^{18}F]fluoroethylated 5-HT$_{1A}$ receptor ligand was provided as injectable solution within 130 min in 25% overall RCY.

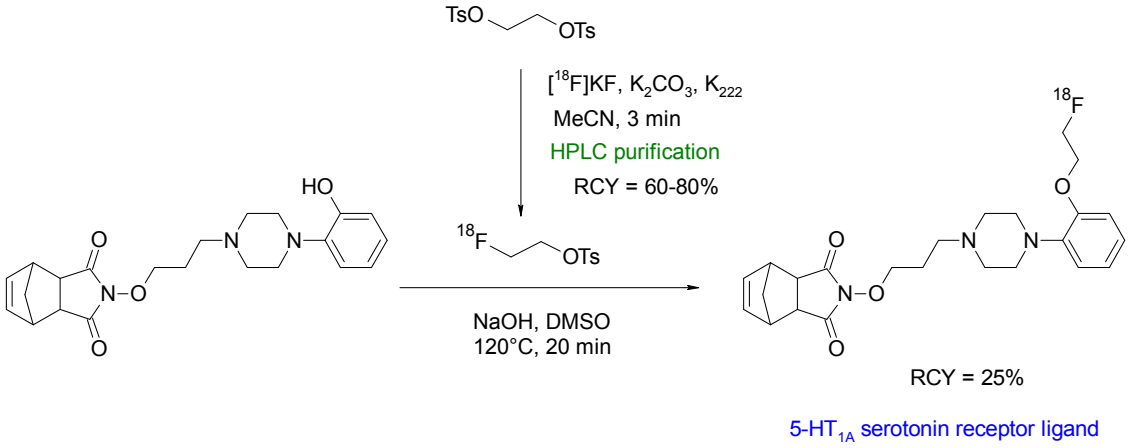

Fig 15. Radiosynthesis of a *O*-[^{18}F]fluoroethylated azatricyclo-dec-8-ene-3,5-dione.[40]

Funke et al. developed ^{18}F-labeled aminocyclohexyl substituted indole carbonitriles having nanomolar affinities to the serotonin transporter (SERT) as important target for PET.[41] The [^{18}F]fluoroethylation was performed as an one-pot two-step procedure. [^{18}F]FETs was added to the dried hydroxyl precursor which was beforehand activated with K_2CO_3 and K_{222} in refluxing methanol. (Figure 16) The HPLC-purified SERT radioligands were obtained within 150 min in 11-22% overall RCY.

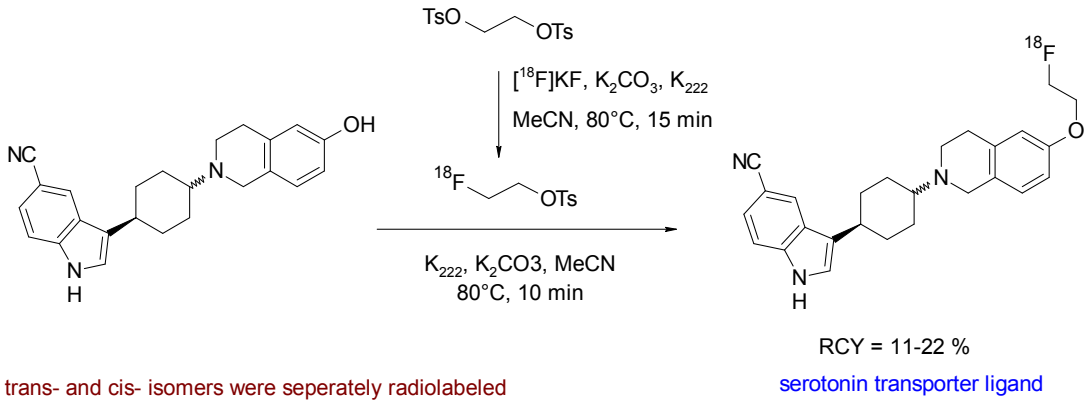

Fig 16. Radiosynthesis of *O*-[^{18}F]fluoroethylated aminocyclohexyl indole carbonitriles.[41]

N-alkylated benzoylpiperidines (e.g. MDL 105725, MH.MZ) represent a compound class targeting the 5-HT$_{2A}$ receptor and have been successfully radiolabeled with [^{18}F]FETs.[42, 43] (Figure 17) The [^{18}F]FETs was purified by SPE prior to the reaction with the hydroxyl precursor which was activated by NaOH in DMSO. Two radiotracers were obtained within 100 min in about 40% overall RCY. Notably, one of the precursors bearing an aliphatic hydroxyl group did not show [^{18}F]fluoroalkylation on the alcohol, indicating that under the standard basic conditions used the phenolic function is more reactive towards [^{18}F]FETs.

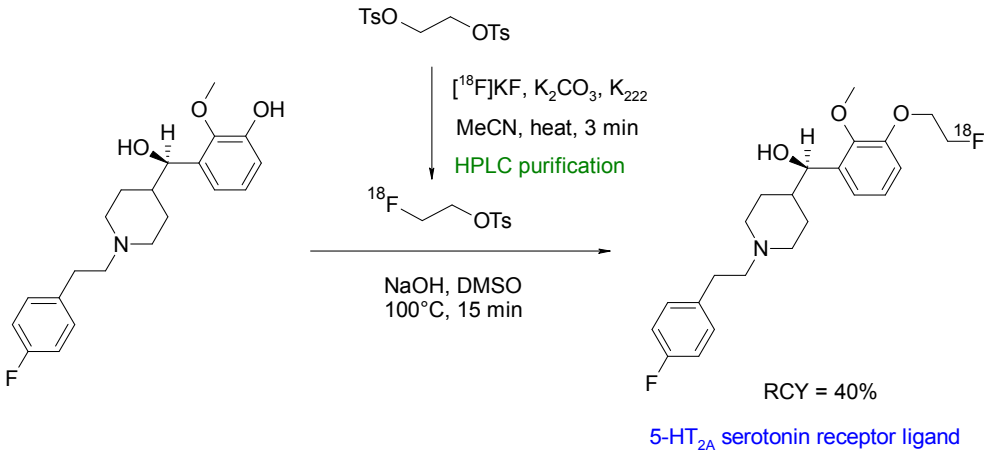

Fig 17. Radiosynthesis of a *O*-[^{18}F]fluoroethylated benzoylpiperidine.[42, 43]

Majo et al. developed phenylpiperazin-substituted 6-aza-uracil derivatives as promising antagonists of the 5-HT$_{1A}$ serotonin receptor.[44] Since the corresponding carbon-11 labeled radiotracer showed a promising *in vivo* behavior but suffered from the usual limitations (short half-live), the authors decided to develop a *O*-[^{18}F]fluoroethylated analogue. Radiosynthesis was performed by a two-step

fluoroalkylation of the phenolate. HPLC-purified [^{18}F]FETs was reacted with the phenol in presence of K_2CO_3 in DMSO at 110°C to provide the [^{18}F]fluoroethylated 5-HT_{1A} receptor antagonist after 60 min in 45% overall RCY. (Figure 18) When aqueous NaOH was used as a base, at temperatures of about 125°C decomposition of [^{18}F]FETs was observed and the RCY dropped to 25%. The authors developed an one-pot modification of their radiosynthesis in which the HPLC purification of [^{18}F]FETs was replaced by SPE-based purification. After drying and elution of the [^{18}F]FETs with diethyl ether the ether layer was removed and [^{18}F]FETs reacted with the phenol precursor. However, by application of this protocol the radiotracer was obtained in only 20-25% overall RCY suggesting that HPLC-purified [^{18}F]FETs is more reactive compared to the SPE purified batch.

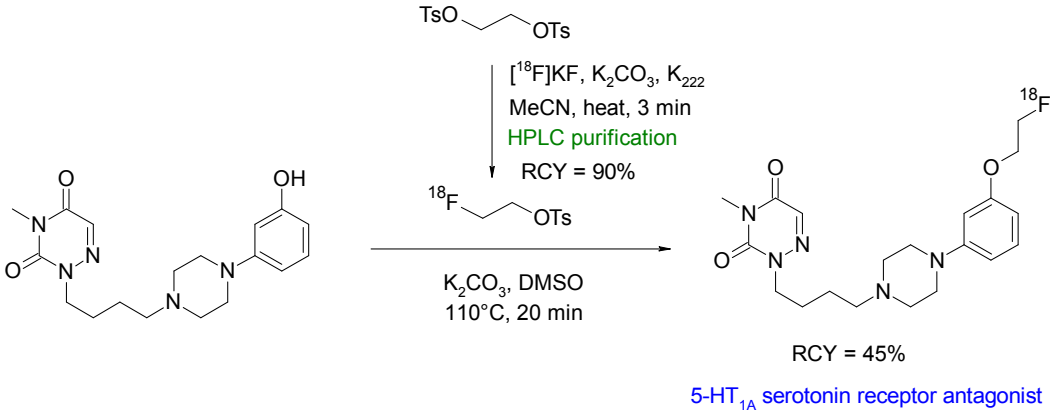

Fig 18. Radiosynthesis of a *O*-[^{18}F]fluoroethylated phenylpiperazin-substituted 6-aza-uracil.[44]

N-Methyl-*D*-aspartate (NMDA) receptors represent another important target in neurological and psychiatric disorders and hydantoin-substituted indole carboxylic acids represent one class of lead compounds for the development of radiolabeled NMDA receptor ligands. The group of Rösch developed a series of *ortho*-, *meta*- and *para*-(2-fluoroethoxy)phenyl-substituted derivatives of this class and performed *O*-[^{18}F]fluoroethylation at different positions.[45] (Figure 19) The initial reaction of the free carboxylic acid with HPLC-purified [^{18}F]FETs in aprotic solvents and NaOH as base resulted in [^{18}F]fluoroethylation RCY < 4% connected with the formation of two unknown by-products. Hence, the authors decided to start from the corresponding ethyl esters. Under optimized conditions using the ethyl ester precursor in DMSO and NaOH at 100°C 25-40% [^{18}F]fluoroethylation was achieved within 5 min. A prolongation of the reaction time resulted in a drop of the RCY caused by cleavage of ester moiety and thermal decomposition. Although different base systems had been tested, the subsequent cleavage of the ethyl ester group with 1 M NaOH was incomplete. Finally, a further HPLC

purification was performed providing the [^{18}F]fluoroethylated NMDA receptor ligand in 5-7% overall RCY.

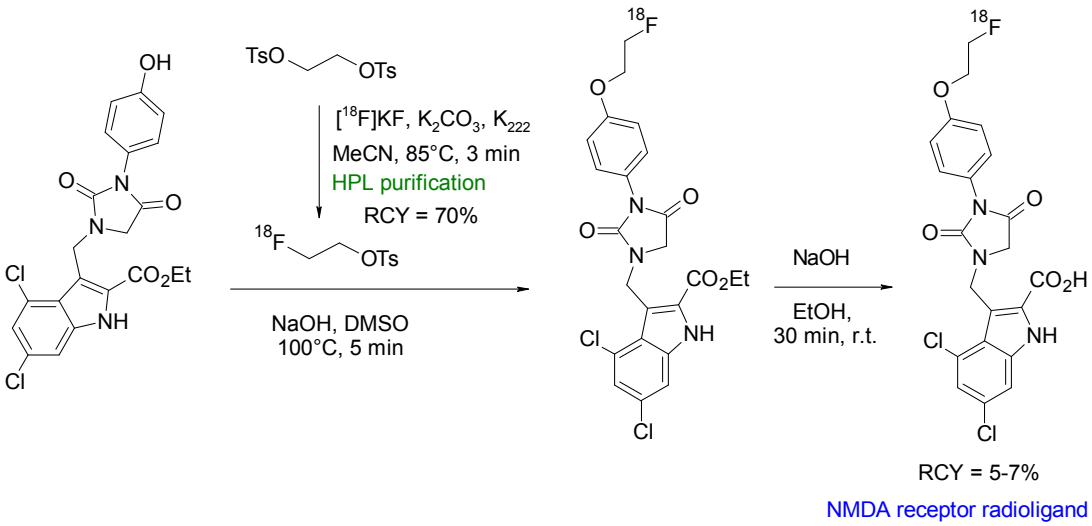

Fig 19. Synthesis of *O*-[^{18}F]Fluoroethylated hydantoin-derivative.[45]

Non-invasive detection of β-amyloid plaques and neurofibrillary tangles in the human brain would help in the early identification of patients with potential Alzheimer's disease. With regard to β-amyloid plaques 2-(4'-(methylamino)phenyl)-6-hydroxybenzothiazole (PIB) is an intensively examined compound owing to its significant accumulation in tissue of high plaque density in the human brain. The corresponding carbon-11 labeled radiotracer [^{11}C]PIB was the first prominent PET radiotracer in this context that had achieved clinical relevance. For obtaining a [^{18}F]fluoroethylated PIB analogue, the free hydroxyl group of PIB was reacted with [^{18}F]FETs in DMSO in presence of K_2CO_3 for 20 min at 100°C, followed by HPLC purification.[46] (Figure 20) The high chemoselectivity of [^{18}F]FETs allowed a selective *O*-alkylation without the need of a protective group on the nitrogen. Although the labeling yields were high for each single step (90 and 85% respectively), the overall RCY of [^{18}F]FE-PIB was only 5-10% attributed to a considerable loss of [^{18}F]FETs during the SPE based purification step.

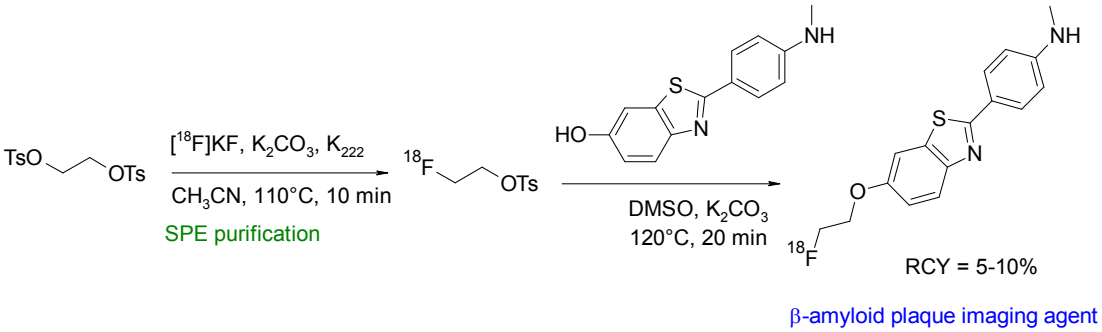

Fig 20. Radiosynthesis of [^{18}F]fluoroethylated benzothiazole.[46]

Recently, the class of imidazo[2,1-*b*]benzothiazoles (IBTs) has been developed as new compound class having high affinity to β-amyloid plaques. An *O*-[^{18}F]fluoroethylated IBT derivative was synthesized and evaluated as PET radiotracer.[47] In that study, direct one-step [^{18}F]fluorination of the corresponding tosylated precursor of IBT as well as a two-step [^{18}F]fluoroethylation at the phenolic hydroxyl group with [^{18}F]FETs was performed. (Figure 21) The one-step procedure provided [^{18}F]FE-IBT in 24% RCY. For the two-step radiofluorination [^{18}F]FETs was purified by HPLC providing the intermediate fixed on SPE cartridge in 45% yield. After drying, [^{18}F]FETs was eluted with DMF to the hydroxyl precursor in DMF, which was deprotonated with sodium hydride beforehand. [^{18}F]Fluoroethylation was performed for 5 min at 90°C and provided the desired [^{18}F]fluoroethylated IBT in 58% RCY.

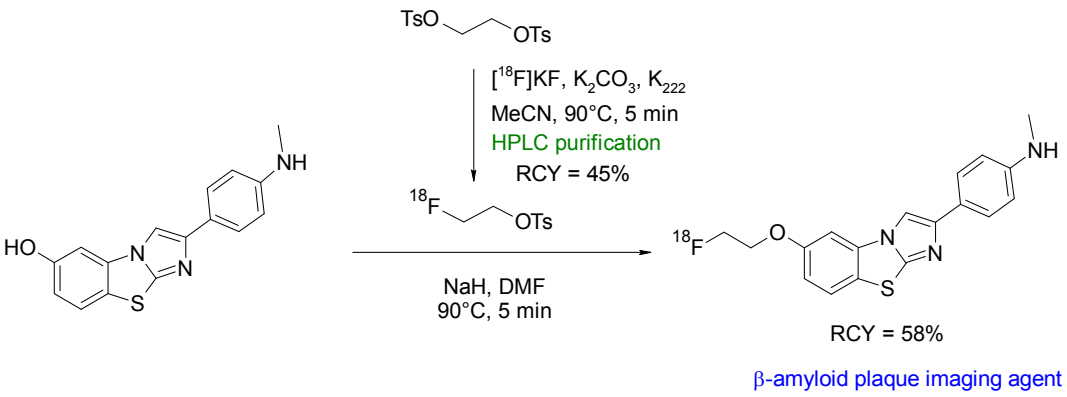

Fig 21. Radiosynthesis of *O*-[^{18}F]fluoroethylated imidazo[2,1-*b*]benzothiazole.[47]

Since sigma receptors are involved in diseases of the central nervous system and have been found to be expressed in some human tumor entities, radiolabeled sigma receptor ligands are of high scientific interest and their role has to be studied in vivo. Among the diversity of structures the

piperazine derivative SA4503 was a promising ligand and has been radiolabeled with carbon-11 for imaging sigma-1 receptors with PET. Consequently, the corresponding *O*-[^{18}F]fluoroethylated radiotracer was synthesized to study its pharmacokinetics *in vivo*.[48, 49] (Figure 22) Elsinga et al. used SPE-purified [^{18}F]FETs to perform [^{18}F]fluoroethylation of the hydroxyl precursor in presence of NaH in DMF. After reaction for 5 min at 125°C [^{18}F]FE-SA4503 was isolated after HPLC purification in 4-7% overall RCY. The low RCY was explained by the authors with the relative large dilution of [^{18}F]FETs in the DMF solution (1 mL) in relation to the small amount of hydroxyl precursor (0.2 mg).

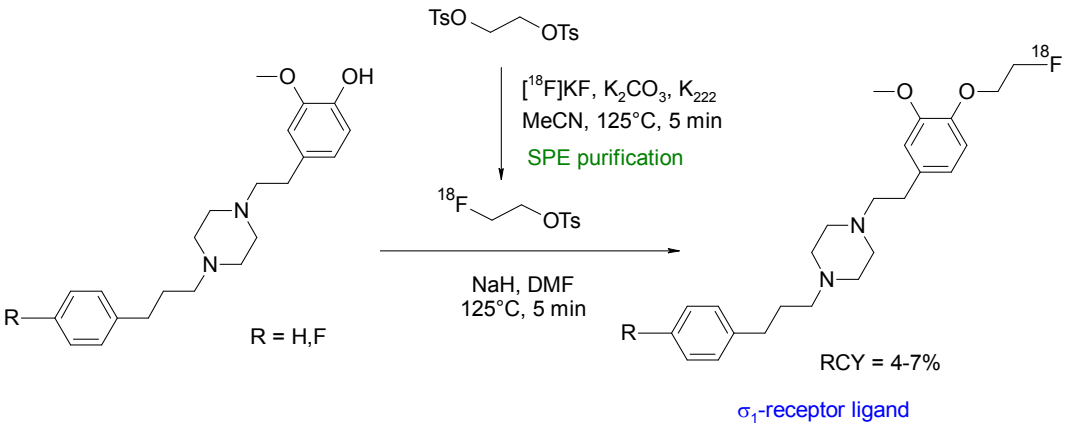

Fig 22. Synthesis of *O*-[^{18}F]fluoroethylated SA4503.[48, 49]

Recently spirocyclic piperidine derivatives were found to possess nanomolar affinities for the sigma-1 receptor subtype and two compounds have been selected for radiotracer development via [^{18}F]fluoroethylation with [^{18}F]FETs.[50, 51] (Figure 23) The radiolabeling of the spirocyclic piperidine was performed in an automated synthesizer by a one-pot two-step procedure without purification of [^{18}F]FETs. After preparation of [^{18}F]FETs the corresponding hydroxyl precursor with sodium hydroxide in DMSO[50] or with Cs_2CO_3 in DMF[51] was added and [^{18}F]fluoroethylation was performed by heating at 110°C for 10-20 min. Final HPLC purification provided the *O*-[^{18}F]fluoroethylated spirocyclic sigma-1 ligands within 100 min synthesis time in 15-20% overall RCY.

