NEW METHODS FOR HALOGENATION OF [^{18}F]-FLUORINATED BENZYL DERIVATIVES

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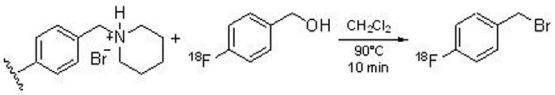
[^{18}F]fluorobenzyl halide derivatives are key intermediates for the synthesis of various radiopharmaceuticals such as 4-[^{18}F]fluoro-L-phenylalanine, 6-[^{18}F]fluoro-L-dopa or 2-[^{18}F]fluoro-L-tyrosine. These compounds are produced in our laboratory through a nca nucleophilic, multistep radiosynthesis approach [1]. So far gaseous HBr was used for the nucleophilic substitution of the [^{18}F]fluorobenzyl alcohol derivatives. Due to the fact that this reagent is quite cumbersome to handle and leads to poor yields for 4-[^{18}F]fluorobenzyl bromide, new strategies were investigated. In the present study, two new approaches for the preparation of this key substrate are reported.

(i) Bromination of 4-[^{18}F]fluorobenzyl alcohol with polymer-supported reagent.

The first method implies the preparation of a polymer-supported reagent which acts as an anhydrous HBr source [2].

This type of approach seems a good alternative to solve gaseous HBr automatisation's problem because HBr trapped on the resin is less toxic, more convenient to handle and can be easily removed after halogenation by filtration. N-piperidinoaminomethylpolystyrene hydrobromide resin is generated by bubbling gaseous HBr into a suspension of N-piperidinoaminomethylpolystyrene in diethylether at 0°C and proved to be stable for several weeks at 4°C.



N-Piperidinoaminomethylpolystyrene bromide

Halogenation of 4-[^{18}F]fluorobenzyl alcohol in dichloromethane affords the 4-[^{18}F]fluorobenzyl bromide with a radiochemical yield of 90 % (HPLC based on alcohol, corrected, n = 14) (90°C, 10 min.). The purity of the product obtained after filtration allows the following alkylation reaction leading after hydrolysis to 4-[^{18}F]fluoro-L-phenylalanine.

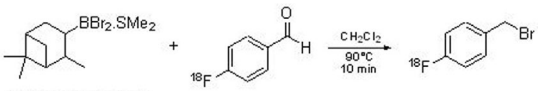
Experiments were also realized with a "homemade" cartridge of the resin, but lower yields were obtained.

2-[^{18}F]fluoro-4-methoxybenzyl alcohol, 6-[^{18}F]fluoro-3,4-dimethoxybenzyl alcohol and benzhydrol were also successfully transformed with this resin with the same yields.

(ii) Reductive bromination with alkylboron dibromides.

The second approach implies the reaction of an isopinocampheylboron-dibromide dimethylsulfide complex with 4-[^{18}F]fluorobenzaldehyde [3].

The isopinocampheyl group is one of the most effective for reducing carbonyl compounds via betahydrogen transfer reactions. The complex is generated by refluxing $HBBr_2(SMe_2)$ with α-pinene in dichloromethane for 3 h. The reductive halogenation proceeds in dichloromethane with 1 eq of complex (90°C, 10 min.) and leads to a radiochemical yield of 90 % (HPLC based on aldehyde, corrected, n = 9). In order to insure a smooth subsequent alkylation reaction, the crude 4-[^{18}F]fluorobenzyl bromide needed to be roughly purified with a simple "homemade" K_2CO_3 cartridge (~ 1 g).



isopinocampheylboron dibromide complex

Further optimizations for the two methods reported here are underway, so far the first one seems to be more interesting because it doesn't remain smelling by-products after purification.

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Keywords: Halogenation, Radiopharmaceuticals, [18F]fluoride

ENZYMATIC RADIOLABELLING OF [^{18}F]5′-FDA AND [^{18}F]5′-FDI

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We have previously reported the successful use of the key enzyme involved in carbon-fluorine bond formation in *Streptomyces cattleya* catalysing the formation of 5′-fluroro-5′-deoxyadenosine (5′-FDA) from fluoride and S-adenosyl-L-methionine (SAM) for its application in ^{18}F labelling[1,2] . The ability to over-express this bacterial enzyme has offered a real biotechnological prospect for developing the utility of this novel approach to radiolabelling. It was our desire to perform further developmental work in order to optimize the radiochemical yield and the time aspect of the rapid-labelling synthesis. We wish to report here the results obtained for labelling [^{18}F]5′-FDA using more concentrated and purer samples of the fluorinase and a one pot two enzymatic step radiolabelling of [^{18}F]5′-FDI from SAM (Scheme 1).

