A GENERAL SYNTHESIS OF [^{18}F]-LABELED a -TRIFLUOROMETHYL KETONES FOR PET IMAGING

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Key words: Fluorine-18, -Trifluoromethyl ketones, Protease inhibitor, PET

Introduction: Alpha-trifluoromethyl ketones (TFMKs) are known to be biologically active compounds. These compounds are potential hydrolytic enzyme inhibitors and cytotoxic agents against human tumor cells, and have potential for radiolabeling with fluorine-18. Traditional preparations of TFMKs are not suitable for synthesis of the [^{18}F]-labeled derivatives. Here we report a novel synthesis of [^{18}F]-labeled TFMKs by fluorination of 2,2-difluoro silyl enol ethers with radioactive fluorine [^{18}F]-F_2.

Methods and Results: *Preparation of 2,2-difluoro-1-aryl-1-trimethylsiloxyl-ethene* ***1a-d*** *(**a**=phenyl, **b**=biphenyl, **c**=naphthyl, **d**=anthryl):* These compounds were prepared from the respective -trifluoromethyl ketones by reaction with metallic magnesium and trimethylsilyl chloride at 0°C in dry tetrahydrofuran. After 1h stirring, solvent and excess reagents were evaporated under vacuum. The residue was suspended in hexane and the insoluble material was removed by filtration. The filtrate was evaporated under vacuum to obtain the desired precursor materials **1a-d** in high yields (80-90%).

*Preparation of [^{18}F]-$_a$-trifluoromethyl ketones (**2a-d**):* 2,2-Difluoro-1-aryl-1-trimethylsiloxyl-ethene **1a-d** (2 L, 10 mol) were separately dissolved in dry acetonitrile (0.5 ml) and cooled to -45°C. [$^{18/19}$F]-F_2 gas was bubbled into the solution for 10 min. The reaction mixture was warmed to room temperature, and the crude product was purified by chromatography either on a silica gel column or HPLC on a reverse phase C_{18} column. The products were reconfirmed by ^{19}F NMR spectroscopy.

These -trifluoromethyl ketones are highly sensitive to water. For example, during HPLC purification with 50% MeCN in water the products (ketones) were converted to their respective hydrated compounds, as a result radiochemical yields were low. However, when purified on a non-aqueous solvent system (10% ethyl acetate/hexane) using a silica gel column the product remained in the keto-form and produced high yields. Pure compounds were analyzed by TLC and found to co-elute with authentic sample when checked by UV and radioactivity monitors. The isolated pure product was further analyzed by HPLC on an analytical column, and hydrated products were observed, which are formed during HPLC analysis.

The radiochemical yields of these compounds were 45-55%, decay corrected (d. c.) in 3 runs/compound. The radiochemical purity was > 99% with specific activities 14.8-22.2 GBq/mmol. The synthesis time was 35-40 min from the EOB. In a representative synthesis of **2c**, 1.1 GBq of labeled product was obtained starting from 2.2 GBq of trapped activity [^{18}F]-F_2.

Conclusion: A convenient synthesis of [^{18}F]-labeled- -trifluoromethyl ketones has been developed. This method appears to be suitable for general syntheses of other [^{18}F]-labeled- -trifluoromethyl ketones of biological interest for PET imaging.

RADIOIODINATION OF BRIDGEHEAD CARBON ATOMS IN ADAMANTANE STRUCTURES.

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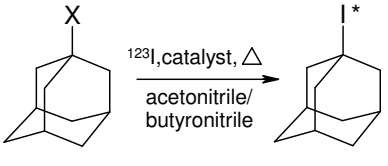
Keywords: Radioiodination, Adamantane, Bridgehead carbon, Catalysis.

Introduction: When a radioiodine atom is incorporated in a radiopharmaceutical, *in vivo* deiodination is often observed. Most common mechanisms for this deiodination are nucleophilic substitution (S_N2) and β-elimination. In order to reduce deiodination, the radioiodine atom is attached to an sp^2 carbon atom in an aromatic or vinylic moiety. A possible alternative is to place the radioiodine atom on a bridgehead carbon atom. Shielding by the ring structure prevents a backside S_N2 attack, while β-elimination is not possible because the ring is too much strained to accommodate an sp^2 carbon atom.

Few examples of the iodination of a bridgehead carbon atom are known in literature. Possible routes in organic chemistry include halogen exchange (1), replacement of a good leaving group (2) or replacement of a hydroxyl moiety using *in situ* prepared trimethylsilyl iodide (3). However, all these reactions are performed under anhydrous conditions. Since radioiodide is only available in water, none of these reactions were performed before in radiochemistry. Recently, we published a method to obtain radioiodide in anhydrous solvents (4), which enables us to perform the previous described reactions. In the preliminary experiments, chemistry was tested on adamantane compounds, as these compounds are relatively easy accessible.

Preliminary results: Radiolabelled 1-iodoadamantane and 1-iodoheptane were tested for their stability towards hydrolysis and nucleophilic attack by ethanethiol. 1-iodo-adamantane proved to be stable up to 50 hours, while 1-iodoheptane was completely deiodinated within 2 hours, proving the stability of the bridgehead carbon-iodine bond.

Table1: *Preliminary results on radioiodination reactions*



X	catalyst	yield (%)
I	silver triflate	75-80
Br	zinc triflate	70-75
Br	ferrocene	75-80
O-Tos	ferrocene	70-75
OH	trimethylsilyl triflate	30-45

As shown in the table, yields using *in situ* prepared trimethylsilyl iodide were limited due to the formation of a side product, which could not be prevented. Halogen exchange and the use of a good leaving group yielded promising results. Both strategies are currently tested for ringsystems other than adamantane, and in the development of new SPECT ligands for the 5-HT_{1A} receptor.

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FLUOROUS PHASE SYNTHESIS; A CONVENIENT NEW RADIOLABELING STRATEGY FOR THE SYNTHESIS AND PURIFICATION OF RADIOPHARMACEUTICALS

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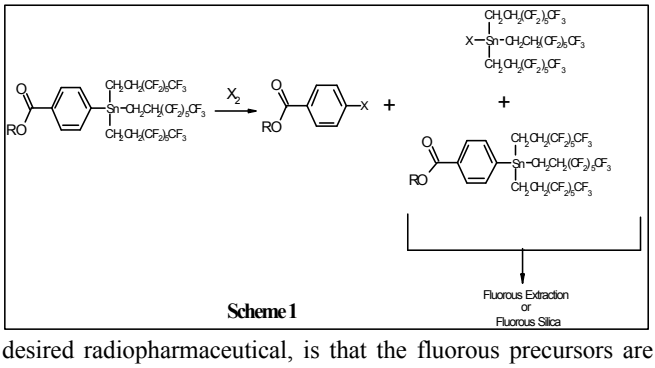
Keywords: Radiolabelling, Fluorous, iodine, carbon-11, fluorine-18

With the increasing use of PET for both clinical studies and basic research, there is a concomitant increase in the demand for new labelling strategies. In particular there is a need to develop radiolabeling methods that are easily automated and that result in products of sufficient purity to avoid the need to use time consuming separation techniques such as HPLC.

Solid phase labelling methodologies, in which the ligand precursor is bound to an immobilized support and released upon exposure to the isotope of interest, are attractive strategies because they remove, for the most part, the need to use HPLC to separate unreacted ligand from the desired product. Unfortunately, this methodology is not without limitations. One of the major stumbling blocks is that the immobilization of the substrate on the support must proceed quantitatively because it is essentially impossible to remove impurities. This problem is further complicated by the fact that it is challenging to identify impurities and evaluate loadings without having to first cleave the substrate from the support.

Taking advantage of the fluorous synthesis methodology pioneered by Hórvath and Rábai [1] and further developed by Curran and co-workers [2], we have engineered a new radiolabeling strategy that has the advantages of both solution and solid phase labelling techniques (**Scheme 1**). In this example the solid support is replaced with a series of long chain fluorocarbons. After labelling the soluble fluorous supported substrate in water or water-alcohol mixtures, the tin-fluorocarbon unit is released and the reaction mixture passed down a Sep-Pak containing a bonded phase that has a high fluorine content. Only those materials with low or no fluorine content, i.e. the radiopharmaceutical, are eluted. The main advantage of this system, besides the convenient means of isolating the desired radiopharmaceutical, is that the fluorous precursors are soluble in a wide range of solvents and can therefore be purified and characterized by traditional means. A further benefit of this strategy is that the target material can be recovered by eluting the Sep-Pak with a fluorocarbon solvent.



Scheme 1

We have successfully utilized the fluorous synthesis strategy to radiolabel tris(perfluorohexylethyl)-tin-benzoic acid derivatives, including N-hydroxysuccinimide esters and benzamides, using a number of different isotopes (^{11}C, ^{18}F and ^{125}I). A description of the methodology, its advantages, limitations and future potential will be presented.

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2-, 3- AND 4-[^{18}F]FLUOROPYRIDINE BY NO-CARRIER-ADDED NUCLEOPHILIC AROMATIC SUBSTITITION WITH K[^{18}F]F-K_{222}

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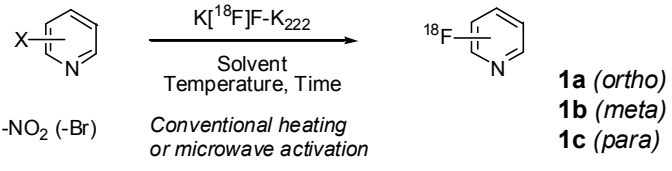
Keywords : Fluorine-18, Fluoropyridine

Nucleophilic substitution by means of cyclotron-produced, no-carrier-added [^{18}F]fluoride ion is the method of choice for the synthesis of radioligands labelled with fluorine-18 (half life : 110 minutes) of high specific activity for Positron Emission Tomography.

Compared to homoaromatic and aliphatic nucleophilic radiofluorinations, only few references can be found describing nucleophilic substitutions with [^{18}F]fluoride ion of hetero-aromatic compounds such as pyridines and only reactions involving fluorination processes at the *ortho*-position (2-position) have been more intensively studied (1) and applied with success to the preparation of radiopharmaceuticals such as [^{18}F]F-A-85380 (2-4). Heteroaromatic nucleophilic substitutions of the pyridine ring at the *para*-position (4-position) has, to our knowledge, never been reported whereas substitution at the *meta*-position (3-position) was only reported once (5).

In the present work, the scope of these nucleophilic aromatic fluorinations at the *meta*- and *para*-position of the pyridine ring with no-carrier-added [^{18}F]fluoride ion as its activated K[^{18}F]F-K_{222} complex has been evaluated and compared to the nucleophilic aromatic fluorinations at the *ortho*-position in this pyridine series. The syntheses of 3- and 4-[^{18}F]fluoropyridines ([^{18}F]-**1b**,**1c**) were chosen as model reactions and compared to the synthesis of 2-[^{18}F]fluoropyridine ([^{18}F]-**1a**).

The parameters studied include the influence of the position of the leaving group at the pyridine ring, as well as the quantity of the precursor used, the type of activation (conventional heating, microwave irradiation), the solvent, the temperature and the reaction time.



Using the corresponding nitro precursor, high yields were obtained at the 2-position (94% yield) using microwaves (100W) for 2 minutes in DMSO. Good yields (up to 72%) were observed at the 4-position using the same conditions while practically no reaction was observed at the 3-position. About 60% yield was also obtained at both the 2- and 4-position using the corresponding nitro precursor at 145°C for 10 minutes in DMSO. In the absence of reactivity towards nucleophilic heteroaromatic substitution at the 3-position, the influence of the nature of the leaving group as well as the influence of the solvent was also studied using 3-bromopyridine as the precursor and DMSO, DMF, acetonitrile and a mixture of DMSO/H_2O (95:5 v:v) as the solvent. Whatever the conditions used, the 3-bromo-derivative was completely unreactive and the desired 3-[^{18}F]fluoropyridine ([^{18}F]-**1b**) could not be detected.

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SYNTHESIS AND PRELIMINARY BIOLOGICAL EVALUATION OF 2-[^{18}F]-FLUOROISONICOTINIC ACID HYDRAZIDE

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Key words: 2-[^{18}F]-Fluoroisoniazid, Bacterial Infection, Tuberculosis.

Tuberculosis present outside the pulmonary system has always posed a diagnostic dilemma. Additionally, central nervous system tuberculosis is becoming a common observation in patients who are intravenous drug abusers with AIDS and AIDS-related complex. MRI and contrast-enhanced CT have been the methods of choice for non-invasive diagnosis of this elusive form of tuberculosis. Nonetheless, these imaging methods have been limited in cases where the lesions are less than 5mm.

Isonicotinic acid hydrazide (Isoniazid) is one of the most effective antituberculosis agents known. Passive diffusion has been the proposed mechanism by which the drug gains access into the bacterial cell. Once inside the cell, enzymatic transformation occurs converting the isoniazid to the acid. The pH of the intracellular milieu causes ionization of the acid and hence resulting in metabolic trapping within the cell. This mechanism implies that radiolabeled isoniazid derivative may be of some value in non-invasive detection and localization of these forms of tuberculosis.

2-[^{18}F]-Fluoroisonicotinic acid hydrazide was synthesized by nucleophilic displacement reaction on ethyl-2- (trimethylammonium)-isonicotinate precursor in acetonitrile. Kryptofix 222 was used as the phase transfer catalyst. The intermediate fluorinated ethyl ester reacted with hydrazine hydrate to produce the hydrazide in excellent radiochemical yield. The overall radiochemical yield was greater than 70% with total synthesis time of approximately 60 minutes.