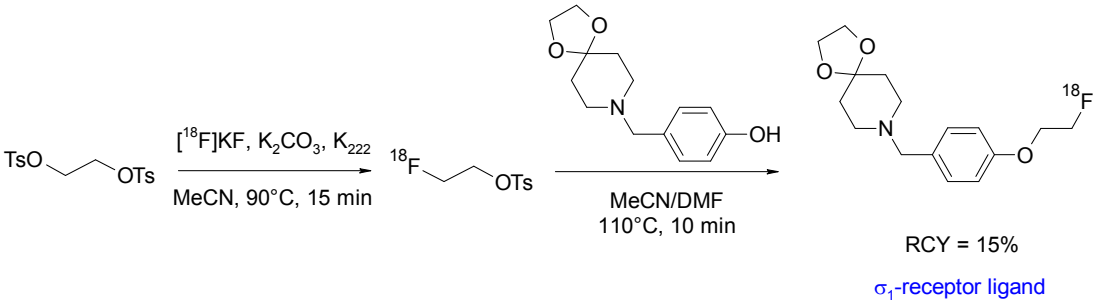

Fig 23. Synthesis of a *O*-[^{18}F]fluoroethylated spirocyclic piperidine.[51]

The translocator protein (TSPO) is an established target for the imaging of neuroinflammation with PET. To develop radiolabeled TSPO ligands Wadsworth et al. performed [^{18}F]fluoroethylation of diaryl-substituted acetanilides.[52] (Figure 24) For the radiolabeling reaction HPLC purified [^{18}F]FETs was used that was heated with the phenol precursor in acetonitrile in presence of Cs_2CO_3 at 120°C for 15 min. After HPLC purification the ^{18}F-fluoroethylated TSPO-ligand was isolated in 16 % overall RCY within 120 min synthesis time.

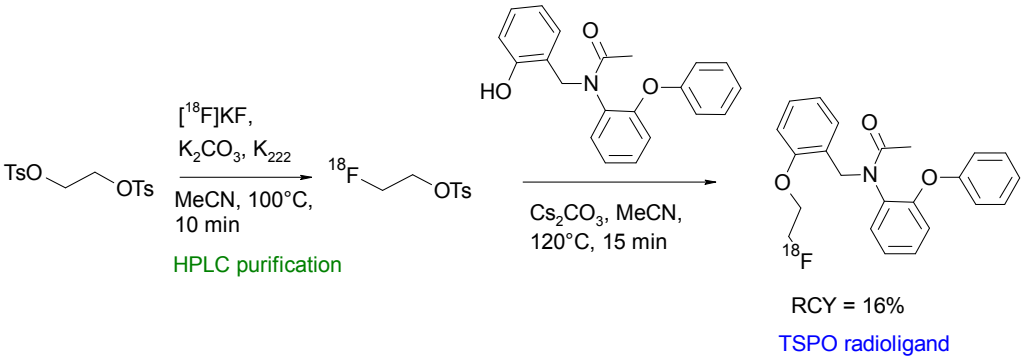

Fig 24. Radiosynthesis of *O*-[^{18}F]fluoroethylated diaryl-substituted acetanilide.[52]

Adenosine A_2 receptors are further attractive therapeutic targets for the treatment of neurodegenerative disorders and radiolabeled AA_2 ligands could be used to assess changes in receptor density. SCH442416 is a furanyl-substituted pyrazolo[4,3-e][1,2,4]triazolo-[1,5-c]pyrimidine compound with high affinity to the AA_{2A} receptor. Radiotracer design aimed at the replacement of a methoxy group of SCH442416 towards a ^{18}F-fluoroethoxy moiety.[53] (Figure 25) [^{18}F]FETs was purified by SPE and reacted with the phenol precursor in acetonitrile and 10µL of 40% aqueous $N(Bu)_4OH$ solution at 115°C for 15 min. The *O*-[^{18}F]fluoroethylated AA_{2A} receptor ligand was obtained within 114 min synthesis time in 7% overall RCY.

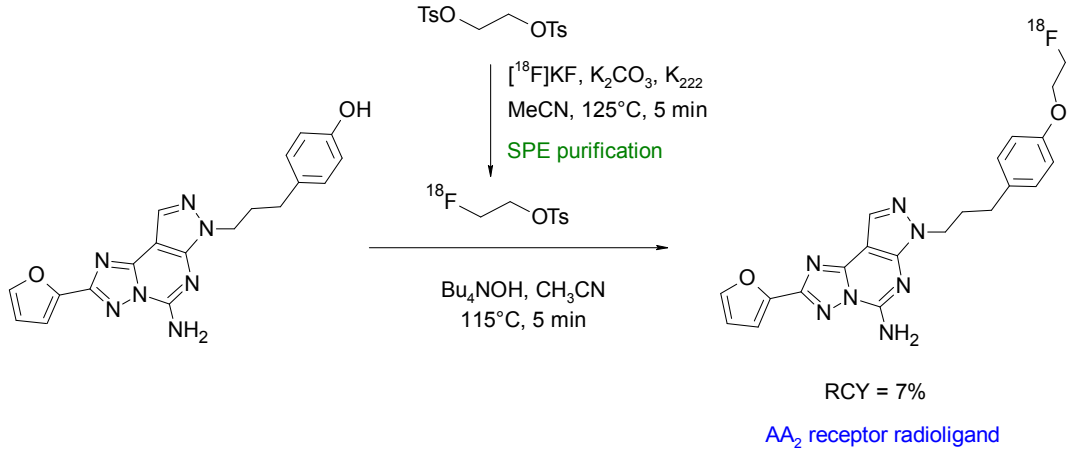

Fig 25. Radiosynthesis of *O*-[^{18}F]fluoroethylated SCH442416.[53]

Because the enzyme monoamine oxidase (MAO) is involved in psychiatric and neurodegenerative disorders, treatment with MAO inhibitors is a common therapeutic approach. For the *in vivo* determination of MAO levels in the brain radiotracers have been developed, most of them radiolabeled with carbon-11 as e.g. [^{11}C]harmine. In the effort to come to a ^{18}F-radiolabeled harmine derivative Schieferstein et al. performed *O*-[^{18}F]fluoroethylation of harmine with [^{18}F]FETs.[54] (Figure 26) [^{18}F]FETs was synthesized using a homemade automated synthesis unit and after SPE purification reacted with harmol and NaOH by heating for 10 min at 130°C. Final HPLC purification delivered the 2-[^{18}F]fluoroethyl-harmol in 47% overall RCY within 100 min synthesis time.

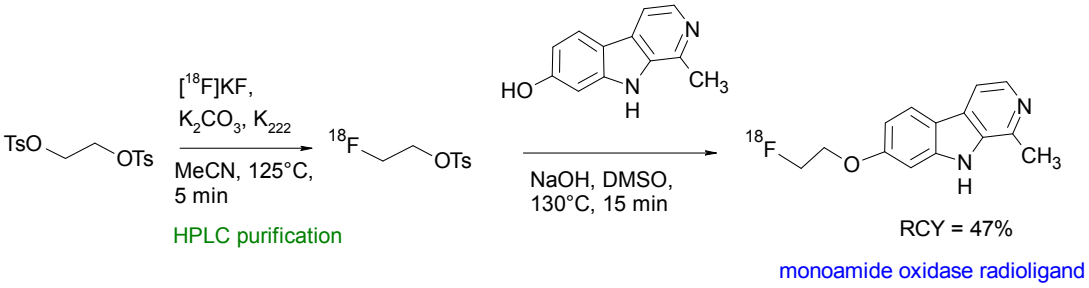

Fig 26. Radiosynthesis of *O*-[^{18}F]fluorethylated harmine.[54]

The non-invasive imaging of the endocrine pancreas with suitable radiotracers might be a useful tool in the diagnostic of e.g. diabetes mellitus. Sulfonureas which can block pancreatic ATP-sensitive potassium channels, located at the β-cells of the islets of Langerhans have been found to act as antidiabetic compounds, therefore fluorine-18 labeled sulfonureas as glyburide may serve as β-cell imaging agents. Shiue et al. developed 2-[^{18}F]fluoroethoxy-glyburide as potential radiotracer.[55] The

corresponding phenol served as precursor for the *O*-[^{18}F]fluoroethylation to form the 2-[^{18}F]fluoroethoxy-glyburide by a one-pot two-step reaction. (Figure 27) [^{18}F]FETs was reacted without purification with hydroxyl-glyburide in DMSO and NaOH 30 min at 90°C. 2-[^{18}F]fluoroethoxy-glyburide was isolated in 5-10% overall RCY after HPLC purification within 100 min synthesis time. The corresponding 2-[^{18}F]fluoroethoxy-5-iodoglyburide was later synthesized by the same group in the same manner and in comparable RCY.[56]

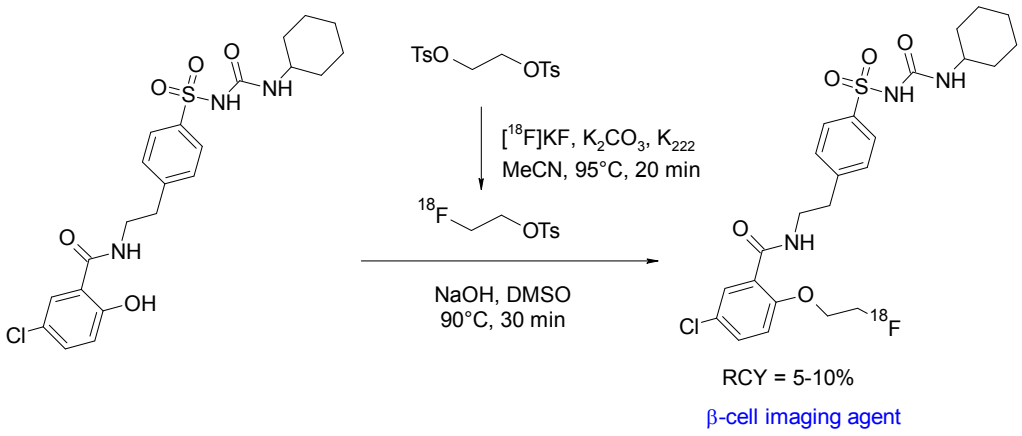

Fig 27. Radiosynthesis of 2-*O*-[^{18}F]fluoroethoxy-glyburide.[55]

Repaglinide is a non-sulfonylurea based glucose regulator and in clinical use to block ATP-sensitive potassium channels to regulate the secretion of insulin. An ^{18}F-labeled repaglinide derivative was developed by Wängler et al. for the in vivo visualization and quantification of human pancreatic β-cells.[57] Since the chemical structure of repaglinide is characterized by the presence of an ethoxy group, a radiolabeling strategy comprising [^{18}F]fluoroethylation was suggested. Radiolabeling was performed as a two-pot procedure with HPLC-purified [^{18}F]FETs which was after a drying step dissolved in DMSO and added to the hydroxyl precursor in presence of NaOH solution. (Figure 28) The carboxylic function of des-ethoxy-repaglinide was protected beforehand by a methylester group to avoid formation of [^{18}F]fluoroethyl ester. *O*-[^{18}F]fluoroethylation was performed for 10 min at 150°C and the intermediate product was purified by HPLC and deprotected with 1 M NaOH at 80°C for 35 min. The *O*-[^{18}F]fluoroethyl-repaglinide derivative was isolated in an overall RCY of 20%. The fact that the [^{18}F]fluoroethyl moiety was unharmed during the relative harsh deprotection step gives evidence for the stability of this labeling group.

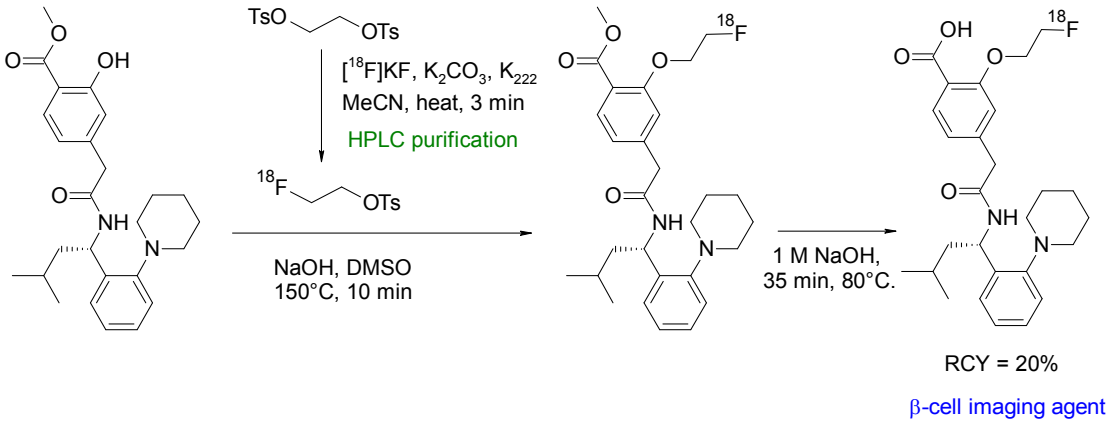

Fig 28. Radiosynthesis of *O*-[^{18}F]fluoroethylated repaglinide.[57]

In humans the peripheral beta-adrenoceptors is involved in the neuronal regulation of the epithelial, glandular and muscular lung function. Fenoterol represents a highly affine subtype-specific β2 receptor antagonist showing three phenolic groups, hence Schirrmacher et al. developed a fluorine-18 labeled derivative of fenoterol via *O*-[^{18}F]fluoroethylation.[58] The radiolabeling was achieved using (*R*,*R*)(*S*,*S*)fenoterol and [^{18}F]FETs by heating in DMF at 130°C for 8 min in presence of 1 M NaOH. (Figure 29) The RCY of [^{18}F]FE-fenoterol was 60% referring to [^{18}F]FETs. As a side-reaction, the formation of the corresponding *N*-[^{18}F]fluoroethylated compound was observed in 40% yield. Taking into account the difference in acidity of the hydroxyl groups, it turned out that the hydroxyl function on the phenol moiety was easier deprotonated than that of the resorcinol; hence a selective [^{18}F]fluoroethylation was achieved using 0.9 equivalents of NaOH. The use of on additional equivalent of base resulted in the formation of an [^{18}F]fluoroethylated resorcinol derivative as a side product. The purification of the radiotracer was performed by HPLC whereby the *O*-[^{18}F]fluoroalkylated radiotracer [^{18}F]FE-(*R*,*R*)(*S*,*S*)fenoterol was delivered in 20% overall RCY . In conclusion, this reaction gives a good illustration of the chemo selectivity of [^{18}F]FETs by a selective labeling of different acidic phenols avoiding the need of protective groups.

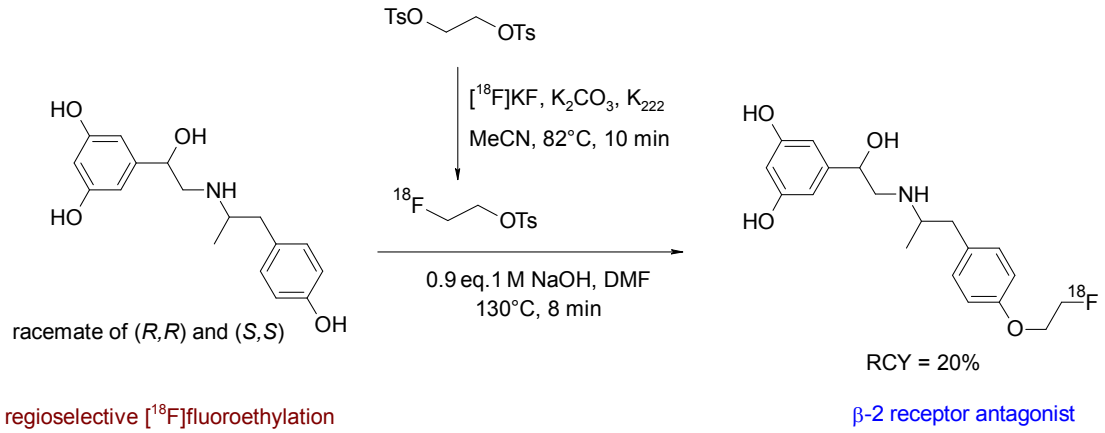

Fig 29. Radiosynthesis of *O*-[^{18}F]fluoroethylated fenoterol.[58]

Tyrosine kinases are playing pivotal roles in the pathogenesis of cancer and tyrosine kinase inhibitors (TKIs) represent promising candidates for therapeutic intervention. Accordingly radiolabeled TKIs would be useful tools to assess the status of cancer disease and help to determine the optimal course of treatment. The class of aminoquinazolines is part of many drugs with antibacterial, antiviral and anticancer activity, so approved TKIs like gefitinib, lalatinib and erlotinib are consisting of this important pharmacophore. Chen et al. developed a series of ^{18}F-radiolabeled aminoquinazoline derivatives as potential tumor imaging tracers by radiolabeling the appropriate phenol precursors with [^{18}F]FETs.[59] The radiotracers were formed via one-pot two-step procedure without isolation of the intermediate labeling agent. The final *O*-[^{18}F]fluoroethylated aminoquinazolines were provided in 21-24% overall RCY (not decay corrected). (Figure 30)

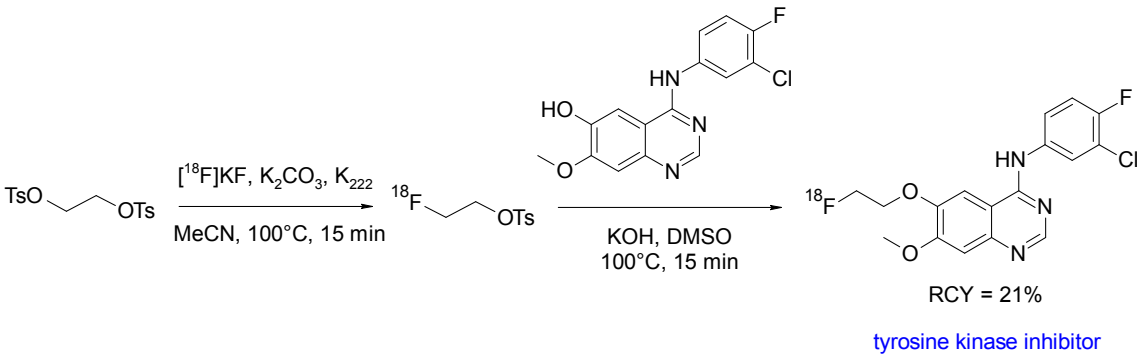

Fig 30. Radiosynthesis of a *O*-[^{18}F]fluoroethylated aminoquinazoline.[59]

Poly-*N*-(2-hydroxypropyl)methacrylamide (HPMA) is a promising nontoxic, non-immunogenetic and biocompatible drug for anticancer treatment and recently lauryl methacrylate segments were added

to HPMAs to improve the delivery of these drugs through the blood brain barrier.[60] For tracking the distribution and accumulation of such laurylmethacrylate-substituted HPMAs *in vivo*, these drugs have been converted into radiotracers by ^{18}F-radiolabeling with [^{18}F]FETs.[60, 61] Radiolabeling was accomplished in two steps comprising synthesis and HPLC purification of [^{18}F]FETs and subsequent attachment to the hydroxyl group of the linker tyramine in DMSO in presence of NaOH. (Figure 31) The radiolabeled polymeric substances were purified by size exclusion chromatography. Altogether the yield of ^{18}F-incorporation did not exceed 4% and the labeling efficiency decreased with higher molecular weight of the polymers, most likely caused by the decreased accessibility of the tyramine linkers.