Aqueous [^{18}F]HF (in a solution of [^{18}O]H_2O) was incubated with the enzyme(s) and SAM. At various time-points samples from the reaction mixture were collected, processed and analysed using a high-performance liquid chromatography (HPLC) system coupled to a radioactivity detector.

Using a mixture of fluorinase preparation (20mg/ml, 100ml) and L-amino acid oxidase as methione scavenger [^{18}F]5′-FDA can be prepared at 25°C with a 32% RCY in 40 min (Fig. 1). When the reaction time was extended to 2 and 4h, RCY increased to 56% and 82%, respectively. In contrast, RCY of 41% was measured at 2h when the assay was performed at 35°C and yield further decreased to 17% when the oxidase was omitted. It was previously established that 5′-FDI can be obtained from a hydrolytic reaction of 5′-FDA mediated by an 5′-adenylic acid deaminase [3], hence we replaced the oxidase with the deaminase and we repeated bioconversion studies of ^{18}F-fluoride and SAM with the fluorinase. Over the course of the experiments only 2 products were observed, [^{18}F]5′-FDA and [^{18}F]5′-FDI, indicating that the conversion of [^{18}F]5′-FDA to [^{18}F]5′-FDI can be achieved enzymatically in a time efficient manner (Fig. 2). At 2.3h [^{18}F]5′-FDI RCY was high (82%) when experiment was performed at 35°C, in contrast the yield was 42% when the assay was carried out at 25°C, indicative of a faster conversion rate at the higher temperature tested. A full description of the methodology, the results and future potential will be presented.

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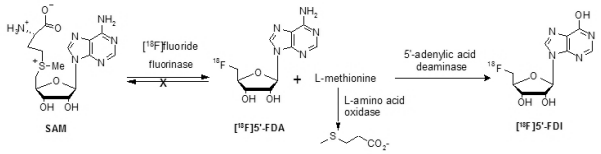

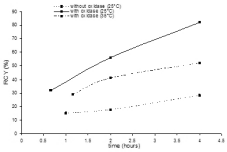
Figure 1

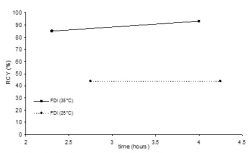
Figure 2

Keywords: Fluorine-18, Enzymatic Radiolabelling, [18F]5′-FDA and [18F]5′-FDI

FLUORINE-18-LABELING OF PEPTIDES AND PROTEINS USING [^{18}F]FPyME, A [^{18}F]FLUOROPYRIDINE-BASED MALEIMIDE REAGENT

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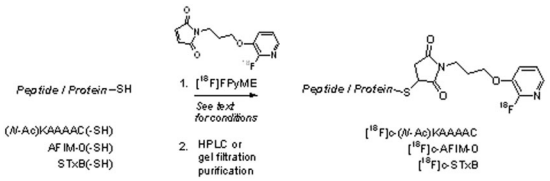
Objectives. Complex high-molecular-weight bioactive chemical structures, such as peptides and proteins are more and more often proposed as radiopharmaceuticals and their applications are rapidly gaining importance in nuclear medicine. Labeling of these macromolecules has been investigated using[^{18}F]FPyME (1-(3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl)pyrrole-2,5-dione), a new [^{18}F]fluoropyridine-based maleimide reagent designed for prosthetic labeling via selective conjugation with a thiol function as borne by cysteine residues.

