## P036 AUTOMATED SYNTHESIS OF 18F-LABELLED ANALOGUES OF ETOMIDATE, VOROZOLE AND HARMINE USING COMMERCIAL PLATFORM

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**Objectives:** Ethyl 1-[(1R)-1-phenylethyl]-1H-imidazole-5-carboxylate (etomidate), 6-[(S)-(4-chlorophenyl)-(1H)1,2,4-triazole-1-yl)methyl]-1-methyl-1H-benzotriazole (vorozole) and 7-methoxy-1-methyl-9H-beta-carboline (harmine) (Figure 1) are inhibitors of three different enzymes 11beta-hydroxylase, aromatase and monoamine oxidase respectively. $^{18}$F-Labelled analogues of these three tracers etomidate[1], vorozole[2] and harmine[3] have recently been developed and evaluated as useful PET tracers for different biological applications. An automated synthesis of a tracer is important for making it useful clinically and this report describes the fully automated synthesis of these three useful PET tracers using the commercially available platform TRACERLab $FX_{FN}$.

**Methods:** The solutions of potassium carbonate (3.5 mg in 500 $\mu$L water), Kryptofix K2.2.2. (10.0 mg in 1000 $\mu$L acetonitrile) and precursor (5.0 mg in 500 $\mu$L anhydrous DMF) were loaded into reservoirs 1, 2 and 3 respectively of the platform (Figure 2). Reservoirs 4, 7 and 8 were filled with HPLC eluent (5 mL), water (10 mL) and absolute ethanol (1 mL) respectively. The target water containing $^{18}$F was passed through a preconditioned QMA cartridge connected into the platform where the $^{18}$F was trapped. The $^{18}$F- was released from QMA cartridge by passing $K_2CO_3$ solution from reservoir 1 through the cartridge and allowed to enter into the reactor. Kryptofix solution from reservoir 2 was added into the reactor and the whole mixture was dried first at 65 °C for 7 min followed by 120 °C for 5 min. The precursor solution from reservoir 3 was added with the dried $^{18}$F ion complexed with potassium and Kryptofix.The reaction mixture was heated at 110 °C for 10 min. In case of FVOZ and FHAR the reaction was performed at 140 °C for 15 min and 160 °C for 15 min respectively. The mixture was cooled to 50 °C, diluted with HPLC eluent from reservoir 4 and loaded into the in-built preparative HPLC system for purification. The appropriate fraction was collected, diluted with water (25 mL) and passed through a C18 SPE cartridge. The purified product was trapped on C18 cartridge, washed with water from reservoir 7 and eluted with absolute ethanol (1 mL) from reservoir 8.

**Results:** Three $^{18}$F-labelled potential PET tracers FETO, FVOZ and FHAR were prepared using fully automated commercial platform with 16-23% isolated decay corrected radiochemical yield. The radiochemical purities were more than 99% in all cases. Nucleophilic fluorination of the corresponding tosylates (FETO and FVOZ) or bromide (FHAR) was used to synthesize the compounds. The reaction was performed in an aprotic polar solvent DMF and in presence of potassium carbonate and Kryptofix K2.2.2.

**Conclusions:** This report describes a fully automated synthesis of three biologically important PET tracers FETO, FVOZ and FHAR using commercial synthesis platform. The implementation of these tracers into a commercial platform will make them accesssible to routine production for clinical applications.

**Research Support:** This work was supported by Uppsala Applied Science Laboratory, Ge Healthcare.

**References:** [1] Erlandsson M, Karimi F, Langstrom B, Nucl Med Biol 2009, (accepted for publication). [2] Erlandsson M, Karimi F, Takahashi K, Langstrom B, J Label Compd Radiopharm 2008; 51: 207-212. [3] Blom E, Karimi F, Eriksson O, Hall H, Langstrom B, J Label Compd Radiopharm 2008; 51: 277-282.

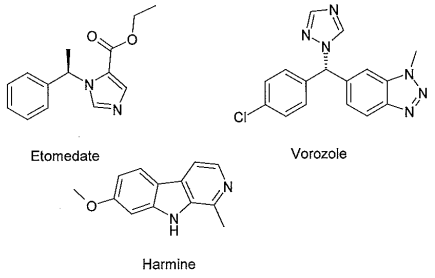


Figure 1: Structures of etomidate, vorozole and harmine

## P037 18F LABELED GALACTOSYL-NEOGLYCOALBUMIN FOR IMAGING THE HEPATIC ASIALOGLYCOPROTEIN RECEPTOR

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**Objectives:** Asialoglycoprotein receptors (ASGP-R) are well known to exist on the mammalian liver , situate on the surface of hepatocyte membrane. Quantitative imaging of asialoglycoprotein receptors could estimate the function of the liver. $^{99m}$Tc labeled galactosyl-neoglycoalbumin(NGA) and diethylenetriaminepentaacetic acid galactosyl human serum albumin(GSA) have been developed for SPECT imaging and clinical used in Japan. We labeled the NGA with $^{18}$F to get a novel PET tracer [$^{18}$F]FB-NGA and evaluated its hepatic-targeting efficacy and pharmacokinetics.

**Methods:** NGA was labeled with $^{18}$F by conjugation coupling with N-succinimidyl-4-$^{18}$F-fluorobenzoate ([$^{18}$F]SFB) under a slightly basic condition. The in vivo metabolic stability of [$^{18}$F]FB-NGA was determined. Ex vivo biodistribution of [$^{18}$F]FB-NGA and blocking experiment was investigated on normal mice. Images were acquired with microPET after injection of the radiotracer (3.7MBq/rat).

**Results:** Starting with $^{18}$F Kryptofix 2.2.2./$K_2CO_3$ solution, the total reaction time for [$^{18}$F]FB-NGA is about 200 min. Typical decay-corrected radiochemical yield is about 8%. After rapid purified with HiTrap Desalting column, the [$^{18}$F]FB-NGA get high radiochemical purity(>99%, determined by RP-HPLC). [$^{18}$F]FB-NGA was metabolized to produce lipophilic molecule in urine at 30 min. Ex vivo biodistribution showed that the liver accumulated 79.18±7.17% and 13.85±3.10% of the injected dose per gram at 5 and 30 min after injection, respectively. In addition, the hepatic uptake of [$^{18}$F]FB-NGA was blocked by preinjecting free NGA as blocking agent(18.55±2.63%ID/g at 5 min p.i.), indicating the specific binding to ASGP receptor. MicroPET study obtained quality images of rat at 5 and 15 min postinjection.

**Conclusions:** The novel ASGP receptor tracer [$^{18}$F]FB-NGA was synthesized with high radiochemical yield. The promising biological properties of [$^{18}$F]FB-NGA afford potential applications for assessment of hepatocyte function in the future. It may provide quantitative information and better resolution which particularly help to the liver surgery.

**Research Support:** This work was supported in part by grants from the National Natural Science Foundation of China (20871020).

| | 5min | 5min inhibit |
|---|---|---|
| Heart | 1.03±0.23 | 5.93±0.68 |
| Liver | 79.18±7.17 | 18.55±2.63 |
| Lung | 1.95±1.01 | 18.74±4.95 |
| Kidney | 2.79±0.37 | 10.38±1.28 |
| Spleen | 1.27±0.41 | 5.13±0.65 |
| Stomach | 0.26±0.07 | 0.49±0.14 |
| Blood | 3.41±2.03 | 41.69±6.40 |
| Bone | 0.99±0.55 | 4.15±1.69 |
| Musle | 0.37±0.24 | 0.61±0.41 |
| Intestine | 0.53±0.30 | 1.20±0.25 |

Table 1The biodistribution of the [$^{18}$F]FB-NGA in normal mice at 5 min p.i.compare with at 5 min p.i. after blocking by preinjecting free NGA. Expressed as % injected dose per gram(%ID/g). n=5.

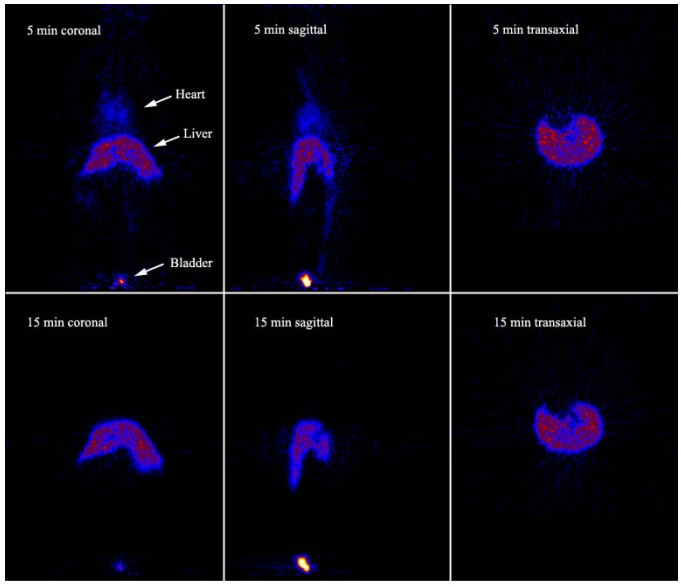


Fig 1 MicroPET images of rat: The liver is visualized 5 and 15 min p.i. as coronal, sagittal, and transaxial section. Radioactivity concentration is also seen in the urinary bladder and myocardium.

## P038 [18F]FEANGA: A PET TRACER FOR EXTRACELLULAR BETA-GLUCURONIDASE

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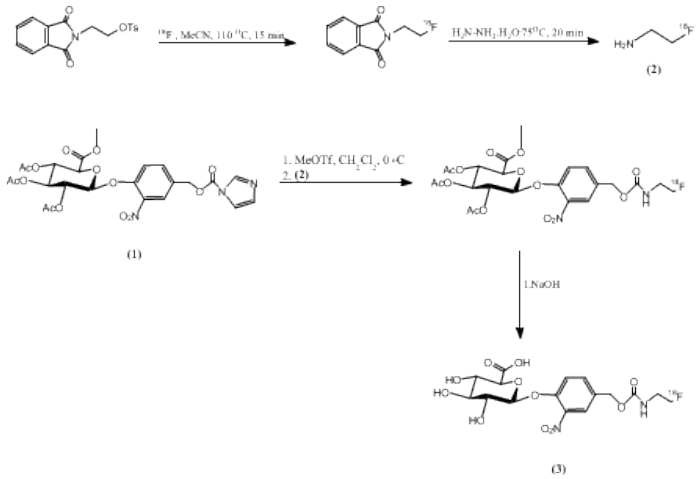
**Objectives:** High levels of extracellular β-glucuronidase (GUS) are found in e.g. tumors, infections and arthritis, where the enzyme a.o. breaks down the extracellular matrix, kills bacteria and produces tissue damage. The enzyme has been exploited to convert nontoxic prodrugs into cytostatic drugs at the target site. DOX-GA3 is an example of such a prodrug, in which the cytostatic drug doxorubicin is linked to a glucuronic acid moiety.[1] To optimize GUS-based prodrug therapies, PET imaging could be a useful tool. Here we describe the first PET tracer for extracellular GUS, [$^{18}$F]FEAnGA(3), which a 2-[$^{18}$F]fluoroethylamine ([$^{18}$F]FEA (2)) group is bound to a glucuronic acid via a nitrophenyl spacer. GUS is expected to cleave the glucuronic moiety from the tracer and release [$^{18}$F]FEA, which is supposed to be trapped in tissue by non-specific binding.

**Methods:** [$^{18}$F]FEA was labeled by fluorination of N-[2-(toluene-4-sulfonyloxy)-ethyl]-phtalimide, followed by deprotection of the amine with hydrazine.[2] [$^{18}$F]FEAnGA(3) was obtained by alkylation of precursor (1)[3] with [$^{18}$F]FEA, followed by deprotection of the sugar moiety with NaOMe/NaOH (Scheme). The product was purified by HPLC. The stability of the tracer in PBS and rat plasma was determined by radio-TLC. The cleavage of tracer (3) by either E. Coli or bovine liver GUS was measured with UV spectrophotometry and radio-TLC to determined $V_{max}$ and $K_m$. Unlabeled p-nitrophenyl β-glucuronide (PNPG) was used as a reference compound in the enzymatic studies.




**Results:** Radiolabeling of the β-glucuronidase tracer was achieved by synthesis of [$^{18}$F]FEA in 25-40 % decay-corrected yield, followed by conversion of this intermediate into [$^{18}$F]FEAnGA. After HPLC purification, [$^{18}$F]FEAnGA was obtained in 10-20% overall radiochemical yield (corrected for decay, based on [$^{18}$F]fluoride) with a total synthesis time of 150 min.The lipophilicity of [$^{18}$F]FEAnGA was shown to be about 10-fold lower than the cleavage product [$^{18}$F]FEA (log P -1.61 ± 0.01 and -0.69 ± 0.02 respectively). Because of the low lipophilicity, the tracer is expected not to penetrate the cell membrane and thus to be selective of extracellular β-glucuronidase. In vitro, [$^{18}$F]FEAnGA was stable in PBS and rat plasma for at least 3 h. FEAnGA is a substantially better substrate of both E-coli and bovine liver GUS than the reference compound PNPG (Table 1). Radiolabeled [$^{18}$F]FEAnGAwas also rapidly cleaved by either E. Coli or bovine liver GUS, resulting in complete cleavage of the tracer into[$^{18}$F]FEA within 10 to 30 min of incubation.

| Enzyme | substrate | $K_m$(μM) | $V_{max}$ (μmolmin$^{-1}$mg$^{-1}$) | $k_{cat}$ ($s^{-1}$) | $k_{cat}/K_m$($10^6M^{-1}s^{-1}$) |
|---|---|---|---|---|---|
| E.Coli GUS | PNPG | 322 | 670 | 3236 | 10 |
| E. Coli GUS | FEAnGA | 206 | 7538 | 36415 | 176.5 |
| Bovine liver GUS | PNPG | 114 | 5 | 24 | 0.2 |
| Bovine liver GUS | FEAnGA | 15 | 28 | 136 | 8.8 |

**Conclusions:** [$^{18}$F]FEAnGA was efficiently labeled with fluorine-18 in good yield. In vitro, [$^{18}$F]FEAnGA was stable and proved to be a good substrate of both E. Coli and bovine liver GUS. These results warrant further evaluation of [$^{18}$F]FEAnGA in animal studies.

**Research Support:** The authors wish to thank for financing support from University Medical Center Groningen (UMCG)

**References:** 1. Haisma et al. Br J Cancer. 2001; 84(4):550. 2. Tewson TJ. Nucl Med Biol 1997; 26:1891. 3. Duimstra et al. J Am Chem Soc 2005; 127:12847.

## P039 AUTOMATED SYNTHESIS OF 18F-LABELED CELL-PENETRATION PEPTIDE AS PET TRACER

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**Objectives:** Cell-penetrating peptides (CPPs), also known as protein transduction domains (PTDs) or membrane transduction peptides (MTPs), or membrane-penetrating peptides(MPP), are of interest due to their ability to translocate across cellular membranes. In order to study biologic activity of CPPs, $^{18}$F-labeled Tat Cell-penetrating peptide ([$^{18}$F]CPP) as PET tracer is synthesized. Also, automated synthesis of [$^{18}$F]CPP is performed in a modified TRACERlab $FX_{FN}$ synthesizer.

**Methods:** Automated synthesis of N-Succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB) and [$^{18}$F]CPP was carried out in the modified TRACERlab $FX_{FN}$ synthesizer. [$^{18}$F]SFB was prepared by a convenient three-step, one-pot procedure. [$^{18}$F]Fluorination of the precursor ethyl 4-(trimethylammonium triflate)benzoate gave ethyl 4-[$^{18}$F]fluorobenzoate. Saponification of the ethyl 4-[$^{18}$F]fluorobenzoate with aqueous tetrapropylammonium hydroxide yielded the corresponding 4-[$^{18}$F]fluorobenzoate salt ([$^{18}$F]FBA) which was then treated with N,N,N,N'-tetramethyl-O-(N-succinimidyl)uronium hexafluorophosphate (HSTU) to obtain [$^{18}$F]SFB. The purified [$^{18}$F]SFB was used for the labeling of Tat membrane-penetrating peptide (containing the Arg-Lys-Lys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Pro-Leu-Gly-Leu-Ala-Gly-Glu-Glu-Glu-Glu-Glu-Glu-Glu sequence, [$^{18}$F]CPP) through [$^{18}$F]fluorobenzoylation of amino groups in lysine.

**Results:** The uncorrected radiochemical yields of [$^{18}$F]SFB were as high as 25-35% (based on [$^{18}$F]fluoride (n=10) with a synthesis time of ~ 40 min. [$^{18}$F]CPP was produced in uncorrected radiochemical yields of 10-20% (n=5) within 30 min (based on [$^{18}$F]SFB). The radiochemical purities of [$^{18}$F]SFB and [$^{18}$F]CPP were greater than 95%.

**Conclusions: [**$^{18}$F]CPP is produce with a modified TRACERlab $FX_{FN}$ synthesizer. It can be used to study the in vivo PET imaging

**Research Support:** This work was supported by the National High Technology Research and Development Program of China (863 Program, No. 2008AA02Z430), Sun Yat-Sen University (No. 80000-317313), and NSFC

## P040 SIMPLIFIED AND AUTOMATED PREPARATION METHOD FOR 18F-FLUOROMETHYLCHOLINE USING A NEW SYNTHESIS MODULE

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**Objectives:** The aim was development of a fast, reproducible preparation method of $^{18}$F-fluoromethylcholine with a new synthesis module developed by Veenstra Instruments. The method should be simplified as much as possible while maintaining reproducibility and high yields for clinical studies. Critical steps to be solved were radiochemical yield of $^{18}$F-fluoromethylbromide and its conversion to the corresponding $^{18}$F-fluoromethyltriflate. The reactivity of both $^{18}$F-synthons with dimethylethanolamine to $^{18}$F-fluoromethylcholine was investigated (Figure 1).

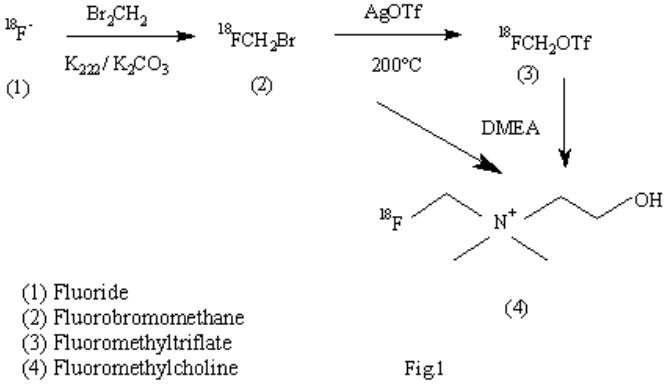

Fig1

**Methods:** The production of $^{18}$F-fluoromethylcholine[1,2] consists of 4 steps: 1) Work-up of $^{18}$F-fluoride; 2) Preparation of the intermediate $^{18}$F-fluorobromomethane; 3) Conversion to $^{18}$F-fluoromethyltriflate and 4) Reaction with the choline precursor dimethylethanolamine (DMEA) to $^{18}$F-fluoromethylcholine. These 4 steps were evaluated and optimised. The elution and drying of $^{18}$F-fluoride was simplified to a single step resulting in a faster azeotropic evaporation. The reaction of $^{18}$F-fluoride (1) with (50-60 ml) dibromomethane in 1 ml acetonitrile gave $^{18}$F-fluorobromomethane (2) which was purified by distillation through a series of 4 Silica SepPak plus cartridge with a 100ml/min flow. $^{18}$F-fluoromethyltriflate (3) was obtained by passing (2) through a silver triflate column with similar flow. The compound (2) or (3) was trapped on a C18 plus cartridge containing 50 ml DMEA. Reaction to $^{18}$F-fluoromethylcholine (4) was instantaneous and at RT. After washing the C18 plus and CM accell light cartridge with 10 ml ethanol and 10 ml water the product was eluted with 5 ml NaCl 0.9% solution and passed through a 0.22-$\mu$m sterile filter (Millex LG; Millipore). Radiochemical purity was measured by analytical HPLC (Astec C18, 250 x 4.6 mm, 50mM Sodium borate / 100mM Sodium hydroxide (45/55) 1 ml/min, retention time = 4.4 min).

**Results:** The elution and drying of (1) was simplified resulting in a 5 min reduction of synthesis time. The yield of the (2) was 40-55 %.The conversion of (2) to (3) was accomplished with an efficiency > 95%.The overall yield of (4) by reacting directly from (2) was 2-4%. The yield of (4) formed from DMEA and (3) had an overall yield from 30–40 %. Total synthesis time was 29 – 32 min. Radiochemical purity > 95%. The concentration of DMAE was ± 50 mg/l and pH= 6.5 – 7.

**Conclusions:** The Veenstra $^{18}$F-fluoromethylcholine synthesis module produced $^{18}$F-fluoromethylcholine in a fast and simple manner according to figure 2. The overall yields of the module are high. A single synthesis-run can produce a multi-dose vial product up to 10 GBq. The synthesis module can be used as universal fluoromethylating synthesis module. Production of $^{18}$F-fluoromethionine and $^{18}$F-fluororaclopride is currently under investigation.

**References:** [1] Iwata R, Pascali C, Bogni A, Furumoto S, Terasaki K, Yanai K. Appl Radiat Isot. 2002 Sep;57(3):347-52. [2] DeGrado TR, Coleman RE, Wang S, Baldwin SW, Orr MD, Robertson CN, PolascikTJ, Price DT. Cancer Res. 2001 Jan 1;61(1):110-7.

**Production scheme for [$^{18}$F]-fluoromethylcholine**

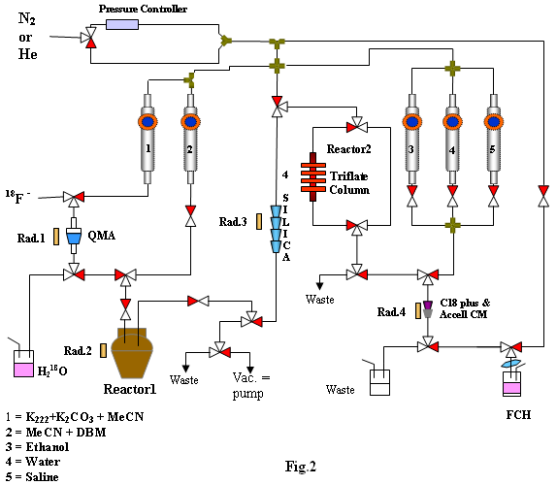

Fig.2

## P041 [18F]SiFA-ISOTHIOCYANATE IS AN EFFICIENT LABELING AGENT FOR PROTEINS

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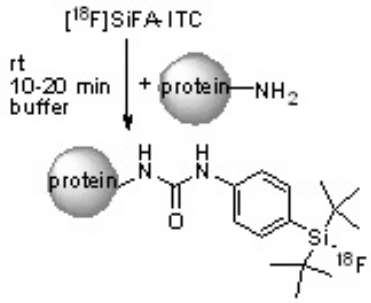
**Objectives:** Fluorescein isothiocyanate (FITC) is the most commonly used fluorescent labeling agent for proteins due to its easy reaction with lysine residues. Currently, there is no radioactive counterpart to FITC with a similar ease of introduction available for the radioactive labeling of proteins for in vivo imaging purposes. The objective of this study was to investigate the protein labeling potential of [$^{18}$F]SiFA-isothiocyanate ([$^{18}$F]SiFA-ITC) targeting primarily lysine residues. The SiFA-labeling technique based on an isotopic $^{19}$F-$^{18}$F exchange reaction has been previously introduced by our group and co-workers [1]

**Methods:** [$^{18}$F]SiFA-ITC was synthesized by isotopic exchange using the corresponding $^{19}$F-compound and purified by means of solid phase extraction thereby omitting cumbersome HPLC purification. The so purified [$^{18}$F]SiFA-ITC was reacted with proteins of different sizes, i. e. rat serum albumin (RSA, 66 kDa), apotransferrin (76-81 kDa) and bovine IgG (144 kDa). The purification of the final labeled proteins was achieved by HPLC, small size exclusion columns (NAP-5) or centrifugal filters. To investigate the in vivo stability of the [$^{18}$F]SiFA-protein conjugation, $^{18}$F-SiFA-labeled RSA (5 MBq) was injected into a healthy rat and subjected to an animal PET scan.

**Results:** The efficacy of the synthesis of [$^{18}$F]SiFA-ITC by isotopic exchange under very mild conditions depended on the precursor concentration ([$^{19}$F]SiFA-ITC) and on the basicity of the reaction mixture. Generally, the dried $^{18}$F (37-74 GBq) was dissolved in 900 µL DMSO and used as a stock solution. For each 20-60 µL of this solution (ca. 2-3 GBq), 10-12 nmol of [$^{19}$F]SiFA-ITC were needed to obtain optimal radiochemical yields (>90%). The calculated specific activities of [$^{18}$F]SiFA-ITC were 100-160 GBq/µmol (2700-4500 Ci/mmol). The conjugation of purified [$^{18}$F]SiFA-ITC to the protein depends on many factors namely the pH, the lysine content of the protein, and the protein concentration. At pH 9.0 we highest conjugation yields (80%) for the RSA protein could be achieved within 10 min. The apotransferrin and the bovine IgG reacted slower, possibly as a result of less lysine residues in the proteins, or to less accessible ones. The maximal conjugation yields of 30-45 % for those two proteins were obtained after 10-20 min. [$^{18}$F]SiFA-RSA (5 MBq) was injected into a healthy male Sprague Dawley rat and the subsequent PET scan revealed only minor radioactivity uptake in bone pointing out the stability of the [$^{18}$F]SiFA-RSA conjugate.

**Conclusions:** We have demonstrated that the [$^{18}$F]SiFA-label is stable under in vivo conditions and particularly suitable for the labeling of proteins. [$^{18}$F]SiFA-ITC might become an applicable radioactive analog to FITC, allowing the reliable and convenient introduction of F-18 into proteins containing lysine residues.

**References:** [1] Schirrmacher et al. Angewandte Chem. Int. Ed. 45 (2006) 6047-6051




Scheme. Conjugation of crude [$^{18}$F]SiFA-ITC (10-12 nmol, 2-3 GBq) with protein in buffer at pH 9.0 at room temperature with yields of 30-80% for the tested RSA, apotransferrin and bovine IgG.

## P042 RADIOFLUORINATION VIA STAUDINGER LIGATION

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**Objectives:** The chemical labeling of biomolecules continues to be an important tool for the study of the cellular fate.[1] In particular the introduction of $^{18}F$ into higher molecular weight compounds like peptides, proteins, oligonucleotides or antibodies represents a special challenge. Usually they can not be labelled with $^{18}F$ at high specific activity directly due to the rough reaction conditions. To circumvent this problem prostetic groups were used for $^{18}F$ labeling of peptides and proteins. Therefore $^{18}F$ labeled small organic molecules were synthesised capable of being linked to peptides, proteins or antibodies under mild conditions. Only a handful of reactions are known for the selective introduction of these labeling agents.

**Methods:** Although several bioconjugation techniques are available for preparation of bioconjugates substituted with a limited number of functional groups, truly chemoselective ligation reactions are rather limited. Most ligation reactions rely on the reaction of an electrophile with a nucleophile. As biological systems are rich in diverse electrophilic and nucleophilic sites, only a few functional groups are available that exhibit orthogonal reactivity to the functional groups present. Bioorthogonal reactions like the [3+2] Huisgen cycloaddition[3] were applicated for the selective radiolabeling[4] of biomolecules. The Staudinger Ligation introduced by Saxon and Bertozzi[5] exploits the smooth reaction between an azide and a phosphane to form an amide bridge between the labeling agent and the respective biomolecule.

**Results:** Substituted phosphanes were used as synthons for the Staudinger Ligation. The preparation of these compounds succeeds via Pd-catalyzed P-C cross coupling of iodophenyl benzoates with $HPPh_2$. No extensive protection group chemistry is needed for this coupling reaction.[2] The radiofluorination proceeds under standard conditions and the subsequent ligation reaction occurs under mild conditions (20 min, 50 watt microwave) that affords the respective Staudinger products in high yields.

**Conclusions:** Our first promising results show the potential of this labeling method for the radiofluorination of various organic model compounds and biomolecules. The experimental details will be presented.

**References:** [1] J. A. Prescher, C. R. Bertozzi, Nature Chem. Biol. 2005, 1, 13-21. [2] C. Mamat, A. Flemming, M. Kckerling, J. Steinbach, F. R. Wuest, Eur. J. Org. Chem. 2009, submitted. [3] C. Mamat, T. Ramenda, F. R. Wuest, Mini-Rev. Org. Chem. 2009, 6, 21-34. [4] T. Ramenda, R. Bergmann, F. Wuest, Lett. Drug Design Disc. 2007, 4, 279-285. [5] K. L. Kiick, E. Saxon, D. A. Tirrell, C. R. Bertozzi, Proc. Natl. Acad. Sci. U.S.A. 2002, 99, 19-24.

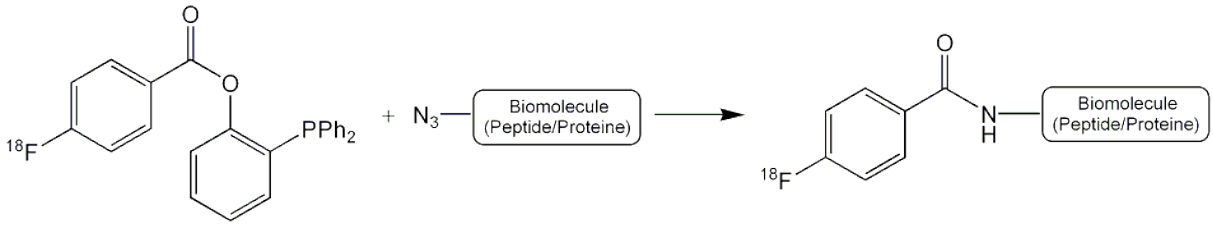


**Figure 1.** Radiolabeling of biomolecules via Staudinger Ligation

## P043 SYNTHESIS AND BIOEVALUATION OF [18F]FB-MPPPA AS POTENTIAL BRAIN IMAGING AGENT

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2. Capital Medical University, PET Center of Xuanwu Hospital, Beijing, China

**Objectives:** Over the past decades, several $^{99m}$Tc radiopharmaceuticals, such as $^{99m}$Tc-HMPAO and $^{99m}$Tc-ECD, have been used in the clinic for the assessment of brain perfusion. Since these agents are far from ideal, the development of new brain perfusion imaging agent remains a subject of great interest in the radiopharmaceutical field. In this study, we report the synthesis and characterization of $^{18/19}$F-labeled MPP ((2-methoxyphenyl)piperazine) derivative [$^{18/19}$F]FB-MPPPA, and furthermore the radiolabeled [$^{18}$F]FB-MPPPA was evaluated as a potential brain imaging agent.

**Methods:** 2-(2-methoxyphenyl) piperazine propylamine(MPPPA) was synthesized by coupling with(2-methoxyphenyl) piperazine, 2-(3-aminopropyl)-1H-isoindole-1,3(2H)-dione. [$^{19}$F]FB-MPPPA was synthesized by coupling MPPPA with 4-fluorobenzoyl chloride. [$^{18}$F]FB-MPPPA( N-(2-(4-(2-methoxy)phenyl) piperazin-1-yl)propyl p-$^{18}$F-fluorbenzoamide) was synthesized by coupling N-succinimidyl- 4-$^{18}$F-fluorobenzoate([$^{18}$F]SFB) with MPPPA. The stability and hydrophilicity of [$^{18}$F]FB-MPPPA were investigated respectively. Biodistribution study of [$^{18}$F]FB-MPPPA was performed in normal mice.

**Results:** Starting with $^{18}$FKryptofix 2.2.2./$K_2CO_3$ solution, the total reaction time for [$^{18}$F]FB-MPPPA, including final high-performance liquid chromatography purification, is about 3 h. Typical decay-corrected radiochemical yield is 18.4%. The radiochemical purity was >99%. [$^{18}$F]FB-MPPPA remained stable over 3h in saline at RT. The log$P_{o/w}$ of which was 1.81. [$^{19}$F]FB-MPPPA was confirmed by IR, $^1$H-NMR, and ESI-MS, respectively.( 1H NMR (CDCl3) δ: 7.82(m, 2H, F-phenyl-CO), 6.87-7.03(m, 4H, $CH_3O$-phenyl-N), 6.81 (m, 2H, F-phenyl-CO), 3.80(s, 3H, $OCH_3$), 3.53(m, 2H, CO-N- $CH_2$ ), 2.74-3.08(m, 8H, N($CH_2$ $CH_2$)N), 2.66(m, 2H, $CH_2$- piperazine), 1.82(m, 2H, $CH_2$ $CH_2$ $CH_2$). ESI-MS calculated for C21H26FN3O2: 371.2; Found 372.2. Biodistribution of [$^{18}$F]FB-MPPPA in mice showed that the brain accumulated 6.59±0.77% of the injected dose per gram at 2 min post-injection time and the brain-to-blood ratio was reached the highest 3.67 at 15 min after injection.

**Conclusions:** [$^{18}$F]FB-MPPPA was synthesized with high radiochemical yield, and showed high initial uptake in the brains of mice. It will be evaluated furthermore as a potential brain imaging agent in the future.

**Research Support:** The Project Sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry and supported in part by grants from the National Natural Science Foundation of China (20871020).

Table 1 The biodistribution of the [$^{18}$F]FB-MPPPA in normal mice. Expressed as % injected dose per gram(%ID/g). n=5.

| | 2min | 15min | 30min | 60min |
|---|---|---|---|---|
| heart | 10.01±1.25 | 3.27±0.35 | 1.60±0.12 | 1.17±0.59 |
| liver | 5.42±0.52 | 21.64±2.79 | 31.83±2.17 | 39.75±3.11 |
| lung | 31.37±3.23 | 8.66±2.02 | 4.43±1.28 | 2.68±0.61 |
| kidney | 27.81±5.08 | 11.46±2.71 | 5.67±0.39 | 3.94±0.93 |
| spleen | 8.25±1.99 | 7.60±2.17 | 4.40±1.07 | 2.67±1.01 |
| muscle | 3.85±0.46 | 2.03±0.34 | 1.20±0.28 | 0.86±0.22 |
| bone | 3.66±0.51 | 3.16±0.55 | 1.75±0.24 | 1.28±0.33 |
| brain | 6.59±0.77 | 4.37±0.85 | 2.20±0.34 | 0.84±0.08 |
| blood | 3.78±0.37 | 1.19±0.28 | 0.68±0.15 | 0.32±0.05 |
| Br/Bl | 1.74 | 3.67 | 3.24 | 2.63 |

## P044 FLUORINE-18 LABELING OF PHOSPHOPEPTIDES: A CONVENIENT APPROACH FOR THE EVALUATION OF PHOSPHOPEPTIDE METABOLISM IN VIVO

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**Objectives:** Phosphopeptides are very useful reagents to study signal transduction pathways related with cellular protein phosphorylation/dephosphorylation. Phosphopeptides also have been identified as important drug candidates to both inhibit and stimulate intracellular signaling mechanisms through targeting phosphotyrosine, phosphoserine or phosphothreonine residue-binding protein domains. In this work we describe a convenient method for the mild and sufficient radiolabeling of phosphopeptides with the short-lived positron emitter fluorine-18 to allow radiopharmacological studies on phosphopeptide metabolism in vivo by means of positron emission tomography (PET).

**Methods:** Peptide syntheses were performed on peptide synthesizer (Syro I, MultiSynTech, Germany) using standard Fmoc chemistry. Radiolabeling was accomplished via conjugation of the N-terminus of Polo-box domain (PBD)-binding phosphopeptide H-Met-Gln-Ser-pThr-Pro-Leu-OH 1 and its unphosphorylated analog 2 with the bifunctional labeling agent N-succinimidyl-p-[$^{18}$F] fluorobenzoate ([$^{18}$F]SFB) (Fig. 1).

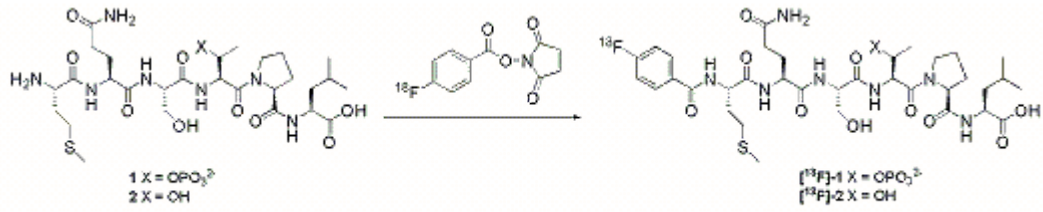


Fig. 1: Peptide labeling with [$^{18}$F]SFB

The radiolabeled phosphopeptide [$^{18}$F]FB-Met-Gln-Ser-pThr-Pro-Leu-OH [$^{18}$F]-1 and its unphosphorylated analog [$^{18}$F]FB-Met-Gln-Ser-Thr-Pro-Leu-OH [$^{18}$F]-2 were subjected to radiopharmacological evaluation involving investigation of metabolic stability in vitro and in vivo, cell uptake studies in human adenocarcinoma (HT-29) and squamous cell carcinoma (FaDu) cell lines, and small-animal PET studies in Wistar rats and NMRI nu/nu HT-29 tumor-bearing mice.

**Results:** Radiolabeling was achieved via $^{18}$F-fluorobenzoylation using the Bolton-Hunter-type reagent N-succinimidyl-p-[$^{18}$F] fluorobenzoate ([$^{18}$F]SFB). The optimized radiosynthesis was conducted in a 0.05 M $Na_2HPO_4$ buffer solution (pH 9.0) at 40°C within 30 min using low amounts of peptide precursor (0.5 mg) to afford reproducible radiochemical yields (25-29%) and high radiochemical purity (>98%) within 95-109 min including HPLC purification. Cell uptake studies in HT-29 and FaDu tumor cells revealed only very little radiotracer uptake (less than 0.6% ID/mg protein). Radiolabeled phosphopeptide [$^{18}$F]-1 showed remarkable high metabolic stability in vivo (65% intact peptide after 20 min) compared to the corresponding unphosphorylated peptide [$^{18}$F]-2 (<5% intact peptide after 20 min). A detailed discussion on the radiopharmacological profile in vitro and in vivo of peptides [$^{18}$F]-1 and [$^{18}$F]-2 will be presented.

**Conclusions:** We developed a reproducible synthesis for $^{18}$F-labeled phosphopeptides, and the presented method is a promising approach for studying phosphopeptide metabolism and kinetics in vivo. Furthermore, cell penetrating peptides (CPP) are currently under investigation as potential mediators to enhance cell uptake of the desired $^{18}$F-labeled phosphopeptides.