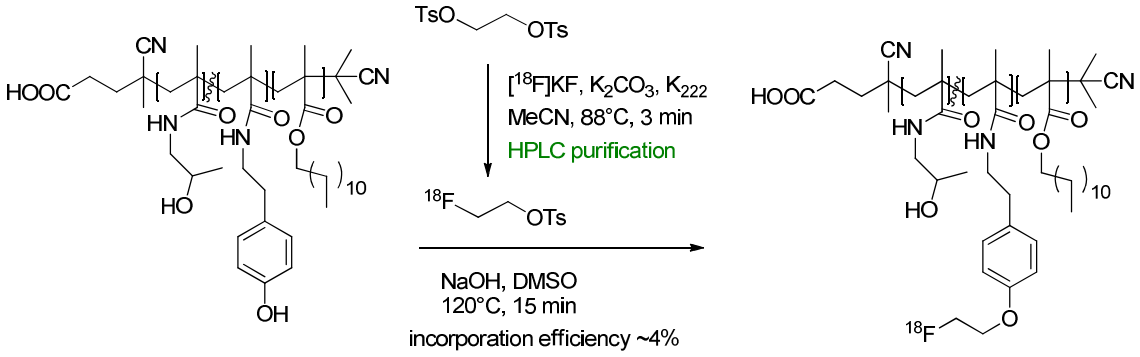

Fig 31. *O*-[^{18}F]fluoroethylation of poly-*N*-(2-hydroxypropyl)methacrylamide.[60, 61]

A relatively new approach for the radiolabeling of bio(macro)molecules is the application of bioorthogonal coupling reactions because they are fast and highly selective. Staudinger ligation is a powerful conjugation reaction in this field using functionalized phosphane analogs to be coupled with an azide-substituted biomolecule. For preparing a prosthetic group suitable as radiolabeling agent in the Staudinger ligation, Mamat et al. has designed a [^{18}F]fluoroethoxy-benzoate substituted phosphane.[62] This new bifunctional labeling agent was prepared in an one-pot method by generation of [^{18}F]FETs and subsequent reaction with the phenol group of 2-(diphenylphosphano)phenyl 4-hydroxybenzoate. (Figure 32) As third step azide functionalized model compounds were added. In this manner the ^{18}F-radiolabeled Staudinger coupling products were formed in 12-17% overall RCY.

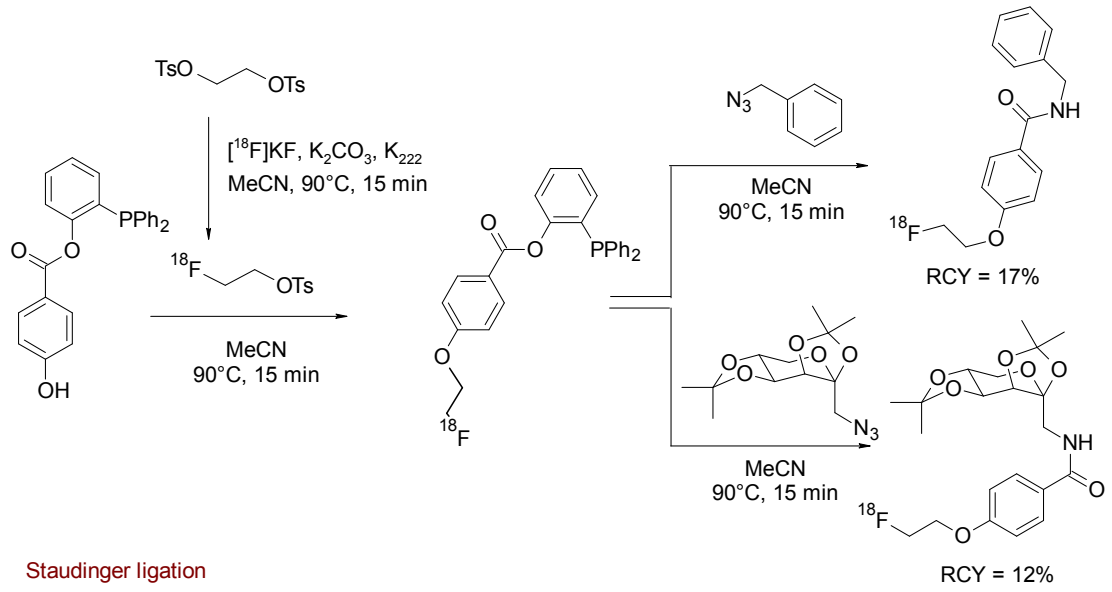

Fig 32. Radiosynthesis of a *O*-[^{18}F]fluoroethoxy-benzoate substituted phosphane.[62]

[^{18}F]Fluoroethylesters

[^{18}F]FETs is not only a suitable reagent for the alkylation of hydroxyl groups, it can also be used as a labeling reagent to form 2-[^{18}F]fluoroethylesters, when a carboxylic acid is provided as precursor as it is demonstrated in the following examples.

Carfentanil, an anilinopiperidine derivative, is a potent agonist of the μ-opioid receptor. A number of ^{18}F-labeled carfentanil derivatives have been evaluated intensively by Henriksen et al. including the 2-[^{18}F]fluoroethyl ester, obtained by the reaction of the carboxylic acid sodium salt with [^{18}F]FETs. (Figure 33)[63, 64] The carfentanil-2-[^{18}F]fluoroethylester was prepared within 100 min in 36% overall RCY. The authors performed a series of optimization experiments to determine the dependency of the RCY on temperature, solvent and addition of NaI. When the [^{18}F]fluoroethylation was performed in DMF at 100°C only 7% radiolabeled product was obtained. Increasing the temperature to 120°C yielded about 20% product. Compared to DMF, DMSO resulted in lower yields under otherwise identical conditions. Addition of NaI to the reaction mixture led to 25 - 55% overall RCY, so the application of 20 mM NaI was optimal.

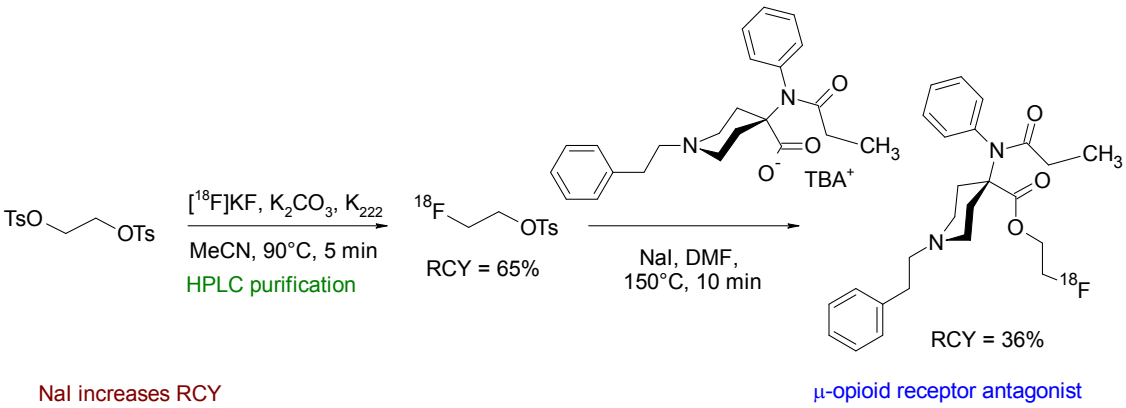

Fig 33. Radiosynthesis of carfentanil-2-[^{18}F]fluoroethylester.[63, 64]

Another example for [^{18}F]fluoroethylester formation was described by Philippe et al. in the synthesis of a new PET ligand for the melanin concentrating hormone receptor-1 (MCHR1) playing a key role in energy homeostasis and is involved in anxiety, diabetes and adiposity.[65] The [^{11}C]methylester of the MCHR1 ligand served as lead for the development of a corresponding 2-[^{18}F]fluoroethylester. For radiolabeling, the carboxylic acid precursor of SNAP-7941, activated by addition of tetrabutylammonium hydroxide, was reacted with the [^{18}F]fluoroethylating reagents [^{18}F]FEBr, [^{18}F]FETs and 2-[^{18}F]fluoroethyl triflate ([^{18}F]FETf) in a vessel based approach. (Figure 34) Surprisingly the authors did not observe any ^{18}F-incorporation into SNAP-7941 for all of these reagents, even the direct [^{18}F]fluorination of a tosylated precursor failed. To overcome this situation, a microfluidic approach was attempted that was finally successful and delivered the 2-[^{18}F]fluoroethyl ester of SNAP-7941 in 44% RCY starting from the tosylated precursor. However the [^{18}F]FETs based method did not work in the microfluidic device either.

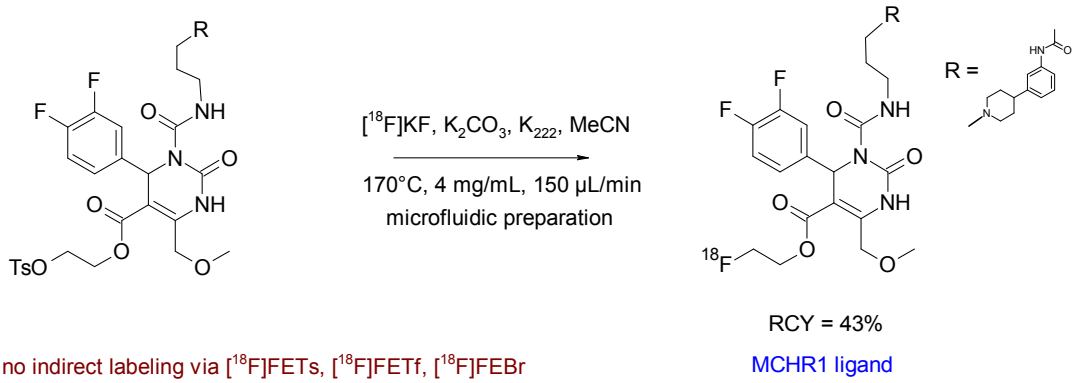

Fig 34. Radiosynthesis 2-[^{18}F]fluoroethylester of SNAP-7941 direct ^{18}F-fluorination.[65]

A third example for [^{18}F]fluoroethylester formation with [^{18}F]FETs was presented by Heinrich et al. who developed the 2-[^{18}F]fluoroethyl ester of rhodamine B for imaging myocardial perfusion with PET.[66] Rhodamines are lipophilic cations which are known to be accumulated in heart tissue and to bind at the mitochondrial membrane. As precursor for [^{18}F]fluoroethylester formation served the lactone of rhodamine B that was heated with the unpurified [^{18}F]FETs in acetonitrile at 160°C in presence of diisopropylethylamine, a base required for inducing the ring opening of the lactone. (Figure 35) The authors found that optimal yields were obtained by slowly evaporating the solvent during the reaction and isolated the rhodamine B 2-[^{18}F]fluoroethylester after HPLC purification in 35% overall RCY within 90 min total synthesis time.

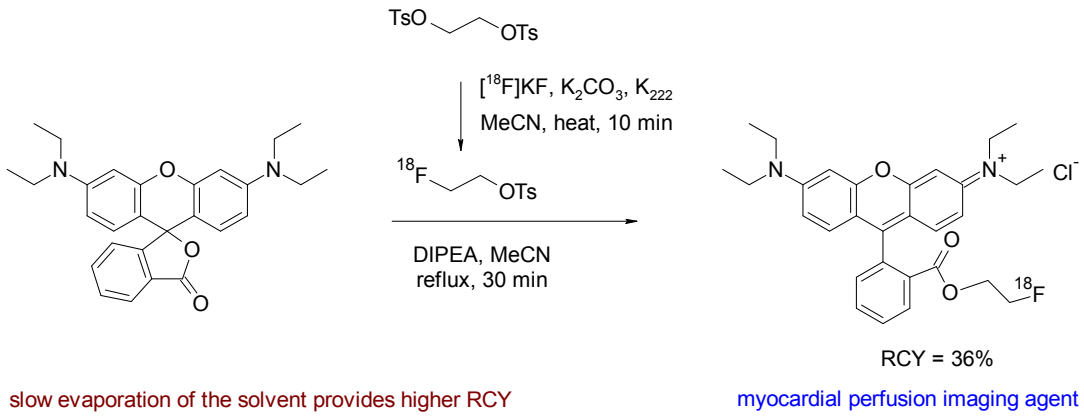

Fig 35. Radiosynthesis of a 2-[^{18}F]fluoroethylester of rhodamine B.[66]

N-[^{18}F]fluoroethylation

2-[^{18}F]fluoroethylcholine

The amine group is an important functionality in numerous biologically active compounds and alkylation of primary and secondary amines is a common and straightforward route in organic chemistry by treatment with alkyl halides or sulfonates. *N*-[^{18}F]fluoroethylation using [^{18}F]FETs or [^{18}F]FEBr stands for simple and efficient radiolabeling strategy, however this method is sometimes limited due to the high basicity of the amine, affording strict aprotic conditions and high temperatures. The following examples may illustrate the potential of [^{18}F]FETs in the radiolabeling of amines.

The finding that radiolabeled choline accumulates in certain tumors cells, e.g. of prostate cancer, because of elevated uptake of choline as precursor of phosphatidylcholine in tumors promoted the use of carbon-11 and fluorine-18 labeled choline as preferred PET tracers for prostate imaging. Hara

et al. firstly described the PET imaging of prostate cancer using [^{11}C]choline and performed in 1997 the first radiosynthesis of 2-[^{18}F]fluoroethylcholine ([^{18}F]FEC) by reacting [^{18}F]FETs with neat *N*,*N*-dimethylethanolamine at 80°C for 20 min.[67] [^{18}F]FEC was obtained in a one-pot procedure after ion exchange based SPE purification in 50% overall RCY. Later an automated synthesis of [^{18}F]FEC was presented including the one-pot two step radiolabeling starting from 1,2-ethylene glycol-bis-tosylate providing the intermediate [^{18}F]FETs, its reaction with *N*,*N*-dimethyl-ethanolamine and subsequent purification by semi-preparative HPLC, and the final trapping of [^{18}F]FEC on a SCX cartridge.[68] (Figure 36) By following this protocol [^{18}F]FEC was obtained after 65 min as hydrochloride in 40% overall RCY in a specific activity of 74 GBq/µmol (EOS).

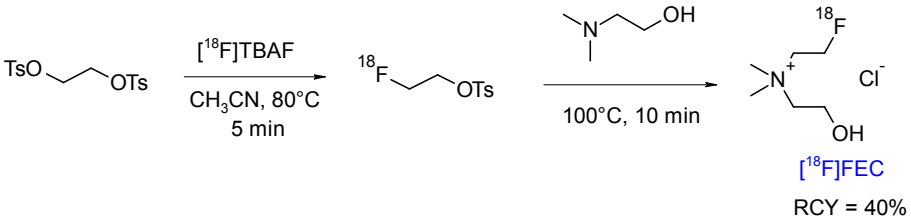

Fig 36. Radiosynthesis of 2-[^{18}F]fluoroethylcholine.[68]

In 2004, Yu et al. published a study comparing the radiosynthesis of 2-[^{18}F]fluoroethyl-, 3-[^{18}F]fluoropropyl- and 4-[^{18}F]fluorobutyl-choline from the corresponding bis-tosylated and bis-brominated alkane precursors.[69] The reactions were performed as one-pot procedure; the radiolabeled choline derivatives were purified by SCX SPE and provided the radiotracers in 18-24% overall RCY. The authors found that the dibromo-substituted alkane precursors provided a considerably lower yield for ^{18}F-incorporation compared to the corresponding bis-tosylated counterparts. Furthermore a one-step radio-fluorination of [^{18}F]FEC was tried by starting from e.g. the *N*-2-acetoxyethyl-*N*,*N*-dimethyl-*N*-2-*p*-toluenesulfonyloxethyl ammonium salt precursors, however this approach was unsuccessful due to supposed ion pair formation between the ammonium cation and the [^{18}F]fluoride anion.

As a further radiotracer for the choline transport Henriksen et al. developed a deshydroxy-[^{18}F]fluorocholine ([^{18}F]FdOC).[70] [^{18}F]FdOC was synthesized by a two-step procedure consisting of the ^{18}F-fluorination of 1,2-ethylene glycol-bis-tosylate and [^{18}F]fluoroethylation of *N*,*N*,*N*-trimethyl amine. (Figure 37) The intermediate [^{18}F]FETs was obtained after reversed-phase HPLC purification in 65% RCY. The final [^{18}F]FdOC was isolated after SCX-based purification within 70 min synthesis time in 20-25% overall RCY.

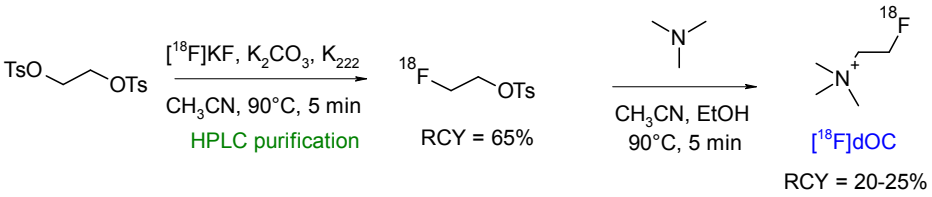

Fig 37. Radiosynthesis of deshydroxy-[^{18}F]fluorocholine.[70]

A reliable, fully automated synthesis of [^{18}F]FEC using a commercially available synthesis unit was published in 2007 by the group of Rösch.[71] Since the authors could show that addition of alkali iodides, especially LiI, to the fluoroalkylating agents [^{18}F]FETs or [^{18}F]FEBr significantly improved the RCY[16] they transferred this method successfully to the synthesis of [^{18}F]FEC. Due to the toxicity of lithium, 6.5 mg of NaI was used instead, as a result higher reaction temperatures (120°C) were needed to obtain [^{18}F]fluoroethylation yields of 85-90%. For the automated radiosynthesis using a two-pot synthesizer, the intermediate [^{18}F]FETs was purified by C18-based SPE while [^{18}F]FEC purification was performed by semi-preparative HPLC. This iodine-promoted protocol provided [^{18}F]FEC after 50 min synthesis time in 30% overall RCY with a specific activity of about 55 GBq/µmol (EOS).

The radiosynthesis of [^{18}F]fluoromethyl-, [^{18}F]fluoroethyl- and [^{18}F]fluoropropyl-choline by the microfluidic approach was reported by Pascali et al. with view on dose-on-demand production of radiopharmaceuticals.[72] The aim of the study was to obtain a single dose of radiotracer in a short time. To enable the two-step ^{18}F-labeling procedure in the microfluidic system additional storage loops and a second micro reactor were implemented. The production of [^{18}F]FETs was performed e.g. by delivering 10 µL of a [^{18}F]KF/K_{222} complex (50-300 MBq) into a 15.6 µL volume micro reactor together with 10 µL of a 1,2-ethylene glycol-bis-tosylate solution (33 mg/mL) and reacting it for 23 s at 150°C. Then 20 µL of the [^{18}F]FETs solution were applied into the second micro reactor and mixed with *N*,*N*-dimethyl-ethanolamine (4-100 µL) at 80°C for 62 s and the ^{18}F-fluoroalkylated choline derivatives were purified by SPE. [^{18}F]FEC was obtained after 15 min in 36% overall RCY with a specific activity of 149 MBq/µmol. Interestingly, [^{18}F]fluoropropyl-choline provided better RCY (49%) than the [^{18}F]fluoroethylated (36%) and the [^{18}F]fluoromethylated (20%) analogs.

N-[^{18}F]fluoroethyl-amines

A *N*-[^{18}F]fluoroethylated L-tryptophan analog was obtained by the reaction of *N*-Boc-L-tryptophan ethyl ester with [^{18}F]FETs and subsequent removal of the BOC and ester protecting groups.[73] (Figure 38) In that protocol SPE-purified [^{18}F]FETs was reacted with the protected tryptophan precursor in

DMSO/NaOH followed by the hydrolysis in hydrochloric acid. The overall RCY of *N*-[^{18}F]fluoroethyl-L-tryptophan was only 0.9%, which was explained by a troublesome purification procedure of the final product.

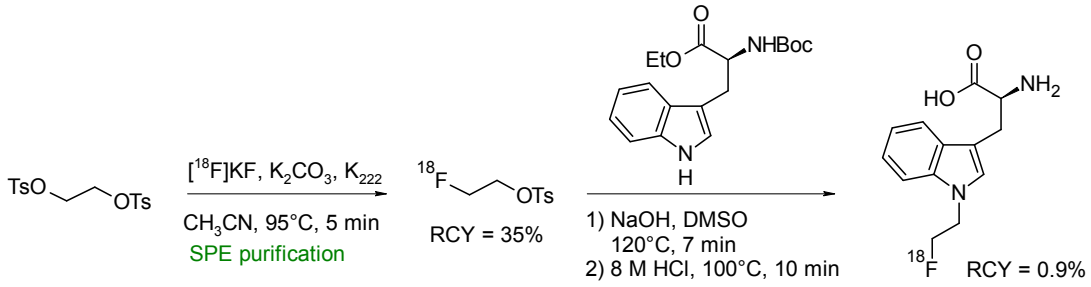

Fig 38. Radiosynthesis of *N*-[^{18}F]fluoroethylated L-tryptophan.[73]

The dopamine transporter is a key protein in the pharmacology and pathophysiology of the brain. Accordingly, a number of radiolabeled dopamine transporter ligands have been developed, among them 2β-carbomethoxy-3β-aryl-substituted tropanes. An *N*-[^{18}F]fluoroethylated tropane derivative ([^{18}F]FECNT) was used as imaging ligand for the dopamine transporter with PET and synthesized by N-[^{18}F]fluoroethylation of nortropane.[74] [^{18}F]FETs, purified via a SiO_2-based SPE was reacted at 135°C for 45 min with the nortropane precursor in DMF without addition of base. (Figure 39) HPLC purification provided [^{18}F]FECNT in 21% overall RCY.