Material and Methods. [^{18}F]FPyME was efficiently prepared using a three-step radiochemical pathway. The developed procedure involves (1) a high-yield nucleophilic heteroaromatic ortho-radiofluorination on [3-(3-*tert*-butoxycarbonylaminopropoxy)pyridin-2-yl]trimethyl-ammonium trifluoromethanesulfonate as the fluorine-18 incorporation-step (70-85%), followed by (2) rapid and quantitative TFA-induced removal of the *N*-Boc-protective group and (3) optimized maleimide formation using *N*-methoxycarbonylmaleimide (64-77%). After being conjugated with a model hexapeptide ((*N*-Ac)KAAAAC) in order to verify the expected chemoselectivity (CH_2-SH versus -CH_2-NH_2), [^{18}F]FPyME was then conjugated with two 8-kD proteins of interest (c-AFIM-0 and c-STxB), currently being developed as tumor imaging agents. Conjugations used optimized, short-time reaction conditions (1/9 (v/v) mixture of DMSO and 0.05M aq. Tris NaCl buffer (pH 7.4) or 0.1 M aq. PBS (pH 8), room temperature, 10 min) and purification conditions (gel filtration using a Sephadex NAP-10 cartridge or a SuperDex Peptide HR 10/30 column), both compatible with the chemical stability of the proteins and the half-life of the fluorine-18.



Results. Typically, 4.8-6.7 GBq (130-180 mCi) of radiochemically pure [^{18}F]FPyME could be obtained after semi-preparative HPLC (semiprep. SiO_2 Zorbax Rx-SIL, Hewlett Packard) in 110 min starting from a cyclotron production batch of 33.3 GBq (900 mCi) of [^{18}F]fluoride (overall RCY, based on starting [^{18}F]fluoride: 28-37% decay-corrected). Conjugation with proteins was achieved in high yields (60-70% non decay-corrected isolated yield) and routinely, 1.11-1.29 GBq of purified conjugated [^{18}F]proteins could be obtained in 25-30 min starting from 1.85 GBq of [^{18}F]FPyME. Radiosynthesis of [^{18}F]FPyME, the conjugation to the proteins and the final LC-purification and formulation lasted 130-140 min. As demonstrated by LC analysis, radiosynthesized labeled conjugated proteins co-eluted with the authentic synthesized reference compounds and were found to be more than 95% radiochemically pure. The preparations were shown to be stable for at least 240 min.

Conclusion. [^{18}F]FPyME represents a new, valuable, thiol selective, fluorine-18-labeled reagent for the prosthetic labeling of peptides and proteins. Conjugation of this reagent to the proteins used optimized, short-time reaction- and purification conditions, compatible with the chemical stability of the macromolecules and the short half-life of fluorine-18. This work was supported by the OLIM European program QLG1-CT-2000-00562 and by the European Molecular Imaging Laboratories network (EMIL; LSH-2004-503569).

Keywords: Fluorine-18, Macromolecule, Prosthetic Labeling

RADIOSYNTHESIS OF A *N*-[^{11}C]METHYL-1,4-DIHYDROQUINOLINE AS CARRIER FOR DRUG DELIVERY TO THE BRAIN

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The delivery of drugs to the brain is often seriously limited by the blood-brain barrier (BBB). Bodor and coll[1] have developed a new concept of drug targeting process, based on a dihydropyridine⇔quaternary pyridinium ion redox system, analogous to the endogenous NADH⇔NAD coenzyme system. However, the main problem encountered with the dihydropyridine derivatives was the short shelf-life time due to hydration and/or oxidation. To overcome these problems, a new carrier system has been suggested, based on a dihydroquinoline ⇔ quaternary dihydroquinolinium ion (Figure 1), dihydroquinolines being more stable than the dihydropyridines. The drug is linked to the dihydroquinoline carrier. After transport across the blood-brain barrier (BBB), an enzymatic oxidation gives the quinolinium salt. The drug is obtained after enzymatic cleavage and can reach its biological target.

In order to demonstrate the concept *in vivo*, we undertook the radiosynthesis of the dihydroquinoline derivative **1**, chosen as model for carrier, with carbon-11 ($t_{1/2}$: 20.4 minutes) using [^{11}C]methyltriflate (Figure 2).