## P045 DIRECT LABELING OF PEPTIDES WITH [18F]FDG

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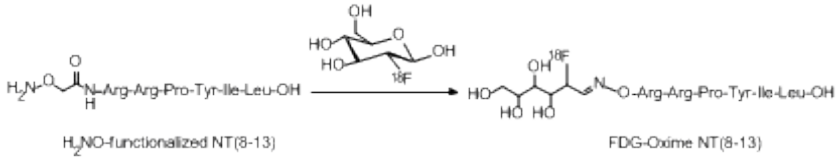
**Objectives:** The routine $^{18}$F labeling of biomacromolecules like peptides and proteins mainly exploits the use of bifunctional labeling precursors, also referred to as prosthetic groups. 2-[$^{18}$F]Fluoro-2-deoxy-D-glucose ([$^{18}$F]FDG) is the most important clinical PET radiotracer, but only very few examples using readily available [$^{18}$F]FDG as a building block for the radiosynthesis of $^{18}$F-labeled compounds. The present study describes the use of [$^{18}$F]FDG as a $^{18}$F building block for the direct labeling of various aminooxy-functionalized peptides via chemoselective oxime formation. The potential of this novel peptide labeling reaction was expemplified by means of various neurotensin NT(8-13) derivatives.

**Methods:** The labeling reaction was performed using a 0.9% NaCl solution of [$^{18}$F]FDG and aminooxy-functionalized peptide at different concentrations in a mixture of MeOH/$H_2O$ at 80 °C for 30 min. The reaction mixture was analyzed by radio-HPLC to determine the radiochemical yield of the conjugation reaction (Fig. 1).




**Results:** Direct labeling of aminooxy-functionalized peptides with [$^{18}$F]FDG was strongly dependent on the amount of used peptide. Monomeric NT(8-13) derivative gave best radiochemical yields of up to 80% based upon [$^{18}$F]FDG. More complex dimeric and tetrameric neurotensin derivatives gave lower radiochemical yields at comparable peptide concentrations. Increase of [$^{18}$F]FDG activity also lowered radiochemical yield due to an increasing competitive reaction with glucose originating from the [$^{18}$F]FDG solution. Depending on the size of the used peptide, separation of [$^{18}$F]FDG-labeled peptide from glucose-labeled peptide is possible by semi-preparative HPLC. The formation of isomers during the aminooxy-aldehyde conjugation reaction between [$^{18}$F]FDG and aminooxy-functionalized peptides in aqueous media leads to the formation of isomers according to literature reports.

**Conclusions:** For the first time, readily available PET radiotracer [$^{18}$F]FDG was shown to be a suitable prosthetic group for direct labeling of aminooxy-functionalized peptides with fluorine-18 under mild conditions. The reaction is especially suitable for small peptides. However, application of larger peptides seems to be limited by increasing separation difficulties of the corresponding glucose-peptide conjugate.

## P046 A FULLY AUTOMATED TWO-STEP SYNTHESIS OF A 18F-LABELED TYROSINE KINASE INHIBITOR FOR EGFR KINASE ACTIVITY IMAGING IN TUMORS

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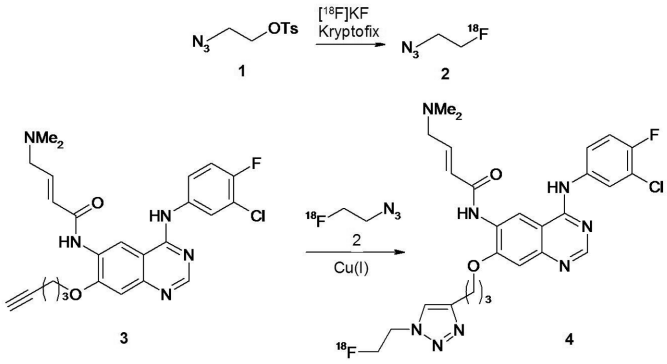
Max Planck Institute for Neurological Research, Dept of Radiochemistry, Cologne, Germany

**Objectives:** Epidermal growth factor receptor (EGFR) overexpression at the kinase level has potential importance for patient selection and monitoring of EGFR-targeted therapies. Radiolabelled tyrosine kinase (TK) inhibitors based on anilinoquinazoline core structure need multi-step radiosyntheses as well as extended reaction time. Herein we report a two-step click labelling approach that was amenable to automation and adopted to a fully automated synthesis module.

**Methods:** 4-Dimethylamino-but-2-en-[4-(3-chloro-4-[$^{18}$F]fluorophenylamino)-7-chinazolin-6-yl]amid ([$^{18}$F]4) was synthesized via Cu(I) ($CuSO_4$/sodium ascorbate) catalyzed Huisgen 1,3-dipolar cycloaddition between 2-[$^{18}$F]fluoroethylazide ([$^{18}$F]2) and the al




**Results:** 2-[$^{18}$F]fluoroethylazide (2) was prepared from tosylate 1 and isolated via distillation (33-35% RCY, decay corrected, n = 3). Cycloaddition between terminal alkyne 3 and azide 2 generated the triazole 4 in yield of (10-21%, decay corrected, n = 3). The overall yield amounted to 7% with radiochemical purity > 99% and specific activity over 37 GBq/$\mu$mol. The synthesis time was 90 min inclusive purification/formulation. PET images of PC9 tumor xenografts using the novel biomarker showed promising results to visualize EGFR activity.

**Conclusions:** The established synthesis approach facilitates the accessibility of potential biomarkers suitable for imaging of tyrosine kinase activity in EGFR expressing tumors.

## P047 SYNTHESIS AND PRECLINICAL IMAGING OF 18F-HX4: A NEW TRIAZOLE CONTAINING 2-NITROIMIDAZOLE TRACER FOR IMAGING HYPOXIC TISSUE IN VIVO

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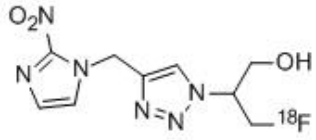
**Objectives:** To synthesize and image an $^{18}$F-labeled triazole containing 2-nitroimidazole derivative and compare its in vivo properties against 18F-MISO.

**Methods:** $^{18}$F-HX4 was prepared on an Explora RN automated synthesis module with average yields of 40-60% (decay corrected) and an average synthesis time of 45 min. Briefly, the precursor was reacted with K$^{18}$F, K222, and $K_2CO_3$ in MeCN at 110ºC for 10 min, followed by deprotection using 1N HCl at 105ºC for 5 min. $^{18}$F-HX4 was purified by RP-HPLC (5% EtOH:95% 21 mM sodium phosphate) and stabilized with ascorbic acid prior to sterile filtration. The specific activity ranged from 5-20 Ci/μmol and the RCP was greater than 95% by RP-HPLC. Pharmacokinetic studies were carried out in white wild type mice at 120 min post IV injection. Metabolic stability analyses were evaluated at various time points up to 120 min. MicroPET studies were carried out using a hypoxic A427, BXPC3 and U87MG murine xenograft model under isoflurane anesthesia capturing either static or dynamic images. Non-radioactive HX4 was co-administered for blocking studies.

**Results:** When compared against $^{18}$F-MISO, $^{18}$F-HX4 exhibits rapid renal clearance and low uptake in several organs, presumably due to the presence of the triazole moiety. In vivo metabolism analyses in mice reveals that the tracer is intact up to 90% after 1 hr. MicroPET imaging results show preferential tumor uptake of $^{18}$F-HX4 in hypoxic xenografts with tumor/muscle ratios between 1.5 - 2.5 :1 after 1 hr. This ratio improved slightly after an additional hour of uptake. Co-administration of HX4 with the tracer did not depress $^{18}$F-HX4 tumor uptake, consistent with $^{18}$F-MISO blocking results. In vitro, HX4 is non-cytotoxic to LS174T, A172, and AML12 cells up to 10 μM.

**Conclusions:** $^{18}$F-HX4, a novel imaging agent for detecting hypoxic tissue, is rapidly excreted via the kidneys, is metabolically stable and affords tumor:muscle ratios of >2.5:1 after 2 hrs in hypoxic tumors. $^{18}$F-HX4 was not cytotoxic in several cell lines up to 10 uM. Given these results, $^{18}$F-HX4 is deemed a viable candidate for detecting hypoxic tissue in vivo.




$^{18}$F-HX4

## P048 FAST AND EFFICIENT RADIOSYNTHESIS OF FLUORINE-18 LABELED SULFONAMIDES USING CLICK REACTION

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1. Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Radiopharmaceutical Research Center, Shanghai, China; 2. China Institute of Atomic Energy, Beijing, China

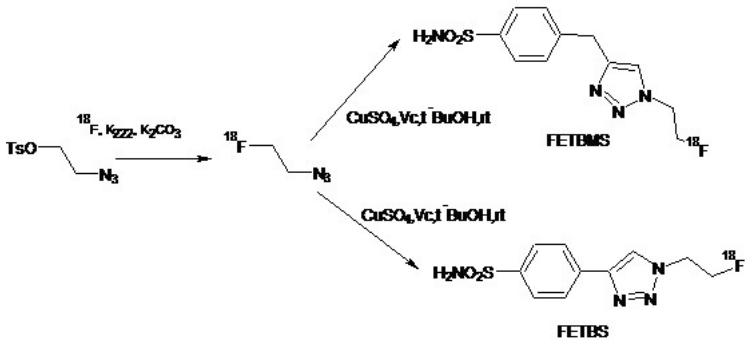
**Objectives:** "Click Chemistry" is a very useful tool in the synthesis and screening of prodrugs and has shown a great potential in the preparation of radiopharmaceuticals, especially for short half life radioisotopes labeled PET probes. To Evaluate the feasibility of "Click Chemistry" in radiosynthesis of $^{18}$F labeled compounds with sulfonamide moieties, two potential $^{18}$F labeled carbonic anhydrase inhibitors were prepared via the "Click" method.

**Methods:** Using "click" reaction, two potential carbonic anhydrase inhibitors 4-{[1-(2-[$^{18}$F]fluoroethyl)-1H-1,2,3-triazol-4-yl]methyl}benzenesulfonamide ($^{18}$F-FETMBS) and 4-[1-(2-[$^{18}$F]fluoroethyl)-1H-1,2,3-triazol-4-yl]benzenesulfonamide ($^{18}$F-FETBS) were prepared with high radiochemical yields and a short synthesis time (Scheme 1). The labeling "intermediate"1-azido-2-[$^{18}$F] fluoroethane was prepared by nucleophilic fluorination of 2-O-tosyldiazonium in acetonitrile under 80 °C with a crude yield of 90%. Without purification 1-azido-2-[$^{18}$F]fluoroethane was added to a mixture solution of precursor 4-{[1-(2-[$^{18}$F]fluoroethyl)-1H-1,2,3-triazol-4-yl]methyl}benzenesulfonamide or 4-[1-(2-tosylethyl)-1H-1,2,3-triazol-4-yl]benzenesulfonamide together with $CuSO_4$ and ascorbic acid. The "Click" reaction performed almost quantitively under room temperature in 15 minutes. After HPLC purification, $^{18}$F-FETMBS and $^{18}$F-FETBS were obtained.

**Results:** The overall radiochemical yields of $^{18}$F-FETMBS and $^{18}$F-FETBS were 51% and 54% respectively. The overall synthesis time (EOS) is shorter than 50 minutes including HPLC purification.

**Conclusions:** The radiochemical yields of the "click" reaction are higher than most of reactions used in thepreparation of $^{18}$F labeled PET agents and the reaction condition is mild and convenient for further purification. These results showed that "Click Chemistry" have special advantages in fast preparation of radiopharmaceuticals and encouraged further studies of its applications in this area.

**Research Support:** This research is supported by National Nature Science Foundation of China (10875163).



## P049 SYNTHESIS AND EVALUATION OF ARYL-SUBSTITUTED DIARYLPROPIONITRILES, SELECTIVE LIGANDS FOR ESTROGEN RECEPTOR β, AS POTENTIAL POSITRON-EMISSION TOMOGRAPHIC IMAGING AGENTS

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1. Inha University, Department of Chemistry, Inchon, South Korea; 2. Korea Institute of Radiological and Medical Sciences, Molecular Imaging Research Center, Seoul, South Korea; 3. University of Illinois, Urbana-Champaign, Department of Chemistry, Urbana, IL; 4. Sogang University, Department of Chemistry, Seoul, South Korea

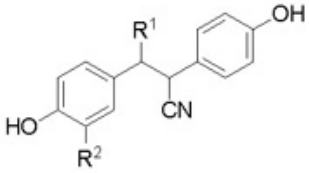
**Objectives:** Radioisotope-labeled ligands for imaging the levels of ERs (ERα and ERβ) in various target tissues by PET or SPECT might be very useful in assessing these receptors as targets for various estrogen therapies and the highly selective radiotracer might also obtain information regarding disease stage and prognosis, and might even be able to predict tumor response to endocrine therapies. Among the various ERs pharmacophores, diarylpropionitrile (DPN, 1a) has good selectivity (72-fold ERβ affinity preference) with full agonistic character. A number of variants on the DPN pharmacophore have been studied, the F-18 labeled DPN analogue, FEDPN (1b), was prepared previously and studied as a potential agent for PET imaging. Its ER-specific uptake in ERβ target tissues such as the ovary, however, was only modest, not enough for [$^{18}$F]FEDPN to be useful for imaging ERβ.

**Methods:** To extend this work, we investigated the novel DPN analogs substituted in the synthetically more accessible meta-position of the distal phenol ring with several substituents of varying size and polarity. These included ones having fluoroethyl and fluoropropyl groups as well as methyl, hydroxymethyl, and halo-substituted DPN analogs to explore the structure-binding affinity relationship at this site. Fluoroalkylated DPN analogs of these would be possible introduce F-18, producing products that might be candidate ERβ-selective PET tracers.

**Results:** In competitive radiometric binding assays with [$^{3}$H]estradiol, most DPN derivatives showed greater ERβ/ERα selectivity than that of DPN with lower binding affinities for both ERα and ERβ subtypes than estradiol and also than DPN. While the ERβ binding affinities of the analogues are around 1 to 6% that of estradiol (100%), because their ERα affinities are all less than 0.025% that of estradiol, their ERβ/ERα selectivities range from 106 to 272. Among these, the meta-fluoro analog, 2d, had the greatest ERβ/ERα selectivity 272 fold, with affinities that were 0.023% for ERα and 6.25% for ERβ relative to that of estradiol.

**Conclusions:** Despite the good ERβ binding selectivities of many members of this series, their absolute binding affinities for ERβ are not good enough for them to be considered useful as potential PET or SPECT imaging agents for ERβ. In addition, preparation of the ortho-fluoro phenol unit in the most favorable compound (2d) in fluorine-18 labeled form would be challenging. Thus, ligands with more optimized ERβ binding affinity and adequate selectivities are likely be required for in vivo imaging of this estrogen receptor subtype.

**Research Support:** This study was supported by Korea Science and Engineering Foundation (KOSEF) and Ministry of Science & Technology (MOST), Republic of Korea, through its National Nuclear Technology Program. We were also supported by the National Institutes of Health (PHS 2R01CA25836 to J.A.K.).




**1a**, $R^1$ = H, $R^2$ = H
**1a**, $R^1$ = $CH_2CH_2F$, $R^2$ = H
**2a**, $R^1$ = H, $R^2$ = $CH_3$
**2b**, $R^1$ = H, $R^2$ = OH
**2c**, $R^1$ = H, $R^2$ = $CH_2OH$
**2d**, $R^1$ = H, $R^2$ = F
**2e**, $R^1$ = H, $R^2$ = Cl
**2f**, $R^1$ = H, $R^2$ = Br
**2g**, $R^1$ = H, $R^2$ = I
**2h**, $R^1$ = H, $R^2$ = $CH_2CH_2F$
**2i**, $R^1$ = H, $R^2$ = $CH_2CH_2CH_2F$

## P050 ONE-STEP 18F-LABELING OF BOMBESIN ANALOGS FOR PROSTATE CANCER IMAGING

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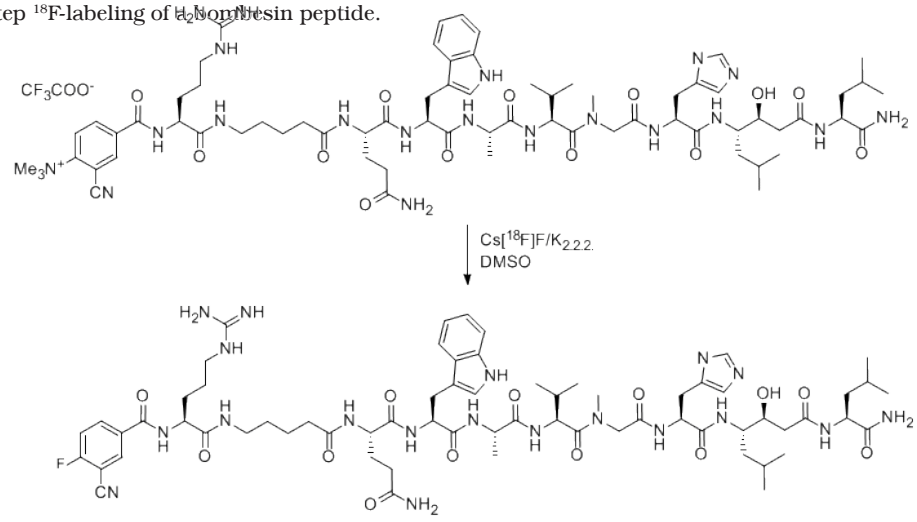
1. Center for Radiopharmaceutical Science of ETH, PSI and USZ, ETH Hoenggerberg, Zuerich, Switzerland; 2. Bayer Schering Pharma AG, Global Drug Discovery, Berlin, Germany

**Objectives:** Peptides such as bombesin (BBS) have gained an increasing attention due to their potential applications for tumor imaging and therapy1. Currently, indirect methods via prosthetic groups are applied for the $^{18}$F-radiolabeling of peptides2. These indirect methods are, however, time-consuming. We here report on a one-step fluorine-18 labeling method which we applied for the radiosynthesis of bombesin peptides.

**Methods:** Bombesin peptides were synthesized according to standard solid-phase peptide synthesis protocols. The TMA-based aromatic moiety was coupled to the peptide for direct $^{18}$F-labeling. Figure 1 depicts the structure and the radiolabeling conditions of one of the peptides.

Figure 1. One-step $^{18}$F-labeling of a bombesin peptide.




**Results:** Several TMA-based bombesin peptides were successfully labeled with fluorine-18 in one step in good radiochemical yields (up to 74% $^{18}$F-incorporation) under mild reaction conditions (50-90°C, 15min). After semi-HPLC purification, up to 29% (d.c) radiochemical yields were obtained. Radiochemical purities of the final products were greater than 98%. Specific activities at the end of the syntheses were in the range of 25-79GBq/$\mu$mol. $IC_{50}$ values of the tested peptides towards gastrin-releasing peptide receptor (GPR) were between 0.6-6.2 nM. Preliminary in vivo results in mice of these labeled peptides for prostate cancer imaging with PET are quite promising.

**Conclusions:** A direct method for the $^{18}$F-labeling of bombesin analogs was successfully demonstrated. Our PET results showed that one of the $^{18}$F-labeled bombesin peptides could be a potential candidate for the PET imaging of prostate cancer.

**References:** 1.Biddlecombe, G. B., Rogers, B. E., de Visser, M., Parry, J. J., de Jong, M., Erion, J. L., Lewis, J. S. Bioconjugate Chem. 2007, 18, 724-730; Schuhmacher, J., Zhang, H., Doll, J., Mcke, H. R., Matys, R., Hauser, H., Henze, M., Haberkorn, U., Eisenhut, M. J. Nucl. Med. 2005, 46, 691-699; Zhang, X., Cai, W., Cao, F., Schreibmann, E., Wu, Y., Wu, J. C., Xing, L., Chen, X. J. Nucl. Med2006, 47, 492-501; Smith, C. J., Volkert, W. A., Hoffman, T. J. Nucl. Med. Biol., 2005, 32, 733-740; Garca-Garayoa, E.;Schweinsberg, C.; Maes, V.; Brans, L.; Blauenstein, P.; Tourwe, D. A.;Schibli R.; Schubiger P. A. Bioconjugate Chem. 2008, 19, 2409-2416. 2. Wester, H. J., Schottelius, M. Ernst Schering Res. Found. Workshop. 2007, 62, 79-111.

## P051 SYNTHESIS AND EVALUATION OF (18F) FNBG, A POTENTIAL PET IMAGING AGENT

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1. Beijing Normal University, Key Laboratory of Radiopharmaceuticals, College of Chemistry, Beijing, China; 2. Xuan Wu Hospital, Center of PET, Beijing, China

**Objectives:** D-glucosamine and some of its derivatives are known to have various biological activities such as antitumor activities and have the potential to kill tumor cells. Recent in vitro investigations have suggested that D-glucosamine and its derivatives have inhibitory effects on human hepatoma cells. We present here our initial investigation on synthesis and biodistribution of N-(2-[$^{18}$F] fluoro-4-nitrobenzoyl) D-glucosaminide ([$^{18}$F] FNBG).

Methods: The direct $^{18}$F-labelling method is based on a [$^{18}$F]fluoro-for-nitroexchange as shown in figure 1. [$^{18}$F]-2 was purified with semi-preparative HPLC and the solvent was evaporated under a stream of $N_2$. The target $^{18}$F-labeled [$^{18}$F] FNBG can be obtained after deprotection in aqueous hydrochloric acid and methanol. The biodistribution of [$^{18}$F] FNBG was studied in Kunming mice bearing S180 tumor.

**Results:** The total radiochemical yield of [$^{18}$F] FNBG was 10-20% decay corrected to EOB, and its radiochemical purity measured by HPLC was higher than 98%. For biodistribution study the [$^{18}$F] FNBG solution was injected to S180 tumor-bearing mice (n=4) and in different interval times 5, 15, 30, 60, 120 minutes organ counts was performed. A normal high accumulation in liver and kidney was observed.The tumor uptake was moderate, and a tumor/muscle ratio was 2.3-2.5 at 30-60min post injection. In addition, further studies on modification of D-glucosamine will be carried out in order to improve the uptake in tumor and blood clearance.

**Conclusions:** [$^{18}$F] FNBG and preliminary biodistribution data suggested that [$^{18}$F] FNBG may be useful in tumor imaging. Further progress will be presented.

**Research Support:** The work was supported by National Natural Science foundation of China (No 20371009 and 20671014) and Beijing municipality-level key subject program. We also thank the PET Centre of Xuan Wu hospital for providing [18F] fluoride activity and technical assistance.

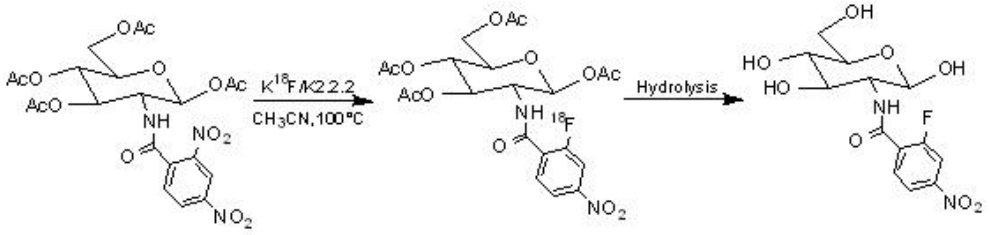


Fig.1. Synthesis [$^{18}$F] FNBG via direct $^{18}$F-labelling method.

## P052 SYNTHESIS OF 18F RADIOLABELED COX189, A SELECTIVE COX-2 INHIBITOR

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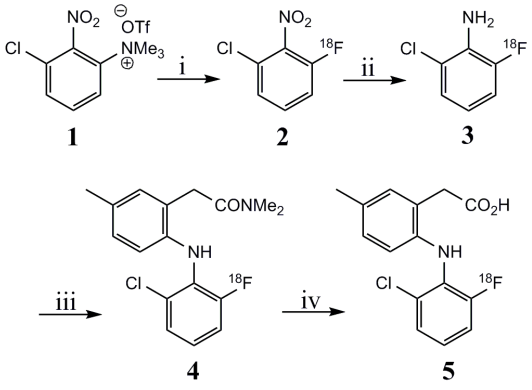
**Objectives:** COX189 (Lumiracoxib) is a highly selective COX-2 inhibitor. It was never approved in the United States[1, 2] and because of potential liver toxicities associated with the administration of the drug, it has been either withdrawn or suspended in several other countries where it had been approved for the treatment of osteoarthritic pain.[2, 3] We herein report the synthesis of $^{18}F$ radiolabeled COX189, which may be helpful in understanding its mechanism of hepatotoxicity by studying its in vivo pharmacokinetics and metabolism.

**Methods:** [$^{18}F$]Fluoride was prepared via (p, n) reaction on [$^{18}O$]$H_2O$. After bombardment, [$^{18}O$]$H_2O$ containing [$^{18}F$]Fluoride was passed through a QMA trap prepared with $KHCO_3$ and water. The trapped [$^{18}F$]Fluoride was then eluted with acetonitrile-$H_2O$ (4:1) containing $K_2CO_3$ and Kryptofix. Volatiles were removed using argon stream at 90 °C (bath temp.). Additional $CH_3CN$ was added and evaporated 4-5 times and the residue was treated with the precursor as given below. The semi-preparative HPLC was carried out using Waters Xterra C18 10x100mm, 5u column and a 60:40 mixture of acetonitrile and 0.1 M ammonium formate as the mobile phase at a flow rate of 2 mL/min.

**Results:** [$^{18}F$]COX189 (5) was prepared in 4 steps (Scheme). The starting material for the introduction of $^{18}F$ label was N,N,N-trimethyl-3-chloro-2-nitroanilinium triflate (1). Treatment of 1 with $^{18}F$-fluoride/Kryptofix/$K_2CO_3$ in DMSO at room temperature for 10 min., followed by C18 Sep-Pak purification gave 2 in 81% yield (n=9). Reduction of the nitro compound 2 to aniline 3 was achieved by stirring with $NaBH_4$/5%Pd-C in MeOH at room temperature for 10 min. (yield 88%, n=9; isolated yield 60%, n=10). The Ullmann coupling of aniline 3 with N,N-dimethyl-2-iodo-5-methylphenylacetamide was carried out at 300 °C for 45 min. in triglyme and in the presence of copper(I) iodide and potassium carbonate to give the coupling product 4 (yield 31%, n=6). Several different conditions were explored for the isolation of 3, for the Ullmann reaction and also for the isolation of the Ullmann product 4. The acetamide 4 was purified using either Alltech C18 cartridges or semi-preparative HPLC as mentioned above. The fraction containing 4 was collected and evaporated to dryness. Hydrolysis of 4 with aqueous potassium hydroxide at 150 °C for 30 min and neutralization gave [$^{18}F$]COX189 (5), which was purified by HPLC using the above conditions. [$^{18}F$]COX189 (5) was isolated in moderate yields (42%, n=10). After evaporation of the HPLC fraction, the product was taken up in saline and filtered through 0.2 micron filter. The total synthesis time was ca. 150 min. (EOB) and overall radiochemical yield was 5-10% (decay corrected, based on starting [$^{18}F$]fluoride).

**Scheme**




i) [$^{18}F$]Fluoride/Kryptofix/$K_2CO_3$, DMSO, rt, 10 min ii) $NaBH_4$, 5% Pd-C, MeOH, rt, 10 min. iii) N,N-dimethyl-2-iodo-5-phenylacetamide, CuI, $K_2CO_3$, triglyme, 300 °C, 45 min. iv) 45% aq. KOH, EtOH, 130 °C, 30 min.

**Conclusions:** In conclusion, [$^{18}F$]COX189 was prepared in 4 steps in quantities and purity suitable for primate and non-primate PET imaging studies.

**References:** 1.http://www.novartis.com/newsroom/media-releases/en/2007/1156327.shtml. 2.Kang P, et al., Chemical Research in Toxicology, 2009, 22, 106-117; and references cited therein. 3.http://www.novartis.com/newsroom/media-releases/en/2007/1169462.shtml.

## P053 EFFICIENT RADIOSYNTHESIS AND EVALUATION OF FLUORINE-18 LABELED BENZIMIDAZOL DERIVATIVES FOR PERIPHERAL TUMOR IMAGING

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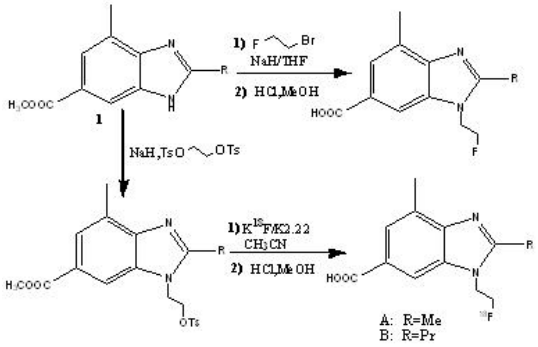
**Objectives:** Benzimidazole derivatives have widespread application in pharmaceutics. Some derivatives of benzimidazole have effect on antineoplastic, such as Bendamustine which can inhibit the growth of human malignant cells. We are developing two novel $^{18}$F-labelling benzimidazole acid derivatives. Herein, we report the synthesis, radiochemical synthesis two bensimidazole acid derivatives and bioevaluation in tumor bearing mice of one benzimidazole acid derivative with $^{18}$F.

**Methods:** The methyl 2, 4-dimethyl-1H-benzimidazole-6-carboxylate (1) was prepared in a 7 step reaction sequence from 3-methyl-4-nitrobenzoic acid. The tosyl precursors were reacted with K$^{18}$F in acetonitrile at 90℃ for 15 minutes. Subsequent hydrolysis in 1N HCl and methanol mixture was carried out at 90℃ for 10 minutes. The product was purified by the semi-preparative HPLC using mixture of water and methanol as solvents. Reaction yields were estimated from the reaction mixture by HPLC. All reference compounds were synthesized and characterized by NMR and MS, and used for the analysis of radioactive product distribution by HPLC. Biological distribution studies on[$^{18}$F] A at 5min, 15min, 30min and 60min was obtained.

**Results:** The overall radiochemical yield of [$^{18}$F] A $^{18}$F] B$^{18}$F] A$^{18}$F] A $^{18}$F] B increases, peaking at 60 min postinjection with value of 1.2 and the tumor to muscle ratio is 3.2.and [ indicates that the activity in both the blood and tumor decreases. While the clearance of activity from the blood and the muscle is faster than the tumor tissue, thus the tumor-to-blood accumulation ratios of [ were both about 50%±5% (based on $^{18}$F-fluoried) and their radiochemical purity exceeded 99%. The biodistribution data of [and [

**Conclusions:** Two benzimidazole acid derivatives have labeled $^{18}$F with high radiochemical yield and purity. The high tumor-to-blood and tumor-to-muscle accumulation ratios of [$^{18}$F] A at 60 min postinjectionsuggests that a good tumor imaging may be observed. Further work will be going on including autoradiography and PET imaging.

**Research Support:** Financial support of this work by National Natural Science foundation of China (No 20371009 and 20671014) and Beijing municipality-level Key Subject Program are gratefully acknowledged. The authors would like to thank the PET Centre of Xuan Wu hospital for providing [$^{18}$F] fluoride activity and technical assistance.



## P054 THE EFFECT OF SOLVENT IN NUCLEOPHILIC 18F-FLUORINATION

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**Objectives:** Polar aprotic solvents like CH3CN, DMF and DMSO are traditionally used in nucleophilic fluorinations. Recently improved fluorination yields were reported with alkali metal fluorides using nonpolar protic solvents, tert-alcohols [1,2]. The aim of this project was to study systematically the effect of solvent for nucleophilic 18F-fluorination reaction in terms of radiochemical yield and regioselectivity. 2/4-Chloro-[4-(chloromethyl)phenyl]benzoates were chosen as inactive precursors as they contain halide leaving group both in aliphatic and aromatic position.

**Methods:** Starting materials for radiosynthesis, 2/4-chloro-[4-(chloromethyl)phenyl]benzoates, were synthesized by esterification of corresponding benzoyl chloride with 4-(chloromethyl)benzyl alcohol. Inactive reference standards were synthesized both for aliphatic and aromatic fluorinated products. All products were purified by column chromatography and their identity/purity was verified by $^{1}$H-NMR and elemental analysis. Radiofluorination was carried out by adding dry K222/K[$^{18}$F]F into a reaction vial containing the inactive precursor dissolved either in anhydrous $CH_3CN$, DMF, t-BuOH or t-amyl alcohol (23% of $CH_3CN$ added for better solubility). The reaction mixture was then heated in a closed vial for 15 min in 80, 120 or 160°C. Labeled products were characterized by radioHPLC. The radiochemical yield was measured based on radioTLC and it was corrected for decay and for possible loss of radioactivity e.g. in the reaction vial.

**Results:** Only radiofluorination in aliphatic position was detected. The best total overall radiochemical yield of both 2- and 4-chloro-[4-([18F]fluoromethyl)phenyl]benzoate was achieved in CH3CN in all reaction temperatures. In the lowest temperature the radiochemical yields were significantly higher in aprotic than in protic solvents (Fig.1). Rising the temperature improved the radiochemical yield in DMF, t-BuOH and t-amyl alcohol. However, higher reaction temperatures resulted in some side-products, especially when t-BuOH was used.

**Conclusions:** Solvent did not have influence on regioselectivity of nucleophilic $^{18}$F-fluorination and the use of protic solvents did not improve the radiochemical yield in this experimental setup. Further results with different leaving groups will be reported in the meeting.

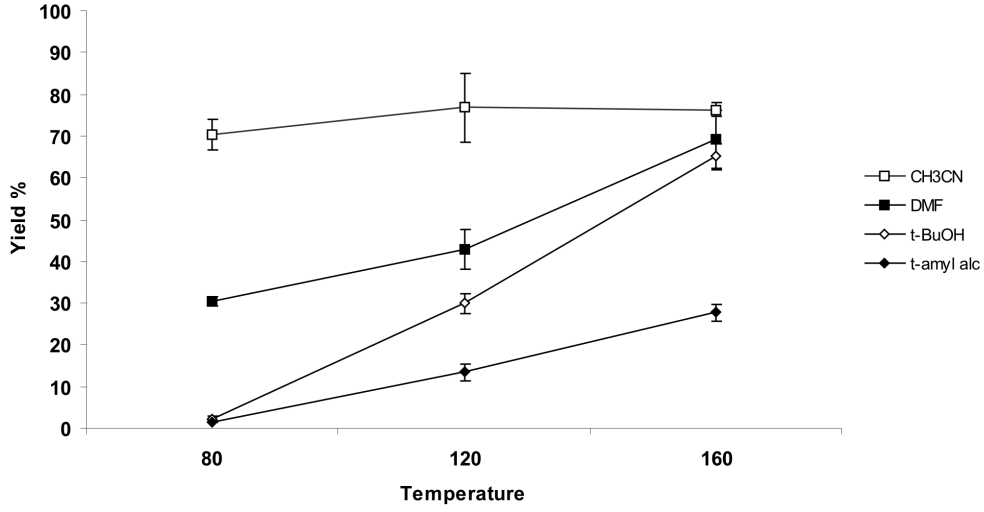


Figure 1. The total overall radiochemical yield (%±SD, n=3) of 2-chloro-[4-([$^{18}$F]fluoromethyl)phenyl]benzoate in different solvents

## P055 RADIOSYNTHESIS AND PHARMACOKINETIC PROPERTIES OF 18F-NIFEDIPINE: A NEW PET TRACER OF CALCIUM CHANNELS

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**Objectives:** [$^{18}$F]Dimethyl 2-(fluoromethyl)-6-methyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate ($^{18}$F-nifedipine) was prepared in no-carrier-added (n.c.a.) for imaging of calcium channels and evaluating the pharmacokinetic properties.

**Methods:** $^{18}$F-nifedipine was prepared form a starting brominated compound in one step at 80°C in Kryptofix2.2.2/[18F]. Pharmacokinetic properties were evaluated by Biodistribution studies of $^{18}$F-nifedipine in normal rats.

**Results:** The chemical and radiochemical purity were 90 and 95% respectidly and total radiosynthesis yield was 3%. The tracer was accumulated in the heart, brain and lungs in the first few minutes due to presence of calcium channels. The radiotracer was metabolized fast in liver but mainly in the intestine after about 1 hour. Protein binding in fresh serum indicates about 50% binding wich is in agreement with other studies using $^{3}$H-dihydropyridines

**Conclusions:** The radiotracer accumulates in the organs that have calcium channels in few minute, so we can use diuretics to decrease radiation dose. This research is also showed that the most important organ for metabolism for the tracer is intestine although the first is liver.

## P056 STRUCTURE OF 2-METHYLPHENYL(2-METHOXYPHENYL)IODONIUM CHLORIDE DIMER

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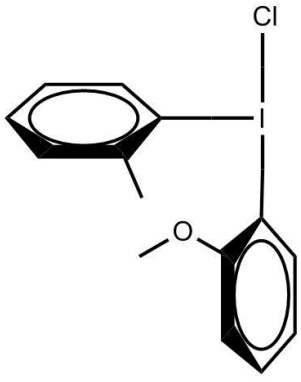
1. National Institutes of Health, Center for Information Technology, Division of Computational Bioscience, Center for Molecular Modeling, Bethesda, MD; 2. National Institutes of Health, National Institute of Mental Health, Molecular Imaging Branch, Bethesda, MD

**Objectives:** Diaryliodonium salts permit the single-step and efficient incorporation of readily available [$^{18}$F]fluoride ion into both electron-rich and electron-deficient arenes [Pike and Aigbirhio, J. Chem. Soc., Chem Commun, 1995, 2218]. This process ($ArI^{+}Ar' + {}^{18}F^{-} \rightarrow Ar{}^{18}F + Ar'I$) is not yet well understood mechanistically. For example,we have found that the radiofluorination of 2-methylphenyl(2'-methoxyphenyl)iodonium chloride (1) surprisingly produces a very high radiochemical yield of [$^{18}$F]2-fluorotoluene accompanied by a much lower yield of [$^{18}$F]2-fluoroanisole. In order to further understand such fluorination reactions and their selectivities at the atomic level, it is necessary to understand the structures of diaryliodonium salts in organic media where the reactions are typically conducted. In this endeavor, we have determined the X-ray structure of salt 1, and then studied its conformations as both monomeric and dimeric forms in MeCN with quantum chemical calculations.

**Methods:** For X-ray crystallography, crystals of 1were grown from MeCN solution by portion-wise addition of water at room temperature. X-Ray data were collected using a SMART Apex CCD diffractometer (Bruker, Madison, WI, USA) with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Quantum chemical calculations at the level of B3LYP/DGDZVP were performed with Gaussian 03.