Biological evaluation was performed in bacterial cells and biodistribution in normal CBA/J mice. The tracer appears to clear relatively fast from the circulation (only about 0.2% of injected dose/ gram at 60 minutes post-injection). The data showed that the major route of elimination was the urine as was reflected by the very high activity in the kidneys after 5 min. However, there was a significant proportion of hepatobiliary retention (the highest activity after 60 min aside from the urine). It was found that the *S. pneumoniae* cells retained the radiotracer in an in vitro assay. The radioactivity associated with the cells appears to increase from 5 to 30 minutes. From this time onwards the value plateau up to 2 hours. For the number of cells used the maximum cell associated radioactivity value was 8-9 % of the applied radioactivity. Additionally the behavior of the tracer in an infection model will be discussed.

APPLICATION OF ISOTOPE EXCHANGE IN PRODUCTION OF 3-[123-I]IODO-a-METHYL-L-TYROSINE

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Keywords: IMT, iodine-123, labeling

3-Iodo-a-methyl-L-tyrosine (IMT) is an artificial amino acid suitable for imaging of different malignant tumors by means of SPECT. Intensive investigations conducted in the last decade have shown that this substance is especially valuable for diagnostics and monitoring of brain tumors.

In comparison with 2-fluorodeoxyglucose, used for the same purposes in positron emission tomography, the background uptake of IMT in normal brain tissue is low, providing high contrast tumor images.

[123-I]IMT is currently produced via reaction of electrophilic radioiodination in presence of such oxidizing agents as potassium iodate, chloramine-T and Iodogen. In order to remove the toxic impurities used in such reaction mixtures a careful purification of the final product should be carried out as well as thorough analysis of the preparation proving adequacy of the purification stage.

Isotope exchange labeling methods using 3-iodo-a-methyl-L-tyrosine as a starting material seems to be more attractive because there is no need in this case for purification of the final preparation.

Two ways of isotope exchange have been investigated: isotope exchange in an acidic media and exchange in presence of copper catalyst. In catalytic reaction the yield of [123-I]IMT did not exceed 60%.

The reaction mixture for isotope exchange in acidic media consisted of IMT solution in hydrochloric acid and 1 – 5 GBq of [123-I]NaI. The yield of [123-I]IMT in this case was 80-85%. A small portion of ion exchange resin was added to the reaction mixture to remove the residual iodide ions. After dilution of the reaction mixture by isotonic solution and filter sterilization the preparation was ready for use. The production procedure takes not more than one hour.

THE FIRST ENZYMATIC METHOD FOR CARBON-FLUORINE-18 BOND FORMATION: RADIOFLUORINATION OF [^{18}F]- 5'-FLUORO-5'-DEOXYADENOSINE

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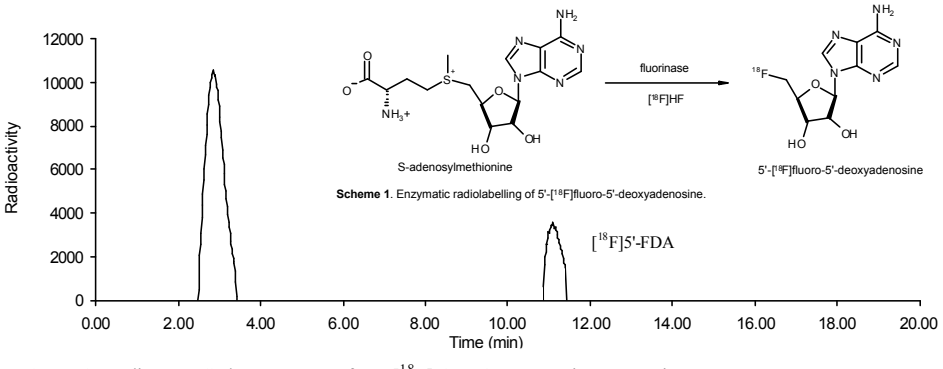
Keywords: Fluorinase, enzymatic radiolabelling, [^{18}F]5'-FDA

New methods for labelling compounds with fluorine-18 are highly desireable in order to expand the possibilities for the development of new radiolabelled PET probes for *in vivo* imaging. Aliphatic nucleophilic displacement of leaving groups with [^{18}F]fluoride-ion is a common procedure for the production of [^{18}F] labelled compounds. Reactivity of the leaving group with [^{18}F]fluoride and compatibility of functional groups with the radiolabelling method will affect the outcome of the radio fluorination experiment. Recent studies with [^{18}F]-5'-fluoro-5'-deoxyadenosine have highlighted the difficulty to introduce [^{18}F]fluoride in the 5'-position when a series of 5'- halo and 5'-sulfonic acid alkyl and aryl esters failed to produce the expected radiolabelled adenosine derivatives in good yield. Recently O'Hagan et al. reported that the key enzyme involved in carbon-fluorine bond formation in *Streptomyces cattleya* catalyses the formation of 5'-fluoro-5'-deoxyadenosine (5'-FDA) from fluoride ion and S-adenosyl-L-methionine (SAM)(Scheme 1). Here we report the exploration of the enzymatic radiolabelling of 5'-FDA with fluorine-18 using this novel biocatalyst.



Scheme 1. Enzymatic radiolabelling of 5'-[^{18}F]fluoro-5'-deoxyadenosine.

Figure 1. Radio-HPLC chromatogram from [^{18}F]5'-FDA enzymatic preparation

In a typical experiment SAM, [^{18}F]HF and the fluorinase enzyme were incubated in aqueous buffer at 30ºC and the course of the reaction was followed using a high-performance liquid chromatography (HPLC) system coupled to a radioactivity detector. Analysis of the aliquots by radio-HPLC showed two radioactive peaks. The retention time of first radioactive signal (Rt=3.6 min) on the reverse-phase column corresponds to the dead volume of the column and is consistent with unreacted [^{18}F]fluoride and/or polar byproducts. The only detectable non-polar product elutes at 11.3 min (Fig. 1). The chemical identity of [^{18}F]5'-FDA was confirmed by coinjection with an authentic 5'-FDA sample. The overall synthesis time was 6hours with a radiochemical yield of 1% relative to E.O.B and corrected for decay.

In conclusion, this work provides the first example of enzymatic carbon-[^{18}F]fluorine bond formation through successfully radiolabelling [^{18}F]-5'-FDA from SAM and [^{18}F]HF in the presence of a fluorinase. Work is in progress to optimise yields obtained in these preliminary studies.

ATTEMPTS ON THE DIRECT RADIOIODINATION OF PHENYL ACETATE AND PHENYL ACETIC ACID WITH IODOGEN METHOD

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Th sodium salt of phenyl acetic acid is used as an antineoplastic in cancer research. The phenyl acetate anion has the essential antineoplastic activity in its metabolism. There is no indication in the literature for the antineoplastic activity of phenyl acetate. However, the nomenclature on phenyl acetic acid anion and phenylacetate has been completely confused in the literature by most authors. The same confusion has been also shown between phenyl butyric acid anion and phenylbutyrate. First, the right nomenclature has been recommended by the authors to prevent further confusions in the literature. Second, the radioiodination of phenyl acetic acid and also phenyl acetate were attempted by the use of iodogen method. The radioiodination studies showed that iodogen can be used as a successful radioiodination agent for phenyl acetate, but contrarily is not successful for radioiodination of phenyl acetic acid. Of course, this was a result of activation of the phenyl ring by acetate substituent (C_6H_5-OOC-CH_3), but of deactivation by the acetic acid substituent (C_6H_5-CH_2-COOH). In this study, some work on the radioiodinated phenyl acetate with iodine-131 was additionally performed to test the preliminary biological activities of phenyl acetate.

PREPARATION OF HIGH SPECIFIC ACTIVITY ^{99m}Tc SULFUR COLLOID

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Key Words**:** sulfur colloid, specific activity, lymphoscintigraphy

It has been reported that using high concentration ^{99m}Tc Nanocoll (Eur J Nucl Med 2001 28:1450) can increase the measured counts in sentinel nodes (SN). ^{99m}Tc sulfur colloid (SC) is the only imaging agent used in the U.S. for lymphoscintigraphy. Base on the proposed active uptake by antigen presenting cells (APCs) and phagocytosis mechanism, we hypothesized that high specific activity (SA) SC may improve the detection of SNs in a similar fashion, and we set out to prepare a high SA SC.

CIS-US SC kits were used in the studies. Same amount (ca. 125 mCi, 24h in-growth) of ^{99m}Tc pertechnetate was used for each preparation. The regular SA SC was prepared exactly according to the procedures written on the package insert. The high SA SC preparation was achieved using only 1/8 of the kit contents. The SC kit, which contains 2 mg of sodium thiosulfate, was first reconstituted with 1 ml of saline. A part of this solution (0.125 ml) was then withdrew and used for the labeling. In order to improve the labeling efficiency, the vial was heated at 100°C for 8 min as heating for only 5 minutes occasionally produced lower labeling efficiency. The radiolabeling efficiency was determined by ITLC. The radiolabeling efficiency of high SA SC was 99% (n=11) when reacted at 100°C for 8 min. The particle size distribution was characterized by filtration.

Since the preparation of high SA SC used only 1/8 of the kit contents and ca. 125 mCi of ^{99m}Tc pertechnetate, this was equivalent to about 1 Ci of ^{99m}Tc pertechnetate activity in a whole SC vial. It was interesting to find that the average particle size of high SA SC is smaller than that of regular SA SC. It has been suggested that the formation of SC deals with a nucleation process. Technetium sulfide nuclei formed first, and then the sulfur deposited in part on them and in part on its own nuclei. The smaller particle size of high SA SC might be attributed to the less amount of sodium thiosulfate (only ca. 0.25 mg) used in the preparation. 23 and 28 consecutive patients were studied using filtered (0.2 μm) regular and high SA SC preparations, respectively. The count ratio of SN to injection site (SN/IS) was calculated for these two groups. The mean and median SN/IS ratios in the high SA group were 2.9 and 2.7 times higher than that in the regular SA group, respectively. High SA SC improves the relative counts in SNs and facilitates their detection. This is attributed to the combined effects of the high specific activity and smaller average particle size of the high SA SC preparation.

[^{18}F]FLUOROMETHYL SULFONATE ESTERS FOR INTRODUCTION OF THE FLUOROMETHYL GROUP - PREPARATION OF [^{18}F]FLUTICASONE PROPIONATE.

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Keywords: ^{18}F, fluoromethylation, ([^{18}F]fluoromethyl) *p*-toluenesulfonate, Fluticasone propionate

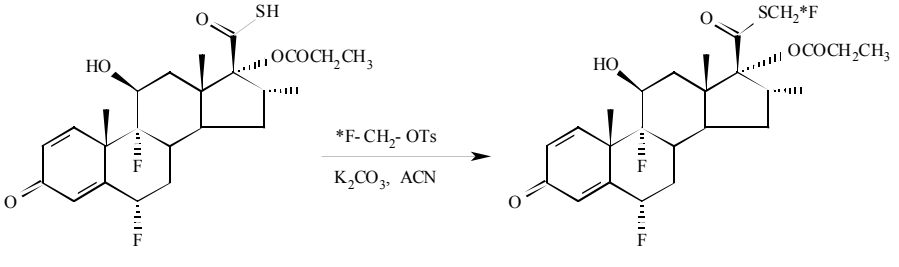
With the goal of developing an improved method for the preparation of [^{18}F]Fluticasone propionate ([^{18}F]FP), we reexamined [^{18}F]fluoromethyl sulfonate esters for introduction of the fluoromethyl group. The preparation of [^{18}F]fluoromethyl mesylate and tosylate has been reported by Block and Coenen (R = methyl and *p*-tolyl below). They also reported that reaction of *p*-chlorophenol with ^{18}F-CH_2-OMs and ^{18}F-CH_2-OTs produced [^{18}F]fluoromethyl *p*-chloroanisole (Nu = *p*-chlorophenoxy below) in 1% and 23% yield, respectively.

$$^{18}F? + CH_2\text{-}(OSO_2R)_2 \longrightarrow {}^{18}F\text{-}CH_2\text{-}OSO_2R + Nu? \longrightarrow {}^{18}F\text{-}CH_2\text{-}Nu$$

Consistent with these results, ^{18}F-CH_2-OMs could be prepared in good yield, but subsequent reaction with nucleophiles failed to produce more than a trace of labeled product.

We found that reaction of ^{18}F? and CH_2-$(OTs)_2$ resulted in the formation of ^{18}F-CH_2-OTs and a less polar byproduct, as indicated by TLC analysis. The nature of the byproduct is under investigation. The byproduct predominated when using *tetra*-butylammonium bicarbonate or low levels of K_2CO_3/[2.2.2] Kryptofix (K-Fix) in acetonitrile (ACN), but ^{18}F-CH_2-OTs was the major labeled product when a larger amount of K-Fix was used. After heating at 100°C for 10 min, the concentrated reaction mixture was passed through a column of alumina. The formation of the byproduct is not of concern because most of it was eluted with 10% dichloromethane in hexanes and ^{18}F-CH_2-OTs was eluted with 5% ethyl acetate in hexanes. Excess ^{18}F? and CH_2-$(OTs)_2$ were not eluted under these conditions. Even if the byproduct was carried on to the next step, it did not react with nucleophiles nor interfere with purification of the desired product. Consistent with results reported by Block and Coenen, other solvents and reaction conditions failed to improve the yield. We now routinely produce ^{18}F-CH_2-OTs in 25-40% decay-corrected yield, based upon ^{18}F?.

