[^{18}F]Fluoroform - a versatile building block for PET tracer synthesis

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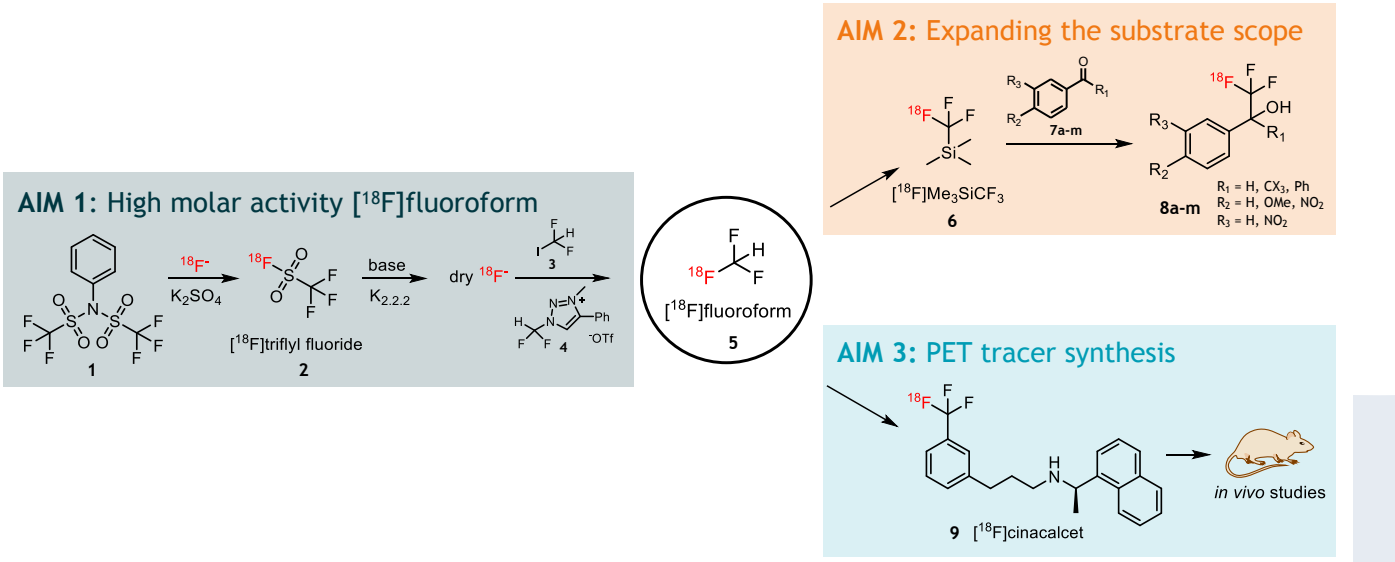
The functional imaging technique positron emission tomography (PET) is based on the use of radiolabelled compounds, so called PET tracers. They consist of a molecular structure, e.g. a ligand binding to a specific target, and a positron-emitting radionuclide that is detected by the PET scanner. One of the most used radionuclides for PET is fluorine-18, because of its convenient half-life (110 min) and good decay characteristics. To introduce fluorine-18 into the molecular structure of interest, different strategies can be pursued. Our strategy is the use of [^{18}F]fluoroform, a fluorine-18 labelled building block, to introduce radioactive CF_3 groups into the tracer molecules (^{18}F-trifluoromethylation). The CF_3 group is a common motif in drugs and therefore, ^{18}F-trifluoromethylation potentially gives access to a huge variety of new PET tracers. However, to be able to fully exploit this building block for PET tracer synthesis, two main shortcomings needed to be addressed: A) [^{18}F]fluoroform typically shows a low molar activity, and B) there is only a limited spectrum of ^{18}F-trifluoromethylation strategies available so far, meaning that the radioactive CF_3 group cannot be introduced in a wide array of molecular structures.

High molar activity [^{18}F] fluoroform

Fluorine-18 is most commonly obtained as aqueous [^{18}F]fluoride from a cyclotron and needs to be activated before its use in the radiolabelling reaction. The activation step typically entails the use of substantial amounts of base and cryptand, which remain present in the subsequent radiolabelling reaction. [^{18}F]Fluoroform precursors however are unstable in presence of base,



Scheme 1. Schematic overview of the content of this thesis.

causing the low molar activity typically observed for [^{18}F]fluoroform. We therefore developed an alternative [^{18}F]fluoride activation strategy based on [^{18}F]triflyl fluoride that enabled the use of less base and cryptand. Gaseous [^{18}F]triflyl fluoride can be formed by reacting bistriflate **1** with aqueous (non-activated) [^{18}F]fluoride and can be distilled into a dry organic solvent containing base and cryptand where it releases reactive [^{18}F]fluoride (see Scheme 1). The excellent reactivity of the obtained [^{18}F]fluoride was demonstrated in various model reactions with aromatic and aliphatic precursors (not shown). Besides being quick (5 min) and high yielding (up to 95%), the method proved to be highly flexible in terms of type and amount of base, cryptand and organic solvent.