A similar tropane-based dopamine transporter ligand was presented in 2004, the labeling approach was likewise a [^{18}F]fluoroethylation on a secondary amine.[75] (Figure 39) In contrast to the above mentioned publication, the use of 5 mg of precursor resulted in only 2.7% RCY. By using 10 mg of nortropane precursor for [^{18}F]fluoroethylation, the final radiotracer [^{18}F]β-CFT-FE was formed in 6% overall RCY. Riss et al. developed a two-step radiosynthesis of [^{18}F]FE-β-CIT, another dopamine transporter imaging agent based on 3-phenyltropane.[76] [^{18}F]FETs was produced using an automated synthesis module with integrated semi-preparative HPLC purification. Subsequent reaction with the nortropane derivative in DMSO at 120°C for 20 min resulted in a [^{18}F]fluoroethylation yield of 64%, whereby the purified [^{18}F]FE-β-CIT was obtained after 100 min in 25% overall RCY. (Figure 39) The authors studied the role of reaction time and temperature and found that 120°C resulted in higher RCY than 105°C and that 20 min were superior to 10 min. Notably, the addition of LiI did not lead to improved RCY because in that case the reaction temperature had to be kept at 80°C to avoid evaporation of the volatile intermediate 2-[^{18}F]fluoroethyl iodide.

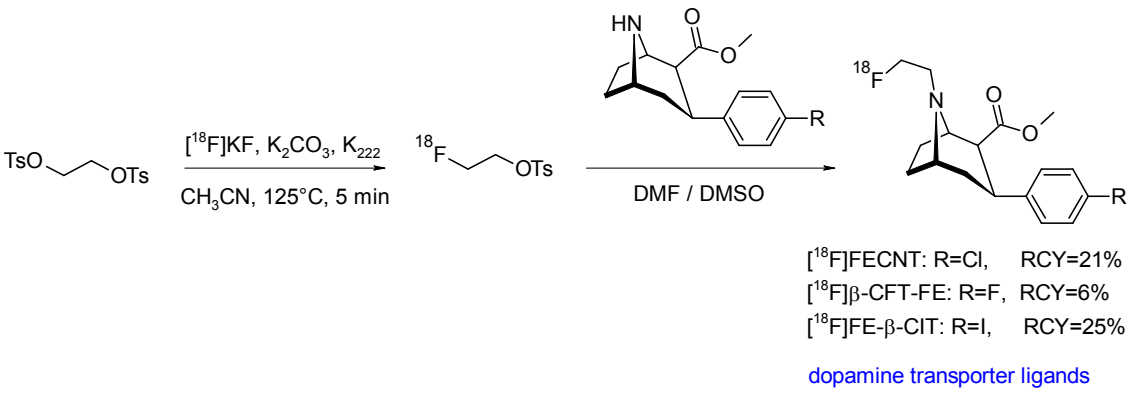

Fig 39. Synthesis of *N*-[^{18}F]fluoroethylated tropane derivatives.[74-76]

[^{18}F]Fluoroethyl-4-piperidyl benzilate [^{18}F]4-FEPB was reported to be a suitable antagonist of the muscarinic acetylcholine receptor (mAChR) and its radiosynthesis was performed by a *N*-[^{18}F]fluoroethylation of the corresponding piperidyl precursor.[77] (Figure 40) [^{18}F]FETs was formed by reaction of ethylene glycol-1,2-bistosylate with [^{18}F]tetrabutylammonium fluoride in acetonitrile at 80°C for 10 min and was purified using a silica based SPE. *N*-alkylation was performed in DMSO at 120°C with the piperidyl precursor within 90 min total synthesis time and provided HPLC-purified [^{18}F]4-FEPB in 20-40% overall RCY. The [^{18}F]4-FEPB synthesis has been re-evaluated by Skaddan et al. in 2001. In addition, [^{18}F]fluoroethyl-3-piperidyl benzilate and [^{18}F]fluoroethyl-3-pyrrolidyl benzilate have been synthesized as possible radioligands for the mAChR.[78] The ^{18}F-fluoroethylation was performed as a two-step procedure in one pot just by adding the amine precursor dissolved in DMSO to the [^{18}F]FETs. By this method the HPLC-purified mACh receptor ligands were isolated in 14-24% overall RCY.

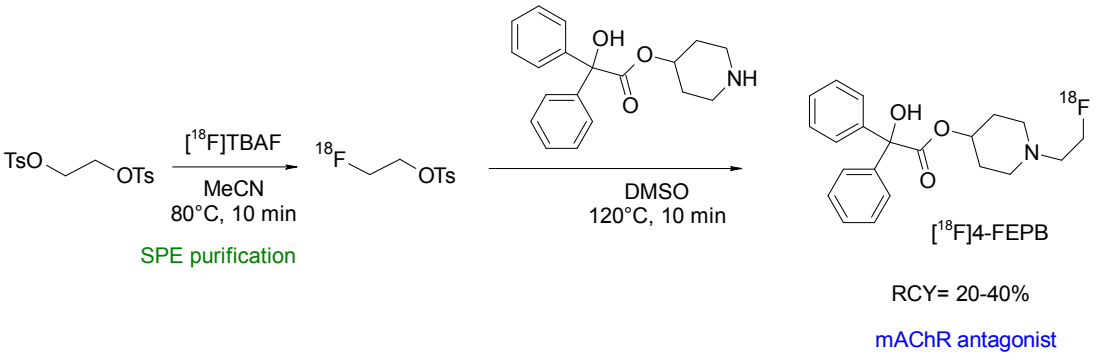

Fig 40. Synthesis of *N*-[^{18}F]fluoroethylated 4-piperidyl benzilate.[77]

Caillé et al. has reported the synthesis and ^{18}F-radiolabeling of two benzodioxane derivatives as new serotonin 5-HT$_4$ receptor antagonists.[79] [^{18}F]Fluoroethylation was performed at the secondary amine; i.e. [^{18}F]FETs was reacted with a piperidine derivative in DMF in presence of different bases (Cs_2CO_3, K_2CO_3, NaOH, Bu_4NOH) at 130°C. (Figure 41) Unfortunately, the *N*-[^{18}F]fluoroalkylation of piperidine was difficult and only the application of DMF/Cs_2CO_3 led to the radiolabeled 5-HT$_4$ receptor antagonists, although in only 1.0% overall RCY. The authors could not achieve any improvements neither by using HPLC-purified [^{18}F]FETs nor by application of a one-pot labeling procedure or by using NaI in DMSO as recommended by the literature.[16] Despite of the low yield the radiosynthesis delivered sufficient amounts of radiotracers to perform PET imaging studies. Noteworthy, [^{18}F]fluoroethylation was the only successful labeling approach because ~~a~~ direct [^{18}F]fluorination was impossible due to the failure in the synthesis of a corresponding ethyl-tosylated precursor.

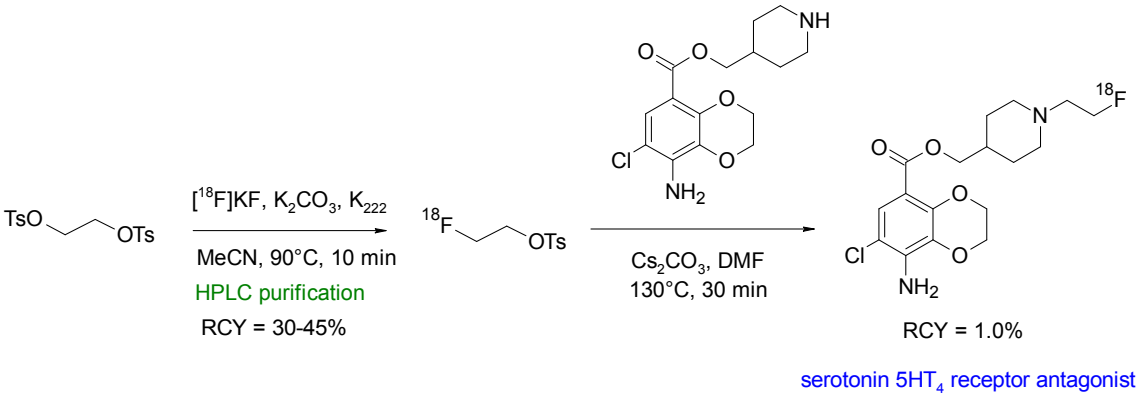

Fig 41. Radiosynthesis of a *N*-[^{18}F]fluoroethylated benzodioxane.[79]

Brain cholinesterase activity has successfully been determined with radiolabeled *N*-[^{18}F]fluoroalkylated piperidine and pyrrolidine esters.[80] The [^{18}F]fluoroethylation was performed as an one-pot reaction without purification of [^{18}F]FETs. The free bases were prepared beforehand from the hydrochloride salts of the piperidine or pyrrolidine precursors by treatment with aqueous $NaHCO_3$ in diethyl ether, drying of the organic phase with sodium sulfate, filtration, DMF addition and ether removal. The activated precursors dissolved in DMF were added to the reaction mixture containing the [^{18}F]FETs and the radiolabeling was performed at 120°C for 15min. (Figure 42) After workup and HPLC purification, the corresponding radiolabeled substrates of cholinesterase were obtained within 90 min in 8-11% overall RCY.

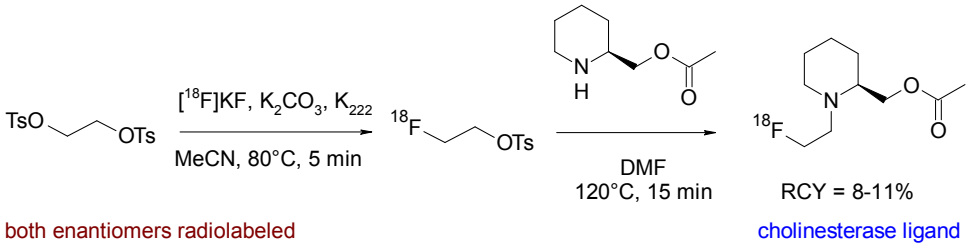

Fig 42. Synthesis of *N*-[^{18}F]fluoroethylated piperidine esters.[80]

N-Methyl-*D*-aspartate (NMDA) receptors represent an important target in neurological and psychiatric disorders. The discovery of compounds providing nanomolar affinities to NMDA receptors, among them *trans*-5,7-dichloro 4-substituted 2-caboxytetrahydroquinolines, has triggered the development of adequate radiotracers. One example is the *N*-[^{18}F]fluoroethylation of a piperazine derivative as shown in Figure 43.[81] The radiolabeling was performed by using HPLC purified [^{18}F]FETs which was added to the piperazine precursor in DMSO in absence of a base. The mixture was stirred at 140°C for 25 min and subsequently purified by HPLC. For removal of a methylester protecting group the radiotracer was stirred with LiOH in methanol, followed by a SEP-based purification of the final product. Despite of the complex 3-step radiosynthesis containing two HPLC-runs, the NMDA receptor ligand was isolated within 110 min in 35% overall RCY.

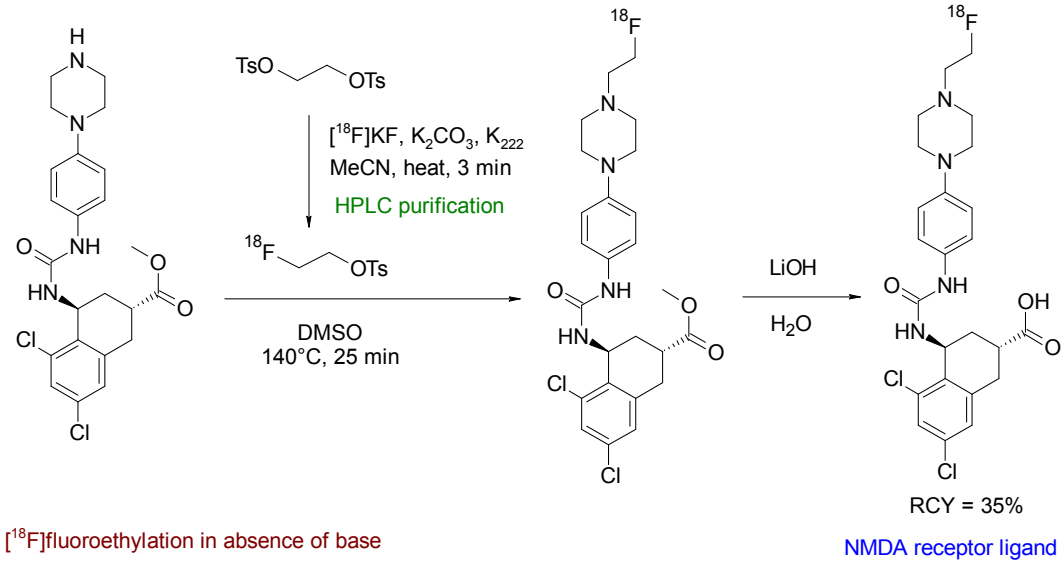

Fig 43. Synthesis of a *N*-[^{18}F]fluoroethylated piperazine.[81]

For non-invasive detection of β-amyloid plaques and neurofibrillary tangles in the human brain a number of radiolabeled biomarkers have been developed during the last decade. The SPECT tracer 6-

[^{123}I]iodo-2-(4'-*N*,*N*-dimethylamino)phenylimidazo[1,2-*a*]pyridine ([^{123}I]IMPY) has been identified to have a high uptake in mouse brain. Therefore the *N*-[^{18}F]fluoroethylated analogue was developed for PET.[82] A one-pot radiosynthesis was performed based on the generation of [^{18}F]FETs and followed by [^{18}F]fluoroethylation of the corresponding *N*-methyl aniline precursor at 135°C for 45 min without addition of a base. (Figure 44) HPLC purification and formulation in saline delivered [^{18}F]fluoroethylated IMPY in 26-51% overall RCY. Notably, also the D_4-[^{18}F]fluoroethylated analog was prepared to provide higher metabolic stability. However no improvement was found in comparison with the non-deuterated radiotracer.

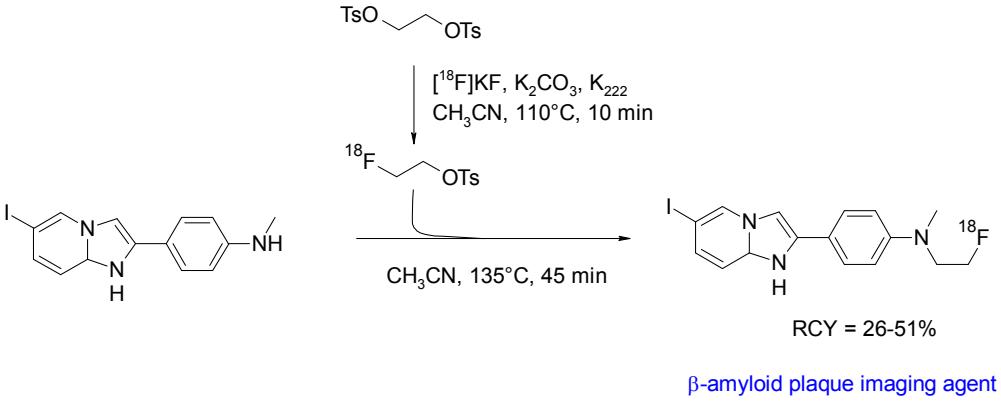

Fig 44. Radiosynthesis of N-[^{18}F]fluoroethylated IMPY.[82]

The tyrosine kinase inhibitor dasatinib is known to be a multi-kinase inhibitor with anti-proliferative and cytostatic action; its structure comprises a hydroxyl-ethyl-substituted piperazine representing a suitable position for *N*-[^{18}F]fluoroethylation. To obtain an [^{18}F]fluoroethylated dasatinib as radiolabeled probe Veach et al. compared *N*-[^{18}F]fluoroethylation with [^{18}F]FEBr and with [^{18}F]FETs in parallel.[83] For [^{18}F]FEBr, 2-bromo-ethyl-triflate precursor was reacted with [^{18}F]fluoride and the labeling agent purified by distillation. Subsequent *N*-alkylation of the piperazine precursor in presence of NaI and Cs_2CO_3 resulted in 10% overall RCY of ^{18}F-labeled Dasatinib. (Figure 45) In contrast, the [^{18}F]fluoroethylation with [^{18}F]FETs was performed as an one-pot method and the piperazine in DMSO was added to the labeling agent. By using this approach the overall RCY could be enhanced to 23%, but, surprisingly, as a drawback the specific activity dropped from 92 to 0.11-0.22 GBq/µmol (EOS) in comparison to the [^{18}F]FEBr method. Hence, in this example [^{18}F]FETs has gained advantage over [^{18}F]FEBr in terms of reactivity but not regarding the specific activity.

A further comparison of [^{18}F]FEBr and [^{18}F]FETs was performed for the radiolabeling of a 4-benzylpiperidine derivative as a model compound.[84] In this work 80% product formation were achieved by using [^{18}F]FEBr in contrast to 10% in case of [^{18}F]FETs.

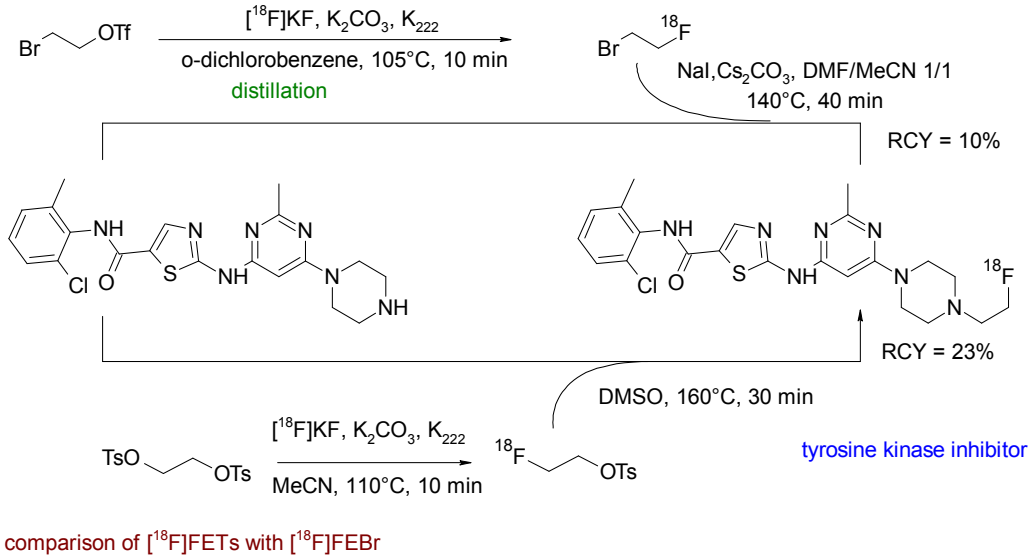

Fig 45. Radiosynthesis of N-[^{18}F]fluoroethylated dasatinib.[83]

Vandetanib is an antiangiogenic TKI also showing a methyl-piperidine moiety and served as lead for the development of *N*-[^{18}F]fluoroethylated radiotracer which was built from a piperidine-substituted quinazoline and [^{18}F]FETs.[85] (Figure 46) Briefly, the labeling agent was purified by SPE technique and subsequently reacted with the freshly prepared sodium salt of the piperidine in DMSO. The radiotracer was isolated in 20% overall RCY after HPLC purification within 75 min synthesis time with a specific activity about 37-74 GBq/µmol (EOS). Here it could be demonstrated that by using SPE-purified [^{18}F]FETs, the [^{18}F]fluoroethylation gave an adequate RCY and satisfying specific activity.