**Results:** The X-ray structure of 1 shows a Cl-bridged dimer crystallized as a pair of conformational enantiomers (2). The I-Cl bonds in 2 are primarily ionic since their lengths are about 0.7 Å longer than in solid $PhICl_2$ [Archer and Schalkwyk, Acta Cryst., 1953, 6, 88; Carey, et al., J. Chem. Res., 1996, 348, 358]. The ionic nature of the I-Cl bond in monomeric 1 allows a secondary bonding interaction [Alcock, Adv. Inorg. Chem. Radiochem., 1972, 15, 1; Landrum et al., New J. Chem., 1998, 883] to arise, resulting in the crystallization of the Cl-bridged dimer. Quantum chemical calculations suggest that monomeric 1 can have a number of conformations in MeCN due to the rotation of the 2'-anisyl group or the rotation of the 2-tolyl group. Calculation also gives a favorable dimerization energy for 1, which, in turn suggest that salts such as 1, may well exist as a dimer in MeCN.

**Conclusions:** Quantum chemical calculations indicate that 1 predominantly exists as a dimer in MeCN because of a strong charge interaction between the monomers. Thus, the radiofluorinations of salts similar to 1 in organic solvent likely requires the dissociation of dimer before the replacement of Cl with F. Work is in progress to determine whether the dissociation process occurs in a concerted or step-wise manner. This will assist in modulating the aryl ring selectivity of radiofuorination.

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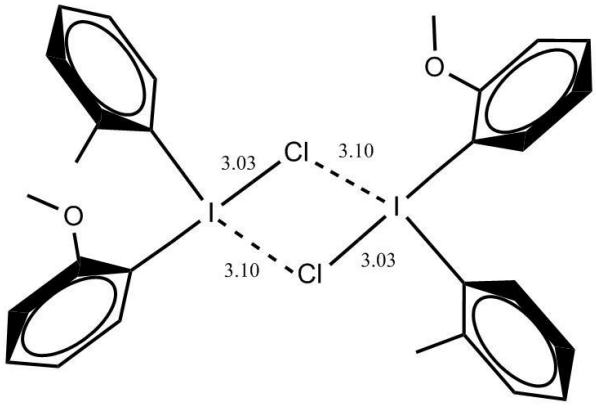


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## P057 TWO STEP ORGANIC PHASE SYNTHESES OF [18F]N-SUCCINIMIDYL-4-FLUOROBENZOATE AND 4-[18F] FLUOROBENZYL BROMIDE

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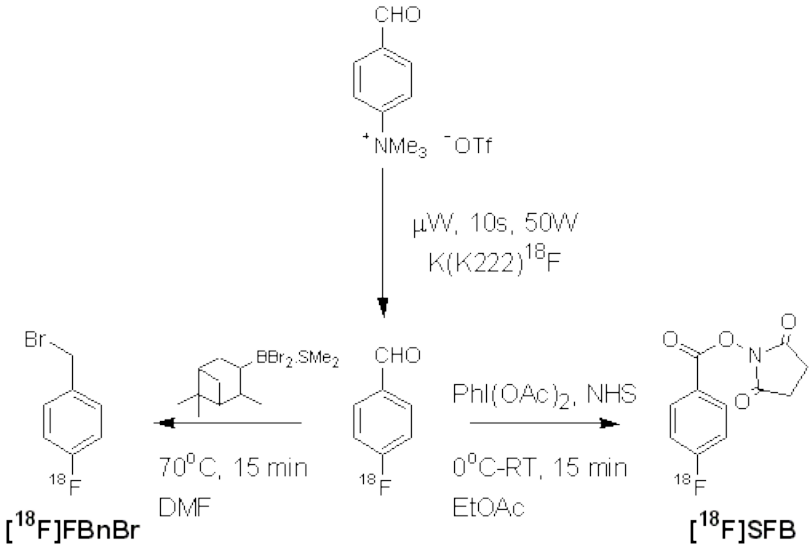
**Objectives:** Radiolabeling small molecules and peptides for applications in molecular imaging frequently requires the rapid preparation of fluorine-18 pre–labelled prosthetic reagents that can be conjugated to small molecule precursors and native or modified peptides. The purpose of this study was to simplify existing synthetic procedures for the preparation of [$^{18}$F] N-succinimidyl-4-fluorobenzoate ($^{18}$F-SFB)[1] and 4-[$^{18}$F]-fluorobenzyl bromide ($^{18}$F-FBnBr).[2]

**Methods:** Starting from K$^{18}$F/$KHCO_3$/K222 and 4-formyl-N,N,N-trimethyl benzeneaminium trifluoromethane sulfonate, 4-[$^{18}$F]fluorobenzaldehyde ($^{18}$F-FBA) was prepared in DMSO using microwave heating. The product aldehyde was next subjected to either a reductive halogenation or oxidation in the presence of N-hydroxysuccinimide to yield 4-[$^{18}$F]fluorobenzyl bromide or [$^{18}$F]N-succinimidyl-4-fluorobenzoate, respectively.

**Results:** Under the microwave conditions employed (10s irradiation at 50 W), $^{18}$F-FBA was prepared in near quantitative analytical yield (94 ± 3 %, n = 4) and following trapping on a silica cartridge $^{18}$F-FBA was eluted using an organic solvent (e.g. DCM, EtOAc, DMF). Typically, $^{18}$F-FBA was isolated in 66 ± 6 % (n = 3) decay-corrected yield with a radiochemical purity >99 % in a total time of 40-45 minutes. At 0°C for 10 minutes followed by warming to room temperature over a further 5 minutes, iodobenzene diacetate oxidation of $^{18}$F-FBA in the presence of N-hydroxysuccinimide in ethyl aceteate afforded $^{18}$F-SFB. The reaction mixture was purified by preparative NP-HPLC providing $^{18}$F-SFB in an overall decay-corrected radiochemical yield of 49 ± 6 % (n = 3) and a radiochemical purity of >99%. In an attempt to remove the HPLC step, solid phase extraction/release of the product from a silica cartridge afforded $^{18}$F-SFB in 77 ± 9 % decay-corrected radiochemical yield (n = 4) albeit with a reduced radiochemical purity of 87 ± 3 % (n = 4). Currently, $^{18}$F-FBnBr can be prepared in 3 steps from [$^{18}$F]fluoride.[2] Following the synthetic method of Kabalka et. al,[3] we hoped to adapt the 1 step reductive bromination of aromatic aldehydes using alkylboron dibromides to prepare $^{18}$F-FBnBr from $^{18}$F-FBA. Using isopinochampheyl boron dibromide in dichloromethane, prepared by reaction of alpha-pinene with dibromoborane-dimethylsulfide, treatment of $^{18}$F-FBA at 70°C for 15 minutes afforded the desired $^{18}$F-FBnBr alkylating agent in 96 ± 3 % (n = 3) analytical yield. Further characterisation of the reductive bromination reaction of $^{18}$F-FBA and subsequent application of $^{18}$F-FBnBr to [$^{18}$F]fluoroalkylation chemistry is currently underway.

**Conclusions:** We have described two straightforward methods for the rapid organic phase synthesis of fluorine-18 prosthetic labelling reagents $^{18}$F-SFB and $^{18}$F-FBnBr. Both routes offer a non-aqueous procedure, employing fewer radiochemical steps than already published methods and both can potentially be readily transferred to automated platforms.

**References:** 1. Synthesis of [$^{18}$F]N-succinimidyl-4-fluorobenzoate, see for example: F. Wust, C. Hultsch, R. Bergmann, B. Johannsen, and T. Henle, Applied Radiation and Isotopes, 2003, 59, 4348. 2. Synthesis of 4-[$^{18}$F]fluorobenzyl bromide, see for example: S. R. Donohue, C. Halldin, M. Schou, J. Hong, L. Phebus, E. Chernet, S. A. Hitchcock, K. M. Gardinier, K. M. Ruley, J. H. Krushinski, J. Schaus and V. W. Pike, J. Label Compd. Radiopharm. 2008, 51, 146152. 3. G. W. Kabalka, Z. Wu and Y. Ju, Tetrahedron Lett., 2000, 41, 5161-5164.



## P058 PROBING THE ORTHO-EFFECT IN THE RADIOFLUORINATION OF DIARYLIODONIUM SALTS

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National Institutes of Health, National Institute of Mental Health, Molecular Imaging Branch, Bethesda, MD

**Objectives:** Diaryliodonium salts ($ArI^+Ar'X^-$) react with various nucleophiles ($Nu^-$) to give ArNu and Ar'Nu as products. An ortho substituent in one of the aryl rings tends to direct the incoming nucleophile to the same ring. This has been dubbed the 'ortho effect', and has been studied only for ortho alkyl. The effect has been rationalized as primarily steric [Lancer et al. JOC,1976, 41, 3360]. Reactions of diaryliodonium salts with [$^{18}$F]fluoride ion provide single-step access to [$^{18}$F]fluoroarenes, including those bearing electron-donating groups (e.g., alkyl, OMe). In view of the growing importance of these reactions for radiotracer preparation, we aimed to study the ortho effect more closely in the radiofluorination of diaryliodonium salts, in order to provide a sound basis for predicting product selectivity.

**Methods:** Ortho-substituted diaryliodonium salts were prepared by treating appropriate aryltriakylstannanes [Pike et al., JCS, Perkin Trans. 1, 1999, 245] or arylboronic acids [Carroll et al., Tetrahedron Lett., 2000, 41, 5393] with [hydroxy(tosyloxy) iodo]arenes. The reactivity of these salts was investigated in a microfluidic apparatus (Advion). The dried [$^{18}$F]fluoride ion complex (0.3–1 mCi for each reaction) and iodonium salt (ca. 10 mM), each in DMF, were delivered simultaneously from two separate reservoirs into a 4-m silica glass micro-reactor held at a specified temperature in the range 90–200 °C. Reaction (residence) times were controlled by setting solution infusion rates to an equal value in the range 4–10 $\mu$L/min. Reactions were quenched by immediate dilution of reactor effluent with MeCN-$H_2O$ (1: 1 v/v) at r.t., and products were analyzed with radio-HPLC to determine decay-corrected radiochemical yields (RCYs). For each iodonium salt, reactions were performed at more than five different temperatures. The selectivity for Ar$^{18}$F vs. Ar'$^{18}$F was calculated for each reaction.

**Results:** Very high RCYs of [$^{18}$F]fluoroarenes were obtained from brief reactions (< 471 s, Table). Product selectivities varied little with temperature. The data revealed the influence of substituents on product selectivities. Thus, the order of influence of substituents in directing the incoming [$^{18}$F]fluoride ion to the same ring was: 2,6-di-Me > 2,4,6-tri-Me > 2-Me > 2-Et ≈ 2-iso-Pr > 2-H > 2-OMe.

**Conclusions:** An ortho-MeO group was the least effective of the examined substituents in directing the incoming [$^{18}$F]fluoride ion to the same ring, showing that bulkiness of the ortho substituent was not the only factor operating on selectivity. An ortho-alkyl group was more effective than H. Increase in size of the alkyl group beyond ortho-Me slightly reduced selectivity. Two ortho-Me groups imparted the greatest selectivity for [$^{18}$F]fluoride ion insertion into the same ring. Introduction of an extra para-methyl group suppressed the selectivity of two ortho-Me groups, perhaps through an inductive deactivation of the ring.

**Research Support:** Intramural Research Program of NIH, NIMH


**Table** RCYs and product selectivities for the radiofluorination of ortho-substituted diaryliodonium salts.

| Entry | Ar | Ar' | T (°C) | RCY (%) Ar$^{18}$F major | RCY (%) Ar'$^{18}$F minor | Selectivity for major product |
|---|---|---|---|---|---|---|
| 1 | 2-Me-$C_6H_4$ | Ph | 130 | 22.9 | 10 | 2.3 |
| 2 | Ph | 2-MeO-$C_6H_4$ | 140 | 59.9 | 6.5 | 9.2 |
| 3 | 2-Me-$C_6H_4$ | 2-MeO-$C_6H_4$ | 140 | 75.4 | 3.7 | 20.3 |
| 4 | 2-Me-$C_6H_4$ | 2-Et-$C_6H_4$ | 170 | 48.6 | 41 | 1.2 |
| 5 | 2-Me-$C_6H_4$ | 2-*i*-Pr-$C_6H_4$ | 170 | 47.6 | 40.2 | 1.1 |
| 6 | 2,6-di-Me-$C_6H_3$ | 2-Me-$C_6H_4$ | 170 | 62.3 | 11.2 | 5.6 |
| 7 | 2,4,6-tri-Me-$C_6H_2$ | Ph | 150 | 62.6 | 19.3 | 3.2 |
| 8 | 2,4,6-tri-Me-$C_6H_2$ | 2-Me-$C_6H_4$ | 130 | 58.6 | 33 | 1.8 |
| 9 | 2,6-di-Me-$C_6H_3$ | 2,4,6-tri-Me-$C_6H_2$ | 190 | 61.4 | 20.5 | 3.0 |

X⁻ is chloride, except for entries 7 and 9 where X⁻ is tosylate; Reaction time is 471 s except for entries 1, 2 and 3 (377 s). Temperatures for highest observed radiofluorination yields are given.

## P059 RADIOFLUORINATION OF UNSYMMETRICAL DIARYLIODONIUM SALT COMPLIES WITH THE CURTIN-HAMMETT PRINCIPLE

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**Objectives:** The radiofluorinations of diaryliodonium salts show unusual features including radiofluorination at aryl rings bearing electron-donating groups and an 'ortho effect', in which an ortho subsitutent, even if electron-donating (e.g., Me), may direct the incoming [$^{18}$F]fluoride ion to the same ring [Cai et al,. Eur. J. Org. Chem., 2008, 17, 2853; Pike and Aigbirhio, J. Chem. Soc., Chem. Commun., 1995, 2215]. Hence the mechanisms of these reactions are intriguing but not yet well understood. Radiofluorination of unsymmetrical diaryliodonium salts (ArI$^+$Ar' X$^-$) may give two radioactive products, Ar$^{18}$F and Ar'$^{18}$F. Here, in order to gain mechanistic insight, we tested whether the product selectivity of this process complies with the Curtin-Hammett principle [Seeman, Chem. Rev., 1983, 83, 84].

**Methods:** Cyclotron-produced [$^{18}$F]fluoride ion in [$^{18}$O]water was mixed with a solution of $K_2CO_3$-Kryptofix 2.2.2 complex in MeCN-$H_2O$ (95: 5 v/v) and dried with microwave heating. The radioactive residue was dissolved in a solution of KF-K 2.2.2 (5 mM) in DMF-$H_2O$ (99.5: 0.5 v/v). For each reaction, the [$^{18}$F]fluoride ion solution and o-methylphenyl(phenyl)iodonium chloride (1) solution (5 mM) in DMF were delivered simultaneously at the same fixed flow rate into a silica glass micro-reactor held at a set temperature [Lu et al. Curr. Radiopharm., 2009, 2, 49]. Residence times in the micro-reactor were controlled by the set flow rate. Exiting reaction mixtures were immediately quenched with MeCN-$H_2O$ (1: 1 v/v) and analyzed by reverse phase radio-HPLC to determine decay-corrected radiochemical yields of products. We postulated that [$^{18}$F]fluoride ion initially attacks the iodine atom of either of two rapidly inter-converting conformers of the diaryliodonium salt, giving one of two possible reaction transition states. We also calculated the structures and energies of these two transition states according to the procedures published elsewhere [Lee et al., J. Phys. Chem. A, 2008, 112, 1604].

**Results:** The radiofluorination of 1 gave two radioactive products, Ph$^{18}$F and 2-Me-$C_6H_4{}^{18}$F, which were in a constant ratio throughout each reaction time-course (see Figure). Therefore, the reaction complies with the Curtin-Hammett principle. Accordingly, we deduce that the selectivity of the reaction was controlled solely by the difference in energies of the two respective transitions states ($\Delta G_{TS1}{}^{\#} - \Delta G_{TS2}{}^{\#}$). From the selectivity value (2.2), ($\Delta G_{TS1}{}^{\#} - \Delta G_{TS2}{}^{\#}$) was 0.58 kcal/mol at 110 °C. This value is in good agreement with that calculated from quantum chemistry for the two postulated transition states.

**Conclusions:** The ring selectivity of the radiofluorination of 1, and by implication of other diaryliodonium salts, is controlled solely by the energy difference between transition states. This finding accords with theoretical analysis of a mechanism involving initial [$^{18}$F]fluoride ion attack at the iodine centers of two rapidly inter-converting conformers.

**Research Support:** Intramural Research Program of NIH, NIMH

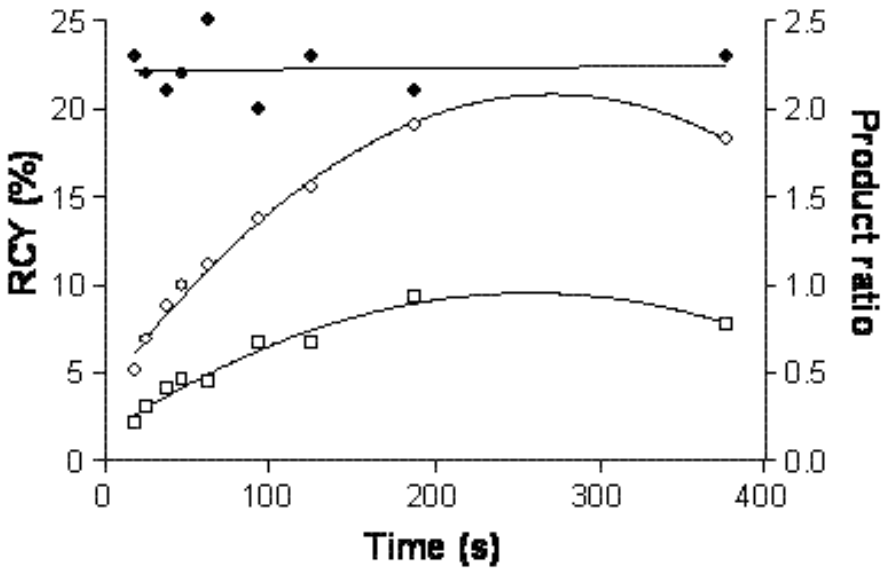


**Figure**. RCYs of [$^{18}$F]fluorobenzene (□), [$^{18}$F]2-fluorotoluene (○) and product selectivity (●) from the radiofluorination of 1 at 110 °C (molar ratio of 1 to KF, 1: 1).

## P060 2-TRIMETHYLAMMONIUM NICOTINIC ACID TFP-ESTER: A NOVEL PYRIDINE BASED SYSTEM FOR THE ONE-STEP LABELLING OF BIOMOLECULES WITH FLUORINE-18

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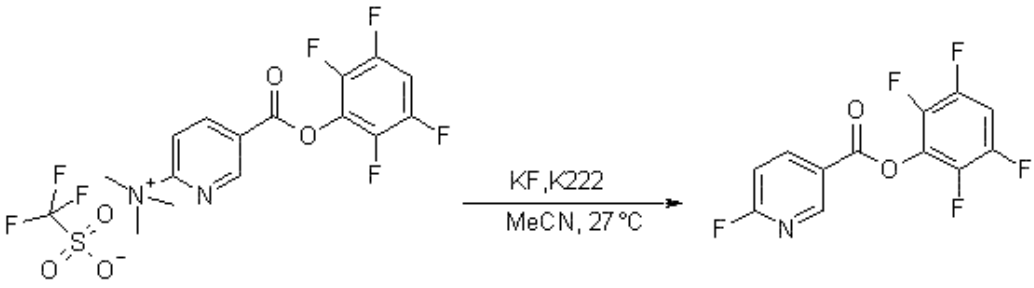
**Objectives:** The purpose of this study was to synthesize active esters of 2-trimethylammonium (as triflate salt) nicotinic acid and evaluate the aromatic substitution of $^{19}F$ and $^{18}F$ for trimethylammonium in the pyridine ring. A one-step labelling reaction and a simple purification step, giving a process amendable for automation, was one of the main goals.

**Methods:** 2,3,5,6-tetrafluorophenol (Tfp) and N-hydroxysuccinimide (NHS) active esters of 2-trimethylammonium nicotinic acid were synthesised in three steps starting from 6-chloro nicotinic acid. The steps included esterification with Tfp or NHS activated by DCC, generation of the trimethylammonium salt by treating the active 6-chloro nicotinic acid-ester in a saturated solution of trimethylamine in THF. Generation to the triflate salt from chloride with silver triflate gave solubility of the precursor in acetonitrile. The synthesized precursors were initially labelled with $^{19}F$ by means of K222 and KF in acetonitrile. The reaction of $^{19}F$ with the most promising ester, Tfp, was studied using $^{1}H$-NMR in acetonitrile-d6 at 27°C to assay reaction kinetics and impurities formed. The fluorinated-Tfp ester was also reacted with an RGD-peptide with a free amino group in carbonate buffer pH 10.

**Results:** Both esters were synthesized in good yields (>50% starting from 6-chloro nicotinic acid) and reacted readily with fluoride in acetonitrile at room temperature. The NHS-ester was more prone to hydrolysis under the reaction conditions than the Tpf-ester and was thus not evaluated further. Studies of the Tfp-ester was studied by following the $^{1}H$-NMR spectrum over 30 minutes and showed rapid incorporation of fluoride at near room temperature, after 2,5 minutes, 32% of the starting material was converted to the desired fluorinated product. In one set of experiments 70% of fluorinated product was obtained in less than 20 minutes. Two nicotinic acid derivates were identified as side-products along with the desired product. Excess precursor can be trapped on an anion exchanger cartridge for purification. Reaction of the fluorinated product with a suitable functionalised RGD-peptide in carbonate buffer pH 10 gave the desired conjugate as analyzed by LC-MS. Further investigations with [$^{18}F$]fluoride are in progress.

**Conclusions:** Labelling of 2-trimethylammonium nicotinic acid Tfp-ester proved to proceed rapidly with fluoride in acetonitrile at room temperature. Minimal hydrolysis of the ester was observed. Conjugation of the fluoride labelled Tfp-ester with an RGD-peptide in carbonate buffer gave the desired peptide conjugate. Further investigations with [$^{18}F$]fluoride are in progress.

**References:** Herman et al,Nucl. Med. Biol. Vol. 21, No. 7, 1005-1010, 1994.



## P061 SITE-SPECIFIC ADDITION OF AN 18F-N-METHYLAMINOOXY CONTAINING PROSTHETIC GROUP TO A VINYLSULFONE MODIFIED PEPTIDE

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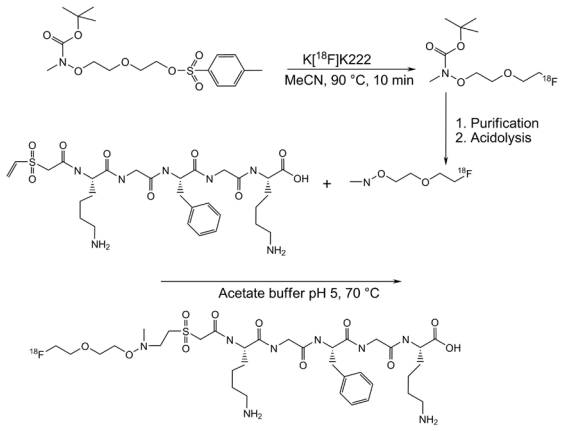
**Objectives:**




Recently, our group reported a new prosthetic group based on the reactivity of the N-methylaminooxy, where it was demonstrated that a model peptide functionalised either with nitrostyrene or maleimide reacted under mildly acidic aqueous conditions to give [$^{18}$F]-fluoro-peptides in moderate to good yields. Here, we study, using a commercial platform, the same model peptide (Lys-Gly-Phe-Gly-Lys) functionalized with vinylsulfonyl acetic acid attached to the N-terminal, which proved to react site-specifically with the prosthetic group O-2[2-(2-[$^{18}$F]fluoroethoxy)ethyl]-N-methyl-N-hydroxylamine.

**Methods:** Synthesis of the precursor and radiolabeling to yield the 2-(2-[$^{18}$F]Fluoroethoxy)ethoxy]methylcarbamic acid t-butyl ester from the corresponding tosylate was done accordingly to previous methods[1]. As the major part of unreacted precursor hydrolysed to the alcohol, which also showed to react with the model peptide functionalised with vinylsulfonyl acetic acid, the Boc-protected [$^{18}$F]-fluorinated precursors were purified with either with reversed phase HPLC or a more simple Sep-Pak approach. After the purification step, the protective Boc-group was removed using hydrochloric acid. Incorporation yield with peptide at 70 °C in acetate buffer using the two purification methods were compared by analysing reaction mixtures by radio-HPLC. Conjugation yields were also studied using reaction time (10 to 70 min) and peptide concentration (7.5, 3 and 0.75 mM) as variabeles.

**Results:** O-[2-(2-[$^{18}$F]fluoroethoxy)ethyl]-N-methyl-N-hydroxylamine reacted in site-selective manner with the vinylsulfone modified model peptide in 0.4 M acetate buffer pH 5 giving only the $^{18}$F-peptide as product. Both purification methods gave good incorporation after 60-70 min using 7.5 mM peptide concentration. The HPLC purified method gave marginally better incorporation yields (84% after 70 minutes) compared to the Sep-Pak base (79% after 70 min) based on the integration of the radioactive peaks of the chromatogram. See table 1 for results. Table 1. Incorporation of O-[2-(2-[$^{18}$F]fluoroethoxy)ethyl]-N-methyl-N-hydroxylamine with model peptide in acetate buffer pH 5 at 70°C.

| | **HPLC purification (% incorporation of prosthetic group)** Peptide concentration (mM) | **Sep-Pak purification (% incorporation of prosthetic group)** Peptide concentration (mM) |
|---|---|---|
| Time (min) | 0.75 / 3 / 7.5 | 0.75 / 3 / 7.5 |
| 10 | 3 / 8 / 28 | 4 / 10 / 27 |
| 30 | 9 / 18 / 61 | 9 / 24 / 55 |
| 50 | 13 / 31 / 75 | 13 / 33 / 69 |
| 70 | 17 / 41 / 84 | 16 / 38 / 79 |

**Conclusions:** The vinylsulfonyl modified peptide, vinylsulfonyl acetyl-Lys-Gly-Phe-Gly-Lys-OH, reacts in a site-selective manner with the prosthetic group O-[2-(2-[$^{18}$F]fluoroethoxy)ethyl]-N-methyl-N-hydroxylamine to give the [$^{18}$F]-peptide conjugate in acceptable to good yields after 60-70 minutes under mild acidic aqueous conditions at 70°C. The synthesis was achieved on a commercial platform. Similar conjugation yields were achiveved using a Sep-Pak based purification step, compared to an HPLC based step, which makes the process more amenable for automation.

**References:** 1. Olberg et al. Bioconjugate Chem. 19 (6), 1301-1308, 2008.

## P062 INFLUENCE OF OXYANIONS AND ALUMINA ON 18F LABELING

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**Objectives:** It has been suggested that oxyanions might influence the labeling of $^{18}F$ substitution reactions [1,2]. Potential sources for oxyanions in the radiosynthesis with $^{18}F$ could originate from borosilicate glassware and silica-based trapping columns (QMA) typically used. The purpose of this study was to investigate how the presence of low levels (ppm) of certain oxyanions and $AlCl_3$ influenced the labeling yield in a typical $^{18}F$ nucleophilic substitution reaction.

**Methods:** The water soluble salts; $Na_2SiO_3$, $KBO_2$ and $AlCl_3$ were added during the radiolabeling of $^{18}F$[Fluoride]-Amino-Cyclobutane-Carbocylic acid (FACBC). The interaction between the salts and its correlation to labeling yield was investigated through a chemiometric design setup (full fractional 2-level design). Radiosynthesis was fully automated.

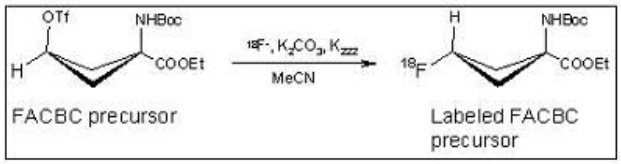
Levels of salts included in the study

| Salt | Levels (ppm) |
|---|---|
| $Na_2SiO_3*9H_2O$ | 50 - 250 |
| $KBO_2*H_2O$ | 4 - 20 |
| $AlCl_3*6H_2O$ | 0.4 - 2 |

**Results:** The additions of either $KBO_2$ or $AlCl_3$ alone showed significant reductions in labeling yield. E.g. 20 ppm $KBO_2$ or 2 ppm $AlCl_3$ reduced the labeling yield 27 ± 2 % and 90 ± 1 % respectively. The presence of $Na_2SiO_3$ alone affected the labeling yield only to a lesser extent. The presence of all three salts together showed a substantial interaction with each other and made a significant impact on the labeling yield at all levels within the design. The clearest result was the proportional reduction in yield correlated to $KBO_2$, no matter the levels of the two other salts. However, it seemed that the negative impact from $KBO_2$ and $AlCl_3$ would cancel out each other when present at similar low ratios.

**Conclusions:** There was a substantial interaction between $Na_2SiO_3$, $KBO_2$ and $AlCl_3$ in how they affected the incorporation of $^{18}F$[fluoride] to the FACBC precursor. The preliminary results from this study demonstrate the potential of water leachable species from a number of sources including borosilicate glassware, silica based cartridges and other surfaces may influence with $^{18}F$[fluoride] nucleophilic substitution.

**References:** [1] Tewson TJ, J. Nucl. Med. and Biol. 16, 533-561, 1989.[2] Nickles RJ et al., Appl. Radiat. Isot. 37, 649-661, 1986.



## P063 THREE-STEP SYNTHESIS OF 2-[18F]FLUORO-L-PHENYLALANINE VIA ISOTOPIC EXCHANGE

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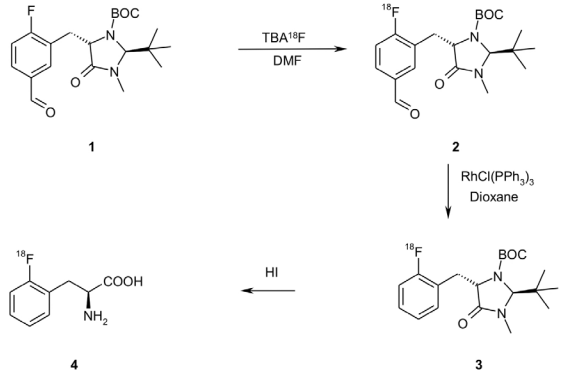
**Objectives:** In nuclear medical diagnosis $^{18}$F-labeled aromatic amino acids are widely used as radiopharmaceuticals for in vivo imaging of tumurs using PET. Two general pathways were developed for the radiofluorination of these arene-derivatives of high electron density. Electrophilic methods are employed, since non-toxic fluoro amino acids can be applied in carrier-added form. However, the electrophilic approach is limited [Hess et al., Appl Radiat Isot. 2002,57:185]. Alternative nucleophilic built-up syntheses using the advantage of large scale production of [$^{18}$F]fluoride are difficult to automate due to their complexity.Recently, an isotopic exchange approach made c.a. 6-[$^{18}$F]fluoro-L-DOPA available in three steps [Wagner et al., J. Nucl Med. 2008, 49, 142P]. For this a precursor was developed containing the chiral amino acid building block (S)-BOC-BMI (Seebach chiral auxiliary) and a formyl group which activates the isotopic exchange. Afterwards, the formyl groupcan alternatively be converted into a hydroxy group or removed by a decarbonylation reaction [Lasne et al., Top. Curr. Chem., 2002, 222, 201]. Here, the versatility of this three-step approach was verified for the second pathway with the radiosynthesis of c.a. 2-[$^{18}$F]fluoro-L-phenylalanine.

**Methods:** The procedure performed relies on the isotopic exchange on tert-butyl 2-tert-butyl-3-(2-fluoro-5-formylbenzyl)-4-oxoimidazolidine-1-carboxylate (1), which undergoes consecutive decarbonylation and hydrolysis reactions. Precursor 1 was $^{18}$F-labeled with TBA$^{18}$F at 130 °C in DMF within 10 minutes. The product was decarbonylated using the Wilkinson's catalyst at 150 °C in dioxane [Plenevaux et al., Appl. Rad. Isot., 1992, 43, 1035]. After 20 minutes the solvent was removed and the residue filtered through a silica gel plug. Hydroiodic acid was added and the mixture was heated to 200 °C for 30 minutes. The final solution was neutralized and the product analyzed by HPLC.

**Results:** The precursor 1 for the preparation of c.a. 6-[$^{18}$F]fluoro-L-phenylalanine was prepared in a 6 steps synthesis, starting from commercially available 3-bromo-4-fluorobenzaldehyde with an overall yield of 48 %. The isotopic exchange reaction produced the labeled compound 1 in 65 ± 5 % radiochemical yield. The decarbonylation procedure using Wilkinson's catalyst proceeded effectively to give compound 2 in 60 ± 10 %. The hydrolysis of the Seebach chiral auxiliary group provided the desired 2-[$^{18}$F]fluorophenylalanine in quantitative yield.

**Conclusions:** A new nucleophilic synthesis to enantiomerically pure 2-[$^{18}$F]fluoro-L-phenylalanine has been developed. The three steps radiosynthetic procedure leads to the desired amino acid in approximately 40 % overall radiochemical yield with high enantiomeric purity of > 96 %. The presented synthetic pathway to [$^{18}$F]fluoro-L-phenylalanine is much easier to be automated than known methods of nucleophilic $^{18}$F-fluorination and thus offers a reliable large scale production procedure.

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## P064 RADIOSYNTHESIS OF A NOVEL ADENOSINE A3 RECEPTOR LIGAND, 5-ETHYL 2,4-DIETHYL-3-((2-[18F] FLUOROETHYL)SULFANYLCARBONYL)-6-PHENYLPYRIDINE-5-CARBOXYLATE ([18F]FE@SUPPY:2)

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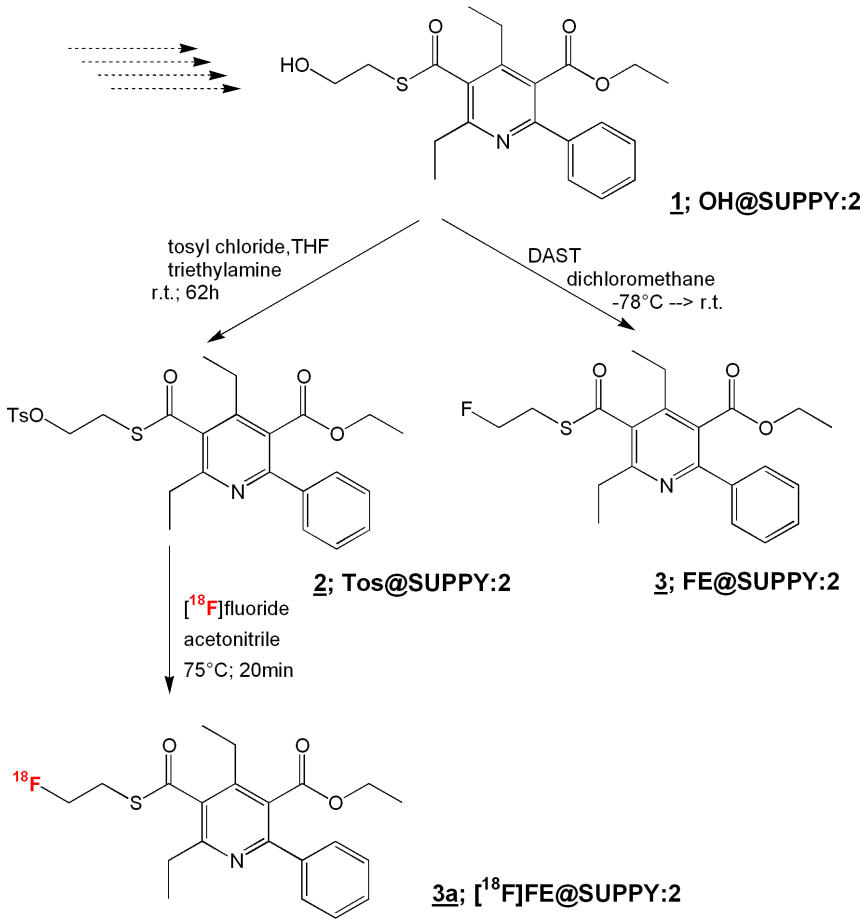
1. Medical University of Vienna, Dept of Nuclear Medicine and PET, Vienna, Austria; 2. University of Vienna, Dept of Drug and Natural Product Synthesis, Vienna, Austria; 3. Medical University of Vienna, Dept of Psychiatry and Psychotherapy, Vienna, Austria

**Objectives:** Since, to date very limited information on the distribution and function of the adenosine $A_3$ receptor is available, the development of suitable radioligands is needed. Recently, we introduced [$^{18}$F]FE@SUPPY (5-(2-[$^{18}$F]fluoroethyl) 2,4-diethyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate) as the first PET-ligand for the A3AR. Regarding the metabolic profile – this class of dialkylpyridines comprises two ester functions, one carboxylic and one thiocarboxylic – one could expect carboxylesterases significantly contributing to cleavage and degradation. Therefore, our aim was the development of [$^{18}$F]FE@SUPPY:2 (5-ethyl 2,4-diethyl-3-((2-[$^{18}$F]fluoroethyl)sulfanylcarbonyl)-6-phenylpyridine-5-carboxylate), the functional isomer containing the label at the thiocarboxylic moiety.

**Methods:** The necessary precursor (5-ethyl 2,4-diethyl-3-((2-tosyloxyethyl)sulfanylcarbonyl)-6-phenylpyridine-5-carboxylate (Tos@SUPPY:2) and reference compound FE@SUPPY:2 were prepared in multi-step syntheses.




, 0.1-20.0mg of Tos@SUPPY:2 (0.2-37$\mu$mol) in 500$\mu$L of acetonitrile were added, the vial was sealed and heated. After cooling to room temperature, the radiochemical yield was determined using analytical radio-HPLC.The crude reaction mixture was subjected to semi-preparative reversed-phase HPLC (column: Merck Chromolith® SemiPrep RP-18e, 100x10mm; mobile phase: acetonitrile/water/acetic acid (60/38.8/1.2 v/v/v; 2.5g/L ammonium acetate; pH 3.2); 10mL/min). The [$^{18}$F]FE@SUPPY:2 fraction was cut and diluted with 80mL water. The resulting solution was then fixed on a C18plus SepPak®. After washing, the product was eluted with 2mL ethanol, sterile filtered and formulated with 0.9% sodium chloride solution (15mL).

**Results:** For satisfactory yields in high scale radiosyntheses, a reaction temperature of 75°C has to be applied for at least 20 minutes using 20 mg of precursor. So far, 6 complete high-scale radiosyntheses were performed. Starting from an average of 51.2 ± 21.8GBq (mean ± SD) [$^{18}$F]fluoride, 5.8 ± 4.1GBq of formulated [$^{18}$F]FE@SUPPY:2 (12.0 ± 5.4%, based on [$^{18}$F]fluoride, not corrected for decay) were prepared in 75 ± 8 minutes.