The isolated ^{18}F-CH_2-OTs was allowed to react with the thioacid analog of FP (FP-SH) and K_2CO_3 in ACN at 110° for about 45 min. Purification by HPLC resulted in >50% yield of FP. We believe the simplicity and overall decay-corrected yield of >10% based upon ^{18}F? represents an improvement over previous methods used to prepare [^{18}F]FP.



FP-SH [^{18}F]FP

We are investigating the reaction of ^{18}F-CH_2-OTs with other nucleophiles and the preparation of other [^{18}F]fluoromethyl sulfonate esters for use as fluoromethylating reagents.

STUDY ON REACTION MECHANISM AND KINETICS OF *p*-[^{18}F]MPPF RADIOSYNTHESIS

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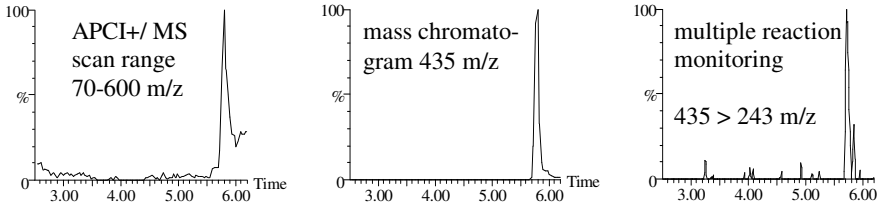
Keywords: radiolabelling, fluorine-18, MPPF, reaction mechanism, LC-MS

Radiolabelling of 4-[^{18}F]fluoro-N-[2-[1-(2-methoxyphenyl)-1-piperazinyl]ethyl-N-2-pyridinyl-benzamide (*p*-[^{18}F]MPPF), a ligand for imaging of serotonergic 5-HT_{1A} neurotransmission with positron emission tomography (PET), has previously been reported (1,2). The aim of our study is to improve the radiolabelling yield of *p*-[^{18}F]MPPF and especially, to study the reaction mechanism and identify the radiolabelled side products in the synthesis.

The synthesis of *p*-[^{18}F]MPPF was based on nucleophilic substitution of corresponding nitro-precursor 4-nitro-N-[2-[1-(methoxyphenyl)-1-piperazinyl]ethyl-N-2-pyridinyl-benzamide (*p*-$MPPNO_2$) with fluorine-18. *p*-$MPPNO_2$ and the fluorinated standard *p*-MPPF were synthesised in a 3- and 4-step procedure, respectively. *p*-$MPPNO_2$ in DMSO was heated with dried [^{18}F]fluoride complex at 150-200 °C for 5-15 min. The crude product was purified by semi-preparative reverse-phase HPLC. Inactive reference standards for *p*-$MPPNO_2$ and *p*-MPPF, and radiolabelled product fractions were identified by TLC, HPLC and LC-MS (Micromass QUATRO II).

The radiochemical incorporation yield of *p*-[^{18}F]MPPF was 28 ± 11 %. Increasing the reaction temperature beyond 170°C and prolonging the reaction time did not increase the yield. The time of synthesis, excluding HPLC-purification, was about 65 min and the overall radiochemical yield was 7.8 ± 2.8 % (EOB, decay corrected). Several labelled products were found in the preparative HPLC-separation, one fraction was identified as *p*-[^{18}F]MPPF, fig.1.

The radiolabelling yield of *p*-[^{18}F]MPPF corresponds to previously reported results with thermal heating (2). The separated *p*-[^{18}F]MPPF fraction could be identified by HPLC and LC-MS, characterisation of other labelled products is under further study. Optimisation of synthesis procedure, including reaction conditions and HPLC-purification will also be continued.



1) 2)

Figure 1. LC-MS analyses of *p*-MPPF[1] and radiolabelled product[2]

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THE [^{18}F]XeF_3^- ION, AN INTERMEDIATE IN $^{18}F^-$ ION EXCAHNGE WITH XeF_2

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Keywords: XeF_2; chemical exchange; fluorine-18, trifluoroxenate(II)

Xenon difluoride (XeF_2) is a versatile fluorinating agent in inorganic and organic chemistry. Fluorine-18 labelled XeF_2 was developed in 1981 (1) and has been used as a fluorinating agent for the regioselective syntheses of ^{18}F-FDG and [^{18}F]6-fluoro-L-DOPA. We have recently shown, by ^{19}F NMR exchange, that fluoride ion exchanges with XeF_2 in CH_3CN solvent under rigorously anhydrous conditions (2). The likely exchange intermediate in this reaction is the trifluoroxenate(II) anion, XeF_3^- (eq 1). Prior to the present work, XeF_3^- had only been characterized in the gas phase by mass spectrometry. In view of the interest in synthesizing [^{18}F]XeF_2 from no-carrier-added $^{18}F^-$ for electrophilic fluorination reactions, the possible intermediacy of [^{18}F]XeF_3^- is of importance in PET radiochemistry.

$$FXeF + {}^{18}F^- \rightleftharpoons [FXeF^{18}F]^- \rightleftharpoons FXe^{18}F + F^- \quad (1)$$

The experimental objectives of the present study were to better define the nature of the transition state in the XeF_2/F^- exchange by NMR spectroscopy and to establish the fluoride ion acceptor properties of XeF_2 by attempting the syntheses of salts containing the XeF_3^- anion. Computational methods were used to explore the nature of the intermediate in the XeF_2/F^- exchange, to arrive at the energy-minimized geometry for XeF_3^-, as well as to determine the gas-phase fluoride ion affinity of XeF_2 relative to those of other main-group binary fluorides.

The enthalpy of activation, $\Delta H^{\ddagger}$, has been determined by use of single selective inversion ^{19}F NMR spectroscopy to be 17.7 ± 1.2 kcal mol^{-1} and 13.6 ± 1.6 kcal mol^{-1} for stoichiometric samples of $[N(CH_3)_4][F]$ and XeF_2 prepared at 0.18 M and 0.36 M concentrations, respectively, in CH_3CN solvent. Although XeF_3^- has been observed in the gas phase by mass spectrometry, attempts to isolate salts containing the XeF_3^- anion in the present work have been unsuccessful and is attributed to the low fluoride ion affinity of XeF_2 (-21.8 kcal mol^{-1}; DFT/SVWN), relative to those of XeF_4 (-57.7 kcal mol^{-1}) and XeF_6 (-77.7 kcal mol^{-1}) which form stable XeF_5^- and XeF_7^- anions. Although a salt containing the XeF_3^- anion has not been isolated, [^{18}F]XeF_3^- is likely to exist as an exchange intermediate in $^{18}F^-/XeF_2$ exchange studies, provided rigorously anhydrous conditions are maintained. The XeF_3^- anion would represent the first example of an AX_3E_3 (E = valence electron lone pair) species under the valence shell electron pair repulsion (VSEPR) formalism. The energy-minimized geometry of the XeF_3^- anion was calculated at the MP2 and DFT levels of theory and found to be planar and Y-shaped (C_{2v} point symmetry). The molecular geometry and bonding have been described and rationalized in terms of electron localization function (ELF) calculations and molecular orbital and VSEPR models.

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INVESTIGATION OF NCA [^{18}F]β-FLUOROETHYL TOSYLATE AS A LABELING AGENT UNDER MICROWAVE CONDITIONS

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Keywords: ^{18}F-fluoroethylation, amine, phenol, ester, microwave

No-carrier-added (NCA) [^{18}F]β-fluoroethyl tosylate has been used as a general [^{18}F]fluoroalkylation agent because it is easy to prepare, has good stability, wide applicability and, as we have shown recently, is suitable for one-pot syntheses. However, the application of this labeling agent is restricted by disadvantages, including lower reactivity compared to some labeling agents (*e.g.* [^{18}F]β-fluoroethyl triflate) and its sensitivity to some solvents and bases that are used in the labeling reactions. The development of microwave-enhanced radiochemistry provides a faster, cleaner, more selective and highly atom-efficient route. The reduced reaction time coupled with other benefits, such as higher purity due to reduced reaction mixture decomposition and the promotion of otherwise sluggish reactions, make it an ideal tool to be explored in radiochemistry with short-lived positron-emitting radioisotopes. It is in this light that we reinvestigated [^{18}F]β-fluoroethyl tosylate as a reagent towards an amine, phenol and carboxylic acid to form a [^{18}F]*N*-β-fluoroethylamine, [^{18}F]β-fluoroethyl ether and [^{18}F]β-fluoroethyl ester.

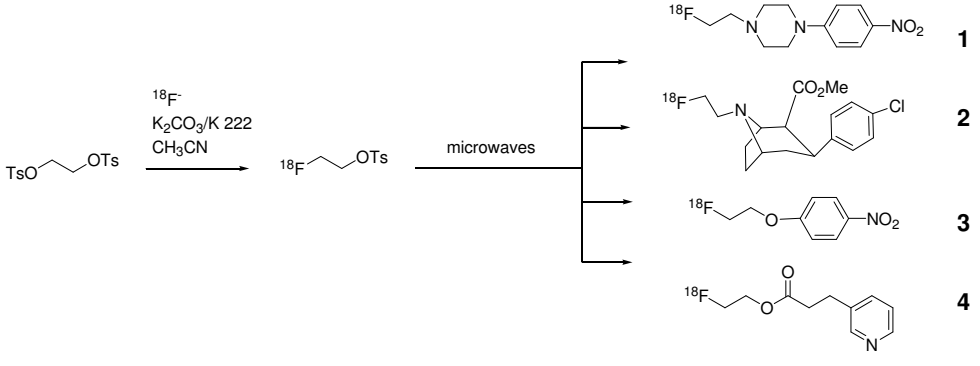

Table 1. Comparison of radiolabeling procedures under microwave and thermal heating conditions (in acetonitrile)

Compound	Base	Microwave		Thermal heating	
		Conditions	RCY*	Conditions	RCY*
1	$NaHCO_3$	300W, 150 ºC, 10 min	51%	-	n/a
2	$NaHCO_3$	300W, 150 ºC, 10 min	78%	120 ºC, 45 min	60%
3	Cs_2CO_3	300W, 6 min	77%	120 ºC, 10 min	59%
4	Cs_2CO_3	300W, 2 min	89%	120 ºC, 10 min	66%

* RCY (decay-corrected radiochemical yield) based on [^{18}F]β-fluoroethyl tosylate

Under microwave conditions, it is possible to heat the reaction mixture rapidly up to 150 ºC in a low boiling point solvent, such as acetonitrile. Thus, the use of high boiling point solvents, such as DMSO and DMF, which are difficult to remove, can be avoided. The microwave-enhanced reaction also gave ~ 20% greater radiochemical yield (decay-corrected) than thermal reactions under similar temperatures and reaction times, and in some cases gave comparable yields in a shorter reaction time (Table 1).

NO CARRIER ADDED NUCLEOPHILIC ^{18}F-FLUORINATION PERFORMED IN AN ELECTROCHEMICAL CELL OF VARIABLE VOLUME

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Keywords: Electrochemical cell, nucleophilic ^{18}F-fluorination, [^{18}F]altanserine, N-methyl-[^{18}F]benperidol

The electrochemical separation of [^{18}F]fluoride from ^{18}O-water combined with a subsequent no-carrier-added nucleophilic ^{18}F-fluorination is an advantageous alternative technical approach[1] in comparison to the commonly performed process including an azeotropic PTC-drying step. Furthermore, this technique leads to an efficient target water recovery without significant decrease of the ^{18}O-enrichment.

The electrochemical cell originally designed has the inherent disadvantage that the reaction volume must be equal to the volume of the target water. However, in order to decrease the amount of educt and phase-transfer-catalyst (cryptate), it is indispensable to diminish the reaction volume.

The aim of this study was to design a modified electrochemical cell which allows to perform the fluoride desorption and nucleophilic fluorination in a volume much smaller than that of the target water.

Based on the original construction a system was established with a moveable centrical platinum canula and a glassy carbon stamp which can be alternatively adjusted inside a glassy carbon cylinder. The diameter of the stamp is about 90 % of that of the cylindrical carbon vessel. Moving the stamp into the cylinder leads to a much reduced inner volume. Accordingly, the resulting slit between the stamp and the inner surface of the glassy carbon cylinder has a (reaction) volume of about 0.3 ml in comparison to 1.3 ml (one target volume) of ^{18}O-water.

Due to the short distance between the cylinder and the stamp (0.5 mm) an electrical field of about 20 V/cm can be obtained with an applied voltage of only 0.1 V between both carbon electrodes. This low voltage allows to desorb the [^{18}F]fluoride after anodic deposition in the presence of educts which are commonly unstable under redox conditions.

The electrochemical system is now routinely used for the synthesis of radiotracers for which there is no need of deprotection after no-carrier-added ^{18}F-fluorination, e.g. [^{18}F]altanserine or the base labile tracer N-methyl-[^{18}F]benperidol, which can directly be labelled in presence of the oxalate cryptate system. For example, in the case of [^{18}F]altanserine the amount of precursor could be reduced to only 3 mg of nitroaltanserine. Thus the HPLC separation of n.c.a. [^{18}F]altanserine from residual precursor is more efficient and the apparent specific activity of the receptor ligand is in the range of 0.2 TBq/µmol. The radiochemical yield of the fluorination step corresponds to about 70% of the desorbed [^{18}F]fluoride activity.