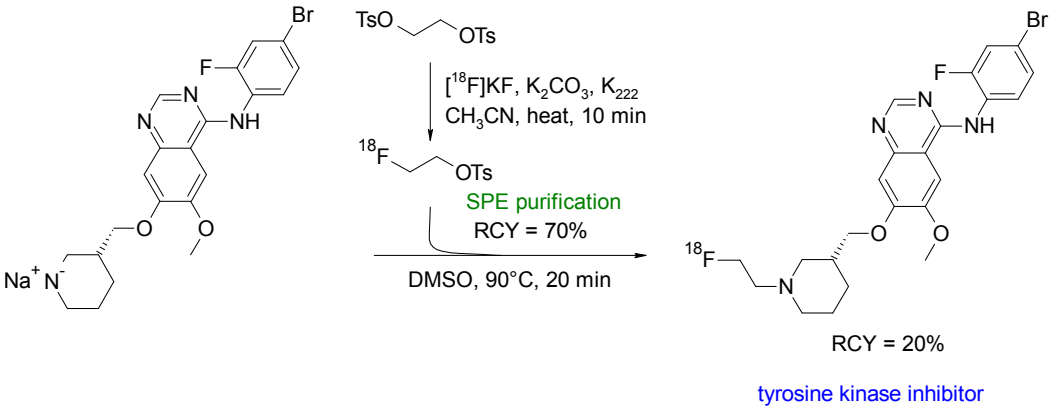

Fig 46. Radiosynthesis of *N*-[^{18}F]fluoroethylated vandetanib.[85]

Malignant melanoma affects an increasing number of patients worldwide and early diagnosis of melanoma is the patient's best opportunity to be cured. In this context, Billaud et al. developed ^{18}F-radiolabeled quinoxaline-carboxamide derivatives targeting melanin containing cells as imaging probes for PET and radioiodinated (iodine-131) derivatives for targeted radiotherapy.[86] In several examples, ^{18}F-radiofluorination was performed directly by nucleophilic substitution of the mesylate precursors with [^{18}F]fluoride. However, in case of 2-hydroxy-ethylamine the synthesis of the mesylate precursor failed so that the authors decided for a *N*-[^{18}F]fluoroalkylation with [^{18}F]FETs. (Figure 47) The [^{18}F]FETs was synthesized in one pot and used unpurified. The secondary amine precursor was added in acetonitrile without base and the mixture was heated at 110°C for 10 min. The [^{18}F]fluoroethylated quinoxaline-carboxamide was provided in 11% overall RCY after HPLC separation. This is lower in comparison with the RCY of the other derivatives obtained by direct [^{18}F]fluorination (16-54%) but [^{18}F]FETs reaction represents in that example the only access to the desired radiotracer. Notably, the amide group of the quinoxaline-carboxamide precursor was unaffected by [^{18}F]FETs due to its lower basic character.

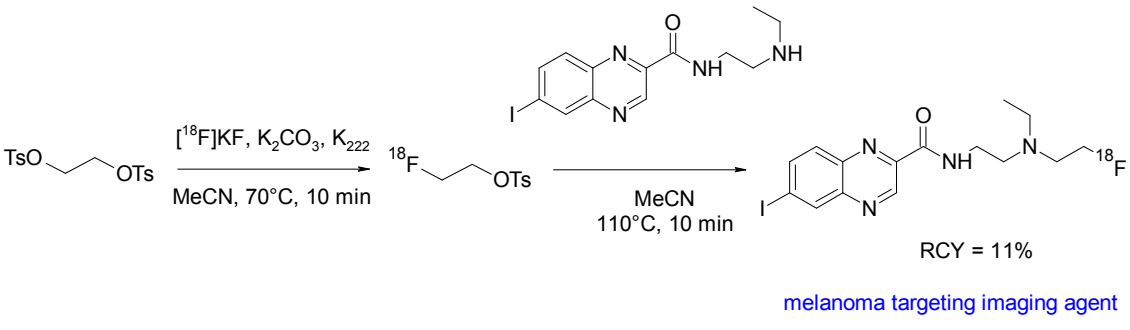

Fig 47. Radiosynthesis of a N-[^{18}F]fluoroethylated quinoxaline-carboxamide.[86]

An interesting contribution for *N*-[^{18}F]fluoroalkylation with [^{18}F]FETs was made by Kopka et al. who developed radiolabeled matrix metalloproteinase inhibitors (MMPIs) as molecular probes for imaging of up-regulated levels of MMPs in the living organism.[87] The chemical structure of the pharmacophore is based on phenyl-piperazine-substituted pyrimidine-2,4,6-triones and [^{18}F]fluoroethylation at the piperazine was the preferred route to obtain the radiotracer. (Figure 48) [^{18}F]FETs was purified by SPE-based method and subsequently reacted with pyrimidine-2,4,6-trione precursor at 120°C for 15 min. After HPLC purification the [^{18}F]fluoroethylated MMPI was obtained in 11% overall RCY in 142 min synthesis time. Remarkably, the pyrimidine-2,4,6-trione precursor showing both amine and amide functions, again the [^{18}F]fluoroalkylation occurred at the amine.

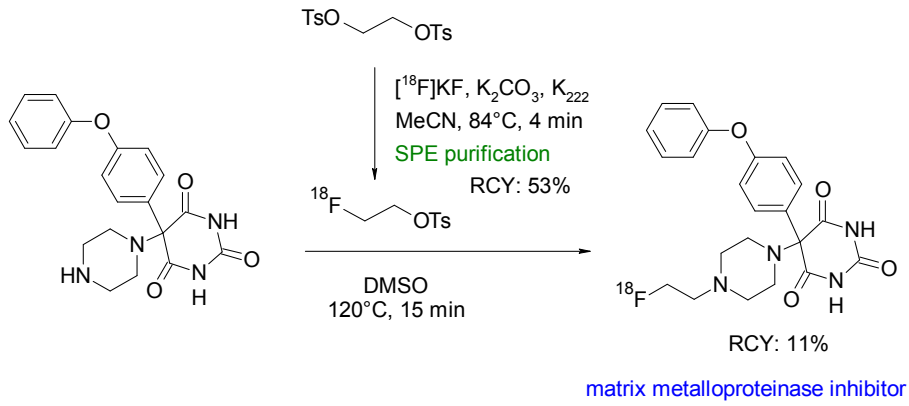

Fig 48. Radiosynthesis of a N-[^{18}F]fluoroethylated pyrimidine-2,4,6-trione.[87]

The appearance of phosphatidylserine (PS) an anionic phospholipide in the plasma membrane may be used as target for imaging of metastatic infectious disease and apoptosis. Li et al. developed an ^{18}F-radiolabeled dipicolylamine derivative showing high affinity to PS as promising radiotracer.[88] In order to find the ideal prosthetic group for the amine functionality, the authors used three ^{18}F-bearing labeling synthons in parallel: 4-nitrophenyl 2-[^{18}F]fluoropropionate ([^{18}F]NFP), *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and [^{18}F]FETs. (Figure 49) Each [^{18}F]fluorine-bearing labeling reagent was produced in a previous reaction and purified prior usage. [^{18}F]fluoroethylation was performed by reacting a solution of [^{18}F]FETs in DMSO with the amine precursor and diisopropylethylamine for 10 min at 100°C. The ^{18}F-fluoroethylated dipicolylamine was isolated after HPLC purification in 76% overall RCY within 65 min. By using the other prosthetic groups [^{18}F]NFP and [^{18}F]SFB, overall RCYs of 68-71% were achieved and the total synthesis time was 105 and 75 min, respectively. Comparing all three labeling agents in terms of labeling efficiency there was no clear benefit for one of them.

However [^{18}F]FETs turned out to be the one that could be prepared easiest, affording a one-step reaction only.

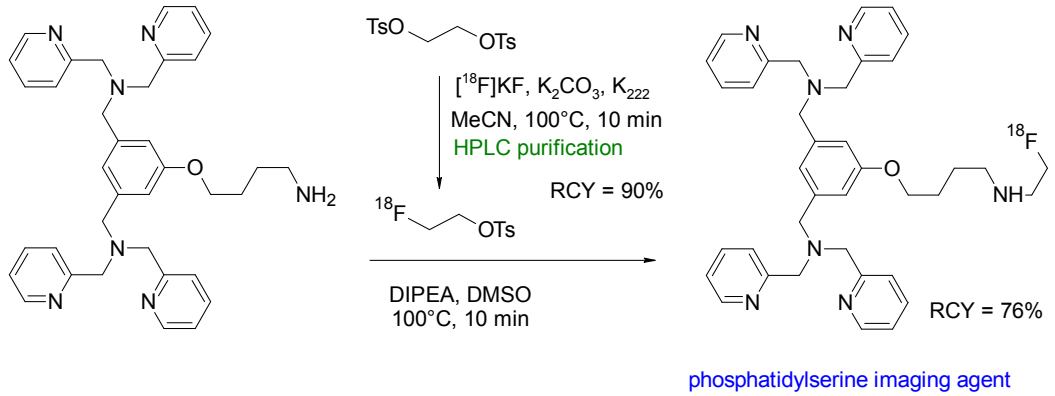

Fig 49. Radiosynthesis of a *N*-[^{18}F]fluoroethylated dipicolylamine.[88]

N-[^{18}F]fluoroethyl amides

The only example for N-[^{18}F]fluoroethylation on an amide functionality was reported by Rösch et al. who developed two radiotracers basing on pirenzepine, a well-established muscarinergic M_1 antagonist that is clinically used in gastric ulcer therapy and as selective proton-pump inhibitor.[89] [^{18}F]FETs prepared by an automated synthesis with integrated HPLC was dissolved in DMSO and added to one of the selected pirenzepine precursors and a base. (Figure 50) [^{18}F]fluoroethylation was performed for 15-20 min at 120°C and the corresponding radiotracers were provided after HPLC purification in 15% and 30% overall RCY, respectively. It is worth to remark that in the case of the diazepine-6-one precursor, that is showing both a amine and an amide function the amide is preferred, that means the *N*-[^{18}F]fluoroethylation occurs exclusively on that position.

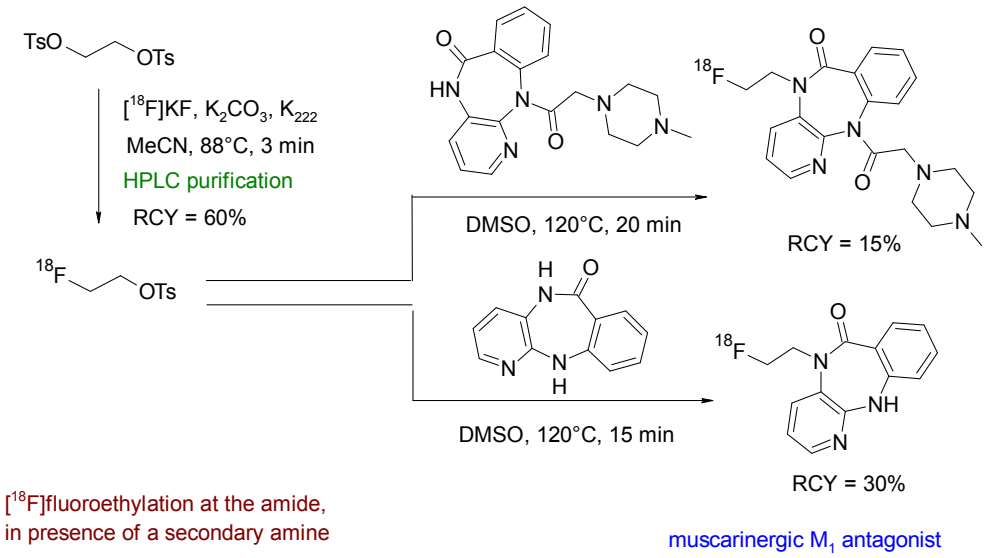

Fig 50. Radiosynthesis of two *N*-[^{18}F]fluoroethylated pirenzepine derivatives.[89]

Direct versus indirect radiolabeling

A leading point in radiotracer design is the radiolabeling approach, which means finding the optimal step in the synthesis route for introducing the radiolabel. The most preferred method, by doing the radiolabeling in the last step of the synthesis, is described as so-called direct radiolabeling. In opposite the application of a prosthetic group or a pre-labeled reagent such as [^{18}F]FETs is called as indirect radiolabeling and often it is difficult to decide what is the method of choice. In this review and with reference on [^{18}F]fluoroethylation with [^{18}F]FETs we found that for a number of radiotracers both strategies were pursued and we took the opportunity of opposing these two approaches.

As we have shown before the *N*-[^{18}F]fluoroethylated dopamine transporter ligand ([^{18}F]FECNT) was synthesized by N-[^{18}F]fluoroethylation of nortropane with [^{18}F]FETs in 21% overall RCY.[74] For simplifying and improving the radiosynthesis Chen et al. and Pijarowska-Kruszyna et al. developed an automated one-step procedure for [^{18}F]FECNT synthesis starting from mesylate precursor and [^{18}F]KF/K_{222} providing the radiotracer in 33±9% RCY what suggests an advantage of the direct radiolabeling. [90](Figure 51)

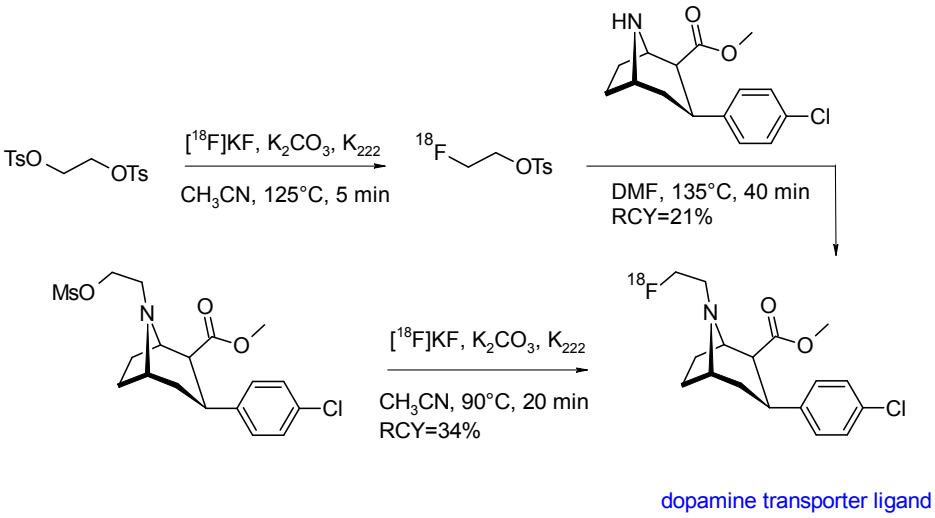

Fig 51. Radiosynthesis of [^{18}F]FECNT [74,90]

For studying the γ-aminobutyric acid (GABA) based neurotransmission radiolabeled GABA transporter ligands have been synthesized. Two radiotracers were reported, available by ^{11}C-methylation and *O*-[^{18}F]fluoroethylation of a triphenylsubstituted piperidine-3-carboxylic acid derivative.[91] The initial reaction of the carboxymethyl ester substituted desmethyl precursor with [^{18}F]FETs in presence of NaOH resulted in 80% [^{18}F]fluoroethylation. However, a subsequent ester cleavage reaction was required to obtain the final radiotracer. To circumvent this deprotection step, the [^{18}F]fluoroethylation with the free carboxylic acid moiety was performed by using a variety of bases and solvents. It turned out that in the presence of NaH in DMSO the highest RCY of about 90% were achieved, whereas in the presence of NaOH and K_2CO_3 under identical conditions only 65% and 40% RCY were obtained. (Figure 52)

In a subsequent paper the authors compared the [^{18}F]fluoroethylation with the direct [^{18}F]fluorination of the ethyl-tosylated precursor with [^{18}F]fluoride. By [^{18}F]fluoroethylation the radiotracer was formed within 5 min in 37-40% RCY whereas direct nucleophilic fluorination with [^{18}F]fluoride provided the radiotracer in only 19% RCY within 40 min.[92] In summary the direct radiolabeling approach is less efficient. Additionally it should be considered that the ethyl-tosylated precursor was difficult to synthesize with yield of only 2.6%.

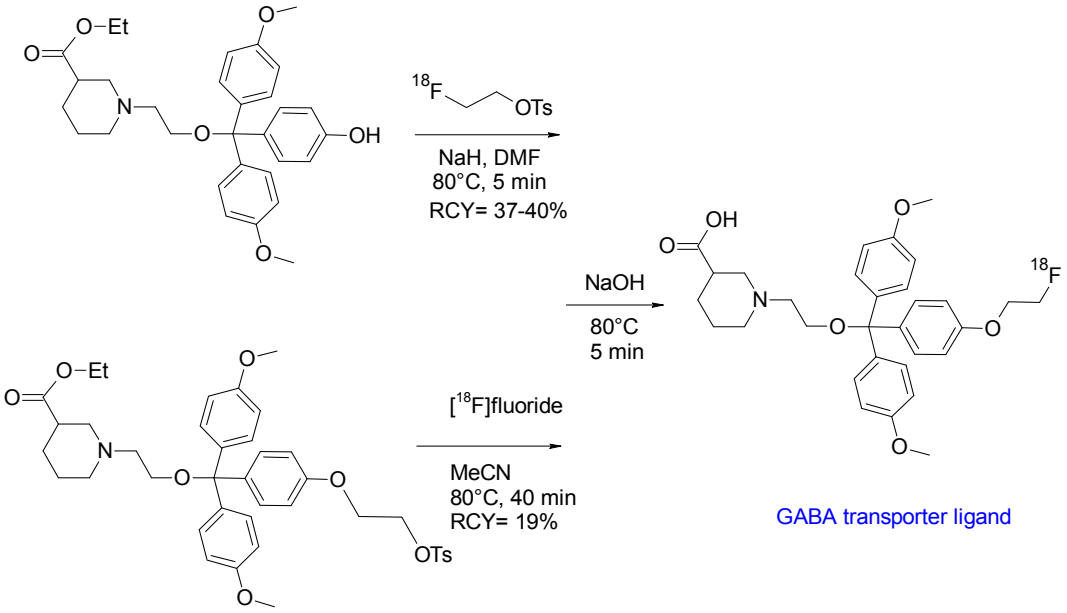

Fig 52. Synthesis of a *O*-[^{18}F]fluoroethylated triphenylsubstituted piperidine-3-carboxylic acid.[91, 92]

Radiolabeled analogs of spiperone and raclopride were the first radiopharmaceuticals targeted towards dopaminergic D_2 receptor. The synthesis of a series of *O*-*ω*-fluoroalkylated derivatives of raclopride was published describing the radiosynthesis of a [^{18}F]fluoroethylated raclopride which was realized via two different approaches.[93] Firstly by using [^{18}F]FETs and secondly, by the direct radiofluorination starting from an appropriate ethyl-tosylated raclopride precursor. (Figure 53) In the indirect one-pot process, the [^{18}F]FETs was not purified and reacted with the hydroxyl precursor by adding the latter to the reaction vessel. The overall RCY of the HPLC-purified [^{18}F]fluoroethylated raclopride was 12%. In the direct procedure, the tosylate precursor of raclopride was reacted with the [^{18}F]KF/K_2CO_3/K_{222} complex to provide the radiotracer in 35% RCY. By comparison of both radiolabeling protocols it turned out that the direct approach is superior to the [^{18}F]fluoroethylation with [^{18}F]FETs due to a shorter reaction time and a higher RCY. But it was pointed out that the HPLC purification of the [^{18}F]FE-raclopride synthesized by the one-step procedure was difficult because the hydrolyzed precursor and the final radiotracer show a nearly identical retention. Furthermore the synthetic efforts for the preparation of the tosylated raclopride precursor are challenging.