**Conclusions:** We were able to achieve the reliable radiosynthesis of [$^{18}$F]FE@SUPPY:2 with satisfactory yields. Therefore, a second PET-tracer for the A3AR has been made available.

## P065 PREPARATION OF ANHYDROUS F-18 FLUORIDE

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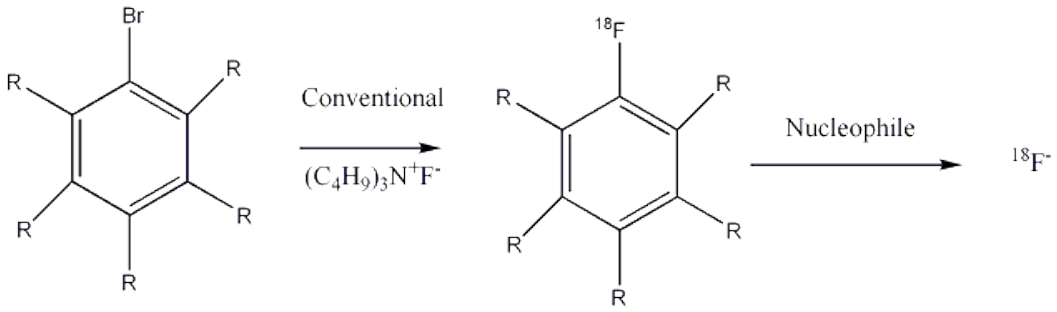
**Objectives:** Conventionally dried fluoride salts always contain some water of hydration which reduces the nucleophillicity and increases the basicity of the fluoride. Recently DiMagno et. al. working with macroscopic quantities of fluoride have shown that nucleophillic displacement of fluoride from aryl fluorides polysubstituted with electron withdrawing groups is a facile reaction and produces tetra-alkyl ammonium fluoride salts which are truly anhydrous and are both more reactive and more selective fluorinating agents than conventionally dried fluoride. It is our objective in this work to adapt DiMagno's procedure to the special requirements of fluorine-18 fluoride to provide a much more selective and reactive fluorine-18 fluorinating reagent.




**Methods:** Bromopentacyanobenzene and hexabromobenzene were reacted, in acetonitrile solution with F-18 tetrabutylammonium fluoride, prepared by proton bombardment of O-18 enriched water, isolated on an anion exchange resin and dried by azeotropic distillation with acetonitrile. The reaction products are passed over an activated allumina column where they are subjected to reaction with the displacing nucleophile. The optimum nucleophile for the displacement reaction is being explored. Tetrabuylammonium cyanide is obtainable truly anhydrous but it is hygroscopic, which makes working under anhydrous conditions on F-18 scale difficult. Tetrabutylammonium azide and phenolate, which are not hygroscopic, are being explored.

**Results:** The reaction of the substituted benzenes with conventional tetabutylammonium fluoride is rapid and proceeds in excellent yields. The reaction products pass over the activated alumina columns and the fluorosubstituted benzenes pass straight through the alumina.

**Conclusions:** The initial steps required for the preparation of anhydrous F-18 fluoride proceed in a straight forward fashion. The optimum nucleophile for the displacement reactions has not yet been established.

**Research Support:** This work is supported in part by grant # DE-FG02-08ER64705 from the Department of Energy

**References:** Haoran Sun and Stephen G. DiMagno,Anhydrous Tetrabutylammonium Fluoride J. Am. Chem. Soc.127, 2050-2051, 2005

## P066 CONVENIENT N-SUCCINIMIDYL-4-[F-18]FLUOROBENZOATE ([F-18]SFB) SYNTHESIS AND LABELING OF BIOMOLECULES

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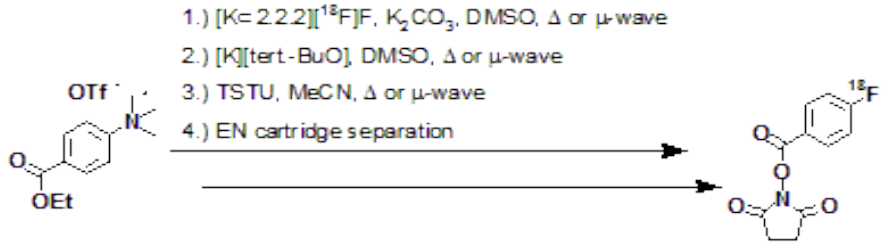
**Objectives:** The many efforts in the past two decades to produce [$^{18}$F]SFB in a convenient and efficient manner have resulted in a number of improvements but the synthesis remains lengthy and complex. In general there are three chemical steps during the synthesis, and as described by Wester et al. several solid phase extraction (SPE) purification steps in between are mandatory. This strategy makes the use of two reactors necessary, which immensely complicates automation. Until now, two approaches have been reported to avoid the use of two reactors. In the first one the reactor is rinsed during the SPE purification. The second one simply leaves out this first purification step. Both strategies make the final purification and formulation of the [$^{18}$F]SFB more demanding. All described radiosyntheses, however, use an aqueous basic solution for the deprotection of the first intermediate ethyl-4-[$^{18}$F]fluorobenzoate to yield 4-[$^{18}$F]fluorobenzoic acid. The water is incompatible with the following reaction with O-(N-succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TSTU) to form [$^{18}$F]SFB. Thus the reaction mixture has to be dried by azeotropic distillation of the eluate of the SPE or passing it through a sodium sulphate cartridge. We present a change of the deprotection strategy that enables an convenient and simple one-pot radiosynthesis of [$^{18}$F]SFB.

**Methods:** Ethyl-4-[$^{18}$F]fluorobenzoate was prepared in DMSO using dry [K<2.2.2][$^{18}$F]F and ethyl-4-trimethylammonium-benzoate triflate as a precursor. Cleavage of the ethyl ester was accomplished by adding a solution of potassium tert.-butoxide and heating. The succinimidyl moiety was coupled by addition of TSTU in acetonitrile at elevated temperature. Heating was either performed in an oil bath or by microwave irradiation in a remote controlled radiochemistry system. Purification of the product was performed by diluting the reaction mixture with 5 vol.% acetic acid and trapping on a Merck EN cartridge. The cartridge was washed with a mixture of acetonitrile and water (1 : 2 ratio). Finally, [$^{18}$F]SFB was eluted using diethyl ether giving an RCY of 40 – 60 % and a radiochemical purity > 95 % according to radio-HPLC and radio-TLC.




The total time of syntheses depends on the heating modality and was significantly shorter with microwave.

**Results:** A simple alternative for the radiosynthesis of [$^{18}$F]SFB using multiple reaction vessels and SPE steps is to change the deprotection strategy of ethyl-4-[$^{18}$F]fluorobenzoate to an anhydrous system. The use of potassium tert.-butoxide in DMSO fulfills this task, and the basicity of the resulting solution of 4-[$^{18}$F]fluorobenzoic acid is sufficient to catalyze the coupling of the N-succinimidyl moiety with TSTU. During SPE purification [$^{18}$F]SFB is eluted with diethyl ether. After evaporation of the solvent the radiotag is dissolved in an aqueous buffer and a biomolecule of choice is added for the labeling reaction.

**Conclusions:** Peptides and diabodies were labeled with [$^{18}$F]SFB according to this procedure and evaluated in vitro and via in vivo micro-PET imaging in mice. The whole process took less than two hours. Most of the procedures are automated or remote controlled.

## P067 RADIOSYNTHESIS OF 1, 3, 4, 6-TETRA-O-ACETYL-2-DEOXY-2-([18F]FLUOROACETAMIDO)-D-GLUCOPYRANOSE FOR IMAGING OF BACTERIAL INFECTIONS

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**Objectives:** Early diagnosis of infection and the capacity to distinguish between bacterial and sterile inflammation is essential to efficiently treat patients and prevent complications of pathology. Localization of bacterial infections by positron emission tomography (PET) has gained interest in recent years, but still few radiopharmaceuticals are available for use. N-acetylglucosamine and its derivatives including hyaluronic acid are unique and obligatory structural components of bacterial cell wall. In this study, 1, 3, 4, 6-tetra-O-acetyl-2-deoxy-2-([$^{18}$F]fluoroacetamido)-D-glucopyranose (TA-[$^{18}$F]FAG), an analog of N-acetylglucosamine, was synthesized and investigated its potential as a bacterial infection imaging agent for PET.

**Methods:** In order to perform one-step radiofluorination, the precursor, 1, 3, 4, 6-tetra-O-acetyl-2-deoxy-2-(bromoacetamido)-D-glucopyranose (TA-BrAG) was synthesized. Radiosynthesis was then performed using microwave (MW) mediated nucleophilic fluorination using no-carrier-added [$^{18}$F]fluoride. To evaluate the metabolism of [$^{18}$F]fluoroacetyl-D-glucosamine, metabolite and biodistribution studies were conducted. In vivo imaging of bacterial infection was performed on model rat using MicroPET.

**Results:** TA-BrAG was prepared from D-glucosamine as starting material with a good yield and more than 98% purity based on HPLC analysis. The radiochemical yields of TA-[$^{18}$F]FAG under different conditions were determined by TLC. The optimized conditions were 25 μmol of the precursor, MW heating for 10 min at 100 °C, giving a labeling efficiency of 75.5 ± 4.2% (n=3). These conditions were used in a semi-automated system to produce after purification 1.3 - 3.5 mCi of TA-[$^{18}$F]FAG for animal experiments. Metabolites and biodistribution studies showed similar patterns to previously reported studies for [$^{18}$F]fluoroacetyl-D-glucosamine. MicroPET imaging showed accumulation in the right posterior extremity of rat bearing E. coli. Hystopathological analysis confirmed the presence of bacterial at site of accumulation of radioisotope.

**Conclusions:** Microwave mediated fluorination of a potential precursor for [$^{18}$F]fluoroacetyl-D-glucosamine was accomplished. The use of protector groups and microwave heating improved the labeling efficiency. This compound showed its usefulness for imaging bacterial infections in animal experiments.•Nevertheless, now our efforts are focused in the synthesis and evaluation of the free sugar derivative.

## P068 RADIOSYNTHESIS OF THE DOPAMINE D4 LIGAND 6-(4-[4-[18F]FLUOROBENZYL]PIPERAZINE-1-YL) BENZODIOXANE AND DERIVATIVES FOR OPTIMIZING THEIR UNSPECIFIC BINDING BEHAVIOR

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**Objectives:** SAR studies have shown that bulky N-arylpiperazines containing a second aromatic moiety are promising lead structures for selective dopamine $D_4$ ligands [Simpson et al., Mol. Pharmacol. 1999, 56, 1116]. Nevertheless, many labeled derivatives based on this structure failed as potential radioligands due to deficient specific binding [Zhang et al., Nucl. Med. Biol. 2002, 29, 233; Oh et al., Bioorg. Med. Chem. 2004, 12, 5505] which is probably a result of the extremely low density of the $D_4$ subtype. The derivative 6-(4-[4-fluorobenzyl]piperazine-1-yl)benzodioxane 1a, however, showed good affinity and selectivity for the $D_4$ receptor ($K_i(D_4)$ = 4 nM vs. $K_i(D_2, D_3)$ > 5000 nM [Hodgetts et al., Bioorg. Med. Chem. 2001, 9, 2207). Therefore, 1a and three further $^{18}$F-labeled derivatives were synthesized in n.c.a. $^{18}$F-labeled form. Based on the known relationship between unspecific binding und lipophilicity derivatives with lower logP values were chosen in order to reduce unspecific binding.

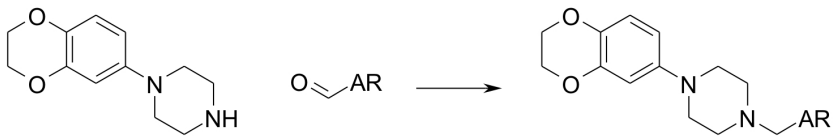
**Methods:** The kind of substituents was selected by simulation of lipophilic properties using the MarvinSketch 5.1.4 software. The presented logP values were determined with both, inactive standards and the $^{18}$F-labeled compounds. For $^{18}$F-labeling coupling reactions based on appropriate aldehydes were used for reductive amination, which could be performed as a one-pot synthesis in DMF or in DMSO. Compounds [$^{18}$F]1a and [$^{18}$F]1d were also prepared by direct nucleophilic substitution using $NO_2$ and Cl as leaving group, respectively.Blocking studies with spiperone and inactive standard were conducted with the $^{18}$F-fluorinated compound [$^{18}$F]1a.

**Results:** The results of the $^{18}$F-labeling and determination of log P values are summarized in the table. The $^{18}$F-labeled ligand [$^{18}$F]1a could not be replaced by one of the used substances.

| compd. | aryl substituend (AR) | $logP_{7.4}$ calc. | exp A[a] | exp B[b] | RCY[c] amination | direct |
|---|---|---|---|---|---|---|
| **[$^{18}$F]1a** | 4-[$^{18}$F]fluoro-phenyl | 3.35 | 2.71 | - | 59 % | 35 % |
| **[$^{18}$F]1b** | 4-[$^{18}$F]fluoro-3-methoxyphenyl | 3.09 | 2.44 | 2.33 | 64 % | - |
| **[$^{18}$F]1c** | 4-[$^{18}$F]fluoro-3-hydroxyphenyl | 2.87 | 1.70 | 1.78 | 72 % | - |
| **[$^{18}$F]1d** | 2-[$^{18}$F]fluoro-pyridin-5-yl | 2.49 | - | 1.81 | 71 % | 14 % |

[a]RP-HPLC determination of inactive standards: system: LiChrospher® 100 RP-8 (5$\mu$m); eluent: phosphate buffer/methanol, 25/75 (v/v).[b]Direct measures from octanol-buffer partitioning detected via Instant Imager™ (Packard Canberra).[c]Overall radiochemical yields for reductive amination and direct $^{18}$F-labeling were detected by radio-HPLC.

**Conclusions:** Four derivatives of compound 1 have successfully been $^{18}$F-labeled by a reductive amination reaction. Two derivatives, [$^{18}$F]1a and [$^{18}$F]1d, could also be obtained by a direct exchange reaction but in both cases the RCY was lower in comparison to the built-up synthesis.[$^{18}$F]1a appears not to be a suitable radioligand due to its high nonspecific binding. Ongoing evaluation of the derivatives [$^{18}$F]1b-d should demonstrate if less lipophilic properties will be a critical parameter for further selection from derivatives of the chosen lead compound.




**[$^{18}$F]1a-d**

## P069 DEVELOPING A RADIOLABELING STRATEGY FOR 2-[F-18]FLUORO-HEAT

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**Objectives:** HEAT: (2-[4-hydroxyphenyl)ethylaminomethyl]-1-tetralone), is a small molecule, potent alpha-1-adrenergic ligand (Kd 48 pM) and this suggests that a fluoro-HEAT analog (F-HEAT) may have potential for selective PET imaging of the brain. We are developing a radiolabeling strategy for [F-18]F-HEAT production and consider the radiolabeling of 2-[F-18]fluoro-tetralone as a key synthetic intermediate, which is amenable to Mannich-based condensation reaction. The original synthesis of HEAT used a single step Mannich reaction between tetralone, paraformaldehyde and tyramine-HCl. Moreover, inclusion of 2-fluorotetralone in the synthesis strategically places a fluorine atom on a chiral, tertiary substituted carbon atom. As a consequence, this should stabilize F-HEAT against metabolic decomposition.

**Methods:** 2-[F-18]fluorotetralone ([F-18]F-TET) was labeled using 2-bromotetralone (30 umol) /Kyrptofix[2.2.2] (40 umol)/ K2CO3 (20 umol)/ [F-18]fluoride in hot DMF (115oC) in an open test tube. Radiochemically pure [F-18]F-TET was obtained after a dichloromethane extraction of the aqueous quenched reaction, alumina(N) SepPak treatment of organic extract and concentration. The Mannich reaction between [F-18]F-TET, (CH2O)x and tyramine-HCl in i-PrOH (115oC, 35 min) was performed in a sealed vial. Progress of reactions was monitored by radio-TLC (silica gel) and radio-HPLC.

**Results:** Labeling of [F-18]F-TET was rapid (4 min) but limited to 17% rcy (max). Prolonged reaction (10 min) led to a decline in yield. Improvement of the labeling yield should be possible. A Mannich reaction between [F-18]F-TET, (CH2O)x and tyramine-HCl in i-PrOH (115oC, 35 min) gave a low yield of [F-18]F-HEAT (10% based on [F-18]F-TET). The low yield of this reaction was due to a low conversion, since [F-18]F-TET was the only other observed activity. In addition, shorter reaction times led to correspondingly lower conversions.

**Conclusions:** The radiosynthesis of [F-18]F-HEAT is feasible but currently impractical for routine production. We are currently working on improvements and will present our progress. This work was supported by NIH (K08-AG023670)

## P070 FLUORINE-18 LABELING OF SUBSTITUTED BENZILS FOR IMAGING CARBOXYLESTERASE

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**Objectives:** Irinotecan is a potent chemotherapeutic used in the treatment of several cancers, including neuroblastoma. Irinotecan itself is a non-toxic prodrug which is converted in vivo to the cytotoxic form, camptothecin, via metabolism by carboxylesterase (CE) enzymes (Yoon, Mol Cancer Ther 2003,2:1171-81). Researchers at St. Jude Children's Research Hospital are taking advantage of CE isoform diversity to design a two-pronged protocol of tumor specific chemotherapy. These complementary approaches combine specific inhibition of human CE activity in normal tissues in an effort to increase drug delivery to the tumor (Wadkins, J Med Chem 2005,48:2906-15), and tumor-specific activation of prodrugs by using neural progenitor cells transfected with a carboxylesterase cDNA (Danks, Cancer Res 2007, 67:22-5). The tumor-selective trafficking of neural progenitor cells allows over expression of CE within the tumor. Both lines of investigation would benefit from in vivo quantification of CE activity in tumors and normal tissues, allowing titration of drug dosing for CE inhibitors and measurement of CE increases selectively at tumor foci in progenitor cell studies. Our laboratory is developing PET radiotracers based on CE inhibitors developed here at St Jude, many of which contain a benzil (diphenylethane-1,2-dione) core structure. To determine whether the benzil structure was sufficiently activated for direct labeling with $^{18}F$, we performed the 4-nitro to 4-fluorobenzil conversion as proof of concept.

**Methods:** Aqueous [$^{18}F$]fluoride was captured on a Waters QMA anion exchange resin and eluted with a mixture of 5 mg Kryptofix and 0.5 mg $K_2CO_3$ in 2:1 acetonitrile/water. The resulting acetonitrile/water azeotrope was evaporated at 85 C yielding the anhydrous Kryptofix-K-$^{18}F$ complex which was then heated at 130 C for 45 minutes with 4-nitrobenzil (5 mg) in anhydrous DMSO. HPLC separation of the 4-[$^{18}F$]fluorobenzil and the unlabelled nitro- and fluorobenzil standards was achieved on ZORBAX Eclipse DBX-C18 column (Agilent, 4.6 x 250 mm, 5μm) eluted with 3:1 MeCN/water at 0.5 mL/min.

**Results:** Nucleophilic aromatic fluorination of 4-nitrobenzil gave 50-65% conversion to the 4-[$^{18}F$]fluorobenzil, as determined by TLC (silica gel, ethyl acetate/hexanes, 3/7) with radiation detection. HPLC analysis of the crude $^{18}F$ product under the stated conditions also showed a radiation peak corresponding to 4-[$^{18}F$]fluorobenzil and good separation from 4-nitrobenzil precursor on the UV chromatogram.

**Conclusions:** These results verify that benzil compounds are adequate precursors for nucleophilic fluorination reactions, although the nitro leaving group requires long reaction times and high temperatures. The ability to directly label such relatively unactivated benzils greatly simplifies the goal of preparing a diverse library of radiotracers with differing CE isoform selectivity. Our lab is currently synthesizing new radiolabeled compounds based on the structure activity relationship scaffold of the benzil compounds, with intentions to design a series of radiotracers with low nanomolar affinity for CE.

**Research Support:** Supported in part by NCI grants CA113446 and CA108775.

**References:** Wadkins, RM; Hyatt, JL; Wei, X; Yoon, KJ; Wierdl, M; Edwards, CC; Morton, CL; Obenauer, JC; Damodaran, K; Beroza, P; Danks, MK; and Potter, PM. Identification and characterization of novel benzil (diphenylethane-1,2-dione) analogues as inhibitors of mammalian carboylesterases. J. Med. Chem. 2005, 48, 2906-2915 Hicks, LD; Hyatt, JL; Moak, T; Edwards, CC; Tsurkan, L; Wierdl, M; Ferreira, AM; Wadkins, RM; and Potter, PM.Analysis of the inhibition of mammalian carbosylesterases by novel fluorobenzoins and fluorobenzils. Bioorg. Med. Chem. 2007, 15, 3801-3817 Yoon, KJ; Krull, EJ; Morton, CL; Bornmann, WG; Lee, RE; Potter, PM; and Danks, MK.Activation of a camptothecin prodrug by specific carobylesterases as predicted by quantitative structure-activity relationship and molecular docking studies. Molecular Cancer Therapeutics. 2003, 2, 1171-1181 Hyatt, JL; Wadkins, RM; Tsurkan, L; Hicks, LD; Hatfield, MJ; Edwards, CC; Ross, CR; Cantalupo, SA; Crundwell, G; Danks, MK; Guy, RK; and Potter, PM. Planarity and constraint of the carbonyl groups in 1,2-diones are determinants for selective inhibition of human carboxylesterase 1. J. Med. Chem. 2007,50, 5727-5734 Danks, MK; Yoon, KJ; Bush, RA; Remack, JS; Wierdl, M; Tsurkan, L; Kim, SU; Garcia, E; Metz, MZ; Najbauer, J; Potter, PM; and Aboody, KS.Tumor-targeted enzyme/prodrug therapy mediates long-term disease-free survival of mice bearing disseminated neuroblastoma. Cancer Res. 2007,67, 1, 22-25 Snyder, SE; Kume, A; Jung, YW; Conner, SE; Sherman, PS; Albin, RL; Wieland, DM; Kilbourn, MR.Synthesis of carbon-11-, fluorine-18-, and iodine-125-labeled GABAA-gated chloride ion channel blockers: Substituted 5-tert-butyl-2-phenyl-1,3-dithianes and dithiane oxides. J. Med. Chem. 1995, 38, 2663-2671 Haka, MS; Kilbourn, MR; Watkins, GL; and Toorongian, SA.Aryltrimethylammonium trifluoromethanesulfonates as precursors to aryl [18F]fluorides;Improved synthesis of [18F]GBR-13119. J. Labelled Compd. Radiopharm. 1989, 27, 7, 823-833

## P071 ON THE SOURCE OF CARRIER IN FLUORINE-18

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**Objectives:** F-18 specific activity has historically been 18-111 GBq (500-3000 mCi) per μmole. The most commonly suspected source of carrier has been O-18 water. Our previous anecdotal data suggested Teflon radiolysis as the major source of carrier. This work tested that hypothesis.

**Methods:** All Teflon components were removed from the target and synthesis systems. F-18 fluoride was exposed to only PEEK, polypropylene, and glass wetted materials. ρ-Fluorobenzaldehyde was radiolabeled in a typical Kryptofix-assisted synthesis. Mass measurement was by HPLC with UV detection at 254nm. Control experiments included benchtop reactions, reactions in the hot cell without fluoride, production with F-18 in the Teflon-purged system and production using Teflon but without target irradiation. F-18 was collected from irradiated O-18 water on Bio-Rad AG-1X8 ion exchange resin and eluted with potassium carbonate. Teflon components - Rheodyne slider valves, transfer tubing, anion exchange resin cartridge, and reaction vessel addition/withdrawal tubing were added in typical configuration. The amount of F-18 radioactivity and time of Teflon exposure was also varied. A range of 5 - 80 GBq (150-2200 mCi) was used. Most experiments used 9 GBq (250 mCi) of fluoride.

**Results:** Approximately 30 nmoles of fluoride was measured in all control experiments. 80 GBq (2200 mCi) fluoride at EOB produced a specific activity of 1,900 GBq (51,000 mCi)/μmole at 60 min EOB, a time chosen as representative of useful products and used for all specific activity values herein. With all Teflon in place, 295 nmoles (average) fluoride were obtained from 5 GBq (250 mCi) EOB; specific activity 22 GBq (600 mCi) /μmole. Each component of the system contributed mass: Transport tubing (8 m), 79 nmole; resin cartridge, 47 nmole; tubing in reaction vessel (2 cm), 169 nmole. Radioactivity was necessary to release carrier, and the time of Teflon exposure to radioactivity was apparently important. As radioactivity was varied during initial syntheses, changes in carrier mass were not significant. However, in later well-controlled experiments there was a correlation between carrier and Teflon exposure time, and between carrier and F-18 quantity. These factors are under continuing investigation. Target water was also investigated as a source. As water is recycled, contamination in handling is the concern, not initial fluoride content. Therefore, carrier KF was added to water and used. The recovered water was reprocessed successively through additional syntheses. All (100%) carrier was removed in the first pass, indicating carrier from water is of no concern.

**Conclusions:** Teflon radiolysis has been proven to be the major source of mass in F-18 syntheses. Elimination of Teflon provided specific activity of 1,900 GBq (51,000 mCi)/μmole 60 min EOB from a one hour irradiation (17-30 fold improvement). Remaining mass (30 x theoretical) is contained in synthetic reagents. Purification of reagents used in synthesis was previously insignificant, but now may reduce product mass further.

## P072 [18F]JHU88868, A NOVEL RADIOLIGAND FOR PET IMAGING OF CANNABINOID CB1 RECEPTORS

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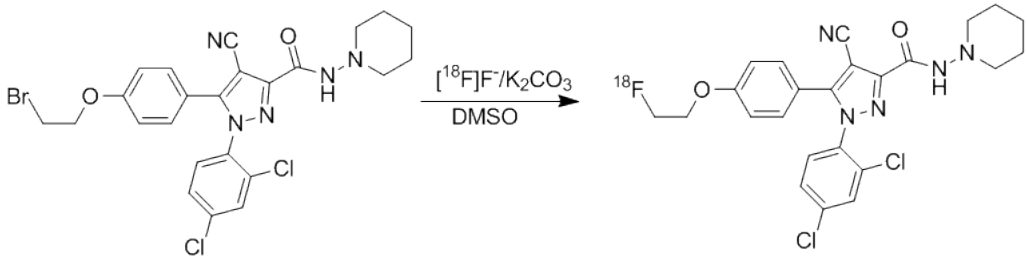
**Objectives:** Cannabinoid type 1 receptors (CB1) have been implicated in many neurological and psychiatric disorders. The ability to image CB1 receptors in human brain would allow the study of these receptors under normal physiological conditions and in disease state and evaluate the effect of treatment of disorders involving CB1 receptors. Recently we have developed a PET radioligand [$^{11}$C]OMAR ([$^{11}$C]JHU75528) that has been successfully used for PET imaging of cerebral CB1 receptors in animals and humans. The short half-life of $^{11}$C has prompted us to develop an $^{18}$F-labelled OMAR derivative [$^{18}$F] JHU88868 (5-(4-(2-[$^{18}$F] fluoroethoxy)phenyl)-1-(2,4-dichlorophenyl)-4-cyano-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide) with longer half-life.

**Methods:** Non-labeled JHU88868 and a precursor for radiolabeling, 5-(4-(2-bromoethoxy)phenyl)-1-(2,4-dichlorophenyl)-4-cyano-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide, were synthesized via multi-step synthesis. Binding affinity of JHU88868 was determined by inhibition binding assay. Radiosynthesis of [$^{18}$F]JHU88868 was performed in one step by a Kryptofix-assisted radiofluorination ofthe brobo-precursorin DMSO solution at 135°C using an FDG radiochemistry box. The final product was purified by HPLC.

**Results:** JHU88868 displayed a binding affinity value of 17 nM. [$^{18}$F]JHU88868was prepared with radiochemical yield 1-5 %, radiochemical purity greater than 95% and the average specific activity of 4000 mCi/mmol at the end-of-synthesis.

**Conclusions:** [$^{18}$F] JHU88868, an [$^{18}$F]fluoroethyl-analog of CB1 radiotracer [$^{11}$C]OMAR, has been synthesized by a one-step radionucleophilic fluorination of corresponding bromo-precursor.

**Research Support:** This work was supported in part by the NIH/NIMH grant MH-079017 (AGH)



## P073 INVESTIGATION OF META-SUBSTITUTED [18F]FLUOROBENZENE REACTIONS

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**Objectives:** The ability to reliably produce radiotracers by nucleophilic [$^{18}$F]fluorination in the meta position, relative to an electron withdrawing group, on a benzene ring remains a chemical challenge. Previous studies focused on [$^{18}$F]fluoro-for-nitro exchange on substituted 3-nitrobenzenes in a glassy carbon vessel (Constantinou et al., 2001] or under "instant fluorination conditions" [Windhorst et al., 2001]. During our recent investigation of methods to produce high yields of [$^{18}$F]fluoromethane, we found that reacting [$^{18}$F]fluoride ion ($K^{18}F$/ $K_{2.2.2}$) with meta-substituted N,N,N-trimethylanilinium triflates in 2,4,6 collidine/ acetonitrile containing 5-10 μL of water gave up to 50% 3-substituted-[$^{18}$F]fluorobenzenes (fluorine incorporation for m-toluene 5%; m-acetophenone 11%, m-benzophenone 10%; m-nitrobenzene 50%). When the fluoride was fully dried the yield for the m-nitro reaction increased to greater than 70%. Thus, we explored the ability to exchange label a variety of m-substituted N,N,N-trimethylanilinium triflates under the same conditions that gave consistent high yields for the nitro substituted precursor.

**Methods:** Aqueous [$^{18}$F]fluoride ion was azeotroped with acetonitrile in a thin walled glass vial, in the presence of base ($KHCO_3$: 1.6 mg or $K_2CO_3$: 1 mg) and kryptofix (6.2 mg), at 100˚C under a vacuum and a stream of nitrogen. The m-substituted N,N,N-trimethylanilinium triflates (5 mg) dissolved in either 2,4,6-collidine: acetonitrile (4:3 v/v; 0.7mL) or DMSO (0.2 mL) was added to the dried [$^{18}$F]fluoride ion. The reactions were heated at 160˚C for 10 minutes. The reactions were analyzed by radio-thin layer chromatography. The chromatograph results were corrected for the amount of [$^{18}$F]fluoromethane produced in the reaction.

**Results:** The results are shown in Table 1. The effect of the solvent and the base on the preparation of 3-[$^{18}$F]fluoro-nitrobenzene is noted. In DMSO the bicarbonate gave a lower yield of the labeled product versus the carbonate. In collidine/acetonitrile the base did not have any effect on the yield. For both bases 3-[$^{18}$F]fluoro-nitrobenzene yield increased significantly upon changing the solvent from DMSO to collidine/acetonitrile. The only other precursor that produced any [$^{18}$F]fluorobenzene product under non-aqueous reaction conditions was the 3-methylketo-N,N,N-trimethylanilinium triflate. Reaction of 1,3 dinitrobenzene in collidine/ acetonitrile gave <5% 3-[$^{18}$F]fluoro-nitrobenzene. Increasing the amount of carbonate from 1-1.5 equivalents reduced the 3-[$^{18}$F] fluoro-nitrobenzene yield to ~54% with a concomitant rise in [$^{18}$F]fluoromethane production from 7 to 23%.

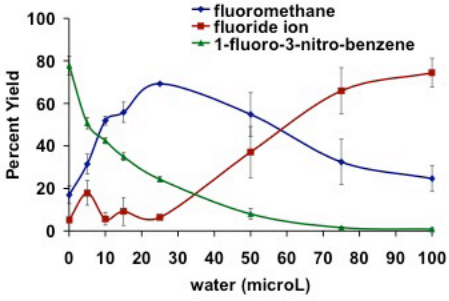
Table 1: Reaction yields for the nucleophilic fluornation reactions

| $NMe_3$ OTf Precursor (base) | 2,4,6-Collidine: $CH_3CN$ (4:3) | DMSO |
|---|---|---|
| m-$NO_2$ ($K_2CO_3$) | 70.9 ± 3.3% | 50.2 ± 4.0% |
| m-$NO_2$ ($KHCO_3$) | 69.2 ± 6.7 | 36.2 ± 1.1% |
| m-$COCH_3$ ($K_2CO_3$) | <2% | |
| m-CN ($K_2CO_3$) | 0% | |
| m-$CH_3$ ($K_2CO_3$) | 0% | |
| m-OH ($K_2CO_3$) | 0% | |

**Conclusions:** No special reaction conditions or vessels were needed to reliably produce high yields of 3-[$^{18}$F]fluoro-nitrobenzene from 3-nitro-N,N,N-trimethylanilinium triflate in collidine/acetonitrile. These yields were achieved using standard resolubilization conditions. These same non-aqueous reaction conditions did not produce any meta-[$^{18}$F]fluoro for precursors with either electron-withdrawing (CN, $COCH_3$) or electron-donating substituents (OH, $CH_3$).

**Research Support:** This reasearch was supported by the Office of Science (BER) U.S. Department of Energy, Grant No DE-FG-08ER64699 and Varian Biosynergy, Inc.

**References:** Constantinou, M.; Shah, F., Pike, V.W. (2001) Radiofluorinations of m-substituted nitrobenzenes. J. Labelled Comp. Radiopharm. 44 S1: S889-891. Windhorst, A. D., Klok, R.P., Koolen, C.L., Visser, G.W.M., Herschied, J.D.M. (2001) Labeling of [$^{18}$F]flumazenil via instant fluorination, a new nucleophilic fluorination method. J. Labelled Comp. Radiopharm. 44 S1: S930-932.




Reaction of [$^{18}$F]fluoride ion with 3-nitro-NNN-trimethylanilinium triflate in 4:3 collidine: acetonitrile, $K_2CO_3$ and $K_{2.2.2}$ with increasing amounts of water. The yield of 3-[$^{18}$F]fluoro-nitrobenzene steadily decreased with increasing amounts of water.

## P074 FLUORINE-18 LABELED GLP-1 PEPTIDES FOR BETA CELL IMAGING

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**Objectives:** Glucagon-like peptide-1 (GLP-1), a 30-amino acid peptide generated by intestinal L-cells, stimulates glucose-dependent insulin secretion from beta cells of pancreatic islets. GLP-1 receptors are expressed on the surface of beta cells, making them a suitable target for in vivo detection of islet mass. We propose that the use of radiolabeled GLP-1 peptides in conjunction with Positron Emission Tomography (PET), or Single Photon Emission Computed Tomography (SPECT) could provide a method of studying islet function. We recently reported a number of modified GLP-1 analogues, containing an indium-DOTA complex, with nM affinity for the GLP-1 receptor. The objective of the current study was to synthesize fluorine-18 labeled GLP-1 derivatives, using the prosthetic group 4-$^{18}$F-fluorobenzoic acid, as potential candidates for in vivo imaging of pancreatic islets using PET.

**Methods:** Peptide analogues were prepared using solid-phase Fmoc methods with piperidine deprotection and uronium-based coupling reagents. Analysis and purification of the synthesized peptides was carried out using ESI-MS and HPLC. The site-specific addition of fluorobenzoic acid was made possible using orthogonal protecting groups, with the non-radioactive derivatives being made using $^{19}$F-fluorobenzoic acid. Binding affinities were determined in vitro with the CHO/GLP-1R cell line. Fluorine-18 labeled fluorobenzoic acid was prepared via nucleophilic radiofluorination of t-butyl-N,N,N-trimethylammoniumbenzoate triflate and subsequent t-butyl deprotection using aqueous HCl, with Sep-Pak© purification. The fluorobenzoic acid was coupled to the resin-bound protected peptide with subsequent cleaving and deprotection followed by HPLC purification.

**Results:** The GLP-1 derivative 37Lys-GLP-1(7-37) was prepared, methyltrityl (mtt) deprotected at position 37 and subsequently coupled to $^{19}$F-fluorobenzoic acid. After cleavage and deprotection, preparative HPLC purification resulted in a 95% pure product. This GLP-1 analogue contained D-Ala-8, a modification known to enhance resistance to degradation by dipeptidyl-peptidase IV. The fluorobenzoic acid coupled GLP-1 (37Lys-FB-GLP-1(7-37)) showed an optimal binding affinity of 56±6 nM for the GLP-1 receptor. Nucleophilic radiofluorination of tert-butyl-N,N,N-trimethylammoniumbenzoate triflate and subsequent hydrolysis of the t-butyl ester using 1M HCl afforded 4-$^{18}$F-fluorobenzoic acid in a 19% decay corrected radiochemical yield. This $^{18}$F-containing prosthetic group was then coupled to the mtt-deprotected 37Lys-GLP-1(7-37) peptide resulting in the formation of the final radiolabeled product. Optimization of the radiolabeling procedures, including automation on a GE tracerlab FX-N, and preliminary in vivo imaging results, will be discussed.

**Conclusions:** Here we report the first example of an $^{18}$F-labeled GLP-1 peptide, which is a promising candidate for PET imaging of beta cells.

**Research Support:** We wish to thank the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Institutes for Health Research (CIHR) and the Ontario Graduate Scholarship in Science and Technology (OGSST) for financial support.

## P075 IMPROVED SYNTHESIS OF 4-[18F]FLUOROBENZYLAMINE: A USEFUL BUILDING BLOCK IN 18F CHEMISTRY

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**Objectives:** 4-[$^{18}$F]Fluorobenzylamine is the key intermediate in the synthesis of the prominent oligonucleotide labeling prosthetic group N-4-[$^{18}$F]fluorobenzyl-2-bromoacetamide. 4-[$^{18}$F]fluoro-benzylamine is prepared in a two step synthesis sequence involving (1) formation of 4-[$^{18}$F]fluorobenzonitrile followed by (2) reduction of the nitrile group, preferentially with $LiAlH_4$ as the reducing reagent. However, the use of $LiAlH_4$ is somewhat troublesome due to the required water-free reaction conditions and the formation of large amounts of aluminium salts.In this work we describe a convenient alternative for the reduction of 4-[$^{18}$F] fluorobenzonitrile to 4-[$^{18}$F]fluorobenzylamine by means of Co(II)-$NaBH_4$ in aqueous solution. The feasibility of this approach was demonstrated by the efficient synthesis of thiol group-selective prosthetic groups 4-[$^{18}$F]fluorobenzyl-2-bromoacetamide and 4-[$^{18}$F]fluorobenzyl-amidopropionyl maleimide.