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OPTIMISATION OF THE SYNTHESIS, QUALITY CONTROL AND METABOLIC ANALYSIS OF [^{11}C]PMP FOR THE IN VIVO EVALUATION OF CHOLINESTERASE ACTIVITY IN HUMANS

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Keywords: PMP, PET, AChE, cholinesterase, Alzheimer

Alzheimer's disease (AD) is a progressive neurodegenerative disorder in which the degeneration of the cortical cholinergic system is one of the most consistent neurochemical changes. Among the several tracers proposed for the *in vivo* assessment of acetylcholinesterase (AChE) with PET, we have chosen 1-[^{11}C]methylpiperidin-4-yl propionate ([^{11}C]PMP) (*1*) for clinical use. The synthesis procedure of [^{11}C]PMP was optimized by replacing DMF by the less toxic solvent acetone and by neutralizing the trifluoroacetic acid salt of the starting material piperidin-4-yl propionate (PP·TFA) *in situ*. Also a simple Sep-Pak procedure was developed for the determination of the metabolic degradation of [^{11}C]PMP.

[^{11}C]PMP was prepared by alkylation of 1 mg of PP·TFA with [^{11}C]methyl triflate in 0.3 ml acetone in the presence of 10 µl 1N NaOH. After neutralization with 1 ml of 15 mM AcOH the acetone was evaporated with a stream of He at 70 °C. Next, the mixture was dissolved in 1 ml of mobile phase and injected on HPLC (Waters µBondapak C18 10 µm, 7.8 mm x 300 mm, NaOAc buffer 0.05M/EtOH 90:10 (V/V), pH=5.5, flow rate 4 ml/min). [^{11}C]PMP eluted at 8'20 and the radiochemical yield was 38% (corrected for decay, starting from [^{11}C]CH_4, synthesis time 35 min). Quality control was performed on a Waters Xterra analytical column eluted with NaOAc buffer 0.05M/EtOH 95:5 (V/V) pH=5.0, flow rate 1 ml/min. The retention time of [^{11}C]PMP was 4'55. The sensitivity of UV and RI detection for PMP and PP was found to be insufficient for the quality control of [^{11}C]PMP. We have developed a radio-LC-MS method which enables the detection of both PP and PMP at concentrations as low as 0.5 nmol/ml. The specific activity at the end of synthesis was higher than 20 MBq/nmol and PP was not detectable.

Separation of [^{11}C]PMP from its metabolite 1-[^{11}C]methyl-4-piperidinol ([^{11}C]HMP) was performed by application of a 1-2 ml plasma sample on a preconditioned C18 Sep-Pak light column, followed by successive elution with 3 ml of 0.05M sodium phosphate buffer pH=7.4 (PB, to elute [^{11}C]HMP) and 5 ml of EtOH (to elute [^{11}C]PMP). The efficiency of the procedure was validated by separation of human plasma samples containing mixtures of [^{11}C]PMP and [^{11}C]HMP.

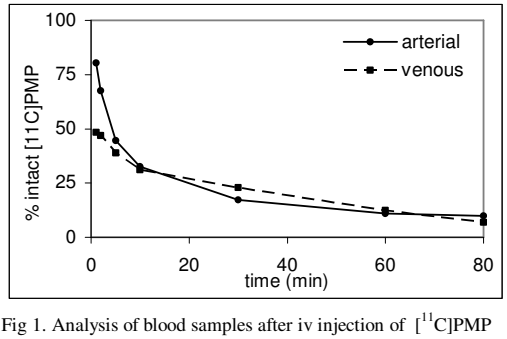

Fig 1. Analysis of blood samples after iv injection of [^{11}C]PMP

In human studies, arterial and venous blood samples were collected in heparinised tubes containing 0.1 mg physostigmine and treated as described above. The plasma and PB fractions were pooled and counted in a sample changer together with the ethanol fraction and the Sep-Pak. A typical result of the blood analysis is given in Fig. 1. Apart from the early phase, the results from venous and arterial blood were comparable.

In conclusion, the described procedure has proven to be a reliable method for the synthesis, quality control and metabolic evaluation of [^{11}C]PMP *in vivo*.

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IMPROVED PRODUCTION OF IODINE-124 AND ITS USE FOR PET

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Keywords: iodine-124, targetry, protein labelling, oncology

Introduction

The long-lived positron–emitting radioisotope iodine-124 ($t_{1/2}$ = 4.2 d, 25 % $^{+}$) can be used for PET studies of biological processes within a wide time window. Furthermore, its half–life allows the isotope to be applied in labs remote from the cyclotron. A reliable method to produce iodine-124 – based on an existing system at IRSL, Hammersmith Hospital – was required.

Methods

After a ^{124}Te(p,n)^{124}I reaction using 12.5 MeV protons [1], the target was transferred automatically into the hot cell for dismanteling and dry distillation.The target consisted of a platinum disk with a depression (i.d. 20 mm, depth 1 mm) containing 1.1 g of isotopically enriched ^{124}Te[TeO_2] (99.3 %). The target was covered with an aluminium foil (0.25 mm thickness) and mounted into a target holder. A quartz glass furnace tube [2] was re–designed to render the device more robust and suitable for semi–automation. The target was heated to 760 ^{o}C while a flow of oxygen (45 ml/min) transferred the volatile radioactive species into a trapping vial containing sodium hydroxide solution (0.4 ml, 20 mM). The manipulations for target handling and distillation have been carried out by remote operation. Both direct and indirect peptide/protein labelling protocols have been applied.

Results

An improved ^{124}Te[TeO_2] solid target was prepared and a remotely operated facility for the safe production of iodine-124 has been established. The distillation process was optimized and sodium [^{124}I]iodide was obtained with a decay–corrected target yield of 9.5 0.8 MBq/ Ah (257 22 Ci/ Ah; n = 24), a specific radioactivity of 27.6 GBq/ mol (746 mCi/ mol) and a radiochemical purity of >99 %. The ^{125}I impurity was found to be 0.053 0.015 % (n = 4) with no ^{123}I detectable. A distillation efficiency of 90 % was reached. The maximum batch yield of [^{124}I]NaI was 5.48 mCi (203 MBq).

The iodine-124 was mainly used for radioiodinations of peptides and proteins of relevance for PET oncology. Examples entail annexin-V, antibodies to vascular endothelial growth factor (VEGF) and insulin.

Conclusion

An improved apparatus for the dry distillation of [^{124}I]iodine was commissioned for routine supply. Iodine-124 was used for labelling bio-molecules with relevance to PET oncology.

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SYNTHESIS AND PRELIMINARY EVALUATION OF [^{18}F]FLUOROETHYL FENOTEROL TO VISUALISE AND QUANTIFY b 2-ADRENOCEPTORS IN VIVO

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Keywords: fenoterol, ^{18}F-fluorination, 2-adrenoceptor, lung

In humans, peripheral -adrenoceptors are widely distributed throughout the lung, with more than 70% of them being of the 2-subtype. The 2 receptors occur for instance in bronchial epithelium, submucosal glands, immune cells and airway smooth muscle fibres [1]. The role of 2-adrenoceptor density for obstructive respiratory diseases (such as asthma or chronic obstructive bronchitis) is still not clarified [2], although changes in -receptor function in the lung have been correlated to chronic obstructive lung disease and cystic fibrosis [3], and although -agonist drugs have been widely applied to treat asthma. For understanding the pathogenesis, therapy and prognosis of such diseases, a non-invasive, quantifiable imaging of 2 density would be valuable. The aim of this study was therefore to label the hydrophilic, highly selective subtype-specific agonist fenoterol with fluorine-18 to image membrane-bound high-affinity state 2-receptors only.

The radioactive labeling was achieved using (R,R)(S,S)-fenoterol liberated from fenoterol hydrobromide (Boeringer Ingelheim, Germany) and the secondary labeling precursor 2-[^{18}F]fluoroethyltosylate. The overall radiochemical yield of (R,R)(S,S)-[^{18}F]fluoroethyl fenoterol ([^{18}F]FEFE) was 20%; isolation of the radiotracer was achieved by HPLC. The resulting liquid phase was diluted with water and passed through a solid phase column (C-18 , Waters) which led to the fixation of the radiotracer. After drying with nitrogen the tracer was eluted from the solid phase with 1 ml of ethanol which was subsequently removed *in vacuo*. The [^{18}F]FEFE was dissolved in an appropriate amount of isotonic NaCl solution for further use.

The receptor binding of the compound and the organ distribution were assessed in Hartley-guinea pigs. Animals were anaesthetized and [^{18}F]FEFE was injected i.v. with an activity of 12-18 MBq. Dynamic PET studies over 60 min were performed either as a baseline study (study group 1, n=3) or as a displacement study (study group 2, n=3; 2 mg/kg (R,R)(S,S)-fenoterol 10 min p.i. of [^{18}F]FEFE). After termination of the PET measurements, animals were sacrificed and multiple organ samples were taken. The tissues were dissolved in KOH (4N) at 75°C for 30 min and the ^{18}F-organ activity was measured. In the control group ^{18}F organ uptake (in %ID/g) of the lung, heart, liver, spleen, kidneys, brain, intestine and blood was 1.16 0.05, 0.49 0.06, 0.95 0.1, 0.84 0.2, 0.70 0.1, 0.02 0.005, 0.77 0.08 and 0.22 0.09, respectively.

[^{18}F]FEFE showed specific binding to pulmonary 2 receptors, which was displaceable by fenoterol (50% of the baseline uptake). These *in vivo* PET results correlated well with the *ex vivo* measurements of the explanted lungs: 1.16 0.05% %ID/g baseline vs. 0.42 0.07 %ID/g displacement. Further evaluations with enantiomerically pure [^{18}F]FEFE are planned to elucidate its use in scientific and clinical studies using quantitative PET.

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SYNTHESIS OF [^{18}F]FLUORINATED a-METHYL-a-AMINO ACIDS BY PHASE-TRANSFER CATALYSIS FOR POTENTIAL PET APPLICATION

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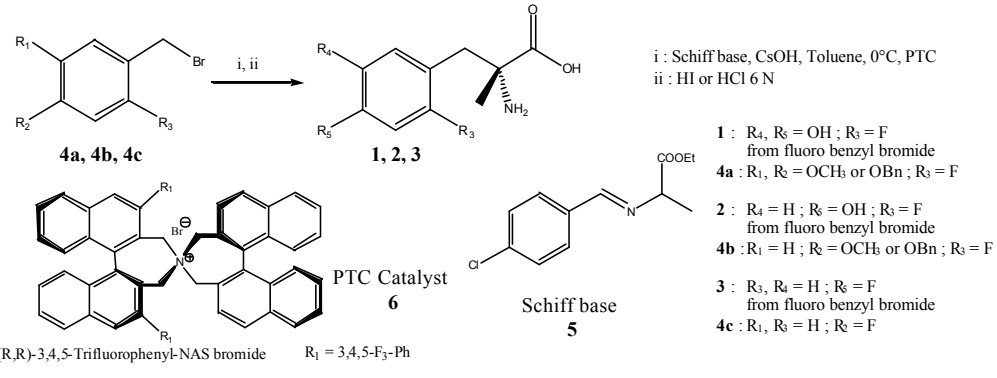
Keywords: -Methyl- -Amino Acids, PET, [^{18}F]fluoride, PTC, Enantioselective Alkylation

[^{18}F]Fluorinated -amino acids are known as potential marker for human investigation by positron emission tomography (PET). For example, 6-[^{18}F]fluoro-L-dopa and 2-[^{18}F]fluoro-L-tyrosine are used for dopaminergic system and protein synthesis studies respectively. Syntheses of these compounds were previously described in our laboratory[(1)]. Using the same approach, the preparation of the corresponding -methyl amino acids (6-[^{18}F]fluoro- -methyldopa (**1**), 2-[^{18}F]fluoro- -methyltyrosine (**2**)) and 4-[^{18}F]fluoro- -methylphenylalanine (**3**) were investigated. In order to validate the radiochemical process, [^{19}F]fluorinated reference compounds were prepared by a multi-step approach from benzylic derivative(s) according to the following scheme.



In the first part of this process, [^{19}F] and [^{18}F] alkylating agents **4a**, **4b** and **4c** were synthesized with high yield as previously described[(1)]. The following step consists of a phase-transfer alkylation between an adequate Schiff base **5** (synthesized in our laboratory) and the fluoro benzyl bromide derivative in presence of the chiral phase transfer catalyst **6**[(2-3)] and a strong base (CsOH or 50% aqueous KOH). Comparatively to our previous procedure[(4)], alkylation step which proceeds at 0°C is nearly quantitative (> 99%) and easy to automated on our Zymark station. Starting from the other catalyst enantiomer ((S,S)-3,4,5-trifluorophenyl-NAS bromide) which is now commercially available, the other configuration of the alkylated product was obtained. The last step, before HPLC purification, is a global hydrolysis of the imine and the phenol(s) protecting group(s). In all cases, the L- or D-[^{18}F]fluoro amino acids were obtained with radiochemical yields (**1** : 20% ; **2** : 35% **3** : 45%, EOB corrected ; n=3) and high enantiomeric excesses (e.e. >97%) determinate by chiral HPLC.

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IN SITU SYNTHESIS OF 2-IODO-1-[^{18}F]FLUOROETHANE

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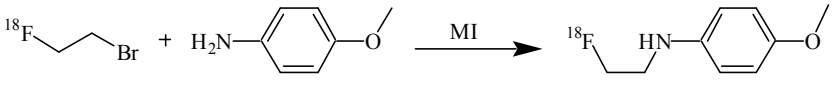
Keywords: ^{18}F-fluoroalkylation, 2-bromo-1-[^{18}F]fluoroethane

Introduction: ^{18}F-Alkylation is an alternative way to introduce a ^{18}F-fluorine label into interesting biomolecules. In comparison to the typical direct ^{18}F-fluorination this route is not adversly affected by acidic groups in the target molecule. The most important ^{18}F-fluoroalkylating agent is 2-[^{18}F]fluoroethyltosylate ([^{18}F]FETos), which was introduced by Block et al. [1], while 2-bromo-1-[^{18}F]fluoroethane ([^{18}F]BFE), firstly synthesized by Chi et al [2], is less commonly used. In this approach we wanted to increase the radiochemical yields of [^{18}F]BFE by an *in situ* formation of 2-iodo-1-[^{18}F]fluoroethane ([^{18}F]IFE).