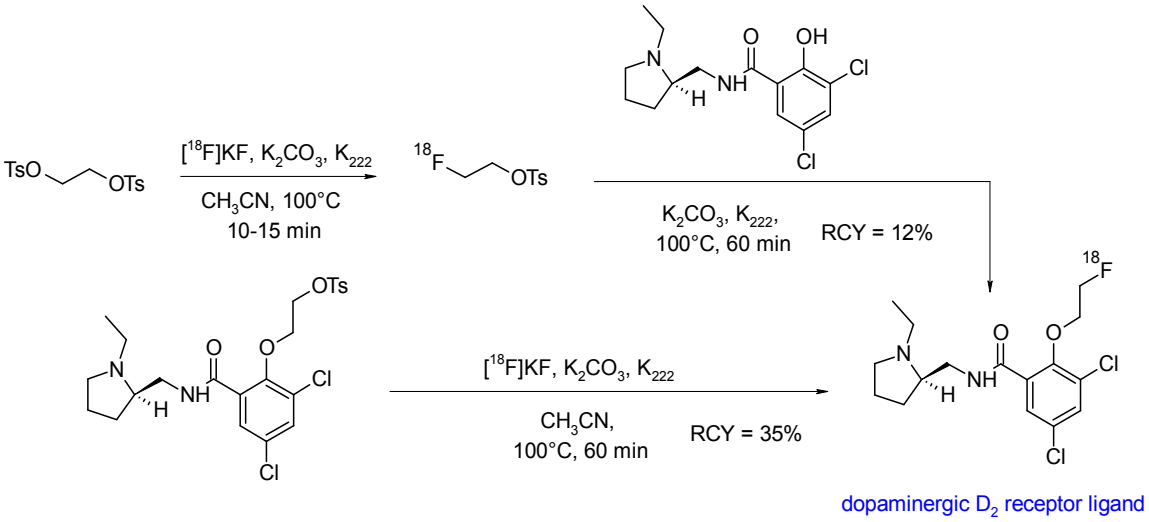

Fig 53. Synthesis of *O*-[^{18}F]fluoroethylated raclopride.[93]

The development of selective PET radiotracers for imaging of neurofibrillary tangles (NFT) is expected to reinforce insights into Alzheimer's disease. Astemizole, having the basic structure of a phenyl-piperidyl substituted benzimidazole, was found to bind to NFT with low nanomolar affinity. For development of a [^{18}F]fluoroethylated astemizole the direct radiofluorination with [^{18}F]fluoride and the indirect *O*-[^{18}F]fluoroethylation with [^{18}F]FETs were investigated.[94] (Figure 54) The direct nucleophilic substitution of the tosylated precursor in acetonitrile at 100°C provided the radiotracer in 29% RCY. The indirect radiolabeling of [^{18}F]fluoroethyl astemizole was performed as an one-pot two-step procedure using an automated synthesizer. [^{18}F]FETs was reacted with the hydroxyl precursor and aqueous KOH in DMF by heating for 20 min at 120°C. Following this route [^{18}F]fluoroethyl-astemizole was isolated in 5-10% overall RCY, accordingly for this radiotracer the direct radiofluorination was found to be superior.

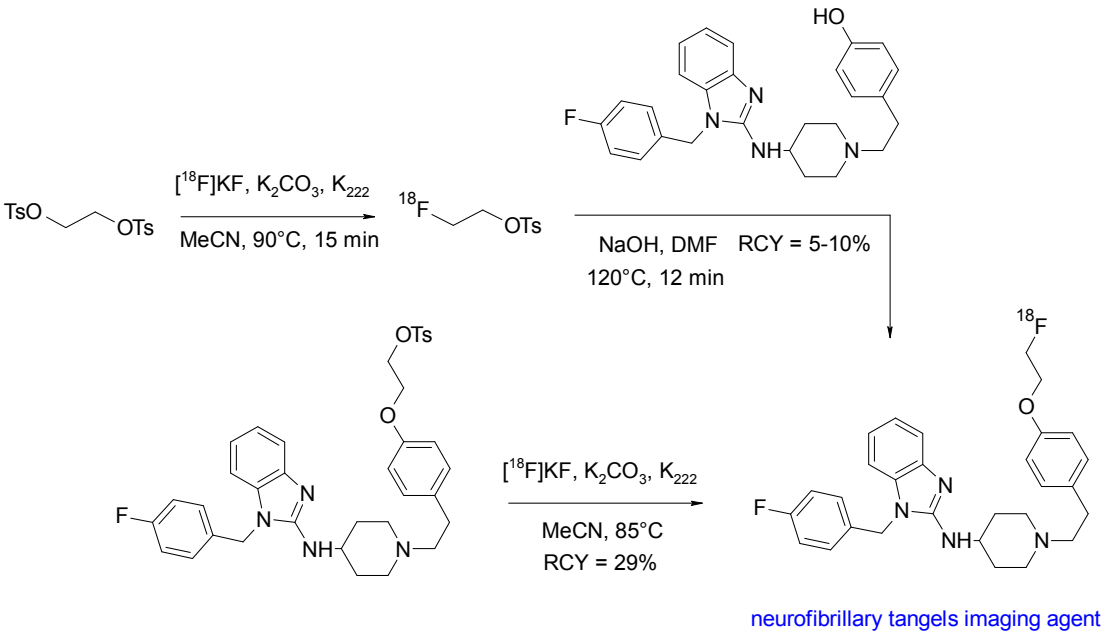

Fig 54. Radiosynthesis of *O*-[^{18}F]fluoroethylated astemizole.[94]

A further contribution in regard to direct and indirect [^{18}F]radiofluorination was made by Skaddan et al. who had developed a PET ligand for imaging of fatty acid amide hydrolase (FAAH) in the brain.[95] The target molecule PF-9811 contains a 2-fluoroethoxy-pyridine moiety and the initially chosen route was the nucleophilic substitution of a tosylated precursor with [^{18}F]fluoride. (Figure 55) However all efforts to produce [^{18}F]PF-9811 by this route from the chosen precursor proved to be unsuccessful, so the authors decided to perform [^{18}F]fluoroethylation by a two-step one-pot process. The desired radiotracer was formed but could not be separated from other chemical impurities by HPLC. To solve this problem, [^{18}F]FETs was purified beforehand by HPLC and reacted with the hydroxyl precursor and K_2CO_3 in DMA but the chemical purity of the radiotracer was not significantly improved. The finally successful route was a three-step one-pot reaction starting with direct [^{18}F]fluorination of a BOC-protected tosylated amine, followed by deprotection and subsequent coupling with phenyl pyrazin-3-yl-carbamate providing [^{18}F]PF-9811 in 11% overall RCY.

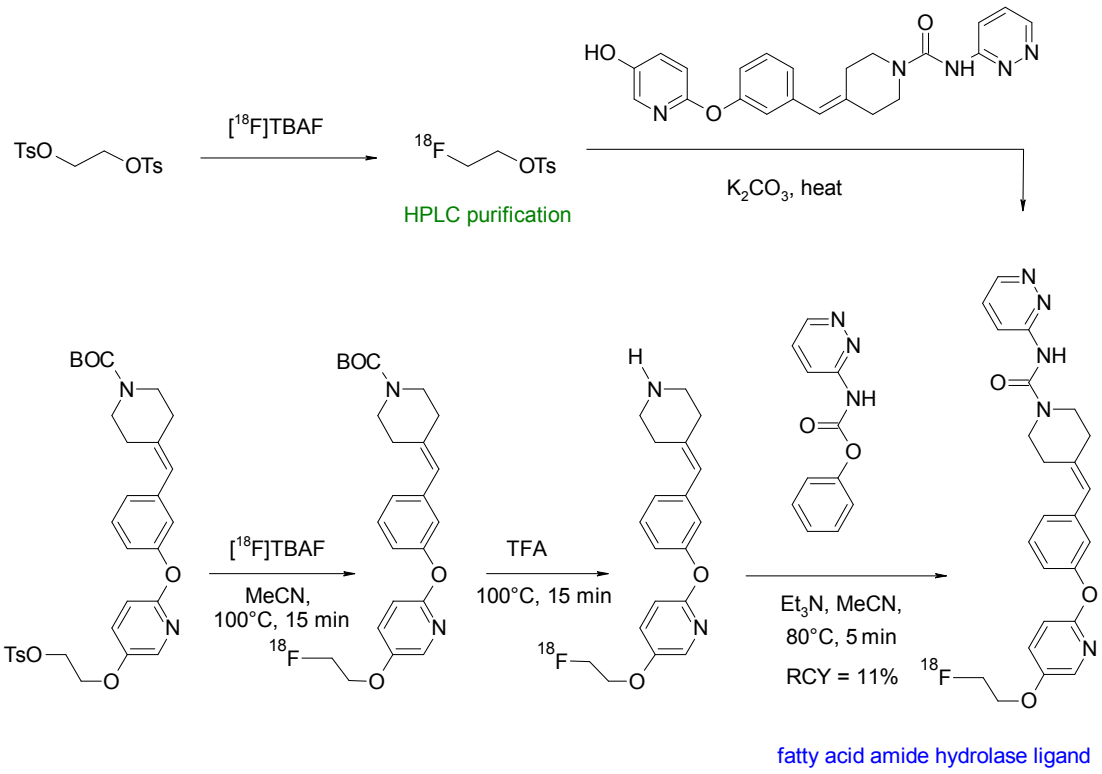

Fig 55. Synthesis of a *O*-[^{18}F]fluoroethylated pyridine.[95]

A ^{18}F-labeled phosphodiesterase 10A (PDE10A) ligand based on 6,7-dimethoxy-4-pyrrolidinylquinazoline was developed by Funke et al.[96] Also in this work the direct and indirect radiolabeling were applied. For *O*-[^{18}F]fluoroethylation with [^{18}F]FETs the labeling reagent was added to the phenolate containing solution, after activating the hydroxyl precursor with K_2CO_3/K_{222} at 60°C. (Figure 56) Interestingly, the solvent showed a significant effect on [^{18}F]fluoroethylation. By using DMF at 110°C only 5% RCY of [^{18}F]FE-PDE10A were observed but changing the solvent to acetonitrile labeling efficiency was improved up to 30-40%. The purification of the final product was difficult due to lipophilic non-radioactive by-products and according to this the authors decided for the development of a direct radiofluorination route of the corresponding tosylated precursor. By using this approach, [^{18}F]FE-PDE10A was formed with a lower part of by-products and could be isolated in 17-40% RCY in high chemical and radiochemical purity.

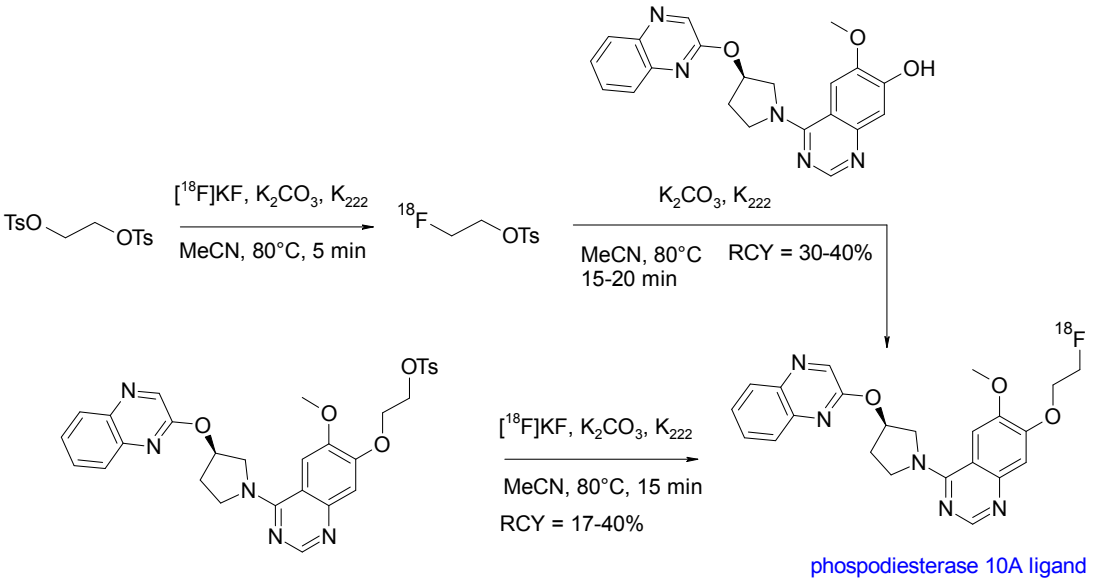

Fig 56. Synthesis of a *O*-[^{18}F]fluoroethylated pyrrolidinylquinazoline.[96]

[^{18}F]NS14490, was synthesized and evaluated recently by Rötering et al as potential radiotracer for neuroimaging of α-7 nicotinic acetylcholine receptors.[97] NS14490 containing a 1,4-diazabicyclo[3.2.2]nonane, an oxadiazole and an indole moiety; the radiolabeling was performed by *N*-[^{18}F]fluoroethylation with [^{18}F]FETs at the indole nitrogen. (Figure 57) By using non purified [^{18}F]FETs as labeling reagent and reacting it with the precursor in acetonitrile at 80°C in a one-pot reaction [^{18}F]NS14490 was formed in 7% overall RCY. In contrast the direct nucleophilic substitution of a tosylated precursor of NS144490 with [^{18}F]fluoride under equal conditions gave the [^{18}F]NS14490 within 2 hours in 36% isolated RCY. The authors did not explore the low yield of *N*-[^{18}F]fluoroethylation, but one reason may be the need for strong deprotonation of the indole hampering sufficient alkylation with [^{18}F]FETs.

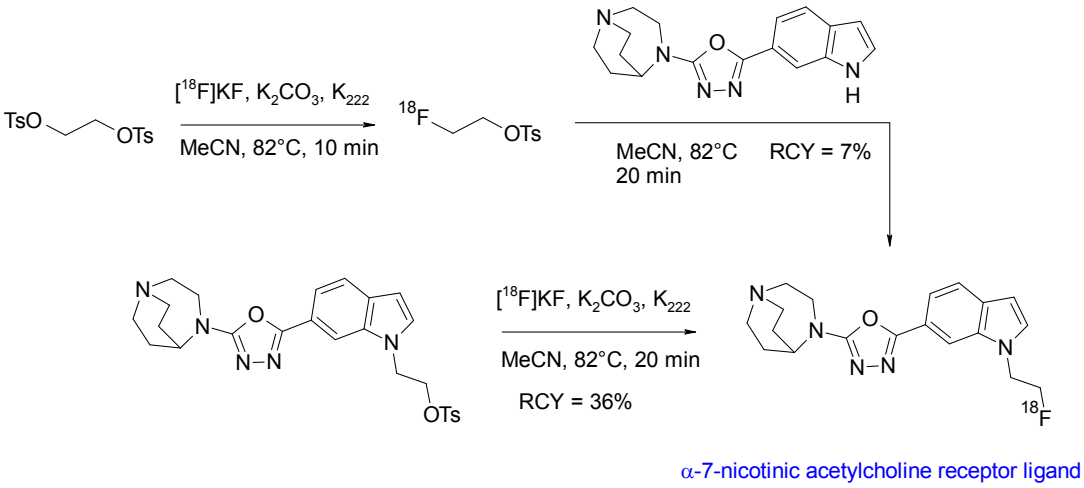

Fig 57. Synthesis of a *N*-[^{18}F]fluoroethylated indole.[97]

Another example of direct and indirect radiofluorination of an indole was described by O'Shea et al. with the synthesis of a radioligand for the translocator protein (TSPO), that is an established target for the imaging of neuroinflammation with PET.[98] For the indirect pathway [^{18}F]FETs was reacted with the indole in DMF in presence of sodium hydride for 10 min at 100°C. (Figure 58) After workup and semi-preparative HPLC purification, the [^{18}F]fluoroethylated TSPO ligand was isolated in 4% overall RCY. In contrast, when the radiosynthesis was performed via direct nucleophilic substitution of a corresponding mesylate precursor for 10 min at 100°C the RCY of the final radiotracer was 18%.

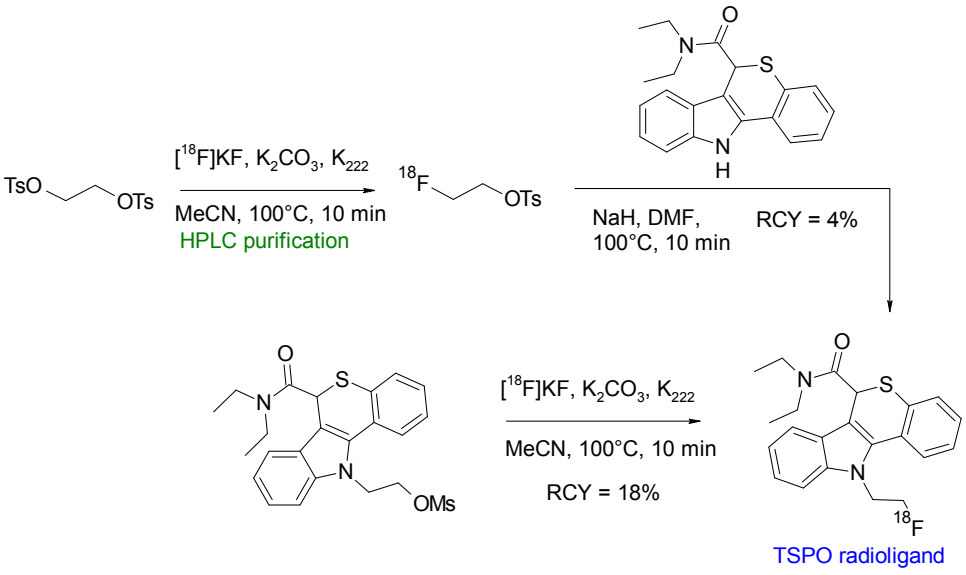

Fig 58. Synthesis of a *N*-[^{18}F]fluoroethylated indole.[98]

For development of radiolabeled tolbutamide to visualize and quantify β-cell concentrations in the pancreas the methyl group of tolbutamide was replaced by a hydroxyl function and *O*-[^{18}F]fluoroethylation and direct [^{18}F]fluorination was applied.[99] (Figure 59) HPLC purified [^{18}F]FETs was used to react with the phenol precursor which was deprotonated beforehand with NaOH in DMF at 80°C. [^{18}F]Fluoroethylation was performed for 10 min at 120°C and the [^{18}F]FE-tolbutamide was isolated after HPLC purification in 45% RCY, what represents an excellent overall yield for this indirect labeling procedure. Notably, the direct nucleophilic substitution by reacting a nitro-precursor of tolbutylamide with [^{18}F]fluoride to build [^{18}F]fluoro-tolbutamide gave only a very low yield with a mixture of by-products.[55] This is caused by the acidic character of the sulfonamide proton due to the unprotected precursor, hampering the nucleophilic substitution with [^{18}F]fluoride.

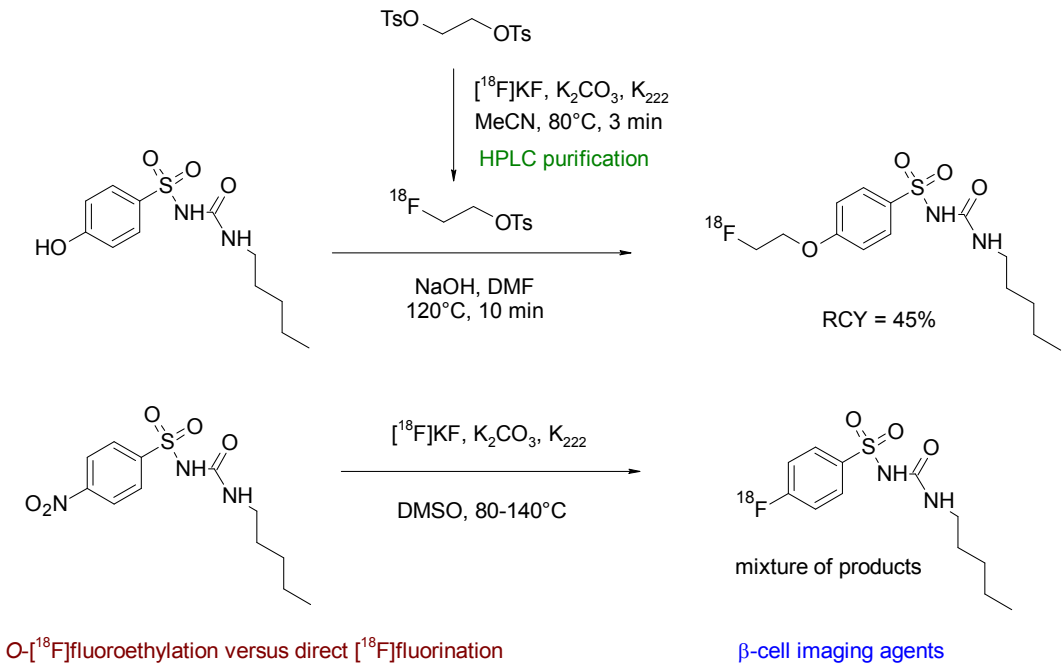

Fig 59. Radiosynthesis of *O*-[^{18}F]fluoroethylated and [^{18}F]labeled tolbutamide.[55, 99]

ICI 89,406 has been reported to be a β1-selective adenoreceptor antagonist and was chosen for the development of new radiotracers. Firstly, iodine-123 and iodine-125 radiolabeled derivatives of ICI 89,406 were developed followed by a carbon-11 labeled radiotracer. But due to the supposed *in vivo* de-iodination and the superior physical decay characteristics of ^{18}F compared to ^{11}C, Kopka et al. developed a [^{18}F]fluoroethylated ICI 89,406 derivative.[100] Also here two synthetic routes for ^{18}F-radiolabeling, the direct and the indirect via [^{18}F]FETs were explored. (Figure 60) The first one started with the corresponding tosylate precursor of ICI89,406 and [^{18}F]fluoride, but by this route the

radiotracer was obtained in poor RCY (3%) and inadequate specific activity caused by incomplete HPLC purification of the product. The indirect labeling approach started with the synthesis and purification of [^{18}F]FETs followed by [^{18}F]fluoroethylation which was performed by reaction of the [^{18}F]FETs with the phenol precursor and NaOH in DMF and resulted in 38% product formation with a specific activity of 40 GBq/µmol (EOS). Finally, an overall RCY of 16% for [^{18}F]FE-ICI89,406 was provided. These results have been reproduced later on by Radeke et al. who had performed [^{18}F]fluoroethylation and [^{18}F]fluoropropylation at 3-and 4-hydroxyl-precursors of ICI 89,406.[101] In that publication the average overall RCY for [^{18}F]fluoroalkylation using the indirect method was 30%. Also in this example the direct labeling process using the corresponding tosylate precursors demonstrated a rather poor chemical efficiency (< 2% radiochemical yield). For this compound class the superiority of [^{18}F]fluoroethylation with [^{18}F]FETs towards direct [^{18}F]fluorination was demonstrated.