**Methods:** 4-Cyano-N,N,N-trimethylanilinium trifluoromethanesulfonate (1-2 mg) in DMSO (400ml) was used as precursor for the radiolabeling with [$^{18}$F]fluoride at 90°C for 10 min. After solid-phase extraction, purified 4-[$^{18}$F]fluorobenzonitrilewas reduced to 4-[$^{18}$F]fluorobenzylaminewith Co(II)/$NaBH_4$ in a THF-water mixture at room temperature within 5 min.4-[$^{18}$F] Fluorobenzylaminewas further converted into prosthetic groups 4-[$^{18}$F]fluorobenzyl-amidopropionyl maleimide and 4-[$^{18}$F] fluorobenzyl-2-bromoacetamide.

**Results:** Radiochemical yields of >80% were obtained for the synthesis of 4-[$^{18}$F]fluoro-benzonitrile. A 1:1 mixture (w/w) of Co(II)/$NaBH_4$ in THF/water gave complete reduction of 4-[ $^{18}$F]fluorobenzonitrile to 4-[$^{18}$F]fluorobenzylamine at room temperature within 5 min. Reaction of 4-[$^{18}$F]fluorobenzylamine with bromoacetyl bromide and N-succinimidyl-3-maleimide-propionate gave the corresponding thiol-reactive prosthetic groups 4-[$^{18}$F]fluorobenzyl-2-bromoacetamide and 4-[$^{18}$F]fluorobenzyl-amidopropionyl maleimide in total radiochemical yields of 75% and 55%; respectively.

**Conclusions:** We have developed a facile method for the convenient conversion of readily available 4-[$^{18}$F]fluorobenzonitrile into 4-[$^{18}$F]fluorobenzylamine. The application of Co(II)/$NaBH_4$ as reducing agent can be extended to the use of borohydride-exchange resins (BERs) as the hydride source, which will further facilitate automation of4-[$^{18}$F]fluorobenzylamine and its broader use as a versatile$^{18}$F labelling building block.

**Research Support:** Acknowledgment: The authors wish to thank M. Grote and J. Schlesinger for their important contributions to this work.

## P076 DEVELOPMENT OF A NOVEL PET OXYTOCIN RECEPTOR BIOMARKER

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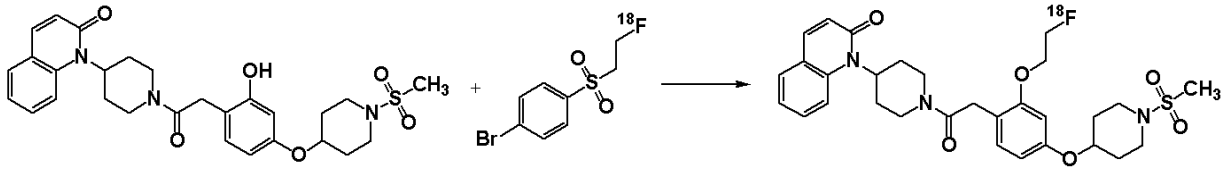
**Objectives:** The development of a PET radioligand to enable pharmacological studies of oxytocin receptors in living human and non-human primates would greatly enhance the ability to understand the neurobiological mechanisms underlying oxytocin behavioral effects. The compound (1-(1-(2-(2,2,2-trifluoroethoxy)-4-(1-methylsulfonyl-4-piperidinyloxy) phenylacetyl)-4-piperidinyl)-3,4-dihydro-2(1H)-quinolinone) has been shown to contain a high affinity for oxytocin receptors in humans. We have developed a fluorinated analog, 1-(1-(2-(2-fluoroethoxy)-4-(1-methylsulfonyl-4-piperidinyloxy) phenylacetyl)-4-piperidinyl)-3,4-dihydro-2(1H)-quinolinone (2), that is a potential radioligand when labeled with fluorine-18 for in vivo PET studies. We report here the synthesis of a precursor to obtain [$^{18}$F]2 in a one step process and autoradiography evaluation of 2.

**Methods:** The PET labeling precursor, 1-(1-(2-(hydroxy)-4-(1-methylsulfonyl-4-piperidinyloxy) phenylacetyl)-4-piperidinyl)-3,4-dihydro-2(1H)-quinolinone (1) was synthesized in 11 steps using ethyl vinyl ether, oxalyl chloride, and 2,4-dihydroxyacetophenone as starting materials. The reference standard of 2, and its radioactive derivative, were synthesized via a reaction of compound 1 with fluoroethylbrosylate or [$^{18}$F] fluoroethylbrosylate. In vitro competition assays were performed with 2 using vole brain tissue known to contain oxytocin and vasopressin receptors.




1 2

**Results:** [$^{18}$F]2 was obtained in 85 min from the start of synthesis with a radiochemical yield of 13% as measured by radiometric HPLC. Biological evaluation via autoradiography demonstrated an $IC_{50}$ of approximately 10nM for oxytocin receptors and 70nM for vasopressin receptors.

**Conclusions:** The preliminary studies suggest that [$^{18}$F]2 could be a potential PET agent used for in vivo PET studies.

## P077 SYNTHESIS OF 18F- RADIOTRACERS IN HIGH SPECIFIC ACTIVITY USING THE FLUOROUS APPROACH

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**Objectives:** Fluorous based purification techniques have gained immense popularity in recent years. The ease, speed and efficiency of these techniques make them particularly attractive in the purification of radioactive compounds with short-lived isotopes, such as the PET isotope $^{18}$F. Here we describe the application of fluorous based purification to the synthesis of $^{18}$F-labeled PET radiotracers with high specific activity.

**Methods:** Several well known $^{18}$F- labeled PET radiotracers are prepared through low-yielding nucleophilic substitution reactions by [$^{18}$F] fluoride ion, which requires the use of large amounts of precursor to obtain reasonable yields of the radiolabeled product. Removal of the excess precursor is critical in obtaining radiotracers with high specific activity.

**Results:** A fluorous solution was developed by using [$^{18}$F] fluoro-2-deoxy-D-glucose ([$^{18}$F]-FDG), which is the most widely used radiotracer in PET applications, and is used to measure glucose uptake in tissues. A fluorous derivative of its mannose triflate precursor was prepared and used for the nucleophilic fluoridation by [$^{18}$F] fluoride. After radiolabeling, the reaction mixture was purified by fluorous solid phase extraction (FSPE). The unlabelled fluorous precursor was almost entirely retained by the fluorous phase, and the desired product was eluted with a mixture of methanol-water.

**Conclusions:** A fluorous approach based on substitution of a highly fluorinated leaving group by [$^{18}$F] fluoride, followed by FSPE, was found to be successful in the effective isolation of [$^{18}$F]-FDG from its unlabeled precursor.

**References:** 1. J. W. McIntee, C. Sundararajan, A. C. Donovan, M. S. Kovacs, A. Capretta and J. F. Valliant, J. Org. Chem., 2008, 73 (21), pp 8236–8243. 2. R. Bejot, T. Fowler, L. Carroll, S. Boldon, J. E. Moore, J. Declerck and V. Gouverneur, Angew. Chem., 2009, 121 (3), pp 594-597.

## P078 FAST AND RELIABLE METHOD FOR THE PREPARATION OF VARIOUS [18F]FLUOROBENZYL HALIDES

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**Objectives:** Radiopharmaceuticals containing a $^{18}F$ atom in an aromatic ring can be prepared by using various substituted (or not) synthons such as [$^{18}F$]fluorobenzaldehydes and [$^{18}F$]fluorobenzyl halides. These synthons have been previously used to perform reductive amination or to alkylate various organic protic function such as imine, amino, thiol or hydroxyl. Very often, the synthesis of the [$^{18}F$]fluorobenzyl halide synthons implies three majors steps: - labeling of an nitro or ammonium triflate aromatic moiety with no-carrier-added (nca) [$^{18}F$]fluoride - reduction of the [$^{18}F$]fluorobenzaldehyde into alcohol with various agent such as $NaBH_3CN$, $LiAlH_4$ or $NaBH_4$. - halogenation of the [$^{18}F$]fluorobenzyl alcohol with reagents such as $SOBr_2$ or $SOCl_2$, gaseous HI or HBr , organic solution of HBr in ether, $P_2I_4$, $PH_3PBr_2$,... However, due to their complexity, none of the numerous processes described in the literature can be easily implemented in a synthesizer.

**Methods:** In the scope of transfering these processes on commercially available synthesizers, chemistry improvement were realized. A special attention was given to the halogenation reaction, the most difficult step to implement on a [$^{18}F$]FDG module. Reactions with reagents on solid support are very attractive. This solid strategy that avoids all conventional work-up and subsequent purification is presently used in our laboratory for the preparation of the [$^{18}F$]fluorobenzyl bromides required for the synthesis of 6-[$^{18}F$]fluoro-L-dopa and 2-[$^{18}F$]fluoro-L-tyrosine. However, although the halogenation step can be conducted with high yield directly on the support with gaseous HBr, the automation of this process with this very corrosive gas is very difficult to automate. Moreover this approach is not well suited for the synthesis of both 2- and 4-[$^{18}F$]fluorobenzyl bromide which are only obtained with very low radiochemical yields (5-20 %). To circumvent these problems, we have evaluated the feasibility of the reaction on the support with aqueous acid such as HCl, HBr and HI.

**Results:** Aromatic nucleophilic substitutions of various nitro and trimethylammoniumbenzaldehyde triflates were realized with nca [$^{18}F$]fluoride. After labelling, the aldehydes were trapped on a SPE cartridge and the subsequent conversion into benzyl bromides realized directly on-line on the support. Firstly, nearly quantitative reduction (>95 %) was performed with an aqueous solution of $NaBH_4$. Secondly, halogenations were conducted on the same support with different aqueous acid (HI 57 %, HBr 47%, HCl 37%).

| Substrates | R.Y. in [$^{18}F$]Fluorobenzyl halides (%, d.c.) Not optimized | | | |
|---|---|---|---|---|
| | X= Cl | X= Br | X= I | T (°C) |
| 2-nitrobenzaldehyde | 16 | 46 | 50 | 60° |
| 4-nitrobenzaldehyde | 17 | 55 | 43 | 60° |
| 6-nitroveratraldehyde | - | 48 | - | RT |
| 6-nitropiperonal | 44 | 42 | 45 | RT |
| 2-formyl-N,N,N-trimethylammonium triflate | - | - | 44 | 60° |
| 4-formyl-N,N,N-trimethylammonium triflate | 62 | 65 | 67 | 60° |
| 2-trimethylammonium-4-methoxybenzaldehyde | 69 | 69 | 68 | RT |
| 2-trimethylammonium-4,5-dimethoxybenzaldehyde | 40 | 40 | 40 | RT |

In most cases, the alcohol conversion into [$^{18}F$]fluorobenzyl halides (X= Cl, Br, I) was almost quantitative in less than 3 min. Except for the 2- and 4-[$^{18}F$]fluorobenzyl halides, halogenation reaction proceeded at room temperature. With this method, various [$^{18}F$]fluorobenzyl halides are obtained both with high specific activity and radiochemical purity.

**Conclusions:** This fast and versatile strategy proceeding either at RT or 60°C allows to synthesize with high radiochemical yields, various [$^{18}F$]fluorobenzyl chlorides, bromides and iodides derivatives which can be eluted from the support with a large variety of organic solvents.

## P079 RADIOSYNTHESIS OF AN [18F]-FLUORO-PEG DERIVATIVE OF TZTP AS A POTENTIAL TRACER FOR THE MUSCARINIC M4 RECEPTOR

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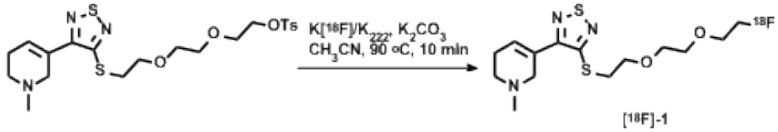
**Objectives:** Muscarinic acetylcholine receptors, of which there are five subtypes ($M_1$-$M_5$), play important roles in several neurodegenerative illnesses, including Alzheimer's disease, Parkinson's disease, and schizophrenia. Development of PET radioligands that exhibit intra-subtype selectivity and specificity offers the potential for quantifying these receptors in the living human brain. To this end, the potent and selective $M_2$ agonist radiotracer, [$^{18}$F]-FP-TZTP, has been developed for imaging Alzheimer's disease in human subjects.[1,2] We have recently reported an improved precursor and automated radiosynthesis of [$^{18}$F]FP-TZTP, with subsequent validation for human use[3] and we aim to exploit this manifold for the efficient labelling of several [$^{18}$F]-labelled derivatives of TZTP with improved intra-subtype selectivity. The objective of the present work is to build upon the established thiadiazolyltetrahydropyridine (TZTP) core.[4] We here report the synthesis of fluorinated alkyl and polyethyleneglycol (PEG) derivatives of TZTP, their in vitro evaluation, and radiolabelling of the most promising candidate with [$^{18}$F]-fluoride.




**Methods:** Several TZTP derivatives (ca. 20 compounds) were synthesized in our lab and then evaluated by the Psychoactive Drug Screening Program (PDSP) of the NIMH for in vitro studies. Based on its promising in vitro evaluation, 3-(2-(2-(2-fluoroethoxy)ethoxy)ethylthio)-4-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,5-thiadiazole (1) was deemed favourable for labelling with fluorine-18, as it offered both specific and selective binding to the $M_4$ receptor. The respective precursor, 2-(2-(2-(4-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,5-thiadiazol-3-ylthio)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate was fluorinated via nucleophilic displacement of the tosyloxy group by potassium cryptand [$^{18}$F] - fluoride (K[$^{18}$F]/$K_{222}$) in $CH_3CN$ at 90 °C for 10 min, and purified by HPLC (Scheme 1).

**Results:** Preliminary in vitro evaluation of compound 1 showed that it bound to the $M_4$ receptor with a $K_i$ of 57 nM, while binding to the $M_1$, $M_2$, $M_3$, and $M_5$ receptors ranged from 202-414 nM. [$^{18}$F]-1 was efficiently and rapidly prepared in 20% radiochemical yield (uncorrected) with specific activity >1000 mCi/mmol at end of synthesis (40 min), and was >99% radiochemically pure.

**Conclusions:** [$^{18}$F]-1 is a promising ligand for imaging the muscarinic $M_4$ receptor and its radiosynthesis is both efficient and rapid.To our knowledge, this represents the first radiotracer developed to target the muscarinic $M_4$ receptor. Biodistribution studies with [$^{18}$F]-1 in rodent models are underway and further derivatives of theTZTPcore are being synthesized in our laboratory in order to develop new [$^{11}$C]- and [$^{18}$F]-labelled imaging agents with increased muscarinic subtype specificity.

**References:** 1. Kiesewetter, D. O., Lee, J. T., Lang, L., Park, S. G., Paik, C. H., Eckelman, W. C., 1995, J. Med. Chem, 38, 5-8 2. Podruchny, T. A., Connolly, C., Bokde, A., Herscovitch, P., Eckelman, W. C., Kiesewetter, D. O., Sunderland, T., Carson, R. E. and Cohen, R. M., 2003. Synapse, 48, 39-44 3. van Oosten, E.M., Wilson, A. A., Stephenson, K. A., Mamo, D. C., Pollock, B. G., Mulsant, B. H., Yudin, A. K., Houle, S., Vasdev, N., 2009. Appl. Radiat. Isot., 67, 611-616 4. Sauerberg, P., Olesen, P.H., Nielsen, S., Treppendahl, S., Sheardown, M.J., Honore, T., Mitch, C.H., Ward, J.S., Pike, A.J., Bymaster, F.P., Sawyer, B.D., Shannon, H.E., 1992. J. Med. Chem., 35, 22752283.

## P080 NEW [18F]-FLUOROAMINES BY RING-OPENING OF AZIRIDINES WITH [18F]-FLUORIDE

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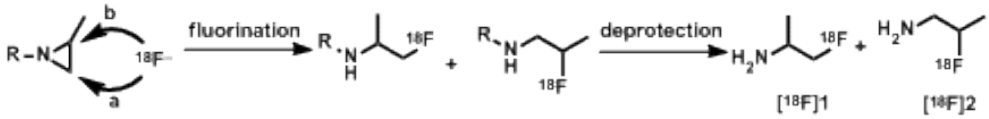
**Objectives:** Aziridines offer a novel methodology to introduce the isotope fluorine-18 ($^{18}$F, $t_{½}$ = 109.7 min) into PET radiopharmaceuticals. New [$^{18}$F]-fluoroamines would be useful in complementing electrophilic [$^{18}$F]-labelled synthons such as [$^{18}$F]-fluoroalkyl halides. Exploitation of aziridine chemistry is a plausible route to synthesize novel amine synthons as nucleophilic ring-opening of aziridines is well documented.[1] However, in contrast to other halide nucleophilic additions to aziridines, there have been relatively few reports describing the ring-opening of aziridines with nucleophilic sources of fluoride. Examples of the [$^{18}$F]-fluoride ion to ring-open aziridines[2–4] are even more sparse. Since attack of an unsymmetrical aziridine such as N-protected 2-methylaziridine can occur at either the secondary or tertiary carbon (Scheme 1), our goal is to control the regioselectivity and efficiency of nucleophilic ring-opening by varying a host of conditions, namely the nitrogen substituent, fluoride source, solvent, and temperature.




R = Bz, Ts, CBz, Boc, Bn

**Methods:** Ring-opening of N-protected 2-methylaziridines was carried out with potassium cryptand [$^{18}$F]-fluoride, followed by deprotection to yield the desired [$^{18}$F]-labelled amines. Characterization of both regioisomers was performed by in situ benzoylation of the mixture followed by HPLC identification. Furthermore, a wide range of nucleophilic fluoride sources, as well as a range of solvents and temperatures, were used to ring-open various N-substituted 2-methyl aziridines in an effort to optimize regioselective ring-opening with fluoride.

**Results:** We here report the regioselective ring-opening of benzyloxycarbonyl (Cbz)-protected and benzoyl (Bz)-protected 2-methylaziridine with [$^{18}$F]-fluoride to prepare the synthetically versatile amines, [$^{18}$F]1 and [$^{18}$F]2, respectively.[5] N-Cbz-2-methylaziridine was successfully ring-opened with [$^{18}$F]fluoride to obtain [$^{18}$F]1 as the dominant regioisomer (85%) via nucleophilic attack at the 2° aziridine carbon (route a, 60% conversion). Conversely, N-Bz-2-methylaziridine was ring-opened to afford [$^{18}$F]2 as the dominant regioisomer via nucleophilic attack at the 3° aziridine carbon (route b), in similar regioselectivity and radiochemical yields. Reactions with $^{19}$F sources of fluoride (including TBAF, KF□$H_2O$, and anhydrous HF) have also shown promising results. For example ring-opening of N-Cbz- and N-Bz-2-methylaziridine with anhydrous HF afforded fluoride attack at the 3° aziridine carbon with >99% regioselectivity in yields of 55-75%.

**Conclusions:** This work demonstrates the feasibility of preparing [$^{18}$F]fluoroamines by ring-opening of N-protected aziridines and presents rare examples of regioselective fluoride attack at the 2° carbon of an unsymmetrical aziridine. Extension of this chemistry to symmetrical aziridines and other unsymmetrical aziridines to create new [$^{18}$F]fluoroamines and radiopharmaceuticals is ongoing in our laboratory.

**References:** 1. Fokin, V. V.; Wu, P. In Aziridines and Epoxides in Organic Synthesis, Yudin, A. K., Ed.; Wiley-VCH: Weinheim, 2006; pp 443477. 2. Farrokhzad, S.; Diksic, M.; Yamamoto, L. Y.; Feindel, W. Can. J. Chem. 1984, 62, 21072112. 3. Gillings, N. M.; Gee, A. D. J. Labelled Compd. Radiopharm. 1997, 764765. 4. Lehel, Sz.; Horvath, G.; Boros, I.; Mikecz, P.; Marian, T.; Szentmiklosi, A. J.; Tron, L. J. Labelled Compd. Radiopharm. 2000, 43, 807815. 5. Vasdev, N., van Oosten, E. M., Stephenson, K., A., Zadikian, N., Yudin, A., K., Lough, A., J., Houle, S., Wilson, A., A., 2009, Tettrahedron Lett., 50 (5), 544-547

## P081 [18F]D4-36, A FLUORINE-18 LABELED APTAMER TARGETING THE TRANSMEMBRANE RECEPTOR TYROSINE KINASE RET

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**Objectives:** Within our intensive programs of development of oligonucleotide-based probes for PET-imaging, novel aptamers targeting the transmembrane receptor tyrosine kinase RET (REarranged during Transfection) have been isolated from whole living cell SELEX protocols. Of particular interest is one series of aptamers targeted towards $RET^{C634Y}$, a mutated form involved in Multiple Endocrine Neoplasia of type 2A (MEN2A) syndromes and in familial medullary thyroid carcinoma. Within this series, one aptamer, termed D4 (98-mer-, chemically stabilized (2'-fluoropyrimidinyl modified) RNA-aptamer) specifically binds to RET with a Kd of 35 ± 3 nM and blocks RET dimerization-dependent signalling pathways induced either by GDNF or by the C634Y activating mutation. For in vivo 3D-imaging purposes, a shortened version (36-mer only), showing similar binding and selectivity properties, was designed (D4-36) [1] and derivatized at its 3'-end with a thiopropyl motive, thus permitting prosthetic conjugation with maleimide-based reagents. D4-36 (-$(CH_2)_3$SH) was tagged with AlexaFluor680® (AF680) for optical fluorescence imaging and with [$^{18}$F]FPyME (1-[3-(2-[$^{18}$F]fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione), a prosthetic reagent labeled with the positron-emitter fluorine-18 [2] for PET imaging, which latter work is presented herein.

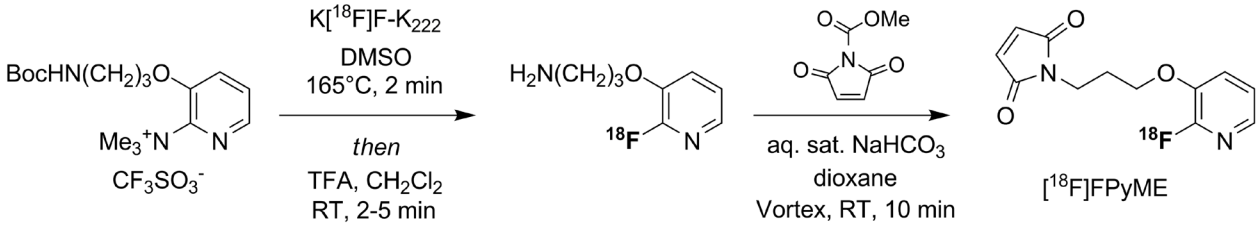
**Methods:** [$^{18}$F]FPyME was prepared using a three-step radiochemical pathway already reported [2]. Briefly, the developed procedure involves (1) a high-yield nucleophilic heteroaromatic ortho-radiofluorination on [3-(3-tert-butoxycarbonylaminopropoxy)pyridin-2-yl]trimethylammonium trifluoromethanesulfonate as the fluorine-18 incorporation-step, followed by (2) rapid and quantitative TFA-induced removal of the N-Boc-protective group, (3) optimized maleimide formation using N-methoxycarbonylmaleimide and final HPLC-purification (semi-preparative $SiO_2$ Zorbax® Rx-SIL, Hewlett Packard). HPLC-purified [$^{18}$F]FPyME was conjugated with D4-36 (90-120 nanomoles) in 1/9 (v:v) mixture (1 mL) of DMSO and 0.1 M aq. PBS (pH 7.5) at rt for 15 min. The product of D4-36 coupling with [$^{18}$F]FPyME (c-[$^{18}$F]D4-36) was purified by size-exclusion chromatography (Sephadex NAP-10 cartridge) and formulated for i.v. injection in aq. 0.9% NaCl.

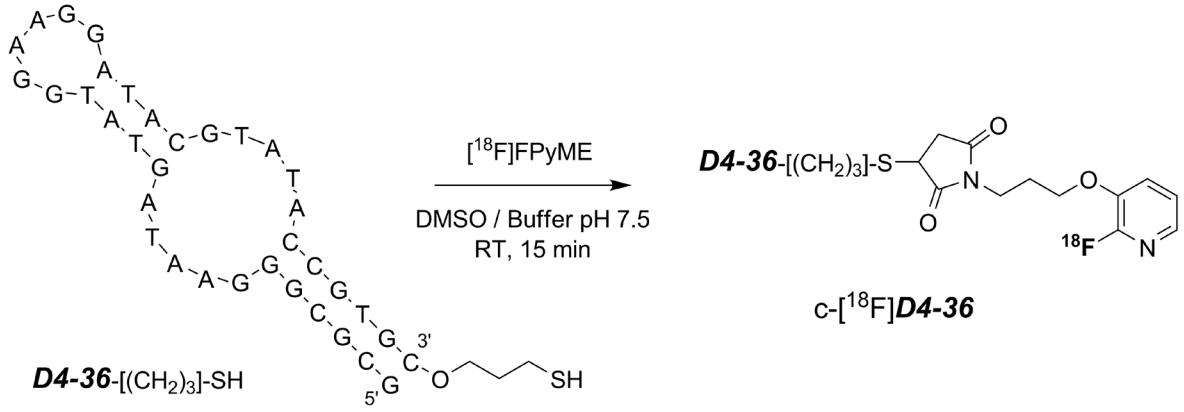
**Results:** 5.2-7.5 GBq batches of radiochemically pure [$^{18}$F]FPyME could be obtained in 110 min (semi-preparative HPLC included) starting from a 37-51 GBq cyclotron-production batch of [$^{18}$F]fluoride (overall decay-corrected RCY: 23-30%). Conjugation of [$^{18}$F]FPyME with the D4-36 aptamer was achieved in good yields (65-80%). Starting from a 1.7-2.0 GBq aliquot of a [$^{18}$F]FPyME batch, 0.9-1.1 GBq of SEC-purified and formulated c-[$^{18}$F]D4-36 could be obtained in 40-45 min. As demonstrated by TLC-, HPLC- and denaturing electrophoresis, c-[$^{18}$F]D4-36 was shown to be > 95% radiochemically pure and shown to be radiochemically stable for at least 180 min in saline at room temperature. Final specific radioactivities ranged from 10-30 GBq/$\mu$mol of aptamer.

**Conclusions:** D4-36 has been successfully labeled with fluorine-18 using [$^{18}$F]FPyME using our Zymate XP robotic system.

**Research Support:** This work was supported by grants from the European Molecular Imaging Laboratory (EMIL) network (EU contract LSH-2004-503569) and l'Agence Nationale de la Recherche (ANR-Emergence ARTIC).

**References:** [1] Cerchia et al. PLoS Biol. (2005), 3(4), e123. [2] de Bruin et al. Bioconj. Chem. (2005), 16, 406-420.



## P082 SYNTHESIS OF A POTENTIAL TYROSINE KINASE INHIBITOR BY KNOEVENAGEL CONDENSATION OF OXINDOLE WITH 4-[18F]FLUOROBENZALDEHYDE

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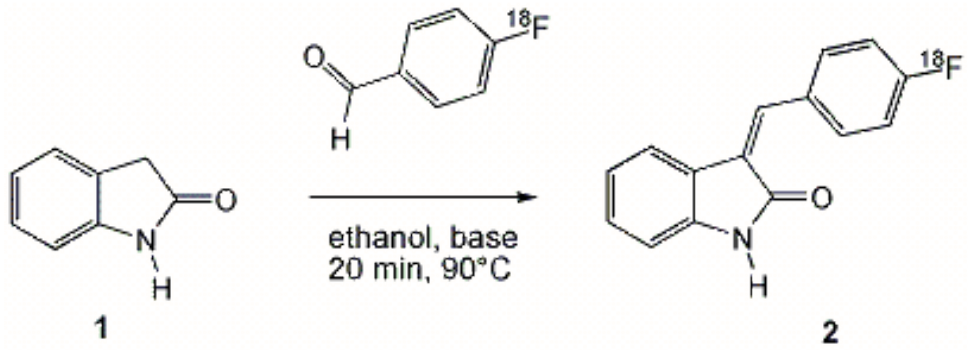
**Objectives:** Receptor tyrosine kinases (RTKs) play an important role in tumour angiogenesis through their involvement in proliferation, migration and differentiation between tumour and endothelial cells. Recently, small molecule tyrosine kinase inhibitors like Imatinib mesylate (Gleevec®), Gefitinib (Iressa®) and SU11248 (Sutent®) have been radiolabeled to study tumour angiogenesis in vivo[1,2,3]. The aim of the present work is the development of a sufficient radiosynthesis of 3-(4'-[$^{18}$F]fluorobenzylidenyl]-indolin-2-one 2 as derivative of potent RTK inhibitor Semaxinib® based on Knoevenagel condensation of 4-[$^{18}$F]fluorobenzaldehyde with oxindole.

**Methods:** Synthesis of 4-[$^{18}$F]fluorobenzaldehyde was performed by reacting [$^{18}$F]fluoride with 4-trimethylammoniumbenzaldehyde triflate in an automated synthesis module (Nuclear Interface). Briefly, 15 mg of precursor dissolved in acetonitrile (1 ml) was heated with dried [$^{18}$F]KF at 90 °C for 10 min. After addition of water (11 ml), 4-[$^{18}$F]fluorobenzaldehyde was purified via solid-phase extraction on a HLB-plus cartridge (250 mg, Waters). Purified 4-[$^{18}$F]fluorobenzaldehyde was eluted from the cartridge with ethanol (3 ml) and transferred into a separate reaction vial containing oxindole 1 (10 mg) and a base. Knoevenagel condensation occurred at 90°C for 20 min (Fig. 1). The radiochemical yield of product 2 was determined by radio-HPLC.




1 2

**Results:** Knoevenagel condensation of oxindole 1 with 4-[$^{18}$F]fluorobenzaldehyde was optimised by screening the influence of different bases on the radiochemical yield (RCY) of the carbonyl olefination reaction. The results are summarised in Table 1. Knoevenagel condensation strongly depends on the used base. Best results could be obtained by using amine bases piperidine (18% to 29%) or diethylamine (48%). Application of stronger amine bases (DABCO, diisopropyl-ethyl amine) or weaker bases (ammonium acetate, 2,6-di-tert.-butyl pyridine) resulted in lower or no product formation. Phosphazane as a very strong base yielded 26% of desired product 2 along with formation of large amounts of non-identified side products.

Knoevenagel condensation between 4-[$^{18}$F]fluorobenzaldehyde and oxindole

| Entry | Time [min] | Base [1) 25mg; 2) 30µl;3) 50µl; 4) 10mg ] | pKa | Temp. [°C] | RCY of 2 [%] |
|---|---|---|---|---|---|
| 1 | 20 | $CH_3COONH_4$ 1) | 2.3 | 90 | -- |
| 2 | 20 | 2,6-di-tbutyl-pyridine 2) | 3.6 | 90 | 14 |
| 3 | 20 | diethylamine 2) | 10 | 90 | 48 |
| 4 | 20 | piperidine 2) | 11 | 90 | 18 |
| 5 | 20 | piperidine 2) | 11 | 100 | 29 |
| 6 | 20 | piperidine 3) | 11 | 90 | 28 |
| 7 | 40 | piperidine 2) | 11 | 90 | 26 |
| 8 | 20 | N-ethyl-diisopropylamine 2) | 12 | 90 | 7 |
| 9 | 20 | DABCO 4) | 18 | 90 | 3 |
| 10 | 20 | phosphazane-base 2) | 40 | 90 | 26 |

**Conclusions:** Knoevenagel condensation of 4-[$^{18}$F]fluorobenzaldehyde with oxindole is a suitable labelling technique for the synthesis of radiotracer 2. Within a series of different bases, diethylamine provides highest radiochemical yields of up to 48%. The Knoevenagel condensation reaction optimised under the object of radiolabeling involving readily available 4-[$^{18}$F]fluorobenzaldehyde should be applicable for the convenient radiosynthesis of other compounds containing a benzylidene motif.

**References:** [1] Kil KE et al. Nucl Med Biol, 2007, 34, 153. [2] Wang JQ et al. Bioorg Med Chem Lett, 2006, 16, 4102. [3] Wang JQ et al. Bioorg Med Chem Lett, 2005, 15, 4380.

## P083 STUDIES ON THE NUCLEOPHILIC AROMATIC SUBSTITUTION USING [18F]FLUORIDE IN METHOXY-SUBSTITUTED ORTHO-NITROBENZALDEHYDES

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**Objectives:** For imaging dopamine metabolism and neutral amino acid transport, continuous interest in $^{18}$F-labeled phenolic amino acids (i.e. [$^{18}$F]FDOPA, [$^{18}$F]fluoro-m-tyrosine) is apparent in PET and recent PET/MRI studies [1]. Owing to the high specific activity and easily available [$^{18}$F]fluoride, nucleophilic aromatic substitution ($S_NAr$) is considered to be the reaction type of choice in order to introduce $^{18}$F at aryl carbons. However, the presence of methoxy groups (as masked phenolic hydroxyl) in the labeling precursors constitutes a challenge for nucleophilic aromatic $^{18}$F-fluorination [2]. Therefore, as model reactions for the introduction of $^{18}$F into phenolic amino acids via $S_NAr$, the replacement of $NO_2$ by [$^{18}$F]fluoride in mono- to tetra-methoxy-substituted 2-nitrobenzaldehydes was investigated systematically.

**Methods:** Nucleophilic aromatic $^{18}$F-fluorination of twelve different methoxy substituted nitrobenzaldehydes was performed in the common K2.2.2/$K_2CO_3$ system (140 °C, 10 mg precursor, 1 mL DMF; $n \geq 3$) and analyzed by Radio-TLC (at 1, 3, 7, 10, 20 and 30 min), Radio-HPLC and LC/MS.

**Results:** Surprisingly, it was found that even the threefold and fourfold methoxylated nitrobenzaldehydes (2,3,4-trimethoxy-6-nitrobenzaldehyde and 2,3,4,5-tetramethoxy-6-nitrobenzaldehyde) reacted in remarkably good yields to the corresponding $^{18}$F-labeled products (see Table; 82 % and 48 %, respectively). In addition, all labeling reactions were monitored by LC/MS and demethylation and intramolecular redox-processes of the precursors were observed. These side reactions and product decomposition decreased the radiochemical yields to some extent the longer the reaction proceeded and became relevant in the case of less reactive precursor molecules. In further studies, we evaluated the dependence of $S_NAr$ reaction parameters on the deshielding of the particular carbon atom bearing -$NO_2$ (chemical shifts in $^{13}$C NMR). A linear correlation between $S_NAr$ reaction rates (k*; determined after one minute of reaction) and chemical shifts of $^{13}$C NMR was observed ($R^2 = 0.89$).

**Conclusions:** The good correlation between the electrophilicity of the leaving group substituted carbon (expressed by its $\delta$ of $^{13}$C NMR) and the reaction rate (k*) after 1 min demonstrates that this reaction parameter plays a major role in the nucleophilic aromatic $^{18}$F-fluorination.

**References:** [1] Pauleit, D., Floeth, F., Hamacher, K., Riemenschneider, M.J., Reinfenberger, G., Muller, H.W., Zilles, K., Coenen, H.H., Langen, K.J. Brain 128 (2005) 678-687. [2] Ding, Y.S., Shiue, C.Y., Fowler, J.S., Wolf, A.P., Plenevaux, A. J. Fluorine Chem. 48 (1990) 189-205.

## P084 PEG-[18F]FPyZIDE AND PEG-[18F]FPyKYNE, TWO NEW FLUOROPYRIDINE-BASED REAGENTS FOR THE FLUORINE-18 LABELING OF MACROMOLECULES USING CLICK CHEMISTRY

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**Objectives:** Click chemistry, and more particularly the Cu-catalyzed 1,3-dipolar cycloaddition, is becoming an new route for the preparation of radiolabeled macromolecules. Numerous examples using fluorescent probes are already described in the literature but application to the field of radiochemistry is still in its infancy. Mild conditions of reaction, high yields and low by-product formation make the click chemistry an interesting alternative to the usual reductive amination, acylation or alkylation strategies for the labeling of macromolecules. As part of our continuous efforts in the development of [$^{18}$F]fluoropyridine-based reagents [1-3], we report herein the preparation of two novel "clickable" reagents, the azide PEG-[$^{18}$F]FPyZIDE (2-[$^{18}$F]fluoro-3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)pyridine, [$^{18}$F]-1a) and the alkyne PEG-[$^{18}$F]FPyKYNE (2-[$^{18}$F]fluoro-3-(2-(2-(but-3-ynyloxy)ethoxy) ethoxy)pyridine, [$^{18}$F]-2a).

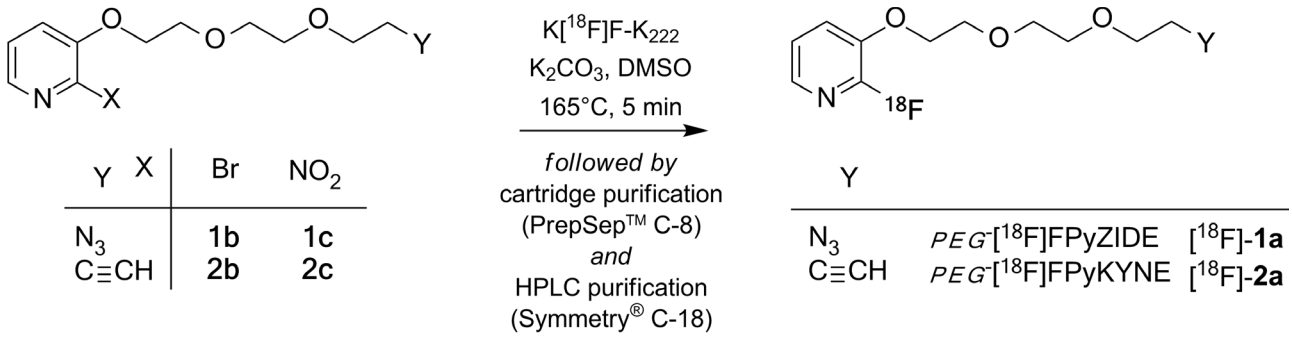
**Methods:** Chemistry : Azide series. Triethyleneglycol ditosylate was reacted with $NaN_3$ in DMF at rt for 15 hrs. After purification, the obtained tosylazide was reacted with 2-bromo-, 2-nitro- and 2-fluoro-3-hydroxypyridine in DMF with NaH or $K_2CO_3$ as base at 80°C for 15 hrs, providing respectively the bromo- and nitropyridine derivatives as precursors for labeling (1b, 1c) as well as PEG-FPyZIDE (1a) as reference compound. Alkyne series. Diethyleneglycol ditosylate was reacted with but-3-yn-1-ol and NaH in DMF at rt for 15 hrs. After purification, the obtained tosylalkyne was reacted with 2-bromo-, 2-nitro- or 2-fluoro-3-hydroxypyridine in DMF with NaH at 80°C for 15 hrs, providing respectively the bromo- and nitropyridine derivatives as precursors for labeling (2b, 2c) as well as PEG-FPyKYNE (2a) as reference compound. Radiochemistry : Fluorine-18-labeling of both target reagents involves: (a) reaction of K[$^{18}$F]F-Kryptofix®222 with about 5 mg of the appropriate precursor for labeling (1b,c for PEG-[$^{18}$F]FPyZIDE and 2b,c for PEG-[$^{18}$F]FPyKYNE) at 165°C for 5 min in DMSO (600 $\mu$L), (b) PrepSep C-8 cartridge pre-purification and (c) semi-preparative HPLC purification. The whole procedure was implemented on our Zymate XP robotic system.