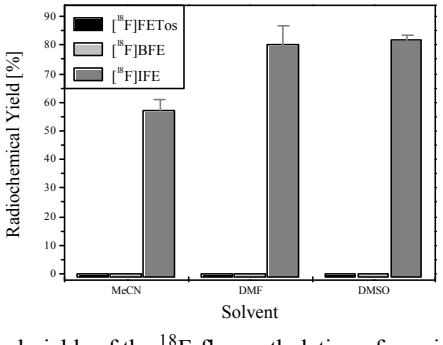
Results: In a systematic examination p-anisidine was alkylated via reaction with [^{18}F]FETos, [^{18}F]BFE and [^{18}F]IFE under identical conditions. [^{18}F]FETos and [^{18}F]BFE were prepared as described elsewhere [1,3], while [^{18}F]IFE was generated *in situ* by addition of [^{18}F]BFE to a solution of an iodine salt and the precursor (scheme 1).



M = Na; K; Cs

Scheme 1: ^{18}F-fluoroethylation of p-anisidine via *in situ* generated 2-iodo-1-[^{18}F]fluoroethane

We compared the radiochemical yields of [^{18}F]FETos, [^{18}F]BFE and [^{18}F]IFE under different reaction conditions, such as solvents, reaction temperatures and reaction times and in case of the [^{18}F]IFE using different iodine salts and varying their concentrations. Shown in scheme 2 are some of the results of the labeling of p-anisidine. As can be clearly seen the addition of NaI to [^{18}F]BFE improves the radiochemical yields up to 80%, while using the established agents [^{18}F]BFE and [^{18}F]FETos no yields were obtained



Scheme 2: Radiochemical yields of the ^{18}F-fluoroethylation of p-anisidine in different solvents (Conditions: 85°C; 3.5 mg/ml precursor; 1 ml solvent; formation of [^{18}F]IFE with 145 µmol NaI)

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A DAY AT THE RACES: THREE MICROWAVE CAVITIES MAKING FDG

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Keywords: microwave synthesis, FDG, PET

The synthesis of PET radiopharmaceuticals generally incorporates a sequence of steps involving heating for the azeotropic removal of water, incorporation of the label by the precursor compound and solvent evaporation. This generally employs oil baths, heating blocks, IR lamps, heated air jets or microwave heating. Heating by microwave corresponds to a constant power input, loosely analogous to designing electronic circuits with current sources. Three commercial, single-mode microwave systems were evaluated in the model reaction leading to 2-^{18}FDG, chosen for its obvious relevance and familiarity among PET chemists. The original (1) synthesis ($K222/K_2CO_3$ and acid hydrolysis) was used in order to exercise more reaction conditions, knowing full well that refinements such as base hydrolysis have increased yields in today's automated "boxes". This contest was intended to pit the three cavities against each other in a tightly constrained steeplechase of manual synthesis, rather than systems that have evolved for the commercial production of FDG.

Three jockeys rode three horses for three races before rotating, for a total of nine races involving 27 syntheses in an effort to uncouple operator skill from cavity performance. Each synthesis started out with 3 equal aliquots of 200 mCi of K222/K+ supported ^{18}F-fluoride from a common QMA trap and release column. Detectors continuously monitored the activity in the reaction vessel, and activity balance was assayed at each node point: end of azeotropic distillation, incorporation, C-18 Sep-pak trapping, dry down of the THF eluate, acid hydrolysis and final purification. The three microwave systems included a Labwell MicroWell 10 (2), an RII 520A (3) and a CEM Discover (4), with a cumulative experience base in our lab (5) of 10, 2 and 1/4 years, respectively. This experience base, in particular the approach to super-heated conditions, demands careful attention to power (transmitted and reflected), argon sweep gas rates, boiling chips, vessel geometry and close visual surveillance to guide a gentle crossing into the boiling state in open vessels. The open geometry of the RII Beenaker cavity offers this advantage, with the reaction vessel mounted on a vertical stage that allows instantaneous control over the RF coupling, and the potential for explosive bumping. As a consequence of this series, the other two units have since been fitted with similar adjustable vessel supports, and provided with illuminated CCTV surveillance of the reaction interior in an effort to level the playing field.

Analysis of the results teased apart the most successful horse, the most successful jockey and the most problematic hurdle. Clearly N=9 is insufficient to support an elaborate statistical inference, but two points stand out: experience has no substitute, and visual feedback is essential to achieve it; the close finish in most races, with yields of =60% EOB, point to a rate-limit that is not related to microwaves. This conservative conclusion belies the optimism that we feel for the future role of microwaves in the synthesis of PET tracers. Our experience with ^{18}F-FLT production confirms the advantages that microwave heating can afford in the research lab, even though designers of automated synthesis modules seem to prefer more conventional thermal sources.

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DETERIORATION OF F-18-FDG PRODUCT QUALITY CAUSED BY HEAT STERILIZATION IN THE CLOSED PRODUCT VIAL ?

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FDG, heat sterilization

Due to the drug laws and the GMP requirements in Germany, we recently had no other choice (besides rebuilding the complete laboratory) than to switch our F-18 FDG- production from terminal sterile filtration over to heat sterilization in the closed injection vial. Our total lab environment has been approved to be of class C (ISO class 7) in operation, which is sufficient only, if terminal sterilization is performed.

Various approaches have been made by others , and even a combined filling and sterilization system is available on the market. Due to room restrictions, we had to choose a stand-alone sterilization system comprising a heavily shielded small size conventional steam autoclave with forced cooling. This system was qualified by external measurements done at a specialized institute. . It operates at 134°C with a 5 min sterilization time. The system is preheated to 90°C, then the samples are introduced to fixed positions, and the process is started. Including cooling time, the process is finished in a still reasonable time of just under 20 min.

Quality control for radiochemical purity is done on a low volume parallel sample, which is treated in the same process. This approach was used to avoid spoiling the integrity of the batches for patient use by taking out analytic samples after sterilization. Standard analytical HPLC methods (Dionex PA10 column, 0,1n NaOH eluent) are used.

Initial validation experiments showed, that a new HPLC-peak appeared after sterilization. This peak is visible as a shoulder preceding the main product peak. Most reasonable and in conjunction with literature values this peak can be attributed to the isomeric F-18-Fluorodeoxymannose. The content can be roughly (due to the unresolved peak) estimated to be 5-8% of the product peak. This is still within the margins set by the monograph in the European Pharmacopeia , which states : FDM content should be less than 10% of the main product peak.

The pH of the solution had been adjusted to 5.5 by formulation with a phosphate buffer concentrate to avoid defluorination. Increase in Fluoride content after sterilization was not observed.

It remains unclear at the moment, whether the observed isomerization is only observed in the analytic sample due to the low volume (2 ml in a 25 ml vial) , which could lead to boiling effects or total vaporization of the sample during the sterilization process. These effects might not occur in the larger volume samples (7-8 ml in the 25 ml vial)

Experiments to compare the purity in the analytic and the patient sample are presently underway and will be presented.

AUTOMATED PRODUCTION OF [BR-76]NH_4BR AND SYNTHESIS OF [BR-76] RADIOPHARMACEUTICALS FOR PET STUDIES

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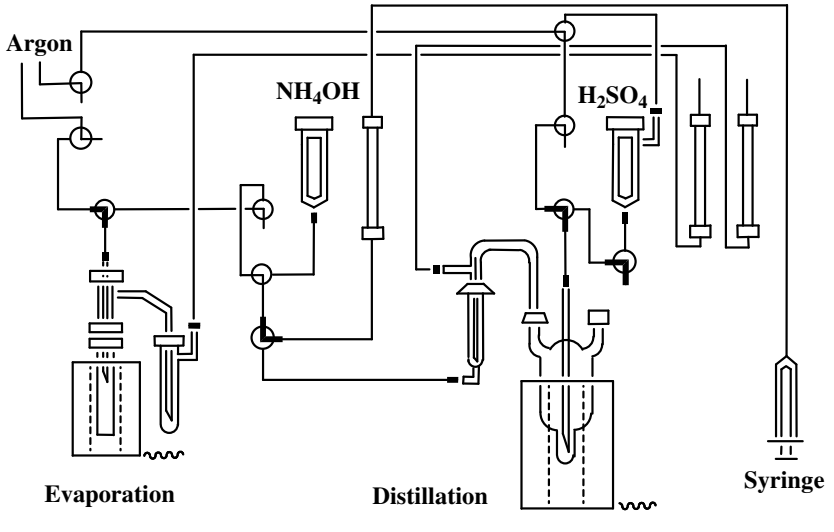
Key words: Br-76, [Br-76] ammonium bromide, [Br-76]FBAU, radioactive mixture, radioassay

Summary: Br-76 with a half life of 16.2h is ideal for PET studies not only of biochemical systems with the slow incorporation of radiotracers, but also those processes where the clearance of non-specific binding is slow. The goal of this work was to install a remote apparatus for the preparation of Br-76 ammonium bromide, suitable for the synthesis of Br-76 radiopharmaceuticals. Br-76 has been produced utilizing the As-75 (He-3,2n) Br-76 nuclear reaction. With a Br-76 production rate of 0.36 ± 0.08 mCi/μAh (25 preparations) we have routinely obtained 43 ± 5 mCi of Br-76 at end of bombardment (EOB) with a six-hour 20 μA particle beam irradiation. Irradiation of arsenic metal resulted in the production of 178 ± 12 % Br-75 ($T_{1/2}$ = 1.6h) and 0.82 ± 0.01 % Br-77 ($T_{1/2}$ = 57h) normalized to Br-76 at EOB (n=8).

[Br-76]NH_4Br was prepared using a remotely controlled automated module consisting of valves and solvent reservoirs, distillation and evaporation vessels as shown in **Figure 1**. The target material was heated at 140°C in an aqueous mixture of H_2SO_4 and ammonium dichromate. Under a flow of argon distilled bromide was trapped as the ammonium salt. [Br-76]NH_4Br was obtained in 78 ± 16 % yield after evaporation of the ammonium solution. For clinical studies [Br-76] 1-(2-fluoro-2-deoxy-β-D-arabanofuranosyl)-5-bromouracil ([Br-76]FBAU) was routinely prepared. Following HPLC purification and product formulation [Br-76]FBAU was obtained in 68 ± 7 % (n=20) radiochemical yield. A minimum of 3.8 ± 1 mCi of [Br-76]FBAU was available at EOS for clinical research studies.

Figure 1. [Br-76]NH_4Br Preparation Apparatus



USE OF 1,8-BIS-(DIMETHYLAMINO)-NAPHTALENE/ H^{18}F COMPLEX AS NEW RADIOFLUORINATING AGENT

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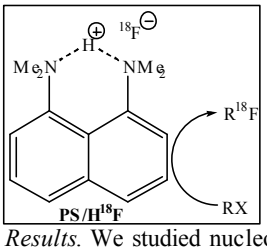
Keywords: Proton sponge, Radiofluorination, Nucleophilic substitution, PET



Introduction. Positron Emission Tomography (PET) is of high impact on both clinical and biomedical research. It mostly relies on radiotracers labeled with positron emitting short-lived radionuclides, such as ^{18}F ($T_{1/2}$ 109 min). New methods for the introduction of ^{18}F atoms into bioactive molecules may expand the types of radiopharmaceuticals available for PET applications. We have explored the use of the complex between 1,8-bis(dimethylamino)-naphthalene (Proton Sponge, **PS**) and H^{18}F in nucleophilic substitution reactions.

Results. We studied nucleophilic substitution on a variety of halogenated aromatic model compounds and a few aliphatic tosylates (OTs) and mesylates (OMs), using both non-radioactive HF and H^{18}F. The following reaction parameters were considered: dehydration of fluoride, stoichiometry, reaction time, solvents (including ionic liquids), and heating conditions (thermal, microwave). GC-MS yields using non-radioactive aqueous hydrogen fluoride were generally very low for aromatic substrates and no fluorination was observed in aliphatic ones. **PS** Nucleophilic aromatic reactions with ^{18}F afforded the labeled products in modest to excellent yields. We optimized reaction conditions for 3 aromatic substrates, 2-chloro-3-nitropyridine (**CNP**), pentamethylbenzyl 4-fluoro-3-nitrobenzate (**FNE**), and 5-chloro-1-phenyl-1H-tetrazole (**CPT**). The optimized conditions require the addition of aqueous ^{18}F (5 to 100 µL) to 7-8 mg of PS in a 1 mL V-vial followed by azeotropic removal of water. Substrate (3-4 mg, 10-20 µmoles) was added followed by addition of 50 µL of 10% H_2O in CH_3CN. The vial was sealed and thermally heated at 100°C for 30 min. The solution recovered by rinsing the vial with CH_3CN was purified by HPLC and the products collected and assayed. Isolated radiochemical yields (decay corrected) are based on initial ^{18}F radioactivity.