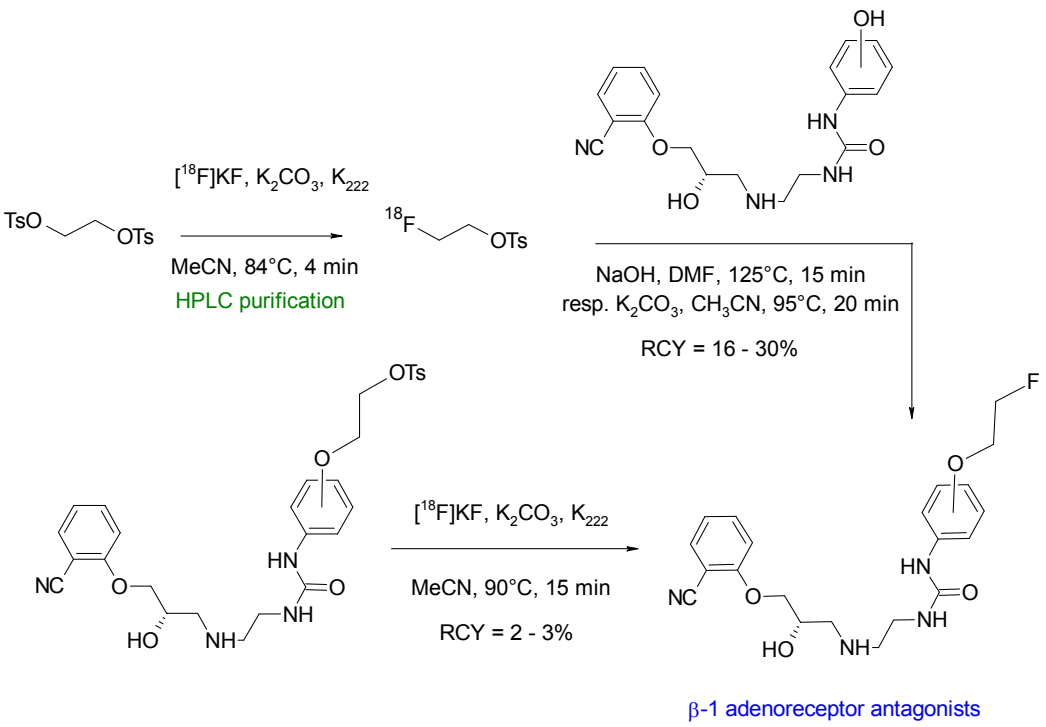

Fig 60. Radiosynthesis of *O*-[^{18}F]fluorethylated ICI 89,406 derivative.[100, 101]

Metomidate is a drug interacting selectively with the mitochondrial cytochrome P-450 species in the adrenal cortex and has been radiolabeled with carbon-11 for adrenocortical imaging. Also the ^{18}F-labeled tracer analog 2-[^{18}F]fluoroethyl-(R)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate ([^{18}F]FETO) has entered clinical studies. Accordingly, Långström et al. developed further ^{18}F-labeled derivatives of

metomidate, some of them via [^{18}F]fluoroethylation with [^{18}F]FETs.[102] The ^{18}F-radiolabeling was performed via two routes, the direct nucleophilic substitution with [^{18}F]fluoride and the indirect approach via [^{18}F]FETs. Here we find another example for *O*-[^{18}F]fluoroethylester formation starting from the carboxylic acid precursor. [^{18}F]FETs was reacted without isolation with the appropriate ammonium salt precursor of the carboxylic acid in DMF at 150°C for 15min. (Figure 61) The corresponding 2-[^{18}F]fluoroethylesters of metomidate were provided in 18-29% overall RCY. In comparison, if the same tracer was produced by the direct ^{18}F-radiofluorination a slightly higher RCY was obtained (27-37%). However, this benefit was diminished by the fact that the synthesis of the tosylated precursors afforded two additional steps with extremely low yields (2-3%) in some cases. In the end it can be noted that [^{18}F]fluoroethylester formation with [^{18}F]FETs is a further effective approach for building [^{18}F]FETO derived radio tracers.

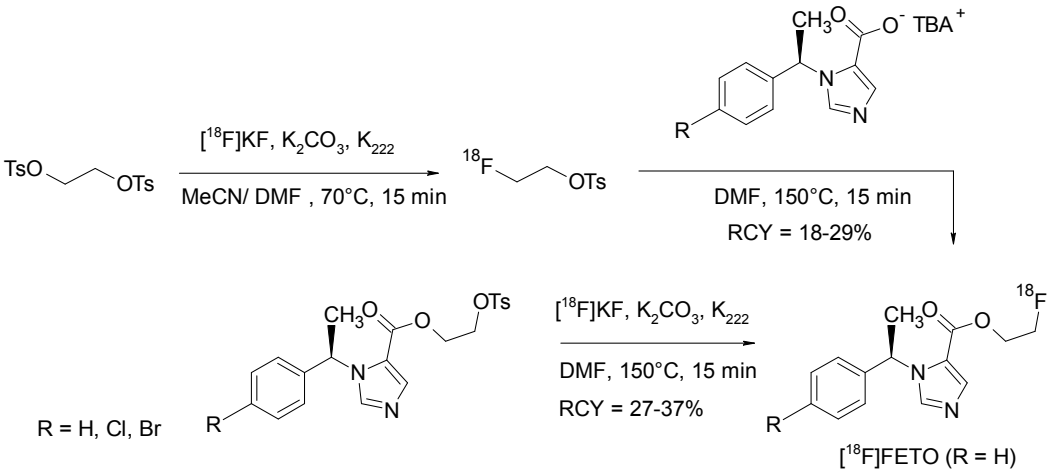

Fig 61. Radiosynthesis of O-[^{18}F]fluoroethylated metomidate esters.[102]

N-[^{11}C]methylvorozole, a substituted benzotriazole was found to be an interesting highly affine aromatase binding radiotracer and, consistently, a corresponding [^{18}F]fluoroethylated derivative was designed.[103] Also for this radiotracer the ^{18}F-radiolabeling was performed via a direct and an indirect approach. (Figure 62) For the latter [^{18}F]FETs and the by an additional ethoxy-group enlarged derivative 2-(2-[^{18}F]fluoroethoxy)ethyl-tosylate ([^{18}F]FEETs) were used, both prepared from the appropriate bis-tosylated precursors by reaction with [^{18}F]fluoride. Subsequently, the benzotriazole precursor in DMF was deprotonated with 1M KOH, added to the previously formed [^{18}F]FETs or [^{18}F]FEETs, and heated at 150°C for 15 min. The overall RCY for the [^{18}F]fluoroalkylated vorozole derivatives was determined to be 11% for the reaction with [^{18}F]FETs and 15% for [^{18}F]FEETs. In contrast provided the radiosynthesis via the direct labeling of the corresponding tosylated or

brominated precursors a significantly higher RCY of 99% and 36%, respectively, but afforded two additional steps for the precursor synthesis with yields between 22-31%.

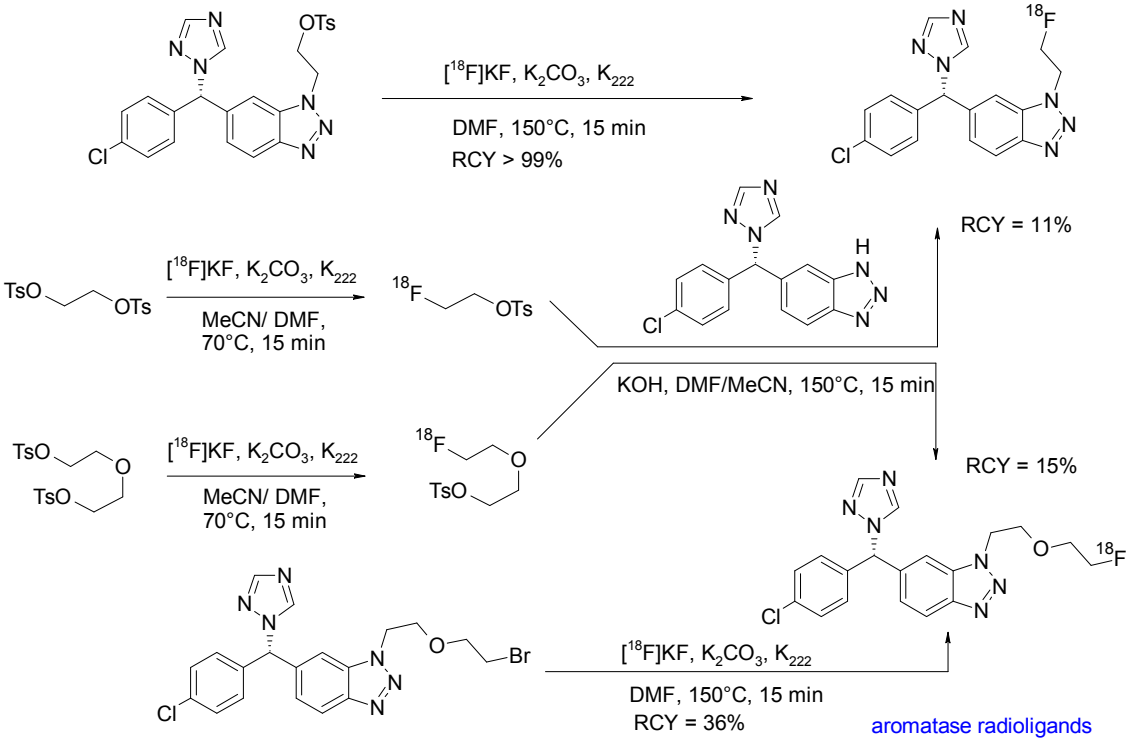

Fig 62. Radiosynthesis of *O*-[^{18}F]fluoroalkylated vorozoles.[103]

The group of Ametamey evaluated the role of L-type amino acid transporters in tumor imaging with the tryptophan isomers L-5-[^{18}F]FEHTP and DL-5-[^{18}F]FEHTP.[104] Both isomers were obtained by [^{18}F]fluoroethylation of the corresponding tryptophan precursors with SPE-purified [^{18}F]FETs in 15% overall RCY. Further evaluation of tryptophan-based radiotracers was carried out recently by a series of ^{18}F-radiolabeled DL-tryptophan derivatives carrying the [^{18}F]fluoroethylation either at position 4-, 6-, or 7- of the indole core.[105] (Figure 63) [^{18}F]FETs was used as [^{18}F]fluoroethylating agent, however only with 7-hydroxy-tryptophan a pure radiotracer was formed. The reaction of 4-hydroxy- and also 6-hydroxy-tryptophan with [^{18}F]FETs resulted in the formation of a more polar radiolabeled by-product, most probably attributed to ^{18}F-fluoroalkylation at the N-1 position of the indole. To circumvent this problem the authors performed the direct nucleophilic substitution of the appropriate BOC-protected mesylate precursors of 4- and 6- hydroxyethyl-substituted tryptophan with [^{18}F]fluoride and obtained 4-[^{18}F]FEHTP and 6-[^{18}F]FEHTP in 13-18 % RCY.

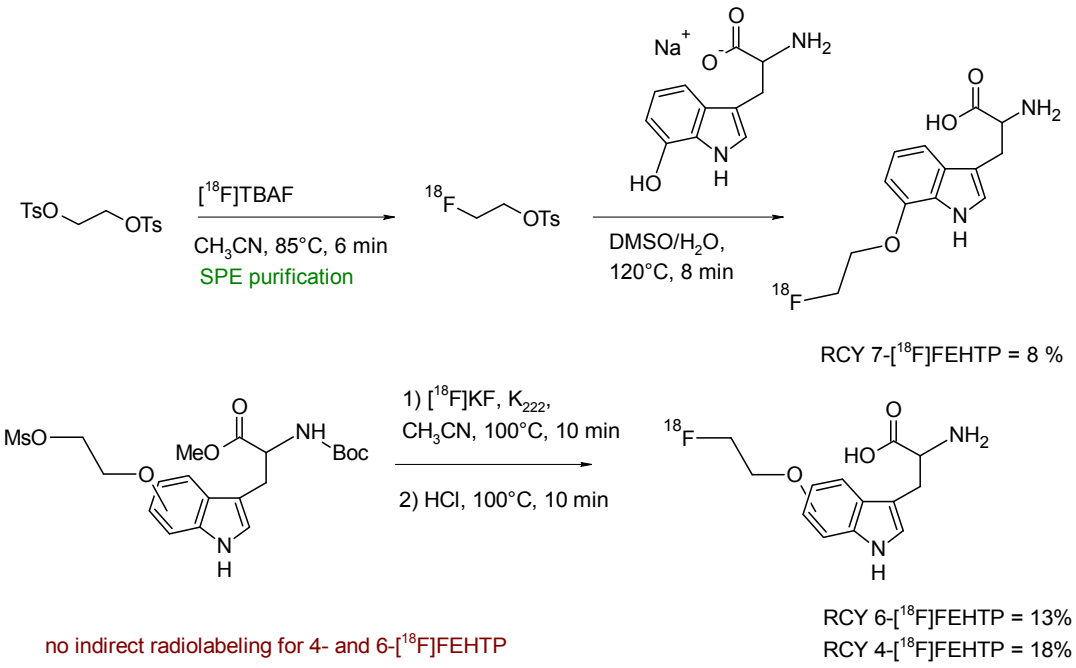

Fig. 63. Radiosynthesis of 4-, 6- and 7-*O*-[^{18}F]fluoroethylated tryptophanes [105]

Sulfur- and phosphoric acid-[^{18}F]fluoroethylation and reactions with [^{18}F]FETs in aqueous medium

In contrast to the often used labeling reaction on the oxygen and nitrogen atom [^{18}F]fluoroethylation is rarely applied to sulfur or phosphorous, probably due to the fact that compounds having free thiol and phosphane groups may represent less suitable precursors for radiolabeling. Nevertheless there exists a number of this kind of [^{18}]fluoroethylations as will be demonstrated on the following examples.

S-[^{18}F]fluoroethyl thioethers

Tang et al. introduced *S*-(2-[^{18}F]fluoroethyl)-L-methionine ([^{18}F]FEMET), a new amino acid analogue of L-[^{11}C]methionine, to overcome the limitations of the short half-life of the carbon-11 labeled radiotracer.[106] [^{18}F]FETs was produced on a functionalized polystyrene SAX resin by direct nucleophilic substitution of [^{18}F]fluoride with 1,2-ethylene glycol-bis-tosylate followed by the reaction of [^{18}F]FETs with L-homocysteine thiolactone hydrochloride in presence of sodium hydroxide and DMSO. (Figure 64) By using a fully automated synthesizing device the new [^{18}F]fluoroethylated amino acid [^{18}F]FEMET was provided in 10% overall RCY.

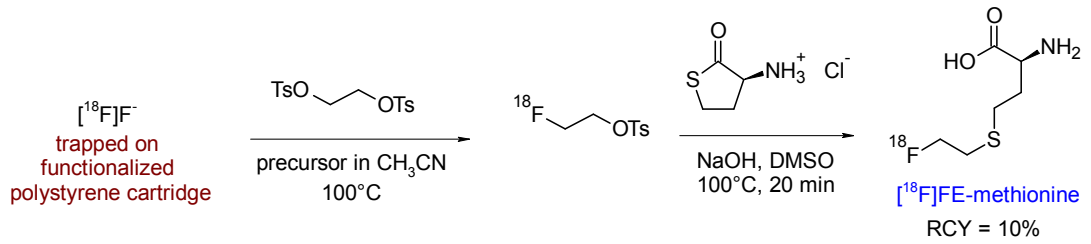

Fig 64. Radiosynthesis of *S*-(2-[^{18}F]fluoroethyl)-L-methionine.[106]

Muscarinic receptors are interesting targets for PET because the receptor density is diminished in patients with Alzheimer's disease. In that context radiolabeled selective muscarinic receptor ligands like [^{18}F]fluoropropyl- and [^{18}F]fluoroethyl-substituted 1,2,5-thiadiazol-4-yl-derivatives such as [^{18}F]FE-TZTP have been synthesized by Kiesewetter et al. by [^{18}F]fluoroethylation of the free thiol group of a tetrahydro-1-methyl-piperidine-thiadiazol.[107] (Figure 65) The radiosynthesis was performed as follows: after preparation of [^{18}F]FETs it was transferred without purification to the thiol precursor in DMF, which was deprotonated with 8 M KOH. After heating at 100°C for 5 min and semi-preparative HPLC purification, [^{18}F]FE-TZTP was provided within 80 min synthesis time in 22% overall RCY.

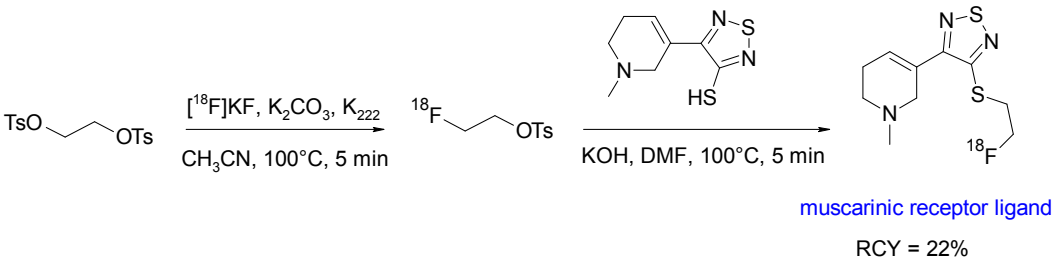

Fig 65. Synthesis of *S*-[^{18}F]fluoroethylated 1,2,5-thiadiazol-4-yl-derivative.[107]

Furthermore diaryl-methyl-guanidine derivatives were found to have a high affinity and selectivity to the NMDA receptor and were suitable to be radiolabeled by [^{18}F]fluoroalkylation by Robins et al.[108] [^{18}F]FETs was used without purification to react with the thiol-substituted guanidine precursor in presence of Cs_2CO_3 in acetonitrile at 110°C. (Figure 66) The low overall yield of the *S*-[^{18}F]-fluoroethylated diaryl-guanidine of only 4-9% RCY obtained after HPLC purification was primarily caused by the low yield of the intermediate [^{18}F]FETs that was obtained according to the authors in no more than 10-17% RCY. Surprisingly, the overall procedure took the authors 3-4 hours for reaction and purification of the radiotracer.