Having developed this new strategy to obtain reactive [^{18}F]fluoride, we focussed on the optimisation of the [^{18}F]fluoroform synthesis towards high molar activity. Stability studies with the labelling precursor difluoroiodomethane **3** showed that reduction of the amount of base and cryptand led to improved precursor stability. We therefore explored the influence of various reaction parameters including base and cryptand on the [^{18}F]fluoroform synthesis and studied the radiochemical yield and molar activity while using [^{18}F]triflyl fluoride as a source of reactive [^{18}F]fluoride. The study revealed that indeed the molar activity of [^{18}F]fluoroform could be improved by reducing the amount of base and cryptand. Furthermore, 80 °C was established as the optimal reaction temperature. Under optimal reaction conditions radiochemical yields of around 40% and molar activities close to 100 GBq/μmol were obtained. The optimised synthesis procedure was automated on a commercially available radiochemistry synthesizer (Neptis® Perform) to facilitate the use of this method in other PET centres.

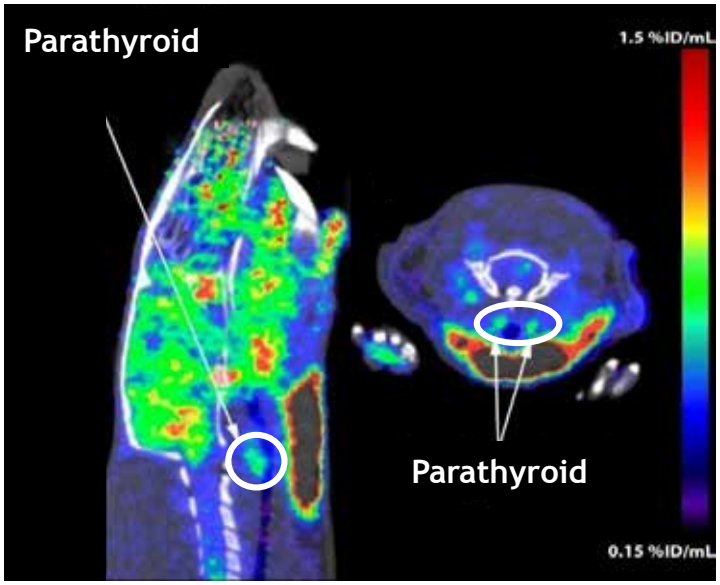


Figure 1. PET/MRI image of [^{18}F]cinacalcet in a healthy Wistar rat.

Besides difluoroiodomethane, we also studied 1-(difluoromethyl)-3-methyl-4-phenyl-1*H*-1,2,3-triazol-3-ium triflate **4** as a new [^{18}F]fluoroform precursor. Stability studies of the precursor and optimisation of the [^{18}F]fluoroform formation showed similar trends as for difluoromethane: reduction of base and cryptand amounts led to increased precursor stability and high molar activities of [^{18}F]fluoroform. The automated synthesis provided [^{18}F]fluoroform with some of the highest molar activities observed so far (153±14 GBq/μmol, dc, EOS). Compound **4** was therefore found to be a valuable alternative to the already established precursors.

Expanding the substrate scope

The Ruppert-Prakash reagent (Me_3SiCF_3) is one of the most important trifluoromethylation agents in organic chemistry and has been used for numerous trifluoromethylation strategies. We therefore envisioned that developing a fluorine-18 labelled version of this important trifluoromethylation agent would be an excellent starting point to translate the trifluoromethylation strategies from organic chemistry to fluorine-18 chemistry and expand the substrate scope of ^{18}F-trifluoromethylation. [^{18}F]Me_3SiCF_3 was synthesised by reaction of [^{18}F]fluoroform with trimethylsilyl chloride and was obtained with radiochemical yields of 85-95% and radiochemical purities of >95%. It was reacted in a simple model reaction with a range of aromatic aldehydes and ketones (**7a-m**) and proved good reactivity and a complementary substrate scope to previously reported methods.

PET tracer synthesis

The final part of my thesis describes the application of the high molar activity [^{18}F]fluoroform strategy in the synthesis of a new PET tracer, [^{18}F]cinacalcet. Cinacalcet is a drug binding to the calcium-sensing receptor of the parathyroid glands, an important regulator of blood calcium levels. In parathyroid hyperplasia the parathyroid glands are overactive and need to be surgically removed, requiring precise pre-operative localisation. Currently used techniques, such as ultrasound, MRI, SPECT/CT, and [^{18}F]fluorocholine PET still show

limited sensitivity in multigland disease. Therefore, we developed [^{18}F]cinacalcet as a tracer to image the overactive parathyroid glands and aid in the localisation of the glands for surgery. The synthesis of [^{18}F]cinacalcet was achieved by aromatic ^{18}F-trifluoromethylation of a boronic acid precursor using high molar activity [^{18}F]fluoroform (see scheme 1). [^{18}F]Cinacalcet was obtained with an overall radiochemical yield of 8±4% (dc) and a molar activity of 40±11 GBq/μmol within 1 hour (n=7). A biodistribution and metabolite study was performed in healthy rats, showing decent uptake in the parathyroid glands (see figure 1) and fast blood metabolism.

In conclusion, we were able to address and overcome the main challenges associated with the use of [^{18}F]fluoroform as fluorine-18 labelled building block for PET tracer synthesis and made a big step towards future clinical application.

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