Substrate	Product	PS Average Yield	K_{222} Yield
CNP	2-[^{18}F]fluoro-3-nitropyridine	30% (22% to 34% n=8)	< 10%
FNE	pentamethylbenzyl 4-[^{18}F]fluoro-3-nitrobenzoate	70% (56% to 88% n=7)	80%
CPT	[^{18}F]-5-fluoro-1-phenyl-1H-tetrazole	35% (28% to 54% n=7)	< 10%

Ionic liquids (a new class of high boiling solvents) were investigated as a co-solvent in CH_3CN, but no yield enhancements could be obtained. Microwave heating resulted in lower labeling yields even though maximum yield was obtained at shorter times. For comparison purposes, the same substrates were radiofluorinated using the conventional conditions (K_2CO_3/K_{222} in acetonitrile); this latter method gave similar yield to the **PS** procedure on **FNE** but lower (<10%) yields on **CNP** and **CPT**.

Conclusion. PS/H^{18}F reagent allows efficient ^{18}F incorporation into **CNP**, **FNE** and **CPT**. It may be superior to K_{222}/K_2CO_3 suggesting that it may prove useful in nucleophilic aromatic radiofluorination.

DEVELOPMENT OF FULLY AUTOMATED MODULE FOR NUCLEOPHILIC ASYMMETRIC SYNTHESIS OF 6-[^{18}F]FLUORO-L-DOPA.

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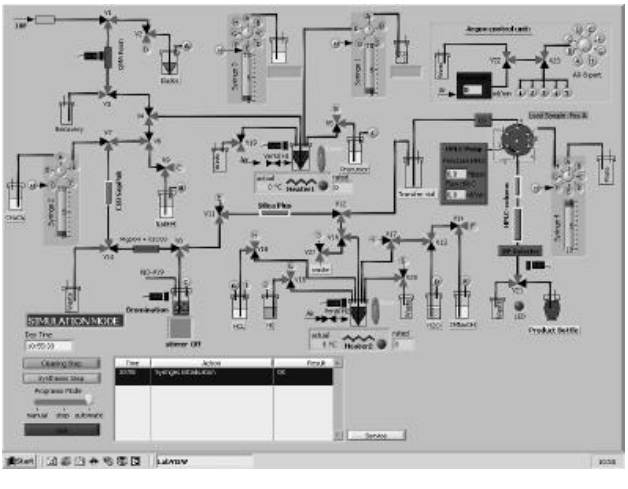
Keywords: automation, 6-[^{18}F]fluoro-L-DOPA, asymmetric synthesis, fluorine-18.

In the last few years considerable efforts have been devoted to automate radiolabeling procedures for the most important PET radiotracers. An automated production of 6-[^{18}F]fluoro-L-DOPA (FDOPA) via an electrophilic process is well established, however the radioactivity obtained is not high enough to fulfill a growing clinical demands. Therefore the development of automated apparatus based on nucleophilic fluorination procedure with higher starting activity of [^{18}F]fluoride is needed. Recent progress in asymmetric synthesis of FDOPA using new stable chiral auxiliaries [1] or phase transfer catalysts [2,3] has opened new opportunity for automation. Here a key chiral alkylation step proceeds under mild conditions without necessity to use strong bases (BuLi) and low temperatures obligatory for chiral glycine enolates.

Based on this approach we have designed a fully automated self-cleaning FDOPA module operated by LabView (National Instruments) as shown on the Fig. All the main components (radiodetectors, heaters, cooling guns etc.) are commercially available and easy to be replaced. Transfer of liquids was performed either by gas flow using a web of Burkert Compromatic valves or by four automated Kloehn syringes equipped with multi port valves. Using this technical decision we were able to clean and dry all the lines by repetitive rinsing followed by flushing under heating of reactive vessels. Careful cleaning was extremely important for successful performance of the reactions sensitive to presence of water: nucleophilic fluorination of 6-nitropiperonal (**I**) in DMSO and bromination of 6-[^{18}F]fluoropiperonyl alcohol (**II**) with Ph_3PBr_2 [1]. Finally, we achieved high incorporation rate of ^{18}F into (**I**) (65±3 %, n=11) and more than 97% of transformation of (**II**) into 6-[^{18}F]fluoropiperonyl bromide. Using Ni (II) complex of a Schiff base of (S)-O-N-[(N'-benzylprolyl)amino]benzophenone and glycine [1] as a chiral inductor we obtained FDOPA in a high enantiomeric purity (>95%) under mild alkylation conditions (5 min, 40°C, CH_2Cl_2). Deprotection was the most difficult for an automation. To prevent evolving of an aggressive hydroiodic acid, reaction mixture in Heater 2 was equally pressurized from five gas inputs. At present the newly build module is under final testing. The apparatus can be applied in a flexible manner to operate complicated synthesis of other radiotracers via [^{18}F]fluorobenzylation.



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ISOTOPIC $^{18}F/^{19}F$ EXCHANGE IN THE FLUMAZENIL MOLECULE USING $K^{18}F$/KRYPTOFIX COMPLEX

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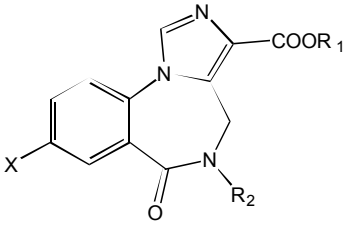
Keywords: isotopic exchange, nucleophilic fluorination, fluorine-18, flumazenil

At present there is a growing interest to ^{18}F-labelled analogues of well known PET radiotracers: due to a longer half-life of ^{18}F they may distributed similarly to FDG. [^{11}C]Flumazenil (Ro 15-1788, (**I**)) is the best radioligand for a PET quantitation of central benzodiazepine receptors. Two ^{18}F-labelled derivatives of flumazenil (**II**, **III**) have been prepared, however they may have a less favourable metabolism (1). The presence of fluorine atom in the molecule of (**I**) is a great challenge to introduce F-18 label into its "natural" position. First attempts to prepare [^{18}F]flumazenil (**IV**) by nucleophilic exchange either with ^{19}F or nitro- leaving groups have shown that special instant fluorination technique had to be applied to achieve a reasonable ^{18}F-incorporation rate into flumazenil (2).



$R_1=C_2H_5$, $R_2=^{11}CH_3$, X=F (**I**)
$R_1=C_2H_5$, $R_2= C_2H_4{}^{18}F$, X=F (**II**)
$R_1= C_2H_4{}^{18}F$, $R_2=CH_3$, X=F (**III**)
$R_1=C_2H_5$, $R_2=CH_3$, X= ^{18}F (**IV**)

As a first approach we aimed to label flumazenil via a ^{18}F for ^{19}F exchange using standard fluorination procedure (3). The [^{18}F]fluoride was eluted from the QMA resin by solution of K2.2.2 and K_2CO_3 in MeCN/H_2O (96/4). After evaporation of solvents 1-2 mg of flumazenil in dry DMSO was added. Reaction was performed under conditions shown in the table. To measure ^{18}F-incorporation rate, a sample of diluted reaction mixture was spotted on the TLC. The identity of **IV** was confirmed by reverse phase, straight phase and cationic HPLC compared to authentic sample.

Molar ratio [K2.2.2/K_2CO_3]/flumazenil	Reaction temperature, °C	^{18}F-incorporation rate, % (from TLC data)	^{18}F-labelled by-products, %
1/1	100	25	
1/1	130*)	62±8 (n=8)	3-4
1/1	160	57,5±0,5 (n=2)	10
1/1	180	68±1 (n=2)	10-15
1/2	130	45±7 (n=3)	3-4
2/1	130	41±2 (n=2)	15-20
4/1	130	16	>50

We found that the most important factor responsible for the fluorination yield belonged to a molar ratio of flumazenil to [K2.2.2/K_2CO_3]. While the high ^{18}F-incorporation rate was achieved with an equimolar ratio, it dropped dramatically under ratio of 4/1. This observation can be explained by instability of flumazenil under basic conditions. In "cold" experiments flumazenil was rapidly degraded in DMSO in the presence of the excess of [K2.2.2/K_2CO_3] under 130-160°C. The increase of reaction temperature over 130°C had no beneficial effect on the performance of the ^{18}F-fluorination, but results in the rise of labeled by-products. Finally, **IV** was purified by standard C18 SepPak procedure, eluted from the cartridge by CH_2Cl_2 and analyzed after evaporation of the solvent. Radiochemical purity was more than 97%, total radiochemical yield (EOB, 60 min synthesis time) was 47±3%, n=5*). Our next step will be to apply this synthetic procedure to a labeling of nitro precursor. We wish to thank Dr. A.D. Windhorst for the discussion of our results.

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RADIOLABELING OF GINKGOLIDE B WITH ^{18}F AND ^{3}H

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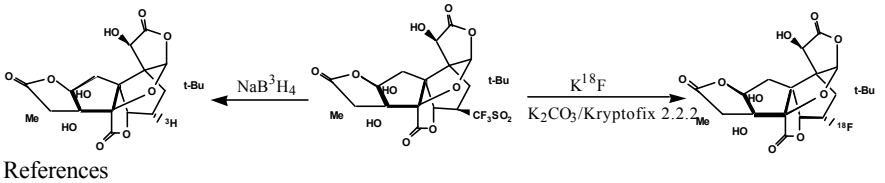
Keywords: ^{18}F-ginkgolide B, PET, ^{3}H-ginkgolide B

Extracts from the Ginkgo tree (*Ginkgo biloba* L.) have been widely utilized as a complementary medication with therapeutic values for various mental diseases such as Alzheimer's disease, dementia, depression and others (1). The well-documented beneficial effects of the *Gingko biloba* extract are believed to be derived from its constituents flavonoids and terpene trilactones, ginkgolides and bilobalide. However, the mechanisms of their bioactivities are not well understood. Mapping action sites of *Gingko biloba* components using their radiolabeled analogs and imaging techniques such as positron emission tomography (PET) and autoradiography will provide unique and valuable information regarding their roles in the brain in both physiological and pathological conditions. Based on this notion, we synthesized an ^{18}F-labeled fluoro analog of ginkgolide B. The radiosynthesis of 7-[^{18}F]fluoro-ginkgolide B was performed via nucleophilic displacement of the triflate leaving group at C7 with [^{18}F]fluoride. The reaction which was carried out in DMF at 120°C in the presence of Kryptofix 2.2.2. proceeded efficiently, and in 10 minutes approx. 18.4±5.8% (n=5) of the radioactivity was incorporated into the ginkgolide B structure. The product was isolated by C-18 semi-preparative HPLC and concentrated with a C-18 Sep-Pak. The radiochemical yield calculated from the radioactivity of the product in the final bottle ready for injection and the decay corrected initial activity was approx. 15.7±6.3% (n=5) with an overall synthesis time including HPLC purification and formulation of 2.3 hours. The specific activity at E.O.S. was approx. 1.1±0.2 Ci/μmole (n=3).

Starting with the same 7-O-trflate precursor, we also labeled ginkgolide B with ^{3}H with a view toward visualization of ginkgolide B action sites by *in vitro* and *ex vivo* autoradiography. 7-[^{3}H]-ginkgolide B was synthesized, according to the literature procedure (2) with modifications, by reacting the precursor with $NaB[^{3}H]H_4$ (100 mCi/μmole) in THF for 2 hours at room temperature and purified by C-18 semi-preparative HPLC. The yield was 5.8% and the specific activity was 19 mCi/μmole. In place of $NaB[^{3}H]H_4$, we also tried $Bu_4NB[^{3}H]H_4$ prepared from $NaB[^{3}H]H_4$ (85 mCi/μmole) and ^{3}H-water. However, the reduction reaction yielded 7-[^{3}H]-ginkgolide B in low specific activity (3.8 mCi/μmole).



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A "ONE-POT" SYNTHESIS OF [^{18}F]FECNT AND ITS IMPLEMENTATION IN A COMMERCIAL AUTOMATED RADIOSYNTHESIS APPARATUS

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Keywords: Fluorine-18, FECNT, Dopamine transporter, Automation

Introduction: ^{18}F-Labeled 2β-carbomethoxy-3β-(4-chlorophenyl)-8-(2-fluoroethyl)-nortropane (FECNT) is a highly effective PET radioligand for the brain dopamine transporter (1). We required our future regular production of [^{18}F]FECNT to be from a commercially available automated system, such as the GE TRACERlab FX_{F-N} (FN) '[^{18}F]nucleophilic substitution module', now offered by GE Medical Systems. However, the original literature radiosynthesis of [^{18}F]FECNT (1) has two steps intermixed with purification procedures that are not easy to integrate within the single-reactor FN apparatus. Thus, we made efforts to design a simplified radiosynthetic procedure and to modify the FN apparatus for automated production of [^{18}F]FECNT, as described here.

Experimental: Two main modifications were made to the FN apparatus: 1) remote introduction of an [^{18}F]fluoride/Kryptofix 2.2.2/K_2CO_3 solution, and 2) remote loading of the HPLC injector and bypass of the existing flow detector-auto-injector. [^{18}F]Fluoride was dissolved with Kryptofix 2.2.2 (5 mg, 13.3 μmol) and K_2CO_3 (0.5 mg, 3.6 μmol) in MeCN-water (95: 5 v/v; 0.1 mL) and dried azeotropically by alternating evaporations and additions of MeCN. Ethylene glycol *bis*-tosylate (2.0 mg, 5.4 μmol) in MeCN (250 μL) was then added and the closed reaction vessel heated at 110 °C for 10 min to generate [^{18}F]2-fluoroethyl tosylate ([^{18}F]F$(CH_2)_2$OTs) (2). *Des-fluoroethyl*-FECNT (2 mg, 7.2 μmol) in MeCN (250 μL) was added to the reaction mixture, which was then concentrated to ~ 50 μL. This residue was heated at 135 °C for 45 min and then diluted one third with water for direct injection onto a HPLC column (Luna C-18; 10 x 250 mm; Phenomenex) eluted with MeCN-10 mM ammonium formate (gradient 40: 60 to 60: 40 v/v; pH 6.0) at 6.0 mL/min, with eluate monitored for absorbance at 229 nm and radioactivity. The radioactive fraction with the same retention time as reference FECNT (12 min) was collected. The collected fraction was analyzed on a Luna column (4.6 x 250 mm; Phenomenex) eluted with MeCN-0.01% H_3PO_4 (35: 65 v/v; pH 3.0) at 1.5 mL/min (FECNT retention time: 6.5 min). Radioactivity and absorbance (220 nm) were monitored to confirm the radiochemical and chemical purity of the [^{18}F]FECNT and to measure its specific radioactivity. Formulation of [^{18}F]FECNT in saline (0.9% w/v; 0.5 mL) and Tween 80 (~ 25 μL) provided a concentrated solution (2–18.4 mCi/mL) for mouse and rat PET experiments.