[^{11}C]Methyl triflate was trapped at room temperature in a solution of quinoline **2** (1 mg, 3.1 µmol) in acetonitrile (0.15 ml). The reduction of the resulting quaternary salt **3** was achieved with sodium dithionite (6 mg, 23 µmol) and sodium carbonate (3 mg, 28 µmol) in water (0.2ml) at room temperature for 5 min. The solution was injected onto a semipreparative HPLC column (nucleosil 100-5 protect 1) using $H_2O/CH_3CN/NEt_3$ (60/40/0.01) as eluent at the flow rate of 4 ml/min. After formulation and sterile filtration, [^{11}C]**1** was obtained in a radiochemical yield of 35-66 % (based on initial [^{11}C]methyl triflate) with a synthesis time of 50 minutes.

Biological evaluation (stability, *in vivo* distribution) of the [^{11}C]dihydroquinoline derivative **1** is now in progress.

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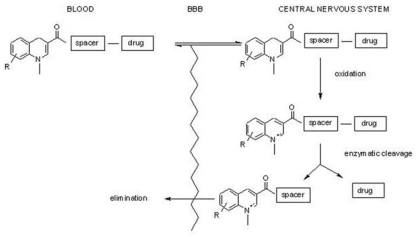

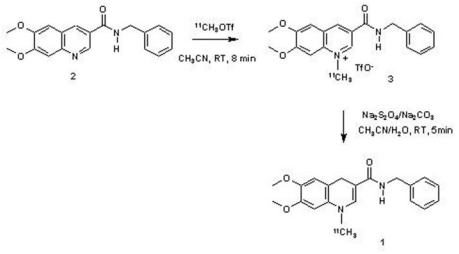

Keywords: Carbon-11, Drug Delivery Systems, Dihydroquinoline

SYNTHESIS, RADIOLABELING AND *IN VIVO* EVALUATION OF S- AND R- *N*-Me [^{18}F]FAMP AS PET TUMOR IMAGING CANDIDATES

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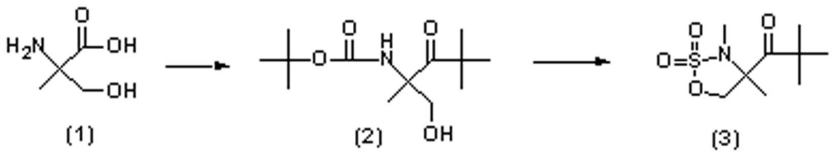
S/R-3-fluoro-2-methyl-2-(methylamino)propanoic acids (S/R-*N*-MeFAMP) were synthesized, [F-18] radio labeled and evaluated *in vivo* as PET tumor imaging agents in 4 types of human tumors in SCID mice model.

The protected *N*-MeFAMP cyclic sulfamidate precursors for radiolabeling were prepared in a simple synthetic sequence, which is shown below. S- and R-α-methyl-serine (1) were treated with di-*tert*-butyl dicarbonate followed by *N,N*-dimethylformamide di-*tert*-butyl acetal to afford S/R-2-*N*-(*tert*-butoxycarbonyl)amino-2-methyl-3-hydroxy-propanoic acid *tert*-butyl esters (2). Subsequent treatment of thionyl chloride and oxidation with sodium periodate followed by selective removal of *tert*-butoxycarbonyl group and *N*-methylation with dimethyl sulfate gave S/R-3,4-dimethyl-1,2,3-oxathiazolidine-4-carboxylic acid *tert*-butyl ester 2,2-dioxides (3) as the radiolabeling precursors.



S/R-*N*-Me[^{18}F]FAMP were obtained by radiofluorination with [^{18}F]KF/K_{222} and hydrolysis with 4N HCl then chromatographic purification. The radiolabeling yields were 69% (n=5) for S-*N*-Me[^{18}F]FAMP, and 82% (n=4) for R-*N*-Me[^{18}F]FAMP, with radiochemical purity over 99% as measured by radiometric TLC.

The *in vivo* biodistribution studies were performed in MDA MB468 (breast), A549 (lung), DU145 (prostate), and SKOV3 (ovary) tumor-bearing SCID mice with tracer injected intravenously. The uptake of radioactivity in tumors and in ten normal tissues of tumor-bearing mice (n=5 each time point) was calculated at 15, 30, 60, and 120 min post injection (*p.i.).* Values were reported as mean percent injected dose per gram tissue (%ID/g). The distributions of radioactivity in tumor and in muscle at 60 min *p.i.* were reported in the **Table 1**. The experiments showed that S/R-*N*-MeFAMP had a rapid and prolonged accumulation in tumors with good tumor to muscle ratio (3.02-11.94, S-*N*-MeFAMP and 1.55-9.89, R-*N*-MeFAMP). Low uptake was found in blood, liver, lung, and bone. These results support the candidacy of S/R-*N*-Me[^{18}F]FAMP as PET tumor imaging agents. Research supported by NIH.