**Results:** All derivatives (1a-c and 2a-c) were synthesized in overall yields ranging from 50% to 70%. Fluorine-18 incorporation provided [$^{18}$F]-1a/[$^{18}$F]-2a in up to 50% radiochemical yields based on radio-TLC. Both [$^{18}$F]reagents could be obtained in non-optimized, non-decay-corrected and isolated 10 to 25% yields after HPLC purification. Typically, starting from a 37 GBq cyclotron-produced [$^{18}$F]fluoride batch, 3.7 to 9.2 GBq of [$^{18}$F]-1a/[$^{18}$F]-2a (>95% radiochemically pure) were obtained within 50-60 min. Best results were observed with the nitropyridines 1b and 2b precursors.

**Conclusions:** PEG-[$^{18}$F]FPyZIDE ([$^{18}$F]-1a) and PEG-[$^{18}$F]FPyKYNE ([$^{18}$F]-2a) were prepared in one single radioactive step in less than 1 hour including HPLC purification. Optimization of the labeling and preparation of trimethylammonium trifluoromethanesulfonates as precursors for labeling are currently in progress.

**Research Support:** Supported by grants from the European Molecular Imaging Laboratory (EMIL) network (EU contract LSH-2004-503569).

**References:** [1] Kuhnast et al. Bioconj. Chem. (2004), 15, 617-627. [2] de Bruin et al. Bioconj. Chem. (2005), 16, 406-420. [3] Kuhnast et al. J. Label. Compds Radiopharm. (2008), 51, 336-342.



# P085 DEVELOPMENT OF [F-18]FLUORINE-SUBSTITUTED 6-ARYL-1,4-DIHYDROBENZO[d][1,3]-OXAZINE-2-THIONES AS PROGESTERONE RECEPTOR IMAGING AGENTS FOR PET

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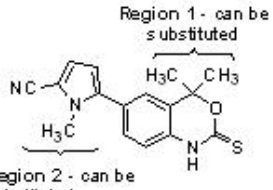
**Objectives:** Progesterone (PR), a member of the steroid receptor family, regulates ovulation and prepares the uterus for pregnancy. Selective PR agonist/antagonists, which have been used for female contraception and hormone replacement therapy, could also be useful in treating breast cancer.[1] Because PR levels are increased by estrogen treatment, measurement by positron emission tomography (PET) of an increase in PR levels in breast tumors after a brief exposure to estrogen might be useful in assessing whether the estrogen receptor is functional and thus capable of mediating endocrine therapies (ET); if effective, ET spares women with breast cancer from radiation and chemotherapy. Recently, Wyeth developed Tanaproget, a non-steroidal progestin agonist with very high PR binding affinity; its relative binding affinity (RBA) value is 150 compared to R5020 (RBA = 100), giving an estimated $K_D$ of ~0.2 nM. If F-18 could be introduced into Tanaproget or an analog, it might be an effective PET imaging agent for PR in breast cancer.

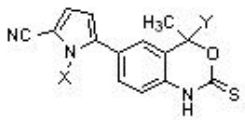
**Methods:** Guided by structure-activity relationships in the Tanaproget series, we prepared analogs bearing fluoroalkyl groups in place of a methyl group at two sites; their structures and binding affinities are shown below.

**Results:** Whereas substitution in region 2 (compound 2) gave a poor binder, a derivative with a fluoroalkyl group in region 1 (compound 1) had higher PR binding affinity than Tanaproget itself. Thus, we selected 4-fluoropropyl Tanaproget derivative (compound 1) for preparation in F-18 labeled form as a potential PET imaging agent for PR in breast cancer. The synthesis of 4-[F-18]fluoropropyl Tanaproget (compound 1) was performed by a modification of a previous report.[2] A methanesulfonate precursor was treated with F-18 TBAF in tert-amyl alcohol at 130 °C for 20 min[3] to give an F-18 labeled intermediate. After deprotection, the carbamate group was converted to the thiocarbamate intermediate with Lawesson reagent, giving the desired product, [F-18] (compound 1), in 40% yield. The radiochemical purity was >95% after reversed phase HPLC, and the overall radiochemical yield was ca. 5% (decay corrected).

**Conclusions:** This fluorine-substituted non-steroidal progestin has a very favorable affinity profile for PR and can be prepared in F-18 labeled form.

**References:** 1. Zang, P.; Terefenko, E. A.; Fensome, A.; Wrobel, J.; Winneker, R.; Lundeen, S.; Marschke K. B.; Zhang, Z. J. Med. Chem. 2002, 45, 4379-4382. 2.Fensome, A.; Bender, R.; Chopra, R.; Cohen, J.; Collins, M. A.; Hudak, V.; Malakian, K.; Lockhead, S.; Olland, A.; Svenson, K.; Terefenko, E. A.; Unwalla, R. J.; Wilhelm, J. M.; Wolfrom, S.; Zhu, Y.; Zhang, Z.; Zhang, P.; Winneker, R. C.; Wrobel, J. J. Med. Chem. 2005, 48, 5092-5095. 3.Moon, Dae Hyuk; Chi, Dae Yoon; Kim, Dong Wook; Oh, Seung Jun; Ryu, Jin-Sook. PCT Int. Appl. 2006 WO 2006065038 A1.



| entry | X | Y | RBA (R5020 = 100) |
|---|---|---|---|
| Tanaproget | $CH_3$ | $CH_3$ | 150±39 |
| 1 | H | $CH_2CH_2CH_2F$ | 190±40 |
| 2 | $CH_2CH_2F$ | $CH_3$ | 19±5.2 |

## P086 SYNTHESIS AND PRELIMINARY EVALUATION OF [18F]FDM FOR TUMOR IMAGING

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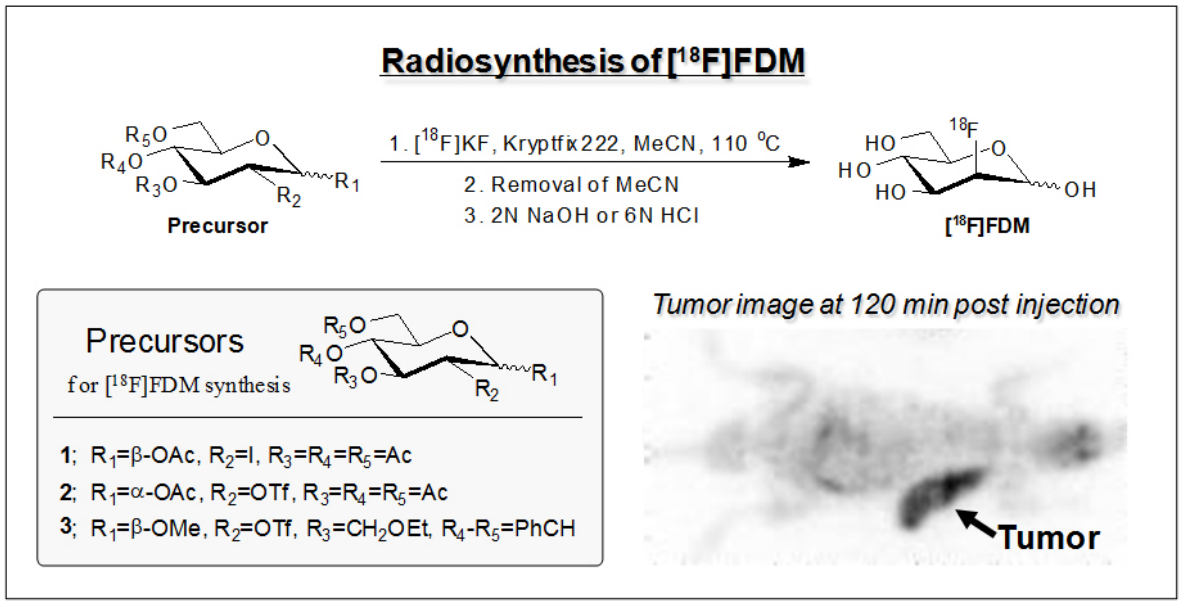
**Objectives:** 2-[$^{18}$F]Fluoro-2-deoxymannose ([$^{18}$F]FDM) is a stereoisomer of 2-[$^{18}$F]fluoro-2-deoxyglucose ([$^{18}$F]FDG) which only differs in the arrangement of fluorine atom on C-2. Considering conformational analogy between them, [$^{18}$F]FDM could also be available for a tumor imaging agent like [$^{18}$F]FDG. Actually, our previous study revealed several notable features of [$^{18}$F]FDM biodistribution including the same high tumor uptake, faster blood clearance, and lower brain uptake in comparison to [$^{18}$F]FDG[1]. These results greatly encouraged us to continue further study to disclose the characteristic and utility of [$^{18}$F]FDM in detail as a tumor imaging agent. To this end, however, we needed a reliable synthetic method of [$^{18}$F]FDM because [$^{18}$F]FDM used in the previous experiments had been obtained as a byproduct of [$^{18}$F]FDG synthesis based on electrophilic fluorination. Thus, we performed this study with an aim to establish a practical method for [$^{18}$F]FDM synthesis and to examine the utility of [$^{18}$F]FDM for tumor imaging.

**Methods:** For [$^{18}$F]FDM synthesis we applied the strategy introducing fluorine atom onto C-2 by $S_N2$ reaction with $^{18}$F-fluoride in the same manner as modern [$^{18}$F]FDG synthesis. We prepared three types of precursors (1, 2, 3) containing a leaving group on C-2. The precursors were radiofluorinated with activated [$^{18}$F]KF/Kryptfix222, followed by removal of the protecting groups on the hydroxyl groups with 2M NaOH (for precursors 1 and 2) or 6M HCl (for precursor 3). [$^{18}$F]FDM was purified by using ion exchange resign, Sep-Pak tC18, and Sep-Pak Alumina N. Radiochemical purity was confirmed by radio TLC. [$^{18}$F]FDM synthesized from precursor 3 was administered into the tail vein of AH109A tumor bearing rats. Biodistribution of [$^{18}$F]FDM was assessed by measuring radioactivity uptakes (%ID/g) of major organs and tumor at 60 and 120 min after injection. Tumor imaging was carried out with a positron planar imaging system (Hamamatsu Photonics, Japan).

**Results:** Radiochemical yields of [$^{18}$F]FDM using precursors 1 and 2 were quite low (0.7 and 1.5 %, respectively). The results of radio TLC analysis of the reactions suggested that fluorinated intermediates from these precursors were not produced stably in the fluorination reaction. By contrast, using precursor 3, [$^{18}$F]FDM was prepared reproducibly in good yield (60%) with excellent radiochemical purity (>98%). In a biodistribution study, the highest uptake of [$^{18}$F]FDM was observed in tumor at 60 and 120 min post injection (2.17±0.32 %ID/g and 2.09±0.20 %ID/g, respectively). Tumor-to-muscle and -brain ratios were 18.8 and 1.90 at 60 min, respectively. In vivo imaging showed [$^{18}$F]FDM to be smoothly excreted into urinary through kidney, leading to clear visualization of tumor with high tumor-to-background ratio.

**Conclusions:** We developed a new precursor suitable for [$^{18}$F]FDM synthesis based on $S_N2$ reaction with $^{18}$F-fluoride. According to this method, [$^{18}$F]FDM was prepared reproducibly with highly acceptable radiochemical yield (60%) and purity (>98%). The [$^{18}$F]FDM showed specific accumulation in tumor and afforded high contrast tumor images. These results warrant further study on the [$^{18}$F]FDM utility for tumor imaging.

**References:** 1) Hiroshi Fukuda, Taiju Matsuzawa, Yoshinao Abe, Satoshi Endo, Kenji Yamada, Kazuo Kubota, Jun Hatazawa, Tachio Sato, Masatoshi Ito, Toshihiro Takahashi, Ren Iwata, and Tatsuo Ido., "Experimental study for cancer diagnosis with positron-labeled fluorinated glucose analogs: [$^{18}$F]-2-fluoro-2-deoxy-D-mannose: a new tracer for cancer detection." Eur J Nucl Med 7: 294-297 (1982).



## P087 INFLUENCE OF METHYL SUBSTITUENTS ON NUCLEOPHILIC 18F-FLUORINATION OF ORTHO-NITROBENZALDEHYDES AND ORTHO-HALOBENZALDEHYDES

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**Objectives:** To investigate presynaptic functions, PET tracers, such as [$^{18}$F]FDOPA and [$^{18}$F]fluorotyrosines, have been developed to measure dopamine synthesis [1]. In general, fluorination of these aromatic amino acids is performed via electrophilic substitution. Nucleophilic substitution offers some major advantages for labeling with $_{18}$F. In order to systematically study radiolabeling aiming at a new synthetic strategy, experiments were carried out with model compounds, where the protected amino acid part of the molecule was modelled by a methyl group, the aromatic ring bearing different leaving groups (LG: -$NO_2$, -F, -Cl, -Br) for the nucleophilic introduction of $^{18}$F.

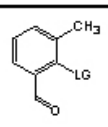
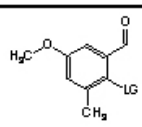
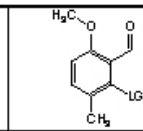
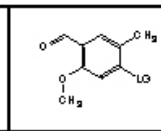
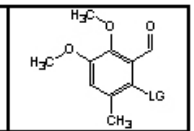
**Methods:** Nucleophilic aromatic $^{18}$F-fluorination of model compounds, mimicking the substitution pattern of precursors for [$^{18}$F]phenylalanine, [$^{18}$F]-m-tyrosine, [$^{18}$F]-p-tyrosine and [$^{18}$F]FDOPA, was performed in the common Kryptofix 222/$K_2CO_3$ system (140 °C (a, see table) or 120 °C (b, see table), 10 mg precursor, 1 mL DMF; n ≥ 3) and reactions were analyzed by Radio-TLC (at 1, 3, 7, 10, 20 and 30 min), Radio-HPLC and LC/MS. The condensation byproduct was synthesized under similar conditions as used in $^{18}$F-fluorination but in the absence of $^{18}$F and K222, isolated and fully characterized.

**Results:** For methylated model compounds (Table), the RCYs were highest for the fluoro substituent as LG. Surprisingly, the RCYs of the nitro compounds varied from high (4-$NO_2$, 69 %) to low (5-$NO_2$, 1 %). For substitution patterns 1, 2 and 5, the nitro precursors showed even lower RCYs than the bromo or chloro precursors. By using LC/MS analytics, every labeling solution of nitro compounds contained a compound with a mass of (2×M(precursor)-47). Two model compounds (1-$NO_2$ and 2-$NO_2$) were chosen as examples in order to synthesize the detected side product in macro scale. The product was formed in the absence of K222, however its presence speeded up the reaction. In analogy to a reaction known in the literature [2], this side reaction (condesation) can be rationalized as an attack of a nucleophilc carbanion on the aldehyde, followed by the denitration through the intermediate alcoholate (Figure). Therefore, RCY was reduced by the following ways: (1) The concentration of precursor was decreased by the coupling between two precursor molecules. (2) The acidic protons formed in the first step of the condensation reaction could also deactivate the $^{18}$F ion.

**Conclusions:** In summary, for compounds bearing a methyl group, the RCY was clearly dependent on the LG and the substitution pattern. The isotopic exchange ($^{18}$F/$^{19}$F) always proceeded easily and in good yields, and fluorodenitration was strongly influenced by the described condensation side reaction. In case of precursors with lower reactivity, this side reaction became dominant, hence resulting in low RCYs.

**References:** [1] Elsinga PH, Hatano K, Ishiwata K. Curr. Med. Chem. 13, 2139-2153 (2006). [2] Kuethe JT, Wong A, Journet M, Davies I. J. Org. Chem. 70, 3727-3729 (2005).

**Table: RCYs (in %) of the precursors modeling $^{18}$F-labeled aromatic amino acids**

| LG |  |  |  |  |  |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** |
| **$NO_2$** | 48 ± 5 [a] | 3 ± 1 [a] | 52 ± 6 [b] | 69 ± 8 [b] | 1 ± 1 [b] |
| **F** | 79 ± 3 [a] | 75 ± 2 [a] | 85 ± 5 [b] | 80 ± 8 [b] | 76 ± 6 [b] |
| **Cl** | 62 ± 9 [a] | 14 ± 3 [a] | 9 ± 2 [b] | 32 ± 3 [b] | 3 ± 1 [b] |
| **Br** | 52 ± 2 [a] | 10 ± 2 [a] | 29 ± 2 [b] | 36 ± 5 [b] | 3 ± 2 [b] |

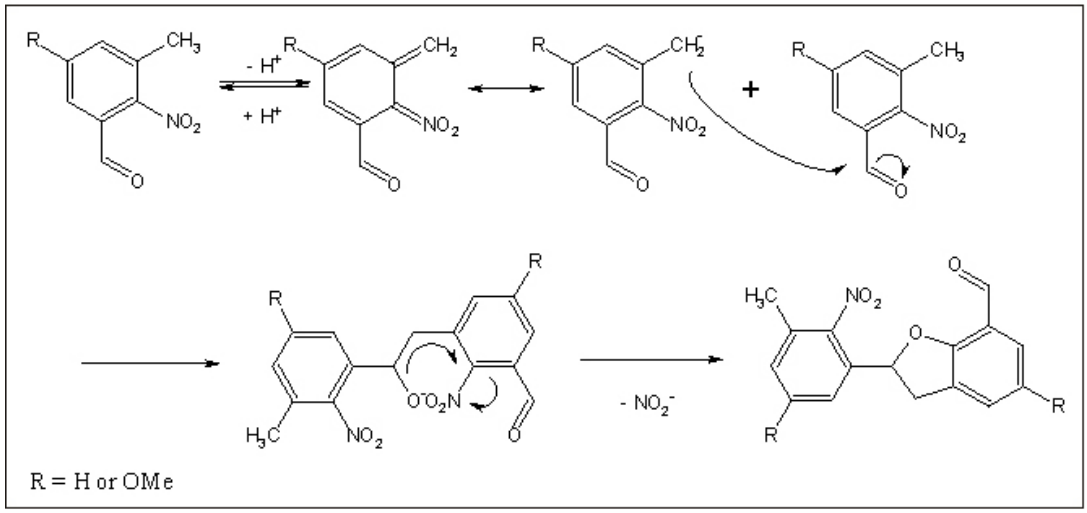



**Figure: Mechanism for the condensation reaction during $S_NAr$**

## P088 MODIFIED AUTOMATED RADIOSYNTHESIS OF N-SUCCINIMIDYL-4-[18F]FLUOROBENZOATE

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**Objectives:** N-Succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB) is a suitable acylation agent for the radiolabeling of peptides, proteins, antibodies, and oligonucleotides. The frequent utilization of [$^{18}$F]SFB for routine applications requires a remotely-controlled synthesis procedure. In this work we describe a modified synthesis of bifunctional labeling agent [$^{18}$F]SFB in a remotely-controlled synthesis unit. [$^{18}$F]SFB was used for the labeling of oligonucleotides functionalized with a terminal amino group.

**Methods:** N-succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB) was prepared using a commercially available automated synthesis unit (General Electric Tracerlab $FX_{FDG}$) which was modified in term of hard- and software. The synthesis is based on a three step/two pot synthesis sequence involving fluoride incorporation into readily available tert.-butyl 4-N,N,N-trimethylammoniumbenzoate, acidic hydrolysis, and subsequent conversion of 4-[$^{18}$F]fluorobenzoic acid ($^{18}$FBA) into [$^{18}$F]SFB. Modifications of the published approach included fluoride drying method, redesign of the reaction train and changes in the methods of reagent addition. The two separate reaction trains of GE TRACERlab $FX_{FDG}$ were linked together by some modifications on valve 21, 23, and 19 in order to transfer synthesized fluorobenzoic acid ($^{18}$FBA) directly from reactor 1 to reactor 2. Tetramethylammonium hydroxide was added directly to reactor 2 instead of to vial 7.

**Results**: It was possible to obtain high radiochemical purity without chromatography by washing the crude $^{18}$F-SFB with 5% acetic acid and water after trapping on a C18 cartridge and final elution of the purified product with acetonitrile. The controlled manufacturing process allowed the convenient synthesis of [$^{18}$F]SFB with an average radiochemical yield of 27% ± 10% (n=6, decay corrected) within 50 minutes for the entire process. The radiochemical purity of [$^{18}$F]SFB was 85% ± 15% (n=6) after solid phase extraction.

**Conclusions:** The modified synthesis procedure provides [$^{18}$F]SFB in reasonable radiochemical yields and radiochemical purities in much shorter reaction times compared with the published method.[1] Preliminary results on the labeling of 5'-amino group functionalized aptamer with [$^{18}$F]SFB will be presented.

**Research Support:** JK: Strategic Training Program in Translational Cancer Research, a partnership between CIHR, the Alberta Cancer Board and the National Cancer Institute of Canada, Alberta Cancer Research Institute Studentship, and Izaak Killam Memorial Scholarship. JM: Alberta Cancer Research Institute.

**References:** [1] P. Mading, F. Fuchtner, F. Wuest. Appl. Radiat. Isotop. 2005, 63, 329-332.

## P089 3D-QSAR, SYNTHESIS AND EVALUATION OF INDANONE DERIVATIVES AS NOVEL ACETYLCHOLINESTERASE INHIBITORS

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**Objectives:** Alzheimer's disease (AD) is a progressive, degenerative disorder of the brain characterised by a loss of memory and cognition, and it is a common form of dementia among the persons over 65 years old .Acetylcholinesterase is an attractive target for anti-Alzheimer's disease drug design[1]. Docking simulation and three- dimensional quantitative structure-actibity relationships (3D-QSAR) analysis were conduced on a series of dual binding site inhibitors of Acetylcholinesterase (AChE).Based on this model, we have designed some new AChEI with good binding affinity and selectivity, as well as the synthesis and biological evaluation in vitro . As the causes of AD have not been clearly understood , so the imaging of Acetylcholinesterase Inhibitors(AChEI) with PET and SPECT could provide a useful tool for understanding how alterations of this system are related to depressive illnesses and other psychiatric disorders, as well as to monitor the treatment of depressed patients.

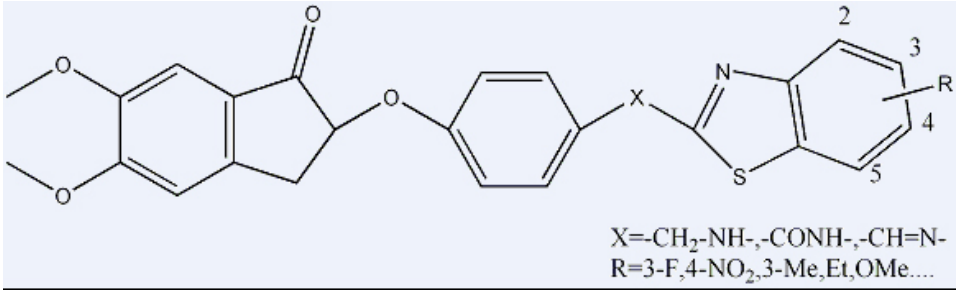
**Methods:** The model date set were selected from published lieratures[2, 3]. The inhibitory activities of AChE were reported as $IC_{50}$ (nm), which were measured by the same method of Ellman[4]. The AutoDock1.4.5 tool was used for the study of finding active conformer. The active conformers of the alignment for the 3D-QSAR study are selected from the binding conformation with the lowest binding energy, but not the conformers with the lowest RMSD values to the template.3D-QSAR models were derived on the basis of protonated molecules with $pIC_{50}$ values spreading over a range of five logarithmic units. All molecular modeling studies were performed using SYBYL 7.0. Gasteiger-Hueckel charges were calculated for all compounds. As we have designed some new indanone derivatives with good binding affinity and selectivity(figure1), the synthesis we designed according to Bischler et al[5]. and the biological evaluation in vitro we use the method of Ellman.

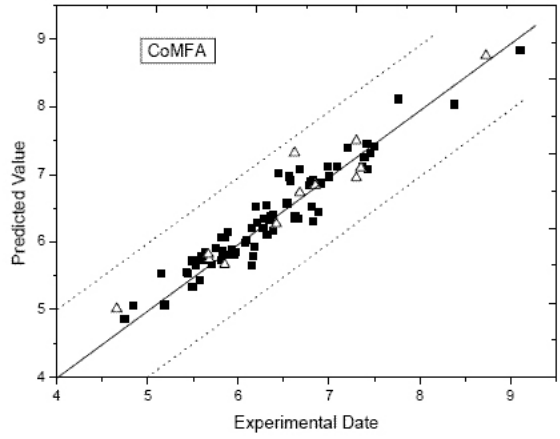
**Results:** The CoMFA model generated from both steric and electrostatic fields exhibited $q^2$ 0.652 with 6 components, $r^2$ 0.917, F-value 139.030, SE 0.245, with 68.2% steric and 31.8% electrostatic field contributions. The CoMSIA model for steric , electrostatic, hydrophobic, H-Donor and H-Acceptor fields yielded $q^2$ 0.641 with 6 components, $r^2$ 0.903, F-value 117.941, SE 0.264, with 5.8% steric, 19.4% electrostatic and 23.7% hydrophobic, 27.8%H-Donor, 23.2%H-Acceptor field contributions. Figure 2. Correlation between the actual and predicted activities of CoMFA CoMSIA models for the training set and test set.(■: compounds of the training set; △: compounds of the test set). The actual and predicted values are show with $pIC_{50}$ value. The preliminary experimental and further radiolabelling of $^{18}F$ and in vivo evaluation are in progress.

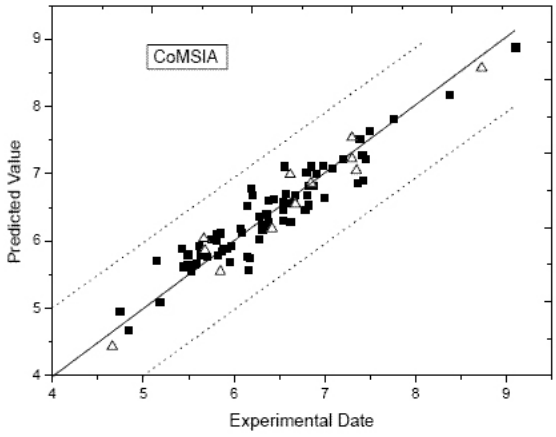
**Conclusions:** Hopefully we wish to find some new AChEI with good binding affinity and selectivity, and then we use the tool of PET to understand the Alzheimer's disease at the molecular level more better.

**Research Support:** This work was supported by the Natural Science Foundation of China (No. 20671013).

**References:** [1]Chemical Physics Letters 458(2008)285-289 [2] Bioorganic & Medicinal Chemistry 16 (2008) 7646-7653 [3] Bioorganic & Medicinal Chemistry Letters 15 (2005) 3834-3837[4] Biochem. Pharmacol. 7 (1961) 88-95. [5] J. Med. Chem. 44 (2001) 1654-1657



## P090 THE SYNTHESIS OF 6-DEOXY-6-[18F]FLUORO-D-FRUCTOSE FOR USE WITH PET

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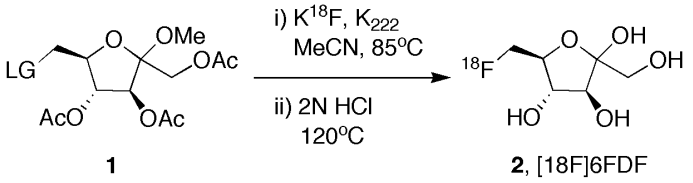
1. University of Alberta, Dept of Physiology, Edmonton, AB, Canada; 2. University of Alberta, Dept of Chemistry, Edmonton, AB, Canada; 3. University of Alberta, Dept of Oncology, Div of Oncologic Imaging, Edmonton, AB, Canada

**Objectives:** Cellular processes depend upon carbohydrates as the main source of carbon and metabolic fuel. Due to abnormally heightened metabolism in cancerous cells, carbohydrates have been a major focus for the development of PET tracers used to image solid tumors. A notable success from these studies is [18F]FDG, the most extensively used PET-radiopharmaceutical in current practice. Although FDG is widely used, there are a number of limitations associated with FDG-PET and these drawbacks have driven researchers to continue the search for improved imaging agents. Recent insights into the mechanism of carbohydrate transport through transmembrane glucose transport (GLUT) proteins have prompted our investigation into the informed design and synthesis of reliable [18F]-labeled fructose analogues for use in tumor imaging. We have developed a synthetic strategy to allow us access to 6-deoxy-6-fluoro-D-fructose (6FDF) as well as the [14C]-labeled 6FDF, both of which have been used to examine transport in MCF-7 and MDA-MB-231 human breast cancer cell lines. Currently, we are focused on designing an efficient synthesis of [18F]6FDF to further confirm the uptake of this potential imaging agent by tumoral cells and examine its potential use as a PET imaging agent.

**Methods:** The nonradioactive 6FDF compound was synthesized using standard organic laboratory techniques and confirmation of the product structure was obtained by examination of infrared and mass spectra, as well as proton (500MHz) and carbon (125MHz) NMR spectra. 6FDF, and its synthetic precursors, were used as TLC reference standards in the radiosynthesis of the [14C]-labelled compound and [18F]6FDF, which was prepared using an automated Eckert & Ziegler Modular-Lab.

**Results:** The synthesis of the 6FDF compound was accomplished in eight steps in 15% overall yield starting with inexpensive and readily available D-fructose. This route can provide a precursor (1) for fluorine installation in gram-scale quantities (Figure 1), although it is best stored as the 6-OH compound since the precursor is not stable to prolonged storage. The Modular-Lab was set up to allow for the facile synthesis of [18F]6FDF utilizing simple Sep-Pak cartridge purification incorporated into the automated system. Preliminary results indicate that [18F]6FDF can be obtained in >90% radiochemical purity. Currently, the total synthesis time is 120 min from the start of the radiofluorination and the overall yield is 43% (dc); however, we anticipate that the time and yield will be improved with optimization of the program.

**Conclusions:** The preliminary data presented herein suggests that we have successfully synthesized [18F]6FDF. Presently, we are focused on optimization of the automated reaction sequence to obtain consistently high quality product that we can investigate further in the context of imaging.




**1** **2**, [18F]6FDF

**Figure 1.** Synthesis of [18F]6FDF

## P091 SYNTHESIS AND EVALUATION OF FLUORINE-18 LABELED 2-FLUOROETHYL-6-PHENYLIMIDAZO[2,1-B] THIAZOLE AS A POTENTIAL MITOCHONDRIAL COMPLEX 1 INHIBITOR

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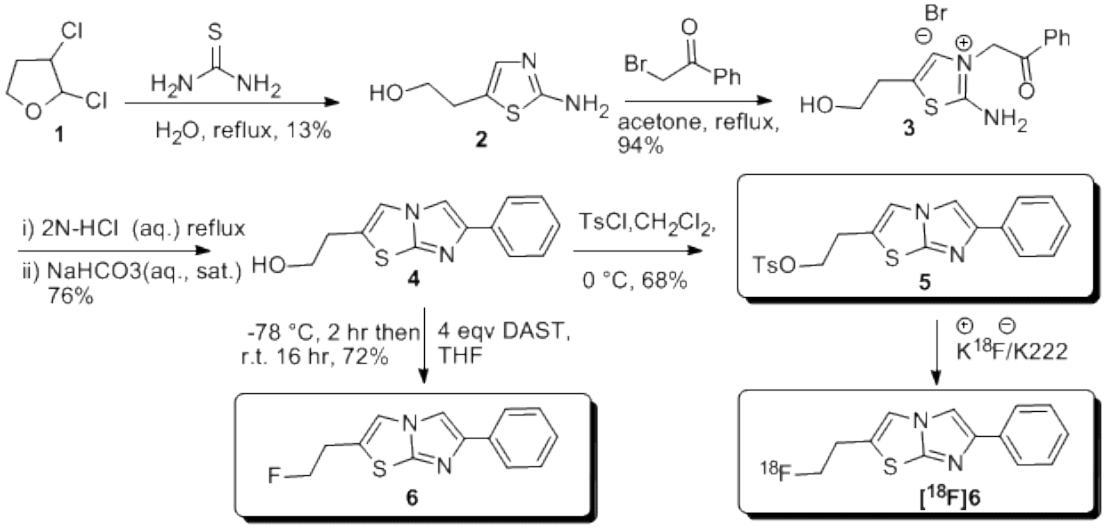
Emory University, Department of Radiology, Atlanta, GA

**Objectives:** The 2-alkyl substituted 6-phenylimidazo[2,1-b]thiazoles are potent inhibitors of the mitochondrial electron carrier enzyme, complex 1 (MC1) which is enriched in tissues consuming large amount of energy such as the heart. We developed a synthesis for fluorine-18 labeled 2-fluoroethyl-6-phenylimidazo[2,1-b]thiazole as a candidate fluorine-18 labeled potent inhibitor of MC1 for the assessment of myocardial perfusion using positron emission tomography (PET).

**Methods:** Compound 6 was synthesized from commercially available 2, 3-dichlorotetrahydrofuran as shown in scheme and $^{18}F$ labeling was performed from tosylate precursor 5 by 18F/K222 reaction.




**Results:** Radiochemical yield and with radiochemical purity over 99% as measured by radiometric HPLC. Biological evaluation in Sprague Dawley rats showed low heart uptake and defluorination.

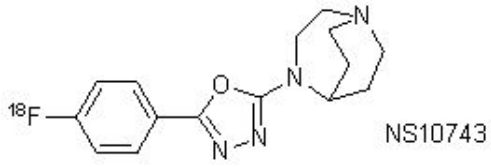
**Conclusions:** A novel potential MC1 inhibitor has been prepared and radiolabeled with fluorine-18, but it did not show pharmacological requirements as a cardio-PET tracer.

## P092 OPTIMISED RADIOSYNTHESIS AND METABOLITE ANALYSIS OF [F-18]NS10743, A RADIOLIGAND FOR NEUROIMAGING OF ALPHA-7 NICOTINIC ACETYLCHOLINE RECEPTOR (ALPHA-7 nAChR)

**S. FISCHER*[1], W. DEUTHER-CONRAD[1], A. HILLER[1], E. OESTERGAARD NIELSEN[2], D. BRUNICARDI TIMMERMANN[2], D. PETERS[2], P. BRUST[1] and J. STEINBACH[1]**

1. Institute of Interdisciplinary Isotope Research, Dept. of Radiopharmacy, Leipzig, Germany; 2. NeuroSearch A/S, Ballerup, Denmark

**Objectives:** The outstanding diversity of cellular properties, mediated by neuronal and non-neuronal α7 nAChR, points to the diagnostic potential for quantitative molecular imaging of this target. However, an appropriate $^{18}F$ labelled radioligand is currently not yet available for clinical applications. We have recently developed a $^{18}F$-labelled diazabicyclononane with promising biological properties for PET imaging: 4-[5-(4-fluoro-phenyl)-[1,3,4]oxadiazol-2-yl]-1,4-diaza-bicyclo[3.2.2]nonane [1] (NS10743; $K_D$ = 7.7 nM). Here we present the radiosynthesis of [$^{18}F$]NS10743 and the characterization of its radiometabolites in tissue samples. “




**Methods:** Two different precursors (I: 4-Br-phenyl; II: 4-$NO_2$-phenyl) were used for $^{18}F$ labelling of NS10743. The prepared K[$^{18}F$]F-K222-carbonate complex was reacted with 5-7 mg I or 2.5-4 mg II in 1 ml DMF using microwaves (75W/150°C for 10-12 min). Purification of the crude product was performed via Sep-Pak RP18 cartridge and semi-preparative radio-HPLC (RP phase, MeCN/water). The required quantitative reduction of remaining precursor II was achieved using Pd/$BaSO_4$ and $HCOONH_4$ in MeOH. After complete removal of the solvent, the radiotracer was dissolved in sterile 0.9% NaCl for injection into mice. To get information about metabolism of [$^{18}F$]NS10743 and suggestions for its pathway, brain, liver, plasma, and urine samples were obtained at 5, 30, 45, and 60 min p.i. Brain and liver samples were homogenised. After precipitation of proteins and extraction with MeCN, the supernatants were analysed by radio-TLC, radio-HPLC (RP mode, gradient method) and in some cases by LC-MS coupling technique using separately prepared reference standards. In all cases recovery rates were controlled.

**Results:** The bromo precursor I led to insufficient results. The labelling yield was ≤10% under varying conditions and the purification was complicated. The optimised microwave assisted radiosynthesis of the nitro precursor II resulted in good, reproducible reaction parameters: labelling yield of 30-40%, RCY of 20-30%, radiochemical purity of >99%, and specific activity of ~150 GBq/μmol. The extraction of the tissue samples was accomplished with high recovery rates of between 85% and 92%. In brain tissue no radiometabolites were observed at 60 min p.i. A single radiometabolite was detected in plasma (30-60%, 30, 45 and 60 min p.i.). Based on gradient HPLC data and LC-MS, the metabolite has been identified as the N-oxide of NS10743.

**Conclusions:** An optimised microwave assisted radiosynthesis of [$^{18}F$]NS10743 was established. In the mouse brain, [$^{18}F$] NS10743 proved to be stable. The single metabolite, identified in plasma, does not cross the blood-brain barrier. These findings suggest that [$^{18}F$]NS10743 is a suitable radiotracer for neuroimaging of α7 nAChR with PET.

**References:** [1] Peters, Dan et al. WO 2007138037, PCT Int. Appl. (2007)

## P093 ARE IONIC LIQUIDS USEFUL FOR FLUORINE-18 LABELING?

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**Objectives:** Ionic liquids (ILs) have been described as particularly convenient solvents for radiofluorinations with [$^{18}$F]F$^-$, increasing the tolerance of the nucleophilic substitution to water. Most of interesting results were obtained in uncapped reactor[1,2]. Here we report such labeling with [$^{18}$F]F$^-$ performed in presence of water in closed reactors. An evaluation of the influence of the losses of water during the labeling step in open reactor is also presented.