Results: A "one-pot" radiosynthesis of [^{18}F]FECNT was developed experimentally and gave radiochemical yields ranging from 26–51% (mean 35%, n = 14), corrected to start of radiosynthesis, and in > 99% radiochemical purity. The subsequent application of the experimental procedure to the FN apparatus afforded [^{18}F]FECNT in 12–46% (mean 32%, n = 17) radiochemical yields, corrected to the start of synthesis, and 5–21% (mean 11%, n = 17) at the end of synthesis (EOS), with a radiochemical purity of > 99%. Specific radioactivities ranged between 2.3–6.8 Ci/μmol at EOS. Improvements to the FN apparatus allowed reliable and consistent introduction of [^{18}F]fluoride solution and transfer of the crude reaction mixture into the HPLC injector.

Conclusions: An automated one-pot radiosynthesis of [^{18}F]FECNT has been accomplished in the FN apparatus. The improvements to the FN apparatus are potentially applicable to the radiosynthesis of many other radiopharmaceuticals from [^{18}F]F$(CH_2)_2$OTs by similar methodology.

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METHODICAL STUDY OF DIRECT FLUORINATION USING [^{18}F]F_2 IN PRESENCE OF AMIDES AND IMIDES

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Keywords: Direct Fluorination, Amides, Imides

An increasing variety of ^{18}F-labelled structures which not necessarily need to be prepared at high specific radioactivity lead us to investigate formally electrophilic labelling procedures using [^{18}F]F_2 in presence of amides and imides. A remarkable selectivity of some of the N-fluoro intermediates involved could be observed especially when equimolar ratios of reactants were applied. This selectivity is supposed to be associated with a formal redox exchange mechanism between the >N-F reagent and the substrate allowing to predict their reactivity from the estimated charge distribution between the >N-F compound and a partially negatively charged C-atom of more or less sp^2 character.

Various amides and imides were reacted with [^{18}F]F_2 mostly in Freon-11 at low temperatures (-40 ^{o}C). Experiments were done within an automated synthesis module originally intended to prepare dedicated compounds from stannylated substrates by introducing [^{18}F]F_2. The module itself as assembled by *raytest* has been found to be suited for variety of procedures consuming [^{18}F]F_2 under controlled temperature and allowed us easily to handle all experiments by download of dedicated synthesis control scripts from the default synthesis control program. The resulting labelled N-[^{18}F]F-products were isolated when possible, or *in-situ* mixed with the respective electron-rich functional carbon atom bearing substrate. The study included known N-fluoro compounds like N-fluoro-*bis*-trifluormethanesulfonimide, N-fluoro-*ortho*-benzodisulfon-amide, N-fluoro-2,3-dihydro-1,2-benzothiazol-1,1-dioxide, N-fluoropyridone, N-fluoropyridinium salts and N-fluorocaprolactame. The novel derivatives, N-fluoro-N-trifluormethyltrifluormethansulfonamide and N-fluorotrifluoromethanesulfonamide, were obtained during fluorination of *bis*-trifluormethanesulfonimide through an unexpected rearrangement.

Successful substrate/reagent combinations for the *in-situ* redox exchange reaction could be identified and are presented in detail. However, the feasibility of >N-F compounds for labelling reactions could not be proved for once. The proposed selectivity of fluorine transfer from >N-F compounds to the functionalized carbon atom seemed to be rather precedent. No significant advantage was observed as compared to the direct addition of [^{18}F]F_2. It seemed that the functionalized carbon atom of the target compound going to be fluorinated exhibited the sensitive and directing driving force in total. The >N-F reagent itself has been found to have almost no influence. In worst cases it prevented the course of the reaction completely by its surprisingly high own chemical stability and virtual inertness.

RAPID SYNTHESIS OF 2-[^{18}F]FDG BY ELIMINATING EVAPORATION STEP

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Keywords: 2-[^{18}F]FDG, Fluorination, Ionic liquid, alkaline hydrolysis

2-[^{18}F]FDG (2-[^{18}F]fluoro-2-deoxyglucose) is the most widely used radiopharmaceutical for positron emission tomography (PET). The most common method to synthesize it utilizes nucleophilic substitution reaction by activated [^{18}F]fluoride to a precursor tetraacetylmannosetriflate [1]. Recently, many interesting researches to ionic liquid have been reported in physical and organic chemistry field. And its usefulness of strengthening the nucleophilicity of weak nucleophiles like fluoride even under hydrous condition has also been reported [2]. The fluorination of organic compound under hydrous condition has a great importance in PET chemistry because labeling time can be shortened by eliminating time-consuming evaporation steps. We have investigated the synthesis of 2-[^{18}F]FDG using ionic liquid, 1-butyl-3-methylimidazolium trifluoromethanesulfonate, in hydrous condition in this study.

[^{18}F]Fluoride was produced by irradiating ^{18}O-water with 13 MeV proton beam. The produced [^{18}F]fluoride was captured on a light QMA SepPak cartridge and eluted by the mixture of 70 l aqueous tetrabutylammonium bicarbonate (40%) and 500 l acetonitrile. The percentage of eluted [^{18}F]fluoride was 93.3 3.0 (n=3). To the eluted [^{18}F]fluoride (3.6-19.9 mCi), 20 mg tetraacetylmannosetriflate, 300 l ionic liquid and 1,000 l acetonitrile were added and reacted for 5 min at 110 C with nitrogen bubbling. The reaction mixture was diluted with 10 ml 0.1 *M* NaH_2PO_4(aq) and passed through the two ^{t}C18 SepPak cartridges. The cartridges were washed with 10 ml saline and filled with 800 l 2 *M* NaOH(aq) for hydrolysis. After 2-5 min, the hydrolyzed product was eluted by 5 ml water (2 times) through IC-H, neutral alumina SepPak and another ^{t}C18 SepPak cartridges and GS filter.

The ^{18}F-labeling efficiency was 80.3 5.3% (n=5). The [^{18}F]fluorinated intermediate (tetraacetyl-2-[^{18}F]fluorodeoxyglucose) was captured on tC18 SepPak cartridges to eliminate ionic liquid, acetonitrile, tetrabutylammonium bicarbonate, and other contaminants and to carry out solid-phase alkaline hydrolysis for its usefulness of short-time process at room temparature without any epimerization [3, 4]. The pH of produced 2-[^{18}F]FDG solution was about 7. The radiochemical yield was 62.2 6.3% (decay-corrected) and the radiochemical purity was 93.3 3.4% (n=5). The synthesis time was about 11-14 min.

The [^{18}F]fluorination in the presence of ionic liquid is a powerful method in the synthesis of 2-[^{18}F]FDG because it saves synthesis time and increases the labeling efficiency and consequently it can make improved yield of the [^{18}F]fluorine labeled radiopharmaceuticals.

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FACTORS AFFECTING THE SPECIFIC ACTIVITY OF [^{18}]FLUORIDE FROM A WATER TARGET

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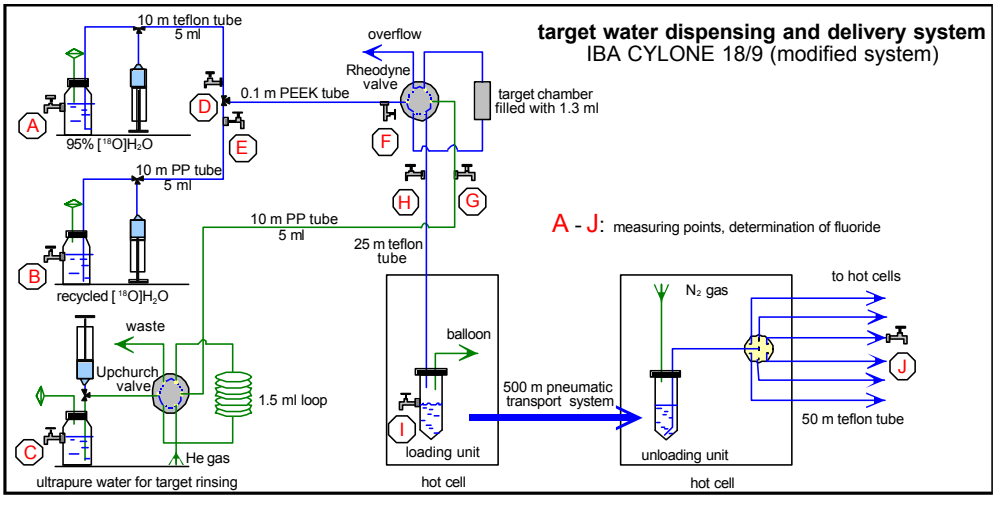
Keywords: [^{18}O]water target, production of ^{18}F, [^{18}F]fluoride, specific activity

The reaction $^{18}O(p,n)^{18}F$ is the method of choice for routine production of n.c.a. [^{18}F]fluoride to synthesize labelled compounds as well as on high activity level and with high specific activity (SA). For quite a few PET radiopharmaceuticals the SA has to be high in order to prevent pharmacological effects at the studied system (e.g. agonistic and toxic effects). We found that the SA shows considerable variation, as also reported in the literature. The SA is an important quality parameter in accordance with the GMP rules and should be reproducible.

The SA of the radiopharmaceutical mainly depends on the SA of the [^{18}F]fluoride used for the syntheses. Initial investigations indicated that the main origin of ^{19}F is the target dispensing and delivery system (see scheme) and is not originated from the starting [^{18}O]water. Detailed investigations were carried out to determine the sources for the ^{19}F.

The table shows the partial contribution of the different constructive parts of the target system to the [^{19}F]fluoride amount. A significant contribution of the irradiation process to the [^{19}F]fluoride amount was not found. The TEFLON tube of the dispensing system (between A and D) is the main origin of [^{19}F]fluoride. The [^{19}F]fluoride amount depends mainly on the radiation dose the tube gets near the target/cyclotron and on the water/tube contact time.

The results will be presented in detail.



sample	95% [^{18}O]H_2O					
	A	D	F	H	I	J
[^{19}F]fluoride, nMol	0.53±0.13	0.5 - 380	0.3 – 17.2	0.8 – 29.8	-	-
sample	recycled [^{18}O]H_2O					
	B	E	F	H	I	J
[^{19}F]fluoride, nMol	0,58±0.34	0.5 – 4.2	0.5 – 6.5	0.9 – 28,5	-	-
sample	ultra pure water					
	C		G	H	I	J
[^{19}F]fluoride, nMol	0.1±0.1		0.1 – 0.8	0.1 – 6.4	2.2 – 20.5	5.3 – 10,6

SYNTHESIS OF [^{18}F]TRIFLUOROMETHANE AND [^{18}F]TRIFLUOROMETHYL BROMIDE

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Keywords: [^{18}F]trifluoromethyl, specific activity

The synthesis of compounds containing a trifluoromethyl group is an active area of organic synthesis development due to their importance in pharmaceutical design based on structure-activity relationship. There is a variety of methods for selective introduction of a trifluoromethyl substituent into organic compounds. Introduction of the [^{18}F]trifluoromethyl group into organic molecules has, however, been unsuccessful in terms of high specific activity. It has been proposed that isotopic dilution originates from superconjugation in case of aromatic substrates (1-4). In esters this has been explained by solvolysis of precursor with subsequent isotopic exchange (5). Fluorine containing alkanes and ethers as well as sulfonic esters have also been proposed to drop F^-, via an carbanion intermediate leading to alkene formation and therefore to isotopic exchange (6,7). These observations, the lack of availability of the high specific activity [^{18}F]trifluorinated building blocks and our broad interest in fluorination chemistry inspired us to explore the synthesis of [^{18}F]trifluoromethyl derivatives.

The reaction of [^{18}F]F^- with dibromodifluoromethane in various solvents yielded [^{18}F]trifluoromethane and [^{18}F]trifluoromethyl bromide in different ratios (Scheme 1).

Scheme 1

$$CBr_2F_2 \xrightarrow[\text{Solvent, 25-110°C}]{[^{18}F]F^-/K_{222}/K_2CO_3} [^{18}F]CHF_3 + [^{18}F]CBrF_3$$

$(CH_3CN, DMF, CCl_4 \text{ or } CBr_2F_2)$

Depending on solvent and temperature the radiochemical yield was 2-40 % (EOS). [^{18}F]trifluoromethane was always the major product. However, when dibromodifluoromethane acted both as precursor and solvent both products were formed almost in equal amounts.

The specific activity of both products was found to be low (in the order of 100 MBq/μmol). The effect of solvent and temperature on the specific activity was slight. Purification of the precursor by distillation from toluene/Na solution did not have an substantial influence to the specific activity. Reduction of the precursor amount, as indicated in earlier studies, leads to an 8 fold increase in the specific activity. This however also leads to reduction in the radiolabelling yield.