Table 1: Biodistribution of S/R-*N*-Me[^{18}F]FAMP in tumor and in muscle of tumor-bearing SCID mice at 60 min *p.i.* (%ID/g)

		MDA MB468	A549	DU145	SKOV3
S-N-MeFAMP	Tumor	3.86	5.05	4.50	8.70
	Muscle	1.29	0.99	0.86	0.73
	Tumor/Muscle	3.02	5.09	5.22	11.94
R-N-MeFAMP	Tumor	2.09	3.76	4.05	12.86
	Muscle	1.35	1.48	1.19	1.30
	Tumor/Muscle	1.55	2.54	3.41	9.89

Keywords: Amino Acids, [F-18] PET, Tumor Imaging

SMALL ANIMAL PET IMAGING OF MCF-7 TUMORS IN MICE WITH PNA-CONJUGATED NANOPARTICLES TARGETING THE UNR mRNA

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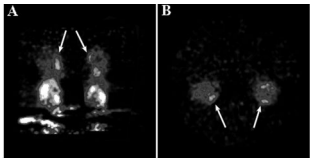
Antisense-based imaging agents, designed according to the expression profile of human cancerous cells, are promising imaging probes for the early detection of cancer (1). Peptide nucleic acids (PNAs) are synthetic DNA mimics, resistant to in vivo enzymatic degradation, that bind complementary DNA or RNA with high affinity and specificity. Using antisense PNAs as molecular imaging probes has some major obstacles in that they have unfavorable biodistributions and are not able to penetrate biologic membranes. These drawbacks may be overcome by using drug-delivery agents that encapsulate and shuttle the PNAs inside the cancer cells in vivo.

In this study, PNAs with high affinity for different binding sites of the unr mRNA, which is highly and uniquely expressed in MCF-7 breast cancer cells, were synthesized and evaluated in vivo (2). The PNAs were conjugated with DOTA and radiolabeled with ^{64}Cu. Despite high uptake in non-target organs (such as kidney or liver), microPET imaging and biodistribution experiments confirmed the ability of antisense PNAs to target MCF-7 xenografts in vivo. In particular, PNA50 exhibited 3.7 and 2.2 times greater tumor/muscle ratio compared to the sense sequence (PNA50s) at 4h and 24h post-injection, respectively.

PNA50 and PNA50s were then conjugated to shell cross-linked nanoparticles (SCKs), nano-carriers prepared by the self-assembly of amphiphilic multiblock polymers and subsequent cross-linking of the hydrophilic shell (3). PTD (a permeation peptide for cell membrane crossing) and DOTA were conjugated to the SCK surface along with the PNAs. As shown in Figure 1, a significantly higher tumor contrast was observed when imaging a MCF-7 xenografted mouse administered with the ^{64}Cu-DOTA-SCK(PTD)-PNA50 construct (left mouse) compared to the SCK bearing the sense sequence PNA50s (right mouse). Post-microPET biodistribution data confirmed that SCK conjugation maintains the PNA specificity for the target. In fact, similar tumor/blood and tumor/muscle ratios were obtained when administering MCF-7 xenograft bearing mice with the PNA-conjugated SCKs or the non-conjugated PNAs. At the same time, SCK conjugation improved the PNA biodistribution profiles since it produced a significantly lower PNA burden in non-target organs.

In conclusion, our studies indicate that antisense PNAs conjugated to SCKs have great potential to be developed as oncogene- or mRNA-specific probes for early diagnosis and treatment of specific cancers.

Figure 1. Nu/nu mice with MCF-7 xenografts in the nape of the neck. MicroPET coronal (A) and transaxial (B) slices, obtained 4h after administration of ^{64}Cu-DOTA-SCK(PTD)-PNA50 (left mouse) and ^{64}Cu-DOTA-SCK(PTD)-PNA50s (right mouse). Tumors are indicated by solid arrows.



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Keywords: Copper-64, PNA, Nanoparticles