**Methods:** [$^{18}$F]F$^-$ was trapped on an anion exchange column and recovered in $K_2CO_3$/K222/$CH_3CN$/$H_2O$. The labeling of 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethane-sulphonyl-α-D-mannopyranose and 2-chloroethyl p-toluenesulfonate were used as tools to evaluate the influence of ILs on [$^{18}$F]F$^-$ incorporation. Six ILs (cation: 1-butyl-3-methylimidazolium or 1-butyl-2,3-dimethylimidazolium ; anion: bistrifluoromethanesulfonimidate, tetrafluoroborate, trifluoromethanesulfonate or mesylate) were evaluated (0,5 mmol/ml in the labeling mixture).

**Results:** In capped reactor and presence of water (5%), the yield of 2-[$^{18}$F]fluoro-1,2,4,6-tetra-O-acetyl-D-glucose synthesis after 15 minutes of labeling at 100°C (precursor 25 mg/ml) was not significantly different in absence or presence of ionic liquid (about 50% of incorporation observed by TLC). Some ILs increased the availability of [$^{18}$F]F$^-$ for labeling by decreasing the losses of radioactivity on the reactor surfaces, while others strongly increased it. In open reactor, the kinetic of 1-chloro-2[$^{18}$F]-fluoroethane synthesis was shown to be correlated to the amount of water effectively present in the labeling mixture: it increased when the water concentration decreased by evaporation. Again, the presence of ionic liquid did not influence these observations.

**Conclusions:** We were not able to confirm the effect of ILs on the tolerance to water of [$^{18}$F]F$^-$ radiofluorination. On the other hand, evaporation of water during the labeling step seems to be essential to reach high yields of incorporation. Therefore, tested ionic liquids are probably not of prime interest in the scope of fluorine-18 labeling in presence of water.

**Research Support:** This research was supported by a grant from European Union's FP6 STRP 516984 MI-Lab-On-Chip. A. Plenevaux is a research associate from FRS-F.N.R.S. Belgium.

**References:** [1] D. W. Kim, Y. S. Choe and D. Y. Chi Nucl. Med. Biolg. 30 (2003) 345-350. [2] H.W. Kim, J.M. Jeong, Y.S. Lee, D.Y. Chi, K.H. Chung, D.S. Lee, J.K. Chung, M.C. Lee Appl. Radiat. Isot. 61 (2004) 1241-1246.

## P094 SYNTHESIS OF 18F-FACPC AND ITS BIOLOGICAL EVALUATION IN 9L GLIOSARCOMA CELLS AND 9L TUMOR BEARING RATS

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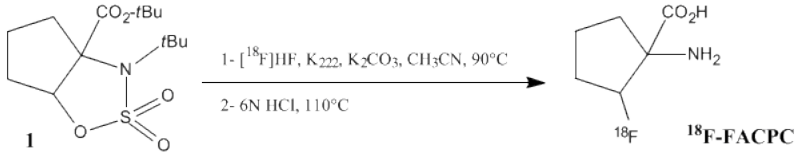
Emory University Center for Systems Imaging WWHC, Radiology Dpt., Atlanta, GA

**Objectives:** Numerous non-naturally occurring labeled amino-acids such as 1-aminocyclopentane-1-[$^{11}$C] carboxylic acid ($^{11}$C-ACPC) have been developed for imaging brain tumors with PET.[1] ACPC is not metabolized nor incorporated into protein in mammals. It is mainly transported across cell membranes by the "L' transporters but has some affinity to the "A" transport system.[2] For PET studies, the 20 min half-life of $^{11}$C limits the applications of $^{11}$C-tracers to hospitals having onsite cyclotron. Herein, we describe the developments toward the design and characterization of (R,S) anti-1-amino-2-[$^{18}$F]fluorocyclopentyl-1-carboxylic acid ($^{18}$F-FACPC), a PET fluorinated analogue of ACPC. The key step in the preparation of $^{18}$F-FACPC involved the synthesis of the cyclic sulfamidate precursor 1.




**Methods:** The radiofluorinated target $^{18}$F-FACPC was prepared by no-carrier-added nucleophilic substitution, from the corresponding precursor 1. The biological behavior of that new radiotracer was evaluated in vitro, via uptake assays using 9L gliosarcoma cells as well as in vivo, through distribution studies in rats bearing 9L gliosarcoma implants and sacrificed at 15, 30, 60, and 120 min after tail injection of $^{18}$F-FACPC.

**Results:** $^{18}$F-FACPC was obtained in an average decay-corrected yield of 61% (n=4) and radiochemical purity of > 99%. The total synthesis time was 60 min from end of bombardment. The in vitro assays performed using 9L gliosarcoma cells in the presence of BCH (2-aminobicyclo[2.2.1]heptane-2-carboxylic acid), MeAIB (methyl α-aminoisobutyric acid), and ACS (6-aminohexanoic acid) (inhibitors of amino acid transport) demonstrated that $^{18}$F-FACPC is a substrate for the L-type amino acid transport system. The biodistribution studies in rats implanted with intracranial 9L gliosarcoma cells, showed high retention of radioactivity in tumor tissue at 15, 30, 60, and 120 min after tail injection of $^{18}$F-FACPC.$^{18}$F-FACPC showed uptake of radioactivity in tumor at 15, 30, 60, and 120 min p.i. of 0.57, 1.68, 1.24, and 0.98 %ID/g, respectively. The tumor versus normal brain ratios of radioactivity were 5:1, 13:1, 13:1, and 10:1 at 15, 30, 60, and 120 min, respectively, after tracer injection.

**Conclusions:** Our findings suggest that the new radiotracer $^{18}$F-FACPC is an attractive candidate for imaging brain tumors with PET. Studies are currently underway to prepare $^{19}$F-FACPC (the cold standard) and to determine the transport properties of the isolated R and S enantiomers of $^{18}$F-FACPC.

**References:** K. F. Hubner, S. Krauss, L. C. Washburn et al. Clin. Nucl. Med., 1981; 6; 249-252 U. Hisao, M. Tadashi, T. Juri et al. Eur. J. Cereb. Boold Flow Metab., 1997; 17; 1239-1253

## P095 RADIOFLUORINATION OF L-THREO-3-(3,4-DIHYDROXYPHENYL)SERINE: SYNTHESIS OF POTENTIAL F-18 LABELLED PET TRACERS FOR NOREPINEPHRINE

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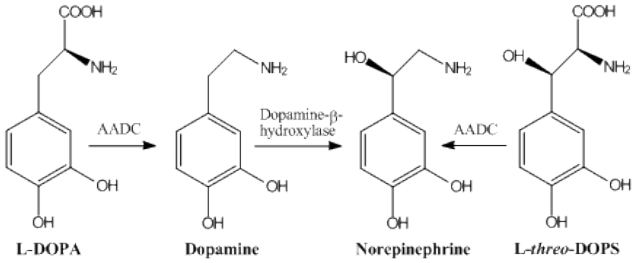
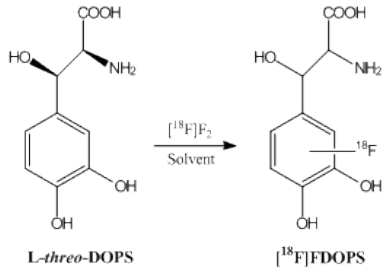
1. Hamilton Health Sciences, Department of Nuclear Medicine, Hamilton, ON, Canada; 2. Centre for Addiction and Mental Health, Toronto, ON, Canada

**Objectives:** Norepinephrine (NE) is a vital neurotransmitter of the sympathetic and central nervous system and its imaging with PET has been investigated since the early 1970's.[1] Norepinephrine is produced in vivo by the side-chain hydroxylation of dopamine by the enzyme dopamine-β-hydroxylase (Scheme 1). Because L-threo-3-(3,4-dihydroxyphenyl)serine (L-threo-DOPS) is a substrate for the enzyme aromatic acid decarboxylase (AADC; Scheme 1) and is capable of crossing the blood brain barrier, it is expected to be a biological precursor for NE that is independent of dopamine biosynthesis. Kirk et. al.[2-5] have published several papers on the synthesis and biological behaviour of L-threo-DOPS and its fluorinated analogues, 2- and 6-F-threo-DOPS. To our knowledge, no attempt has been made to study the reactivity and selectivity of L-threo-DOPS towards fluorine during electrophilic fluorination. In this paper, we report the synthesis of [$^{18}$F]2- and 5-fluoro-DOPS by the direct fluorination of L-threo-DOPS using [$^{18}$F]$F_2$.

**Methods:** Fluorine-18 labelled $F_2$ was produced by the $^{18}$O(p,n)$^{18}$F nuclear reaction using a Siemens 11 MeV cyclotron (RDS 112) by the double-shoot method and was passed through a solution of L-threo-DOPS in suitable solvent (Scheme 2). Products resulting from the fluorination were isolated by preparative HPLC and characterized by $^{19}$F NMR spectroscopy.




**Scheme 1.** Biosynthesis of norepinephrine




**Scheme 2.** Radiofluorination of L-*threo*-DOPS

**Results:** Direct fluorination of L-threo-DOPS was carried out in several solvent media and the effect of reaction conditions on the radiochemical yield and regioselectivity of L-threo-DOPS towards $F_2$ was investigated. The combined radiochemical yield of [$^{18}$F]2- and [$^{18}$F]5-FDOPS was 12% with respect to [$^{18}$F]$F_2$. Under the current HPLC conditions, [$^{18}$F]5-FDOPS was isolated with a radiochemical purity of 95 ±2%, whereas [$^{18}$F]2-FDOPS co-eluted with the precursor.

**Conclusions:** Clinically useful quantity of [$^{18}$F]5-FDOPS was obtained at the end of 60 min synthesis. Work is underway to synthesize [$^{18}$F]6-FDOPS as well as to optimize the HPLC separation between the ring fluorinated isomers of DOPS.

**References:** Fowler, J.S.; MacGregor, R.R.; Ansari, A.N.; Atkins, H.L.; Wolf, A.P. J. Med. Chem. 1974, 17, 246-248. Chen, B.-A.; Nie, J., Singh, M.; Pike, V.W.; Kirk, K.L. J. Fluorine Chem. 1995, 75, 93-101. Herbert, B.; Kim, I.H.; Kirk, K.K. J. Org. Chem. 2001, 66, 4892-4897. Kim, I.-H.; Kirk. K.L. Tetrahedron Lett. 2001, 42, 8401-8403. Borri Voltattorni, C.; Bertoldi, M.; Bianconi, S.; Deng, W.; Wong, K.; Kim, I.-H.; Herbert, B.; Kirk. K.L. Biochem. Biophys. Res. Commun. 2002, 295, 107-111.

## P096 NEW IMPROVEMENTS IN THE ENANTIOSELECTIVE SYNTHESIS OF 2-[18F]FLUORO-L-TYROSINE AND 6-[18F]FLUORO-L-DOPA

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**Objectives:** Nowadays, 6-[$^{18}$F]fluoro-L-dopa and 2-[$^{18}$F]fluoro-L-tyrosine are well established radiopharmaceuticals compounds to study in vivo the presynaptic dopamine metabolism and for realizing oncology investigations with positron emission tomography (PET) respectively. These last years, the need for reliable production of these two radiopharmaceuticals has led to the report of various electrophilic and nucleophilic radiosyntheses. Among these, the nucleophilic one is undoubtedly the most interesting with regards to yield and amount of activity available at the end of the synthesis. The no-carrier-added (n.c.a) [$^{18}$F]fluoride enantioselective synthesis of these two [$^{18}$F]fluorinated aminoacids implies the asymmetric alkylation of a N-(diphenylmethylene)glycine tert-butyl ester (a Schiff base) by a [$^{18}$F]fluorobenzyl bromide derivative using a chiral Phase Transfer Catalyst (PTC) at 0°C (Lemaire, 2004). Presently, before alkylation, the benzyl bromide has to be cooled at 0°C at least 10 minutes. Moreover, the alkylation step must be conducted at this temperature for five additional minutes. Consequently, automation of the enantioselective alkylation step requires a cooling system which complicates the process. Moreover, this cooling step is time and radiochemical yield consuming. It is certainly one of the reason why these [$^{18}$F]fluorinated amino acids are not more often used for clinical investigation. We describe here, one major improvement realized to facilitate this automation.

**Methods:** Seven different chiral PTC catalysts have been synthesized. Five of them are cinchonidine derivatives, one from BINOL and the last has a biphenyl core. The PTC alkylation reaction has been conducted with these catalysts, in presence of the Schiff base described above, CsOH or KOH, and the [$^{18}$F]fluorobenzyl bromide whose synthesis has been recently improved (Lemaire, 2009; Libert, 2009) . Enantiomeric excesses, in toluene or $CH_2Cl_2$, have then been evaluated at different temperature (0°C, RT, 35°C, 50 °C and 80 °C).

**Results:** In all cases, alkylation reactions are fast (<10 min) and nearly quantitative (> 90 %). After hydrolysis and HPLC purification, enantiomeric excesses were determined on a crown pack chiral column (Daicel). From these data, it appears that one catalyst derived from dihydrocinchonidine bearing both a $CH_2$-anthracen-9-yl and a benzyl groups and the catalyst with the biphenyl core afford even at room temperature high enantiomeric excesses (Ee > 97 % at RT).

**Conclusions:** The alkylation step which proceeds now with excellent enantioselectivity either at room temperature or even at slightly higher temperature (50 °C), makes automation of this critical synthesis step now possible without additional equipement. With this major improvement, implementation of the n.c.a. enantioselective synthesis of various [$^{18}$F]fluoro amino acids should be possible in commercially available FDG synthesizer.

**References:** Lemaire C., et al. European Journal of Organic Chemistry (2004), (13), 2899-2904. Lemaire C., et al. Fast and reliable method for the preparation of various [$^{18}$F]fluorobenzyl halides. Abstract, this meeting Libert L., et al. Large scale preparation of [$^{18}$F]fluoromethoxybenzyl bromides, key precursors for 2-[$^{18}$F]fluoro-L-tyrosine and 6-[$^{18}$F]fluoro-L-dopa syntheses. Abstract, this meeting

## P097 SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL FLUORO-18 LABELED POSITRON EMISSION TOMOGRAPHY (PET) IMAGING AGENTS FOR HYPOXIC TISSUES

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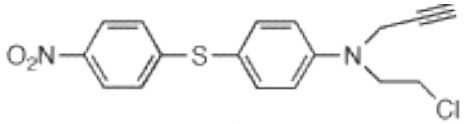
**Objectives:** To synthesise and investigate the labeling of compounds 1 (N-(2-chlorothyl)-4-(4-nitrophenylthio)-N-(prop-2-ynyl)aniline) and 2 (N-(2-chlorothyl)-4-(4-methylsulfinyl) -N-(prop-2-ynyl)aniline) via Fluoro-18 halogen exchange using K[$^{18}$F]-kryptofix or click chemistry using [$^{18}$F]fluoroethylazide. These compounds are being evaluated as potential candidates for hypoxic imaging agents in stroke and tumor models.



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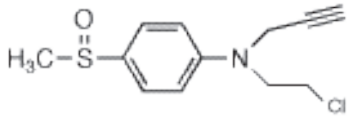

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**Methods:** Compound 1 was prepared from two steps: firstly, the coupling of (4-nitrophenylthio)aniline with propargyl bromide in the presence of base to give 4-(4-nitrophenylthio)-N-(prop-2-ynyl)aniline. Secondly, 2-chloroethyl triflate was reacted with the propagyl aniline under basic conditions to give compound 1. This compound was labeled with K[$^{18}$F]F-kryptofix 2.2.2 complex at 110°C in DMSO. Compound 1 was also subjected to click chemistry following the procedure prescribed by Glaser and Arstad.[1] Compound 2 was prepared by coupling 4-(methylthio)analine with 2-chloroethyltriflate, in the presence of base, followed by the coupling reaction to propargyl bromide in basic conditions to give N-(2-chloroethyl)-4-(methylthio)-N-(prop-2-ynyl)aniline. Oxidation of this compound yielded compound 2, which will subsequently be labeled in the same manner as described above.

**Results:** Fluoro-18 labeled compound 1 was successfully synthesized with 6% yield and is ready to be subjected to in vivo studies. However, labeling of this compound via click chemistry has been proven to be unsuccessful to date, and requires further investigation.

**Conclusions:** The syntheses of compounds 1 and 2 have been successful. The radiolabeling of both compounds and the in vivo studies are in progress.

**Research Support:** Contract sponsor: NHMRC; project grant number: 469002

**References:** [1] Glaser M. and Arstad E., Bioconjugate Chem., 2007, 18, 989-993

## P098 USE OF ORGANIC BASES FOR 18F-FLUORIDE ANION EXCHANGE ELUTION AVOIDING THE CLASSICAL AZEOTROPIC DRYING STEP BEFORE LABELING

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**Objectives:** Nowadays, for PET radiopharmaceuticals labeling, fluorine-18 is certainly the most widely used short-lived radioisotopes ($^{18}F$, $t_{1/2}$= 109.7 min). According to its production mode, the n.c.a. [$^{18}F$]fluoride ion obtained in aqueous solution, is strongly hydrated and unreactive for nucleophilic substitution. Several methods have then been described to increase its reactivity. Currently, the well established procedure requires trapping of the $^{18}F$-fluoride ion onto an anion exchange resin and its subsequent elution with an acetonitrile/water solution containing $K_2CO_3$ and a kryptand. After two or three azeotropic evaporation, the $K^+/^{18}F$/kryptofix complex is solubilized in a polar aprotic solvent ($CH_3CN$, DMSO) suitable for the subsequent labeling reaction (Hamacher, 1986). However, this evaporation step is not compatible with the recent miniaturization of PET radiochemistry systems.

**Methods:** A large variety of organic bases differentiated by strength, nucleophilicity, steric hindrance are now commercially available. These organic bases usually contain nitrogen atoms, which after protonation can lead to highly reactive anions. Based upon this finding, we think that a strong organic base in presence of a compound bearing an acid hydrogen should generated anionic species in solution able to displace the [$^{18}F$]fluoride ion trapped on the QMA support.

**Results:** Preliminary experiments have shown that a dry solvent such as acetonitrile cannot be used for the elution. Nevertheless, if acetonitrile contains a very small amount of water (500 - 25000 ppm) and a strong organic base such as $P_4$tBu (phosphazene base) elution is possible. In this case, more than 95 % of the activity trapped on the support can be eluted with less than 1 ml of eluent. With other organic bases, the percentage of elution with a small volume increases when moving from classical base to super strong bases. Recovery starts when bases with pka around 25 or above are used. When water is substituted by another protic compound such as an alcohol (methanol, isopropanol,…) nearly quantitative elution (> 95 %) without any addition of water in the eluent is still possible. The reactivity of this $^{18}F$-fluoride has been evaluated with the mannose triflate precursor. In presence of one additional organic base such as TMGN, MTBD, $P_1$tBu,…. , high reproducible labeling yields (>80-90 %) are obtained without any previous evaporation step. Moreover, labeling reaction can be conducted at room temperature. Depending upon labeling conditions (amount of base, nature of the base, volume, reaction time,..) yields up to 70 % can be obtained in less than 10 minutes. Besides acetonitrile/water, other solvent mixtures can also be used to eluted the fluoride (i.e toluene/$CH_3OH$).

**Conclusions:** This new strategy avoiding the azeotropic evaporation step eases the automatization and reduces synthesis time. A good selection of solvent, protic additive, organic base and temperature should allow to tune up the reactivity of the [$^{18}F$] fluoride according to the nature of the substrate to label.

**References:** K. Hamacher, H. Coenen, G. Stcklin. J. Nucl. Med. 27 (1986) 235-238

## P099 CLICK CHEMISTRY BASED 18F-LABELING AND GLYCOSYLATION OF AN RGD PEPTIDE FOR PET IMAGING OF αvβ3 INTEGRIN EXPRESSION

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**Objectives:** Glycosylation of peptides influences the pharmacokinetics of peptide based PET radiopharmaceuticals by enhancing bioavailability and in vivo clearance properties. The aim of this study is the development of a novel method for concomitant $^{18}$F-labeling and glycosylation of peptides. Herein, we present the radiosynthesis of 2-deoxy-2-[$^{18}$F]fluoroglucopyranosyl azide ([$^{18}$F]FGA) and its application for Cu(I)-catalized 1,3-dipolar cycloaddition (click chemistry) using the alkyne-functionalized $\alpha_v\beta_3$ targeting c(RGDfPra). Furthermore, we studied the in vitro binding affinity and in vivo biodistribution of the glycosylated RGD peptide ([$^{18}$F]FDG-RGD) using a tumor mouse model.

**Methods:** c(RGDfPra) was synthesized by Fmoc-assisted solid-phase synthesis and analyzed by LC-ESI-MS. Glycosylation of c(RGDfPra) with FGA was carried out using standard click chemistry conditions ($CuSO_4$, sodium ascorbate, PBS, rt). Receptor binding assays were performed using immobilized $\alpha_v\beta_3$ integrin and human glioblastoma cells (U87MG) with $^{125}$I-echistatin. $^{18}$F-labeling of c(RGDfPra) was achieved by glycosylation with [$^{18}$F]FGA in a three-step two-pot reaction including Cu(I)-catalized Huisgen 1,3-dipolar cycloaddition. The product ([$^{18}$F]FDG-RGD) was isolated by semipreparative radio-HPLC. Biodistribution and $\mu$PET studies were carried out using a U87MG cell xenograft nude mice model.

**Results:** $\alpha_v\beta_3$-binding affinity of the glycosylated c(RGDfPra) ($K_i$ ($\alpha_v\beta_3$)=11nM; $K_i$ (U87MG)=395nM) remained uninfluenced in comparison with c(RGDfPra) ($K_i$ ($\alpha_v\beta_3$)=26nM; $K_i$ (U87MG)=1.4$\mu$M) as determined by competition binding studies versus $^{125}$I-echistatin. The ligation of [$^{18}$F]FGA with c(RGDfPra) proceeded quantitatively in 15 min under mild aqueous conditions ($CuSO_4$, sodium ascorbate, PBS, 60°C), yielding [$^{18}$F]FDG-RGD with a specific activity of 210 GBq/$\mu$mol in an overall decay-uncorrected RCY of 19-21% (EOB) within 70 min. In biodistribution and $\mu$PET experiments using U87MG tumor mice, the tumor uptake of [$^{18}$F]FDG-RGD was significantly inhibited by 40-65% in the presence of a blocking dose of c(RGDfV) (10 mg/kg) when compared with the tumor uptake in control animals (60 min p.i.). The tumor to blood ratio was 2.5-4.0 (30-120 min p.i.), allowing clear visualisation of the tumor by $\mu$PET.

**Conclusions:** 2-Deoxy-2-[$^{18}$F]fluoroglucopyranosyl azide ([$^{18}$F]FGA) represents a novel agent for concomitant $^{18}$F-labeling and glycosylation of peptides under mild conditions. This method was successfully applied to the straightforward and high-yielding synthesis of [$^{18}$F]FDG-RGD for imaging of integrin expression in vivo and merits adoption for common use in the synthesis of $^{18}$F-glycopeptides, whose pharmacokinetics could be studied by positron emission tomography.

## P100 ONE-STEP SYNTHESIS OF 18F LABELED [18F]-N-SUCCINIMIDYL 4-FLUOROBENZOATE FOR A20 AND A20-36 PEPTIDES LABELING

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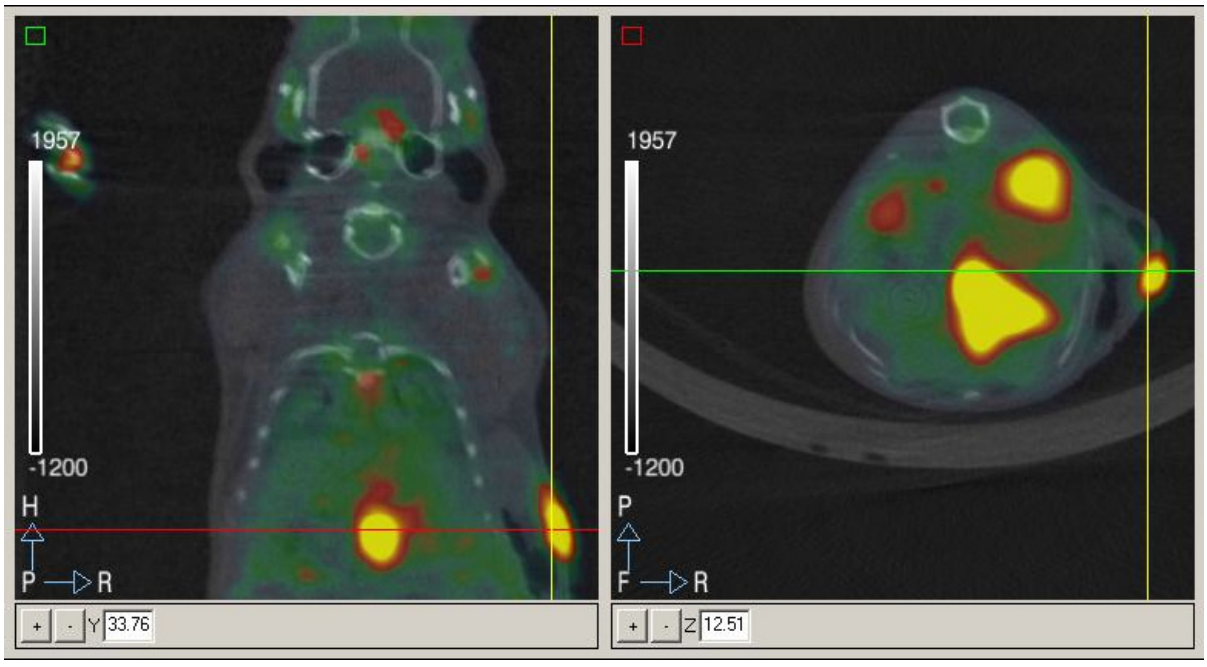
**Objectives:** The application of biologically active peptides labelled with positron-emitting nuclides has emerged as an useful and interesting field in nuclear medicine. Among a number of positron-emitting nuclides, fluorine-18 appears to be the best candidate for labelling bioactive peptides by virtue of its favourable physical and nuclear characteristics. In recent years, various techniques have been developed which allow efficient labelling of peptides with $^{18}F$ without affecting their receptor-binding properties. A20 and A20-36 peptides can be labelled with fluorine-18 as a potential radiopharmaceutical for quantitative in vivo mapping of B Cell receptors,(BCR), in Murine B-cell lynphoma. We used the F-18 N-succinimidyl 4-fluorobenzoate to label the amino group of terminal aminoacid of A20 and A20-36 peptides and we could obtain radiolabelling yields of >15%.

**Methods:** Pentamethyl 4-(trimethylammonium trifluoromethanesulnate)benzoate was reacted with anhydrous $^{18}F$-fluoride in the presence of kryptofix and potassium carbonate in acetonitrile at 105°C for 10 min to yield 18F-pentamethyl 4-fluorobenzoate. The pentamethyl ester was hydrolyzed with 0.1 mL trifluoroacetic acid to yield 18F-4-fluorobenzoic acid which was converted to $^{18}F$-N-succinimidyl 4-fluorobenzoate with N,N-disuccinimidyl carbonate. The final product was purified with the reversed phase semi-prep HPLC column using 25% acetonitrile with 0.15% acetic acid as the eluent running at 6mL/min. The radioactive product collected in HPLC solvent was trapped on a C-18 cartridge and eluted with 1mL dichloromethane collected into a 2mL microtube. A20 and A20-36 peptides (50μg) in 50μL of buffer solution at pH value of 9.0 were transferred to microtube containing the radioactivity, the mixture was allowed to react for 36min at 50°C.

**Results:** Increasing quantities of substrate were incubated with approximately 50mCi of $^{18}F$. Incubation with 2.3mg of substrate produced an yield of 18mCi of radiocompound, while incubation with 3.3mg increased the yield to 32mCi. Incubation with 4.3mg of substrate, however, did not increase the yield any further. The purification of 18F-N-succinimidyl 4-fluorobenzoate was initially performed with 660 mg C-18 cartridge, but the best results were obtained with 800 mg C-18 cartridge.

**Conclusions:** A20 and A20-36 peptides labelled with $^{18}F$-N-succinimidyl 4-fluorobenzoate is presently purfied with gradient analytical HPLC in Vydac column (5μm, 250mmx4.6mm) using ethanol and water as the eluent. This method allows to inject directly the radiocompounds into the animals. This direct injection was not possible when the labelled peptides were purified with gradient analytical HPLC using acetonitrile and water added with 0.15% TFA (trifluoroacetic acid) as the eluent.

**References:** Lang L, Eckelman WC. Labeling proteins at high specific activity using N-succinimidyl-4[18F](fluoromethyl) benzoate. Appl Radiat Isot1997; 48:169-173.



## P101 SYNTHESIS OF 5-[18F]FLUOROISOQUINOLIN-1-OL BY MODIFIED BALZ-SCHIEMANN REACTION

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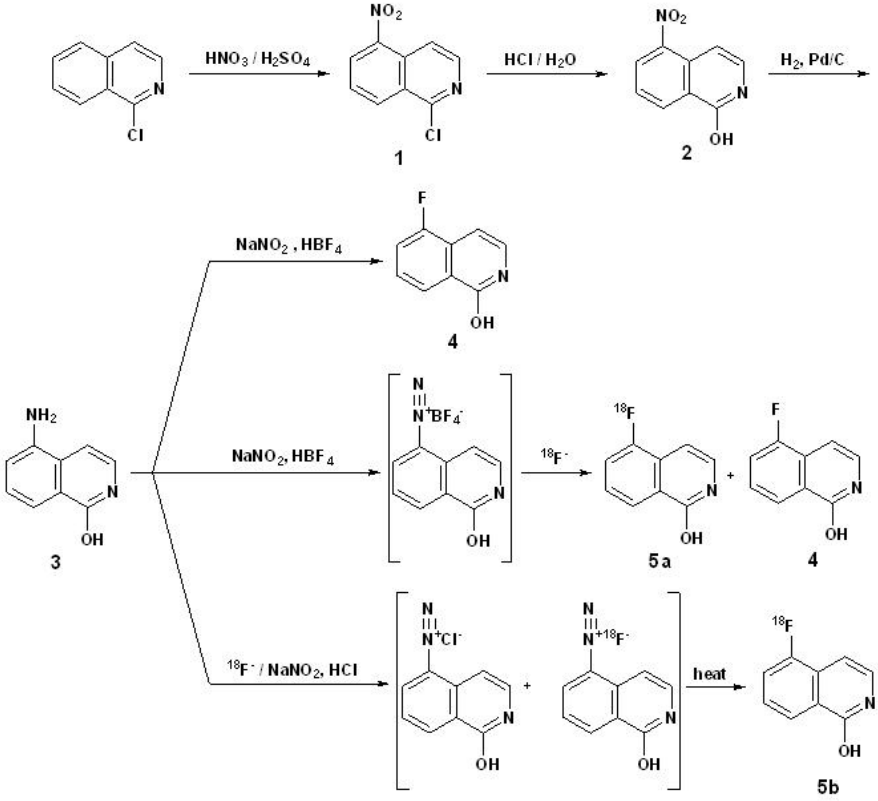
**Objectives:** Poly(ADP-ribose) polymerase-1 (PARP-1) is enzyme involved in Poly(ADP-ribosylation) of DNA-binding protein. PARP-1 plays an important role in regulating DNA repair, gene transcription, cell cycle progression and cell death. Recently, PARP-1 inhibitors have demonstrated the ability to enhance the efficacy of various chemotherapeutic agents against tumors like as glioma, melanoma, breast cancer. In addition, PARP-1 inhibitors reduced VEGF-induced proliferation. PARP-1 inhibitors, therefore, may be considered as a attractive therapy. We selected target compound as 1,5-isoquinolinediol known for effective PARP-1 inhibitor and 5-[$^{18}$F]fluoroisoquinolin-1-ol was synthesized by Balz-Schiemann reaction used for [$^{18}$F]fluoride labeling on isoquinoline compound.

**Methods:** 1 was synthesized by the nitration of the 1-chloroisoquinoline. 1 was treated with hydrochloric acid to give 2 and then reduction of nitro group gave 3. 4 was prepared by Balz-Schiemann reaction using $HBF_4$ with 3. 5a was synthesized by 1-hydroxyisoquinoline-5-diazonium tetrafluoroborate with [$^{18}$F]fluoride. After 3 was mixed with [$^{18}$F]fluoride and HCl, it was treated with $NaNO_2$ to give 5b. [$^{18}$F]fluoride labeling reaction was carried out in the presence of Kryptofix-2.2.2 and $K_2CO_3$ at 120℃ for 15 min.




**Results:** The Balz-Schiemann reaction allowed the introduction of fluoride on aromatic compounds. but In radiofluorination, it had a critical limit due to low specific activity because 4 and 5a coexisted by means of the Balz-Schiemann reaction. We used HCl instead of $HBF_4$ to solve the problem. Particularly, it was an important to add a reagent in sequence, because 5b was not synthesized by [$^{18}$F]fluoride after diazonium chloride was formed. Therefore, it should that 3 was treated with [$^{18}$F]fluoride and HCl simultaneously. 5b was separated and purified by HPLC (stationary phase: Perkin-Elmer SPHERI-5 RP-18 5㎛, 250×4.6㎜ column; mobile phase: $H_2O$/EtOH(70/30) 1mL/min; $R_t$: 15min). The radiochemical yield was about 10% and the radiochemical purity was above 95%.

**Conclusions:** 5-[$^{18}$F]Fluoroisoquinolin-1-ol has been synthesized as a PET imaging probe for the diagnosis of cancer. Modified Balz-Schiemann method was applied to label [$^{18}$F]fluoride on aromatic compounds without a electron withdrawing group. Further investigation is going to be performed with several Lewis acides to enhance radiochemical yield.

**References:** [1] S. Snchez-Fidalgo, I. Villegas, A. Martn, M. Snchez-Hidalgo, C. Alarcn de la Lastra, European Journal of Pharmacology, 2007, 563, 216223. [2] M. Rajesh, P. Mukhopadhyay, G. Godlewski, S. Btkai, G.Hask, L. Liaudet, P. Pacher, Biochemical and Biophysical Research Communications, 2006, 350, 10561062. [3] J. A. De Soto, X. Wang, Y.Tominaga, R. H. Wang, L. Cao, W. Qiao, C. Li, X. Xu, A. P. Skoumbourdis, S. A. Prindiville, C. J. Thomas, C. X. Deng, International Journal of Biological Sciences, 2006, 2(4), 179-185. [4] H. E. Bryant, N. Schultz, H.D. Thomas, K. M. Parker, D. Flower, E. Lopez, S. Kyle, M. Meuth, N. J. Curtin, T.Helleday, NATURE , 2005, 434, 913-917.

## P102 NOVEL 18F-LABELED VALINE DERIVATIVES: SYNTHESIS AND BIODISTRIBUTION IN MICE BEARING S180 TUMOR

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**Objectives:** To find out potential PET imaging agents for tumor detection, a series of amino acid derivatives have been designed, synthesized, labeled with $^{18}F$ and evaluated by our team. Among these compounds, 2-(2-[$^{18}F$]fluoro-4-nitrobenzamido)-3-methylbutanoate ([$^{18}F$]MFNBMB) and its corresponding acid 2-(2-[$^{18}F$]fluoro-4-nitrobenzamido)-3-methylbutanoic acid ([$^{18}F$]FNBMBA), although need structural modification to improve their lipophilic properties and metabolizabilities, showed some obvious advantages to O-2-[$^{18}F$]fluoroethyl-L-tyrosine (L-[$^{18}F$]FET) in biological evaluation executed in female KM mice bearing S180 tumor[1]. In this work, we designed and synthesized 2-(2-[$^{18}F$]fluoro-4-nitrobenzamido)-3,N,N-Trimethyl-butyramide([$^{18}F$]FNTA) and 2-(2-[$^{18}F$]fluoro-4-nitrobenzamido)-3,N-Dimethyl-butyramide ([$^{18}F$]FNDA) based on the mian structure of [$^{18}F$]MFNBMB and [$^{18}F$]FNBMBA, hoping to get compounds with better properties on biodistribution and organ clearance.

**Methods:** The synthesis pathways are represented in figure 1 and the terminal products prepared by a one-step short-time $^{18}F$ nucleophilic substitution were purified by HPLC. Octanol-water partition coefficients were determined by using 2.5ml n-octanol as the organic phase and 2.5 ml 0.01 M PBS (pH 7.4) as water phase. The in vivo biological evaluation of [$^{18}F$]FNTA was executed in female KunMing mice bearing S 180 tumor.

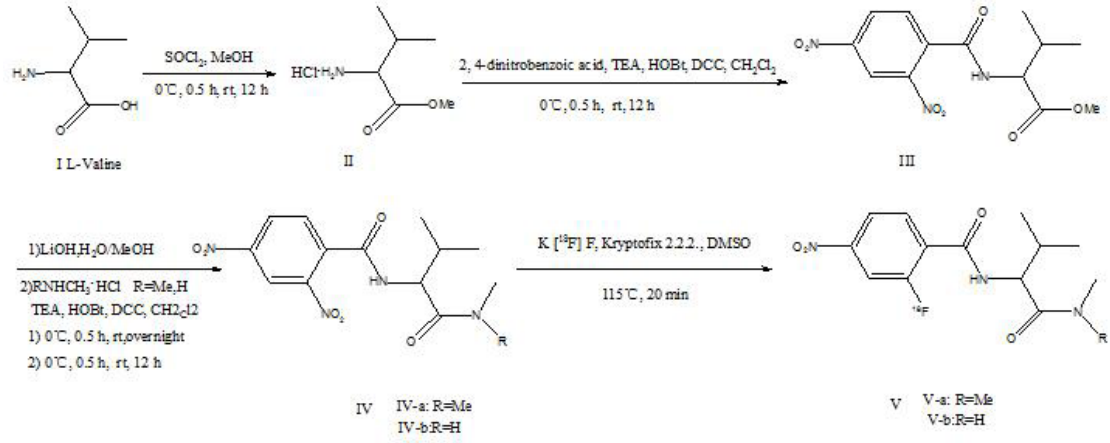


Figure 1 Synthesis of [$^{18}F$]FNTA and [$^{18}F$]FNDA

**Results:** The biological evaluation data of [$^{18}F$]FNTA in mice bearing S 180 tumor showed that [$^{18}F$]FNTA had obviously fast clearance in blood. The uptake of [$^{18}F$]FNTA was 2.79(ID%/g) at 5 min after injection and reduced by half at 30 min after injection. The uptakes in liver and kidney were 8.55(ID%/g),6.57(ID%/g) at 5 min after injection and then decreased to 55%,45% at 60 min after injection. Initial uptake of [$^{18}F$]FNTA in tumor was 2.67(ID%/g) at 5 min after injection. Then it decreased slowly to 90% at 30min after injection. At 60 min, [$^{18}F$]FNTA still remained nearly 70% in tumor. Tumor/blood ratio reached its peak 1.57(ID%/g) at 60 min after injection. Tumor/muscle did not achieve the maximum before 120 min.