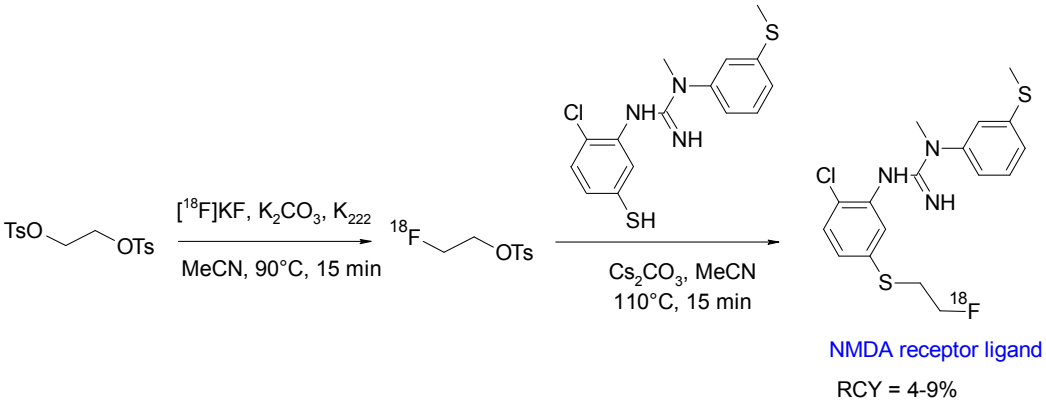

Fig 66. *S*-[^{18}F]Fluoroethylation of a diaryl-methylguanidine.[108]

[^{18}F]fluoroethylated phosphorous compounds

Antisense oligonucleotides (ODN) are molecules having typically 10-25 nucleotides and show potential therapeutic and diagnostic interest because hybridization of antisense ODN with target mRNA can interrupt the translation process and inhibit the expression of a specific gene. Radiolabeled antisense ODNs would represent useful tools for imaging the status of hybridization; likewise the biodistribution and pharmacokinetics of ODNs could be studied *in vivo*. De Vries et al. performed a comparative study for the radiolabeling of adenosine 5'-*O*-thiomonophosphate as model compound using six ^{18}F-labeled alkylating agents, among them *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide, α-bromo-α'-[^{18}F]fluoro-m-xylene and [^{18}F]FETs.[109] (Figure 67)

It should be noted that this is the only example for *S*-alkylation of a thiosphosphate moiety with [^{18}F]FETs. The *S*-[^{18}F]fluoroalkylation with [^{18}F]FETs provided the radiolabeled nucleotide in 9% RCY In contrast, the non-radioactive fluoroethylation for obtaining the reference compound yielded 44% product. As explanation for the low RCY the authors investigated the by-products during the radiofluorination. They observed an unknown volatile side product and postulated the formation of [^{18}F]vinyl fluoride from [^{18}F]FETs as a result of β-elimination.

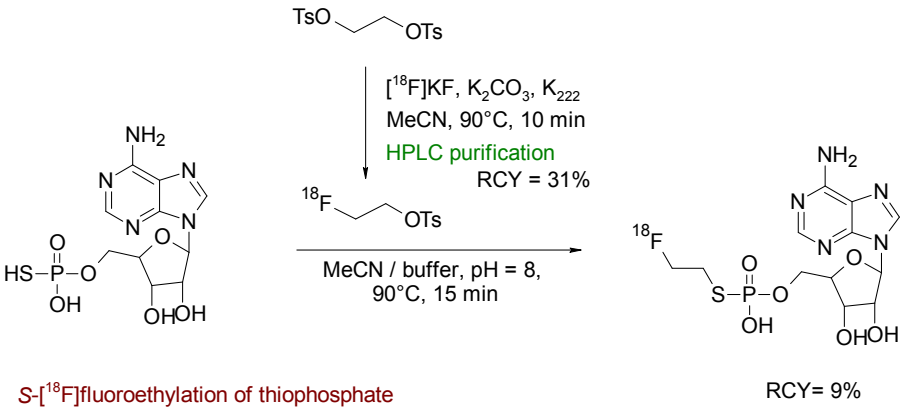

Fig 67. *S*-[^{18}F]fluoroethylation of adenosine 5'-*O*-thiomonophosphate.[109]

Special organophosphorus esters are known to be highly toxic to the nervous system; their action is generally attributed to the inhibition of acetylcholinesterase (AChE) which is responsible for the hydrolysis of the neurotransmitter acetylcholine. For obtaining radiolabeled inhibitors of AChE studies were performed to radiolabel organic phosphorous compounds with fluorine-18. For that purpose, the 4-nitrophenyl ester of methylphosphonic acid was [^{18}F]fluoroethylated by [^{18}F]FETs.[110] (Figure 68) In this solely example of [^{18}F]fluoroethylation of a phosphorus-bound hydroxyl group, [^{18}F]FETs was reacted with the phosphonus-organic precursor in acetonitrile in presence of Cs_2CO_3 and activated molsieve under microwave conditions to provide the radiotracer within 90 min in 6.5% overall RCY.

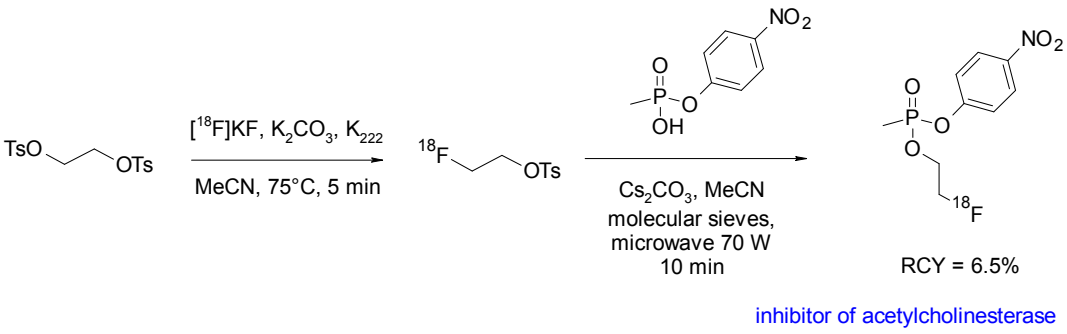

Fig 68. [^{18}F]Fluoroethylation of methylphosphonic acid-4-nitrophenylester.[110]

[^{18}F]fluoroethylation in aqueous medium

Generally all reactions with [^{18}F]FETs were performed under non-aqueous conditions to avoid hydrolysis of alkylating agents, however recently there have been two papers published demonstrating the practicality of [^{18}F]fluoroethylation in aqueous medium.

Quantum dots are semiconductor crystals consisting of a semiconductor core and an outer surface that can be easily functionalized to introduce functionalities having an affinity to specific biological targets. The application of quantum dots based imaging agents in humans is hampered by missing data about their behavior *in vivo,* therefore they have been radiolabeled with carbon-11 and fluorine-18, respectively. The [^{18}F]fluoroethylation of quantum dots was performed by Patt et al. in DMSO/borate buffer, the reaction mixture was analyzed by size exclusion chromatography to determine the radiolabeling yield.[111] Even though DMSO/buffer system is not ideal for the radiolabeling agent [^{18}F]FETs, [^{18}F]fluoroethylated quantum dots could be obtained with up to overall 5% RCY. Notably, amino-functionalized quantum dots gave a slightly better yield compared to their carboxyl-functionalized counterparts. In comparison, ^{11}C-radiolabeling of quantum dots with [^{11}C]methyl iodide delivered 35-45% RCY.[111]

For diagnosis of prostate cancer the most promising target is the prostate specific membrane antigen (PSMA) that is highly expressed in this disease. Beside ^{68}Ga-labeled peptides other small amino acid conjugates based on the glutamate-urea-lysine moiety with high affinity to PSMA have been developed and radiolabeled with technetium-99m and fluorine-18.[112] Recently El Momani et al. synthesized an alternative small ^{18}F-labeled peptide, [^{18}F]FE-Tyr-urea-Glu ([^{18}F]FETUG) as PMSA affine ligand using [^{18}F]FETs as labeling precursor.[113] (Figure 69) This is for indirect labeling of peptides an innovative approach since usually for labeling of peptides prosthetic groups like 6-[^{18}F]fluoronicotinic acid 2,3,5,6-tetrafluorphenylester ([^{18}F]FPyTFP) or N-succinimidyl 4-[^{18}F]fluorbenzoate ([^{18}F]SFB) are used. Briefly HPLC purified [^{18}F]FETs was eluted from the C18 cartridge with 80% aqueous acetonitrile and added to the peptide TUG dissolved in 15µL of aqueous base, the reaction was performed for 15 min at 80°C. The authors found out that the labeling efficiency as well the selectivity of radiolabeling was strongly depended on the choice and concentration of the supporting base. By using a peptide/$NaHCO_3$ ratio of 1/20 a radiolabeling yield of 40% was achieved, reducing the amount of base to 10 equivalents resulted in a RCY of only 7 % of [^{18}F]FETUG together with a radiolabeled by-product, that was supposed to be an ^{18}F-fluoroethylester derivative. Finally by application of 10 M NaOH in a 1/18 peptide/base ratio the RCY of [^{18}F]FETUG was increased to 77%. This efficient radiolabeling of TUG is remarkable since it stands for a successful ^{18}F-fluoroethylation with [^{18}F]FETs in aqueous milieu and is from the best of our knowledge the first example of a peptide radiolabeling with this radiolabeling agent.

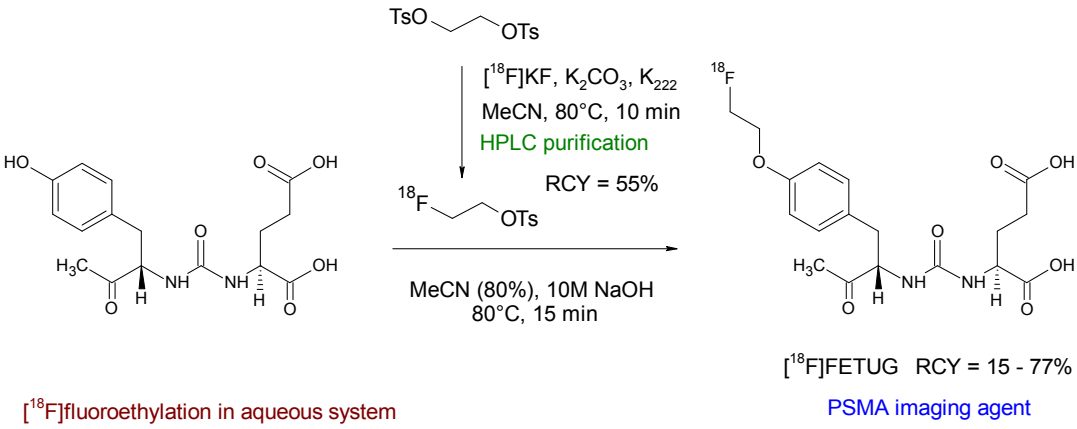

Fig 69. O-[^{18}F]Fluoroethylation of a small peptide.[113]

[^{18}F]FETs as intermediate for building blocks

[^{18}F]FETs is known as [^{18}F]fluoroalkylating reagent and is used for integration of [^{18}F]fluoride into molecules with pharmaceutical interest, however in a few cases [^{18}F]FETs was used as an intermediate to build other building blocks, for example in the formation of [^{18}F]fluoroacetaldehyde as reductive ^{18}F-alkylating agent.[114] In selected cases [^{18}F]Fluoroacetaldehyde is expected to be superior to [^{18}F]FETs because it enables labeling in aqueous solution and thus should be advantageous for the radiolabeling of peptides, proteins and antibodies. Formation of [^{18}F]fluoroacetaldehyde was achieved by oxidation of [^{18}F]FETs in DMSO in presence of K_2CO_3 according to Kornblum conditions. (Figure 70) Briefly, [^{18}F]FETs was synthesized in acetonitrile, the solvent was removed in vacuum, and DMSO was added to the intermediate. [^{18}F]Fluoroacetaldehyde was then generated by heating the mixture to 150°C and separated by distillation under a stream of nitrogen into a vial containing water. The RCY of [^{18}F]fluoroacetaldehyde from starting [^{18}F]FETs was 31-37%. For proof of principle the reaction of [^{18}F]fluoroacetaldehyde with two model compounds was investigated; the formation of the corresponding 2,4-dinitrophenylhydrazone and the reductive alkylation with benzylamine.

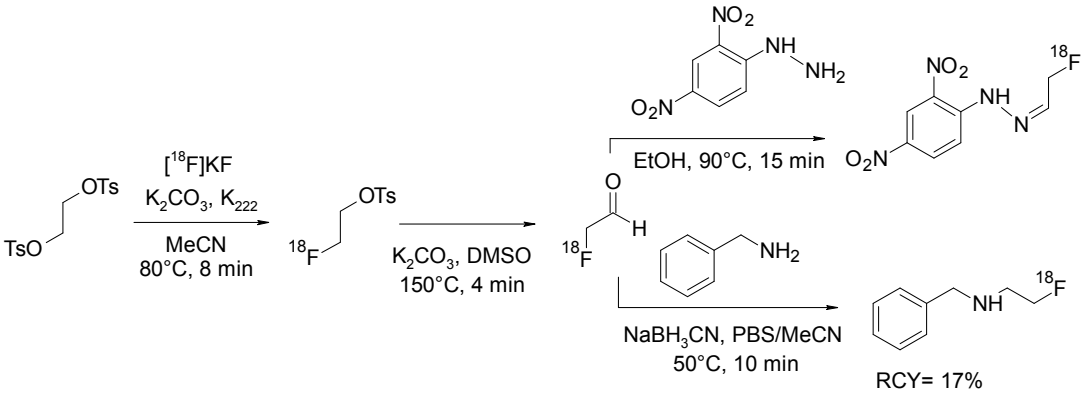

Fig 70. Radiosynthesis of [^{18}F]fluoroacetaldehyde from [^{18}F]FETs and reaction with model compounds.[114]

Summary

[^{18}F]Fluoroalkylation holds growing interest in PET chemistry according to the easy introduction of a ^{18}F-radiolabel into phenolic, thiophenolic, carbocyclic, amine and amide functions and due to the supposed minimal modification of the pharmacologic properties of the lead compound. [^{18}F]FETs is one of the mostly used [^{18}F]fluoroalkylating agents because it is simple to prepare, almost stable and provides a high reactivity together with - for some extent - a chemo-selectivity.

Since the introduction of [^{18}F]FETs in 1987[14] much work was done for optimizing the manufacturing, the purification and to improve the overall radiolabeling yields. An important step was the finding that by addition of alkali iodides the yield of [^{18}F]fluoroethylation could be significantly increased [10] as well as the fact that the reactivity of [^{18}F]FETs could be further improved by introduction of strong electron withdrawing substituents as e.g. for 2-[^{18}F]fluoroethyl-3,4-dibromo-benzenesulfonate ([^{18}F]FEBr$_2$Bs) and 2-[^{18}F]fluoroethyl-4-nitro-benzenesulfonate ([^{18}F]FENs).[19,20] The knowledge that application of HPLC-purified [^{18}F]FETs had a significant impact on labeling yield, chemical and radiochemical purity of the final radiotracer[13,44] has triggered the development of innovative automated methods for production and purification of [^{18}F]FETs and [^{18}F]fluoroethylation.[12,46,48,71]

[^{18}F]FETs has been approved as building block in the synthesis of clinical relevant radiotracers as of 2-[^{18}F]fluoroethylcholine ([^{18}F]FEC)[67-71] and O-(2-[^{18}F]fluoroethyl)tyrosine ([^{18}F]FET)[28-31] and equally in the [^{18}F]fluoroethylation of other amino acids like methionine and tryptophan.[33,73,104-106] In radiotracer building the reaction with [^{18}F]FETs is indispensible as has been demonstrated for a multitude of *O*- and *N*-[^{18}F]fluoroethylations, the radiolabeling of thiols[106,107,109] and the formation of [^{18}F]fluoroethyl-carboxylic esters[63-66,102], -amides[89] and -phosphorous esters.[109,110]

By comparison of [^{18}F]FETs with other [^{18}F]fluoroalkylating reagents as e.g. 2-[^{18}F]fluoroethyl bromide in majority [^{18}F]FEBr showed higher RCY but this benefit is diminished by the need for additional efforts for purification (distillation) of [^{18}F]FEBr.[13,83,84] Generally the application of HPLC-purified [^{18}F]FETs was found to be advantageous[44] but it was also demonstrated that by using SPE-purified [^{18}F]FETs high RCY and sufficient specific activities could be achieved.[12,25,27,74,77,85]

In turns of chemoselectivity phenolic groups are the preferred objects to react with [^{18}F]FETs; and in molecules having both an amino and phenol functionality the [^{18}F]fluoroethylation will normally occur on the phenol.[28,46,47,100] If the target compound shows several phenol groups a regioselective [^{18}F]fluoroethylation can be achieved by the character of the added base[58], otherwise a selective protective group may be used.[35] In presence of a concurrent amino and a carboxylic group [^{18}F]fluoroethylation is preferred on the amine[18]; when amide and amine groups are present there is no clear chemoselectivity observed.[86,87,89]

In a number of protocols besides to the [^{18}F]fluoroethylation with [^{18}F]FETs (indirect approach) the direct radiolabeling of a corresponding mesylated or tosylated precursor with [^{18}F]fluoride has been performed in a parallel approach. By comparison of these two synthesis pathways it is shown on a first view that in several protocols the direct [^{18}F]fluorination with [^{18}F]fluoride provided more than doubled overall yields compared to [^{18}F]fluoroethylation[93-95,97,98,103] suggesting the direct method as the superior one. However in a closer look it turned out that often the RCY of [^{18}F]fluoroethylation was diminished by using e.g. non-purified [^{18}F]FETs or the insufficient production of [^{18}F]FETs. For two examples the total RCY for the direct and indirect approach was almost comparable[96,102] whereas in a notable set of protocols indirect [^{18}F]fluoroethylation with [^{18}F]FETs gave significant higher RCY in comparison to the direct radiofluorination.[36,55,47,79, 87,91,101] The reason is that the synthesis of the corresponding tosylated/mesylated precursors has failed[79,86] or afforded extremely low yields (2-3%).[92,102] Under that circumstances [^{18}F]fluoroethylation is the solely method to come to the desired radiotracer. If the [^{18}F]fluoroethylation may be performed either by direct labeling or by introduction of the radiolabeled precursor [^{18}F]FETs (indirect approach) is dependent on the chemical behavior and reactivity of a particular precursor compound. A prognosis about the optimal reaction route may be very difficult in most cases. For preventing of protective groups and avoiding additional precursor synthesis the indirect way may be favorable.

A further benefit of [^{18}F]FETs as one of the preferred labeling agents is that it is ideal for the easy transfer of promising ^{11}C-labeled radiotracers into their ^{18}F-labeled counterparts as it was shown for several examples.[13,34,39,44,46,48,49,53,54,82,100,102] One main concern by replacing the [^{11}C]methyl by a [^{18}F]fluoroethyl labeling unit is the preservation of the physical and chemical properties of the

radiotracer, i.e. will the affinity to the designated target substantially altered. To answering this question we have extracted for a series of radiotracers the in vitro data assessed for the methyl and their corresponding fluoroethyl substituted derivatives. The results are displayed at Table 1. In most cases there is only moderate alteration in in vitro behavior by substitution of methyl by fluoroethyl, there are even examples where the receptor affinity is improved by the fluoroethyl group.

	-CH_3 substituent	**-CH_2CH_2F** substituent
$GABA_A$ ligand [102]	MTO: IC_{50} = 9 µM [115]	FETO: IC_{50} = 4.8 µM [115]
$GABA_A$ ligand [39]	K_i = 0.29 nM [116]	K_i = 0.52 nM [39]
Dopamin D_3 antagonist [34]	K_i = 0.32 nM [34]	K_i = 0.12 nM [34]
$5HT_{1A}$ SR antagonist [44]	K_i = 1.1 nM [117]	K_i = 0.1 nM [44]
Sigma ligand [48]	IC_{50} σ1 = 17.4 nM [48]	IC_{50} σ1 = 6.48 nM [48]
	IC_{50} σ2 = 1784 nM	IC_{50} σ2 = 2.11 nM
Sigma ligand [49]	IC_{50} σ1 = 33 nM [49]	IC_{50} σ1 = 3.1 nM [49]
	IC_{50} σ2 = 9.5 nM	IC_{50} σ2 = 6.8 nM
MAO inhibitor [54]	K_i = 2.5 nM [54]	K_i = 0.54 nM [54]
AA_2 receptor ligand [53]	K_i = 4.1 nM [118]	K_i = 12.4 nM [118]
β1 AR antagonist [100]	K_i = 0.067/0.288 nM [119]	K_i = 0.049/0.297 nM [100]
β-amyolid plaque imaging [46]	K_i = 4.9 nM [120]	K_i = 0.17 nM [46]
β-amyolid plaque imaging [82]	K_i = 15 nM [82]	K_i = 27 nM [82]

Table 1: In vitro properties of selected drugs comparing methyl- and 2-fluroethyl-substituent

In summary in respect of its broad application in radiolabeling molecules with pharmaceutical relevance [^{18}F]FETs is an absolutely essential building block in ^{18}F-radiochemistry. It enables a rapid radiolabeling and investigation of the potential radiotracer without increased organic synthetic effort for precursor synthesis. That [^{18}F]FETs can also be used for ^{18}F-peptide radiolabeling in aqueous medium was recently successfully demonstrated; a result which opens the door to additional applications of this compound.[113] For the future and due to the fact that a number of reliable protocols for a - preferably automated - synthesis and purification of [^{18}F]FETs nowadays are available, [^{18}F]fluoroethylation with [^{18}F]FETs will stay for a versatile tool for building ^{18}F-based radiotracers.

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