These results can not be explained by superconjugation or base promoted anion formation. Solvation to form a intermediate carbocation and F^- is likely to be an explanation to these results. At this point however the reaction mechanism leading to formation of [^{18}F]trifluoromethane is not known.

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A NEW CONTINUOUS LARGE SCALE METHOD FOR PROTEIN LABELING

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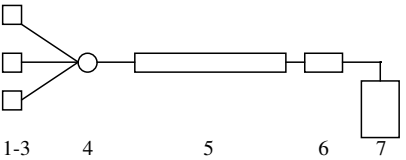
Keywords: Antibodies, Iodination, Chloramine-T, Iodine-131, Radioimmunotherapy

Our goal was to develop a large scale radiolabeling method designed for the pharmaceutical industry. The method has to be able to radiolabel proteins with several GBq in a short production time. The most critical limiting factors for the labeling batch size are the total activity, radiolysis of the product and the volume of the labeling batch.

The new approach to avoid these difficulties was to label the batch of material in a continuous manner with only a small portion of the protein, radiation and oxidation agent mixed together at any given time. The labeling apparatus consists of three different processing segments. The first segment (1-3) of the process utilizes three separate reaction component reservoirs containing: Protein, Chloramine-T and Iodine-131 solution in a local lead shielding. The second segment (4-5) consists of tubing that leads from each of the reagent reservoirs into a single mixing tube where the labeling reaction takes place. The third segment (6-7) of the process consists of an inline purification column and collection device.

Scheme



1-3 Educt Vials
4 Pump
5 Reaction Tubing
6 Anion Exchanger
7 Collecting Vial

In the second segment of the labelling apparatus, the educts from the reagent reservoirs lead into the reaction tube with a three channel peristaltic pump. The reaction time is simple to regulate with the length of the reaction tube and with the pump flow. The free Iodine and oxidation agent can be removed from the process by an in line purification column which is mounted at the end of the reaction tube. The labeled protein solution is collected in a vial already containing stabilizers and additives required for the final formulation.

We have used this method to radiolabel batch sizes up to 487 GBq of I-131 bound to a monoclonal antibody. The entire processing took approximately 40 minutes including reaction time, purification and final formulation with a total yield of 467 GBq. The results of the large scale production are presented in the following table. The cell binding assays proved the high quality of the product.

Batch	Activity	Chloramin-T	Antibody	Total Vol.	Reaction Time	Yield		Free Iodine
	GBq	mg	mg	mL	Min.	GBq	%	%
A	**47.9**	1.8	60	3 x 3.3	10	**42.9**	**91**	**1.4**
B	**25.2**	0.9	30	3 x 3.3	10	**21.4**	**85**	**0.1**
C	**288.2**	25	450	3x20.0	10	**256.5**	**89**	**1.8**
D	**487.3**	30	1000	2x10 + 50	10	**466.8**	**96**	**2.1**

In conclusion the new inline labeling method allows the rapid large scale production of therapeutic antibody doses of high quality.

AN IMPROVED TOTAL SYNTHESIS OF PET HSV-TK GENE REPORTER PROBE [^{18}F]FHPG

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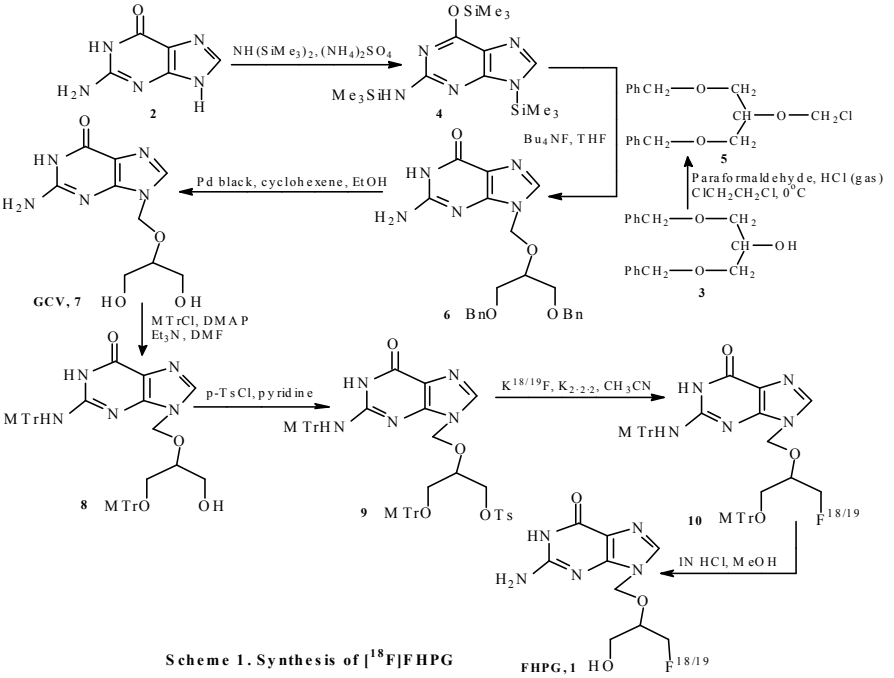
Keywords: HSV-tk gene; reporter probe; positron emission tomography; [^{18}F]FHPG; total synthesis

The objective of this study was to synthesize PET gene reporter probes such as fluorine-18 labeled penciclovir (PCV, 9-[4-hydroxy-3(hydroxymethyl)butyl]guanine) and ganciclovir (GCV, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine) analogs 8-[^{18}F]fluoropenciclovir ([^{18}F]FPCV), 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]FHBG); 8-[^{18}F]fluoroganciclovir ([^{18}F]FGCV), 9-[(3-[^{18}F]fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG) for *in vivo* imaging of herpes simplex virus thymidine kinase (HSV-tk) expression. In our previous work, we have reported an improved total synthesis of [^{18}F]FHBG. In this ongoing study, we report an improved total synthesis of [^{18}F]FHPG starting from 1,3-dibenzyloxy-2-propanol and guanine (**Scheme 1**). The key intermediate GCV was synthesized from these two starting materials in 4 steps with 16% overall chemical yield. The unlabeled standard sample 9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]guanine (FHPG) was synthesized from GCV in 4 steps with 5% overall chemical yields. The tosylated precursor N^2-(*p*-anisyldiphenylmethyl)-9-[((1-anisyldiphenylmethoxy)-3-tosyl-2-propoxy)methyl]guanine was prepared from GCV in 2 steps with 18% overall chemical yield for radiolabeling. The target radiotracer [^{18}F]FHPG was prepared by nucleophilic substitution of the precursor with potassium [^{18}F]fluoride/Kryptofix 2.2.2 followed by a quick deprotection with 1 N HCl and purification with a simplified Silica Sep-Pak solid-phase extraction (SPE) method in 10-15% radiochemical yield, and 70 min synthesis time from end of bombardment (EOB).



Scheme 1. Synthesis of [^{18}F]FHPG

PRODUCTION OF F-18 FLUOROTHYMIDINE: SUBSTRATES AND YIELDS

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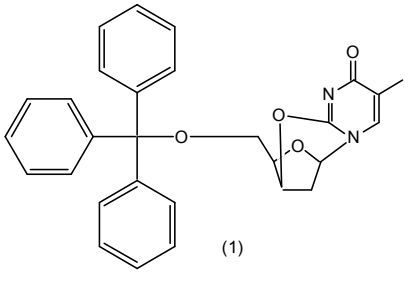
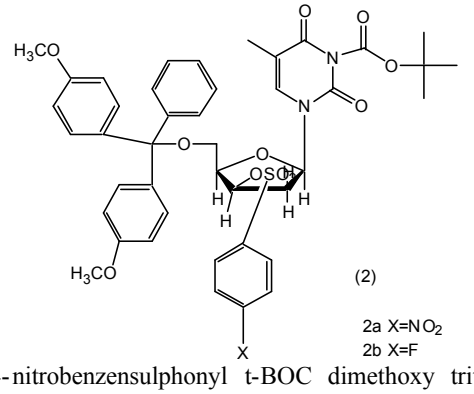
Key words: Fluorine-18, Fluorothymidine, aromatic fluorination, yields

There are a variety of different substrates available for the preparation of F-18 fluorothymidine (FLT). All of them work but none of them are really outstanding. All of the substrates have a leaving group in the *lyxo* configuration on the deoxy pentose and fluorination is via nucleophillic displacement of this leaving group with inversion to give the 3-deoxy-3-fluoro-pentose in the *ribo* configuration. The fundamental problem is that trans elimination from the 2-deoxy position is a favorable reaction under the basic conditions necessary for fluorination and this removes the starting material from the reaction.

We initially used the 3`-anhydro-5`-trityl thymidine derivative (1) as a substrate with low quantities of potassium bicarbonate (1 µmol) as a base. This gave fairly high fluorination yields, ~50% but was accompanied by the production of a volatile fluorine-18 derivative, boiling point below -100ºC. This species, which was due to the low base concentrations and not the substrate, was produced in increasing amounts in successive reactions. It has not been identified. Increasing the amount of potassium bicarbonate prevented the formation of this species but also dropped the fluorination yield to less favorable values, < 10%.



(1)



(2)

2a X=NO_2
2b X=F

With these results we switched to the 4-nitrobenzensulphonyl t-BOC dimethoxy trityl derivative (2). This compound has the advantage that it can be fluorinated in acetonitrile solution with milder conditions than is required by (1). However reaction of (2a) with ^{18}F potassium fluoride/Kryptofix produced two radioactive compounds and on removal of the protecting groups there is an anionic radioactive product that is not fluoride and appears to be 4-fluorobenzene sulphonic acid. We did a macroscopic reaction with ^{19}F fluoride and ran a fluorine NMR on the total reaction mixture. There were two organic fluorine signals in the spectrum, one at -172 ppm that is expected for the 3-deoxy-3-fluorothymidine derivative and a second signal -139 ppm. This is the expected chemical shift for aromatic fluorine and has the expected coupling for a 4-substituted fluorobenzene. There appears to be competition for the fluorination, either at the 3'-position of the ribose to give the required fluorothymidine derivative or at the aryl nitro group to give the 4-fluorobenzene sulphonate derivative (2b). We have not yet prepared an authentic sample of the 4-fluorobenzenesulphonate derivative (2b) to confirm that it is indeed being formed but it appears very likely.

Using 2a as the precursor we start with ~300 mCi's of fluoride and end up with ~20 mCi's of ^{18}F-FLT pure and ready for administration. The specific activity is > 1 Ci/micromole and the UV adsorption peak for FLT is the largest UV peak in the HPLC.

RADIOSYNTHESIS OF 7-[^{18}F]FLUOROINDOLE DERIVATIVES

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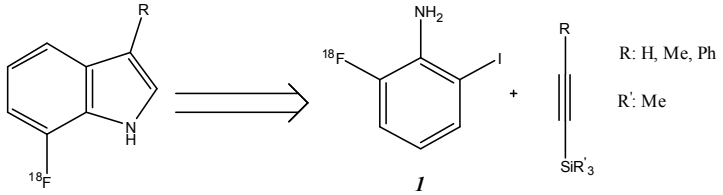
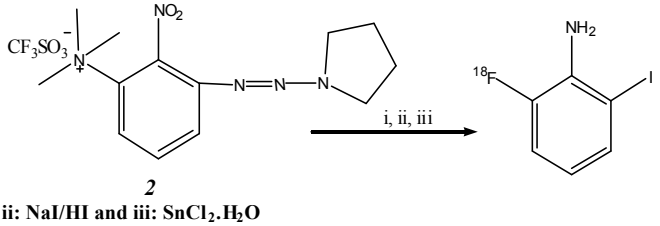
Keywords: Fluorine-18, Indole, Palladium

Although indole is one of the most important heterocycle ring, being involved in numerous critical biological compounds (for example: serotonin), radiochemists paid very little attention to it. A methodology for labeling 7-fluoro-3-substituted indoles from [^{18}F] fluoride was then developed. This strategy is based on a palladium catalyzed coupling reaction between 2-[^{18}F]fluoro-6-iodoaniline and alkynes.(1)



1

In the first approach, we tried to synthesize compound ***1*** through nucleophilic substitution of 2-chloro-6-iodo-nitrobenzene with [^{18}F]fluoride. Unfortunately, this way led to 2-[^{18}F]fluoro-nitrobenzene. The lost of iodine moiety during the labeling reaction prompted us to investigate another radiochemical way starting from compound ***2***.



2

i: [^{18}F]/F, ii: NaI/HI and iii: $SnCl_2.H_2O$

Labeling of the compound ***2*** gave the 2-[^{18}F]fluoro-6-iodoaniline in (25 8%) yield (corrected to EOB) in 85 min. In the cyclisation step, palladium catalyzed coupling reaction of the 2-[^{18}F]fluoro-6-iodoaniline with either trimethylsilylacetylene, 1-(trimethylsilyl)-1-propyne or 1-phenyl-2-(trimethylsilyl)acetylene, followed by protodesilylation under acidic conditions afforded respectively : 7-[^{18}F]fluoroindole (14 2%, n=9),7-[^{18}F]fluoro-3-methylindole (13 6%, n=9) and 7-[^{18}F]fluoro-3-phenylindole (21 1%, n=4).

To validate the radiochemical process, 2-fluoro-6-iodoaniline was synthesized from 2-fluoro-6-iodobenzoic acid (2) and the reference compounds were synthesized according to the radiochemical methodology.

Although the radiochemical yields obtained were low, this strategy showed the feasibility of building up the indole moiety bearing a labeled fluorine atom at position 7.

References:

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2.Stadlwieser J, Barbier P, Taylor, S. *J Helv Chim Acta* 1998; 81: 1088.