**Conclusions:** [$^{18}F$]FNTA had a relatively good initial tumor uptake and reservation and acceptable tumor/blood ratio between 30 min and 120 min after injection. The initial uptakes in liver and kidney were high and the clearance of were not ideal, which may had much to do with the lipophilic property and metabolizability of [$^{18}F$]FNTA. Compared of [$^{18}F$]FNTA, [$^{18}F$]FNDA had a low lipophilic property and the lack of terminal N,N-dimethyl may increase the easiness of metabolizing. The biological evaluation of [$^{18}F$]FNDA is in process.

**Research Support:** We acknowledged with thanks for financial supports from the National Natural Science foundation of China (No 20371009 and 20671014) and Beijing Key Subject Program. We also wish to thank the cyclotron operator team of the PET Centre of Xuan Wu hospital, for providing [$^{18}F$]fluoride activity and technical assistance.

**References:** [1] Y. Qiao, Y. He, S. Zhang, et al. Synthesis and evaluation of novel F-18 labeled fluoroarylvaline derivatives: Potential PET imaging agents for tumor detection. Bioorg Med Chem Lett. Reviesd.

## P103 PRODUCTION OF [18F]-FLUOROETHYLCHOLINE AND [18F]-FLUOROETHYL-L-TYROSINE IN HIGH YIELD ON AN AUTOMATED SYNCHROM SYNTHESIS MODULE

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**Objectives:** $^{18}$Fluoroethyl is a widespread used moiety for labelling PET-radiopharmaceuticals. The synthesis of $^{18}$Fluoroethyl-compounds is often performed by a two step synthesis strategy with the necessity to purify the intermediate $^{18}$Fluoroalkylation agent by HPLC or distillation. The aim of this study was the development of a reliable and high yield production of $^{18}$Fluoroethyl-radiopharmaceuticals on an automatic SynChrom synthesis module using [$^{18}$F]-1-Bromo-2-fluoro-ethane ($^{18}$FEtBr) as intermediate. The purification of the intermediate was realized by SPE thus avoiding a HPLC purification or distillation. The suitability of the used process was shown by production of the two often applied tracers [$^{18}$F]-Fluoroethylcholine (FEC), and [$^{18}$F]-Fluoroethyl-L-tyrosine (FET).

**Methods:** Trapping the [$^{18}$F]-Fluoride on QMA cartridge, elution and azeotropic drying was performed according to standard procedures. The nucleophilic substitution with a new developed precursor bromoethylnosylate was optimized and the intermediate product $^{18}$FEtBr was purified by SPE. The SPE-purification was arranged by 2 cartridges with different lipophilicity. The [$^{18}$F]-Fluoroalkylation step was performed by the addition of the intermediate $^{18}$FEtBr to dimethylaminoethanol and L-tyrosine disodium salt, respectively. The products FEC and FET, respectively, were again purified by SPE.

**Results:** After evaluation of the reaction steps in a manual set up the syntheses of $^{18}$FEtBr, FEC and FET was adapted to the automated, remote controlled SynChrom-synthesis unit. The intermediate product $^{18}$FEtBr could be provided in preparative radiochemical yields of up to 70% including SPE-purification. At this, a cartridge with medium lipophilicity (C18) trapped the precursor bromoethylnosylate, whereas the product $^{18}$FEtBr passed this cartridge and was trapped on a second cartridge with higher lipophilicity. The radiochemical purity was >95% and the precursor was not detectable after the SPE-purification. The subsequent alkylation step by $^{18}$FEtBr was investigated by reactions producing two frequently used PET-tracer FEC and FET. The alkylation of dimethylaminoethanol with $^{18}$FEtBr was optimized with radiochemical yields of 70%. This results in overall radiochemical yields of 40±5%, which only slightly differs from the manual synthesis (50%, unpublished results). The $^{18}$Fluoroethylation of L-tyrosine results in radiochemical yields of 65% FET (alkylation step). Both tracers were purified by SPE and the total synthesis time was approximately 50 min including trap and release on QMA and azeotropic drying of the [$^{18}$F]-Fluoride. The radiochemical purities for both tracers exceeded >95% with an excellent chemical purity.

**Conclusions:** The two step synthesis of FEC and FET was successfully realized using a fully automated, remote controlled SynChrom-synthesis unit based on a SPE-purification of the intermediate $^{18}$FEtBr. This process allows the efficient and reliable production of [$^{18}$F]-Fluoroethylcholine and [$^{18}$F]-Fluoroethyl-L-tyrosine, respectively, in less than one hour.

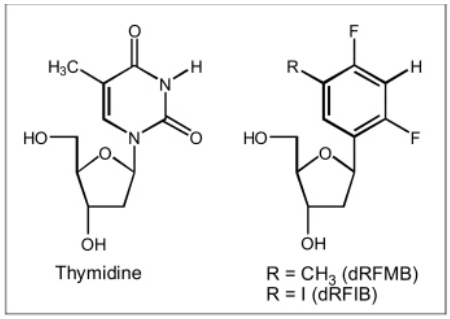


**Figure 1:** Structures of thymidine and the difluorophenyl mimetics dRFMB and dRFIB.

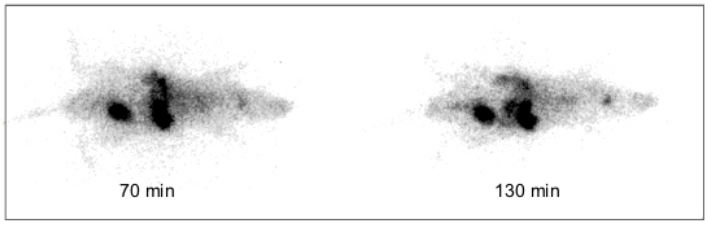


**Figure 2:** Images of a rat (3.9 MBq of dRF[$^{123}$I]IB, 20 min/image, isoflurane anaesthesia).

## P104 TERTIARY ALCOHOLS TO AVOID EVAPORATION IN FLUORINE-18 LABELING

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**Objectives:** In PET radiochemistry, [$^{18}$F]fluoride is classically recovered by concentration on an anion exchange column and elution with a solution of potassium carbonate and kryptofix K222 in acetonitrile and water. Following this elution, the water has to be eliminated by evaporation to finally obtain a solution of [K/K222]$^+$[$^{18}$F]F in a non protic solvent, for example acetonitrile (ACN). Tertiary alcohols were recently described as protic solvents compatible with [$^{18}$F]F radiofluorination[1]. The purpose of this work was to evaluate the possibility to use tertiary alcohols to promote the elution of [$^{18}$F]fluoride from the cationic solid support without the use of any water. This must allow engaging the radioactivity for the labeling without a time consuming evaporation step.

**Methods:** [$^{18}$F]F was trapped on an anion exchange column (Waters SepPak® QMA/carbonate 130 mg). The cartridges were rinsed with 10 ml of ACN dried on molecular sieves and then purged with nitrogen. Tetrabutylammonium hydroxide (TBAOH) 40% in methanol was evaporated at room temperature with a nitrogen flow. Dry ACN (< 100 ppm water) was added several times to perfect the evaporation of the initial solvent and humidity. The residue was recovered in a mixture of dry ACN and 2-methylbutan-2-ol (tROH). Solutions with different proportions of the two solvents were used to recover the radioactivity from the QMA cartridges. The residual concentration of water in the eluted fractions was determined by Karl-Fischer titration. The reactivity of the eluted radioactivity was tested for the labeling of 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethane-sulphonyl-α-D-mannopyranose.

**Results:** Using 1 ml of a TBAOH solution with a high proportions of tROH (>50% V/V), it was possible to recover more than 85% of the trapped [$^{18}$F]fluoride. The water concentration in the eluted solution was less than 5000 ppm. After labeling (precursor 25 mg, 5 minutes, 100°C) and subsequent dilution of the labeling medium with water, the incorporation of more than 85% of the recovered [$^{18}$F]F was observed as 2-[$^{18}$F]fluoro-1,2,4,6-tetra-O-acetyl-D-glucose by TLC analysis.

**Conclusions:** This method allows the rapid recovery of [$^{18}$F]F and its use for radiochemistry without the use of an evaporation step. It opens a potential route for the implementation of high activity [$^{18}$F] radiochemistry into microfluidic devices, in which it is impossible to set up evaporation. Other precursors are now tested to demonstrate the general applicability of this method for the synthesis of aliphatic [$^{18}$F]fluorinated tracers.

**Research Support:** This research was supported by a grant from European Union's FP6 STRP 516984 MI-Lab-On-Chip. A. Plenevaux is a research associate from FRS-F.N.R.S. Belgium.

**References:** [1] D.W. Kim, D.S. Ahn, Y.H. Oh, S.Lee, H.S. Kil, S.J. Oh, S.J. Lee, J.S. Kim, J.S. Ryu, D.H. Moon, D.Y. Chi J. Am. Chem. Soc. 128 (2006) 16394-16397.

## P105 LABELLING OF AN ANTISENSE OLIGONUCLEOTIDE WITH [F-18]FPY5YNE

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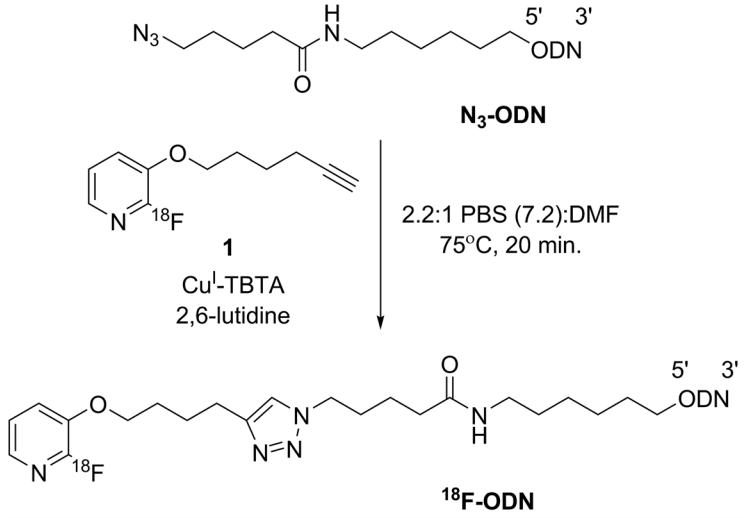
**Objectives:** Recently, our group 1 and others 2 have described the radiosyntheses of 18F- based bifunctional molecules that include 2-substituted pyridines, activated towards efficient nucleophilic heteroaromatic [18F]fluorination, and robust terminal alkyne groups. In our case, [18F]FPy5yne (1) was used to 18F label an azide- modified peptide by way of a CuI- mediated Huisgen [3+2] cycloaddition reaction. In the hopes of expanding this technique to include nucleic acid- based molecular probes, we now report the radiobioconjugation of 1 to a model DNA antisense sequence ( 18F-ODN ), which is antisense to mdr1 mRNA.

**Methods:** [18F]FPy5yne (1) was prepared from the corresponding 2-trimethylammonium triflate according to protocol (K[18F]F/K222 in DMSO, 110 ºC, 15 minutes)1, except the reaction volume was reduced from 0.7 mL to 0.5 mL. After HPLC purification, compound 1 was conjugated to a 5'-azide modified3 20mer (N3-ODN, 200 nmol) in the presence of 2,6-lutidine and CuI-tris(benzltriazolylmethyl)amine.4 The reaction mixture was heated to 75ºC in a solvent matrix of 2.2:1 PBS (pH 7.2, 57 mM):DMF for 20 minutes. HPLC purification, evaporation of the eluent, and desalting on ion-exchange resin afforded radiochemically pure 18F-ODN.

**Results**: Non-decay-corrected, collected yield from start of bioconjugation reaction was 43.2% (57.8% decay- corrected) after HPLC purification. 18F-ODN (4.03 mCi) was formulated in 1 mL nanopure water. Starting from 92.6 mCi at end-of-bombardment, 4.4% non- decay corrected yield (24.9% decay- corrected) of 18F-labelled antisense oligonucleotide was obtained. Total unoptimized synthesis time was 276 minutes from end-of-bombardment. [IMG]




**Conclusions:** The ultimate utility of [18F]FPy5yne for the preparation of nucleic acid- based PET imaging agents will require further improvements to protocol. These may include (a) establishing a minimum effective concentration of biomolecule, (b) finding a solvent matrix that readily dissolves all components, and (c) speeding up the drydown of 18F-ODN in HPLC eluent.

**References:**(1) Inkster, J. A. H.; Guérin, B.; Ruth, T. J.; Adam, M. J. Journal of Labelled Compounds and Radiopharmaceuticals 2008, 51, 444-452.(2) Kuhnast, B.; Hinnen, F.; Tavitian, B.; Dolle, F. Journal of Labelled Compounds & Radiopharmaceuticals 2008, 51, 336-342. (3) Seo, T. S.; Li, Z. M.; Ruparel, H.; Ju, J. Y. Journal of Organic Chemistry 2003, 68, 609-612. (4) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. Organic Letters 2004, 6, 2853-2855.

## P106 TEMPERATURE EFFECT ON THE STEREOSPECIFICITY OF NUCLEOPHILIC FLUORINATION: FORMATION OF [18F]TRANS-4-FLUORO-L-PROLINE DURING THE SYNTHESIS OF [18F]CIS-4-FLUORO-L-PROLINE

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**Objectives:** Fluorine-18 labelled cis-4-fluoro-L-proline (cis-FPro) has been suggested as a suitable PET tracer to study abnormal collagen synthesis occurring in lung fibrosis, tumors and liver cirrhosis.[1] Hamacher has reported the formation of [$^{18}$F] trans-4-fluoro-L-proline (trans-FPro; 7–28%) during the synthesis of cis-FPro using diastereomerically pure (2S,4R)-N-Boc-4-(p-toluenesulfonyloxy)proline methylester (2S,4R precursor) at 85 °C.[2] We have previously reported the synthesis of diastereomerically pure cis-FPro by the nucleophilic fluorination of the 2S,4R precursor at 110 °C.[3] In this paper, we present the temperature effect on the stereospecificity of the nucleophilic fluorination of diastereomerically pure 2S,4R precursor.

**Methods:** Cis- and trans-FPros were produced by cryptate mediated no-carrier-added nucleophilic $^{18}$F-fluorination starting from the corresponding N-Boc-4-tosyloxy-L-proline methylesters. The reaction was carried out at 70–145 °C in a Reacti-Therm™ for 10 min followed by 5 min stirring at room temperature. The resulting reaction intermediate was purified from unreacted $^{18}$F-fluoride using a Silica Sep-Pak®. The final product was obtained after hydrolysis of the reaction intermediate using 2 M triflic acid at 145 °C, followed by purification using an acetate based anion exchange column. The diastereomeric FPros were analyzed using a Waters Spherisorb® $NH_2$ HPLC column and a 0.01 M phosphate buffer solution containing 40% acetonitrile (pH 4.0) at 1.0 mL/min.

**Results:** At lower temperatures (70–110 °C), the nucleophilic substitution was stereospecific and followed $S_N1$ mechanistic pathway that yielded only cis- or trans-FPro starting with the 2S,4R or 2S,4S precursor, respectively. The decay corrected radiochemical yield of the diastereomerically pure FPros was 70 ±3% after 90 min synthesis. At 145 °C, however, nucleophilic fluorination of the 2S,4R precursor resulted in both diastereomers in a 65:35 ratio, with cis-FPro as the predominant isomer. This is indicative of the formation of the diastereomers via both $S_N1$ and $S_N2$ mechanisms at higher reaction temperatures.

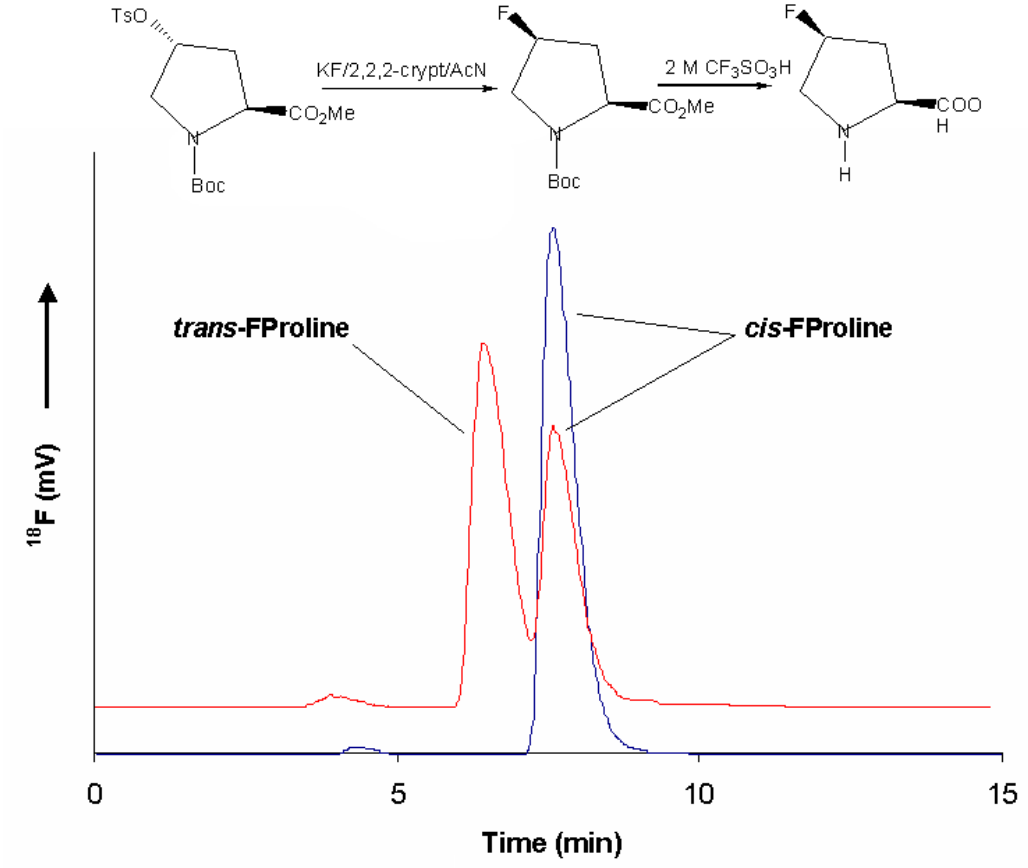


Figure. Typical radio-HPLC traces of the final product solution resulting from the fluorination of 2S,4R precursor at 110 °C (bottom trace) and of a mixture of cis- and trans-FPros (top trace).

**Conclusions:** Nucleophilic fluorination of (2S,4R)-N-Boc-4-(p-toluenesulfonyloxy)proline methylester is stereospecific at 110 °C and occurs with an $S_N2$ reaction mechanism. However, the fluorination of the same precursor at 145 °C appears to follow both $S_N1$ and $S_N2$ pathways. Work is in progress to determine the temperature at which the nucleophilic fluorination loses its stereospecificity. The temperature effect on the fluorination of the 2S,4S precursor is also being investigated.

**References:** 1.Hamacher, K.; Herz, M.; Truckenbrodt, R.; Stocklin, G.; Schwaiger, M.; Senekowish-Schmidke, R. J. Nucl. Med. 1996, 37, 41P. 2.Hamacher, K. J. Labelled Compd. Radiopharm. 1999, 42, 1135-1144. 3.Chirakal, R.V.; Behnam Azad, B.; Ashique, R.; Saab, C.; Farncombe, T.; Wang, I.; Labiris, R. J. Labelled Compd. Radiopharm. 2007, 50, S115.

## P107 COMPARISON OF ALPHA- AND BETA-[18F]FAZDR WITH WELL-KNOWN HYPOXIA IMAGING AGENTS [18F]FAZA AND [18F]FMISO IN A SMALL ANIMAL PET STUDY

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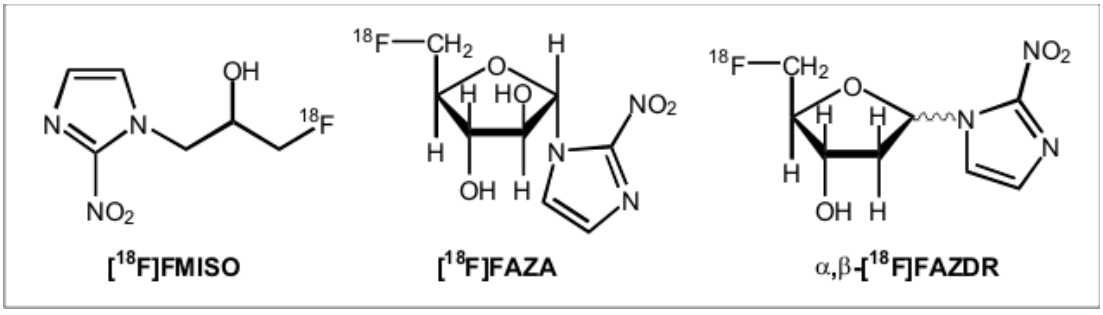
**Objectives:** To date, for imaging hypoxia a number of PET biomarkers based on 2-nitroimidazole (azomycin) have been developed and are under evaluation, e. g. [$^{18}$F]FMISO or [$^{18}$F]FAZA, with azomycin linked to arabinose (alpha-glycosidic). Recently, first results in a mouse tumor model with [$^{18}$F]fluoro-azomycin-beta-deoxyriboside ([$^{18}$F]FAZDR) showed lower uptake compared to [$^{18}$F]FAZA, but high tumor-to-muscle ratios were obtained faster.[1] Here, [$^{18}$F]FAZDR was to be evaluated further, together with its alpha-anomer (α-[$^{18}$F]FAZDR) in comparison to [$^{18}$F]FMISO and [$^{18}$F]FAZA.

**Methods:** Tumor bearing BALB/c mice (CT26, colon CA) were injected intravenously in the tail vein with either β-[$^{18}$F]FAZDR, α-[$^{18}$F]FAZDR, [$^{18}$F]FAZA or [$^{18}$F]FMISO (10-12 MBq). Small animal PET scans (Inveon, Siemens) were performed according to the following protocols: (i) dynamically for 3 h p.i., (ii) dynamically for 1 h p.i. followed by static scans 2 and 3 h p.i. or (iii) statically 1, 2 and 3 h p.i. Static scans were for 10 min. Anesthesia was applied with 1.5 % isoflurane and an air flow of 0.6 L/min. The following quantitative data are provided as %ID/ccm (tumor values at 1h p.i.).

**Results:** All tracers showed a substantial wash-out after 3 h. In static scans, α-[$^{18}$F]FAZDR showed a lower uptake (1.58 ± 0.23 %ID/ccm) in comparison to [$^{18}$F]FAZA (2.23 ± 0.15 %ID/ccm; n = 3). β-[$^{18}$F]FAZDR displayed a significantly lower uptake (0.64 ± 0.21 %ID/ccm) in comparison to [$^{18}$F]FAZA (2.23 ± 0.15 %ID/ccm; n = 3; $P < 0.001$) and [$^{18}$F]FMISO (3.20 ± 0.34 %ID/ccm; n = 3; $P < 0.001$) and also in comparison to α-[$^{18}$F]FAZDR (1.58 ± 0.23 %ID/ccm; $P < 0.01$). Interestingly, tumor (and muscle) uptake of β-[$^{18}$F]FAZDR was higher in dynamic scans (4.48 ± 0.55 %ID/ccm), compared to static scans (0.64 ± 0.21 %ID/ccm; n = 3; $P < 0.001$). There was a significant difference in tumor uptake for scans 3 h p.i. according to (i) (2.36 ± 0.04 %ID/ccm), (ii) (1.08 ± 0.37 %ID/ccm) and (iii) (0.29 ± 0.04 %ID/ccm; n = 3; $P < 0.05$).

**Conclusions:** The uptake mechanism for alpha- or beta-[$^{18}$F]FAZDR seems to be different from [$^{18}$F]FAZA and [$^{18}$F]FMISO, with the latter two uptake and clearance were independent from the sleep-wake-cycle of the mice (dynamic vs. static scans). Both [$^{18}$F]FAZDR isomers showed a lower uptake in comparison to [$^{18}$F]FAZA, thus implying that the sugar moiety (2-deoxyribose or arabinose) plays an important role for pharmacokinetics. The differences between beta-[$^{18}$F]FAZDR and alpha-[$^{18}$F]FAZDR indicate the influence of the position of the 2-nitroimidazole at the anomeric carbon.

**References:** [1] Reischl G et al., J. Nucl. Med. 49, 292P (2008)



## P108 NOVEL PROSTHETIC GROUP FOR F-18 LABELING OF BIOMOLECULES

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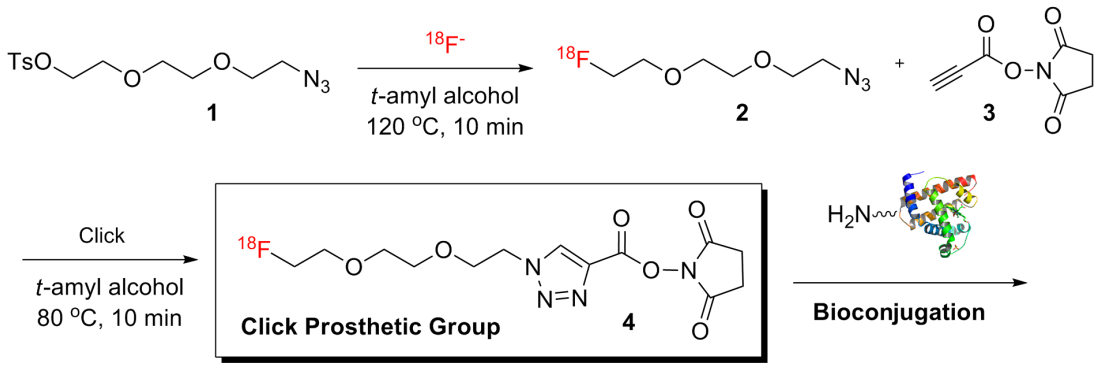
**Objectives:** Although $^{18}$F-labeled bioactive peptides such as small synthetic peptides, monoclonal antibody fragments, and even intact whole proteins have great promise as potential diagnostic PET imaging agents, efficient radiolabeling of those peptides with F-18 is still challenging. Usually, $^{18}$F-labeled N-succinimidyl esters have been utilized as $^{18}$F-labeled prosthetic group for F-18 labeling of peptides via amide bond formation. N-Succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB) is known as the most common prosthetic group, and have driven radiochemists to develop efficient preparation methods. However, it requires three-step synthesis from methyl benzoate precursor including treatment with base and acid in sequence. Herein, we report the two-step synthesis and basic applications of a novel $^{18}$F-labeled prosthetic group.

**Methods:** We used a copper-free click chemistry for the labile N-succinimidyl ester not to be broke. Radio-TLC was used for reaction optimization. Decay-corrected radiochemical yield (RCY) was calculated after HPLC separation. Amidation of several amines was monitored by radio-TLC and identified by coinjection of cold authentic to HPLC.

**Results:** We designed a novel N-succinimidyl ester to avoid interim base and acid treatment, which is thought to make the manual preparation of [$^{18}$F]SFB tedious and difficult. By using click chemistry in the second step, such laborious handling would be eliminated. As shown in Scheme 1, $^{18}$F-labeled azido compound can react with N-succinimidyl propiolic ester to give the desired $^{18}$F-labeled active ester. Noteworthy, we also found that electron-deficient terminal alkyne can make 1,2,3-triazole with azido compound under slight heating and copper-free condition (not published result). It also provides regioselective 1,4-regioisomer almost exclusively. Typical click condition using Cu(I) was found to break N-succinimidyl ester bond to be carboxylic acid due to slightly basic environment. Therefore, copper-free 1,2,3-triazole formation is essential factor for this study.




Scheme 1. Two-step preparation of a click prosthetic group.

[$^{18}$F]Fluorination of azido tosylate (1) under typical F-18 labeling condition yielded F-18 labeled azido intermediate (2) in 95% radio-TLC conversion. The reaction mixture was filtered with a short silica column to remove inorganic base and salts. Copper-free click reaction of $^{18}$F-labeled azido compound was performed with N-succinimidyl propiolate in small volume of t-amyl alcohol and THF at 80 °C to give excellent radio-TLC conversion. The resulting novel $^{18}$F-labeled prosthetic group was purified by HPLC in 70% RCY (n=3) and then reacted with several simple amines to validate amide formation.

**Conclusions:** Novel designed $^{18}$F-labeled prosthetic group was successfully synthesized in two steps and in excellent RCY with high specific activity. It is expected to be better alternative to [$^{18}$F]SFB.

## P109 POLYMER-SUPPORTED PRECURSORS VIA CLICK LINKAGE FOR SIMPLE PURIFICATION OF F-18 LABELED COMPOUNDS

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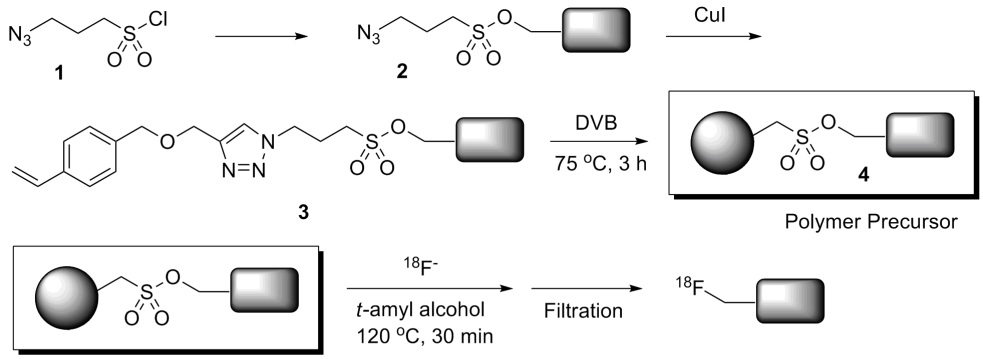
**Objectives:** Diverse polymers have assisted $^{18}$F-radiochemistry and $^{18}$F-radiopharmaceuticals for various purposes, e.g., efficient capture-release of F-18, on-column/resin [$^{18}$F]fluorination reaction, and fast purification. Especially, polymer-supported precursors were developed by several groups for better purification of $^{18}$F-labeled compounds. However, the resulting product solution obtained after filtration of polymer residue contains numerous byproducts showing many peaks in HPLC-UV profile. Such many undesired compounds might be produced from polymer precursor by unexpected side reactions. Recently, we suggested tertiary alcohol solvents such as t-butanol and t-amyl alcohol as more effective reaction media, which dramatically reduce the side reactions by forming hydrogen bond with [$^{18}$F]fluoride and base. Encouraged by this reaction specificity in tertiary alcohol solvents, we designed novel styrene-based precursor monomer and prepared a series of polymer-supported precursors.

**Methods:** 3-Azidopropanesulfonyl chloride (1) was prepared from 1,3-propanesultone in two steps in overall 95% yield. Polymer used in this study was grinded and sorted with sieves to be 100-200 mesh particle.

**Results:** Firstly, we developed a new sulfonyl chloride reagent 1 to attach precursor (naphthalene model compound) to styrene-based monomer via click reaction. Generally, it is not ease to make chemical bond between precursors and polymeric supports. As shown in Scheme 1, precursor-attached monomer 3 could be readily synthesized by using 3-azidopropanesulfonyl chloride (1) and click reaction. Subsequently, radical polymerization of precursor monomer 3 and divinylbenezene produced the corresponding precursor-containing polymer 4 in excellent yield. After checking cold fluorination of polymer 4, [$^{18}$F] fluorination was confirmed using 50 mg of polymer precursor in t-amyl alcohol at 120 °C. After 30 min, the reaction solution was filtered to remove polymer residue and washed with ethyl acetate. Although 20% of radioactivity remained on polymer residue, radio-TLC analysis of filtrate solution showed 85% [$^{18}$F]fluorination. More importantly, the HPLC-UV showed not only simpler mass pattern, but also very small amount (several mg calculated by internal standard) of total mass compounds. With the successful result from naphthalene model compound, we applied this method to the synthesis of [$^{18}$F]FLT and [$^{18}$F]FMISO.




**Scheme 1**. Preparation and [$^{18}$F]fluorination of polymer-supported precursor.

**Conclusions:** We could make a series of polymer-supported precursors in excellent yields. [$^{18}$F]Fluorination of these polymeric precursors was performed to give good radiochemical yield with very small amount of byproducts. These polymeric precursors can be used for simple purification.

## P110 IN VIVO EVALUATION OF m-(3-[F-18]FLUOROPROPYL)-BENZYLGUANIDINE ([F-18]FPBG) FOR PET IMAGING AND METABOLISM IN THE HEART

**J. S. KIM*[1], B. S. KIM[1], J. Y. SON[1], S. K. HONG[1], S. H. KANG[2], S. K. KIM[2], B. S. MOON[3], H. S. KIL[3], D. Y. CHI[3], Y. K. KIM[1], W. W. LEE[1], B. C. LEE[1] and S. E. KIM[1]**

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**Objectives:** To investigate adrenomedullary radiotracer for PET imaging as an alternative m-iodobenzylguanidine (MIBG), we designed [$^{18}$F]fluoropropylate benzylguanidine ([$^{18}$F]FPBG) and describe its F-18 labeling with biological evaluation as the potential myocardial PET imaging.

**Methods:** [$^{18}$F]FPBG was prepared in two steps from mesylated propylbenzylguanidine as a precursor: the labeling step of fluorine-18 and the deprotection step of two Boc groups. Biodistribution of [$^{18}$F]FPBG was carried out in normal SP rat and 6-OHDA treated SD rat to evaluate whether this radiotracer undergoes an in vivo uptake mechanism similar to that of MIBG. Additionally, dynamic microPET study was performed in SD rat and reserpin treated SD rat for 180 min, respectively. In metabolism studies, [$^{18}$F]FPBG was injected via tail vein and then heart and bladder were collected at 30 and 60 min post-injection. The heart samples were homogenized and centrifuged, whereas urine samples were filtered through membrane. These samples were analyzed by reverse phase HPLC and confirmed with authentic compounds.

**Results:** The radiosynthesis of [$^{18}$F]FPBG was accomplished by n.c.a. nucleophilic substitution and deprotected by HCl. The radiochemical yield is 17-30% with high radiochemical purity (97%). Tissue distribution of [$^{18}$F]FPBG in rat showed high radioactivity accumulation in heart and heart to blood uptake ratios were high about 19.5 and 10.5 times at 60 and 180 min, respectively. The highest uptake showed in the adrenal gland where adrenal gland to blood uptake ratios were 28.8 and 25.4 times at 60 and 180 min, respectively. In reserpin pretreatment rats by microPET/CT, the ratio of heart to liver was decreased about 41% for 20 min to 130 min. In addition, only [$^{18}$F]FPBG was observed in heart sample at 30 and 60 min in metabolism studies. In contrast, two identified metabolites and [$^{18}$F]FPBG were appeared in urine. These results are comparable to the uptake of MIBG in normal rat.

**Conclusions:** Our results demonstrated that [$^{18}$F]FPBG may be useful for myocardial imaging. Further biological evaluation for myocardial imaging to assess the adrenergic nerve activity will be present.

## P111 AMMONIUM SALT-SUPPORTED PRECURSORS VIA CLICK LINKAGE FOR SIMPLE PURIFICATION OF F-18 LABELED COMPOUNDS

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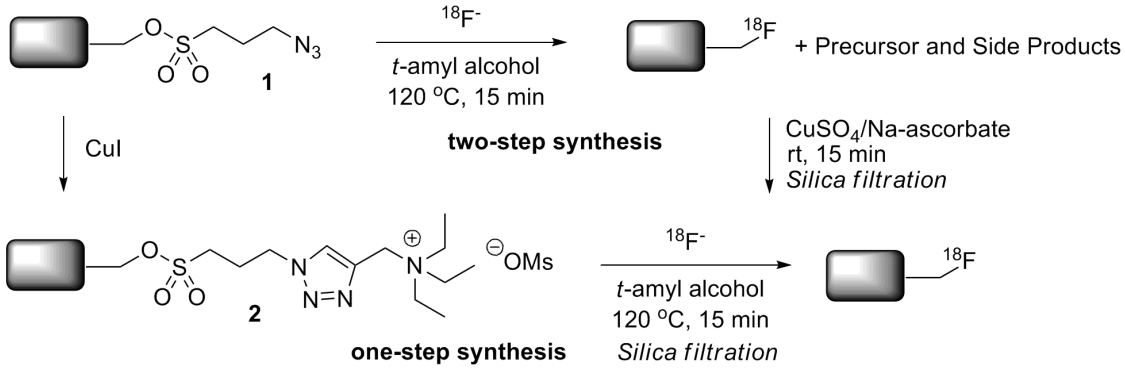
**Objectives:** Nucleophilic aliphatic [$^{18}$F]fluorination usually produces a variety of side products as well as $^{18}$F-labeled compound. These side products often not only make purification problem, but also reduce specific activity. Moreover, in case of secondary $^{18}$F-radiotracers such as [$^{18}$F]FDG and [$^{18}$F]FLT, larger amount of secondary sulfonate precursors (20-30 mg) are necessary for sufficient [$^{18}$F]fluorination because of their less reactivity. Such large amount of side products including unreacted precursor becomes a great burden to HPLC purification. So far, many efforts have been made to reduce the amount of side products for efficient purification. We prepared a series of ammonium salt-supported precursors using 3-azidopropanesulfonyl chloride and click reaction. We expected that most byproducts might be removed by water extraction or short silica filtration before injection to HPLC.

**Methods:** 3-Azidopropanesulfonyl chloride was prepared from 1,3-propanesulfone in two steps in overall 95% yield. Purification ability was evaluated and roughly quantified by HPLC-UV and internal standard.

**Results:** For this study, we prepared two sulfonate precursors 1 and 2. Azide-containing sulfonate precursor 1 was synthesized by the reaction of alcohol compound and 3-azidopropanesulfonyl chloride. The [$^{18}$F]fluorinated compounds were prepared using 3 mg of precursor 1 by two-step synthesis i.e., [$^{18}$F]fluorination and click reaction. [$^{18}$F]Fluorination of 1 was performed in t-amyl alcohol solvent at 120 °C for 15 min, and then propargyl triethylammonium mesylate (6 mg), 0.2 M aq. $CuSO_4$, and 0.2 M aq. Na-ascorbate were added to the reaction mixture for the next click reaction. After 15 min at room temperature, the mixture was filtered with a short silica column and washed with ethyl acetate. Compared to direct HPLC analysis, additional click reaction offered the great clearance of byproducts based on HPLC-UV profile. We also synthesized ammonium salt precursor 2 using precursor 1 and click reaction. Through one-step synthesis, [$^{18}$F]fluorinated compounds was also obtained in excellent radiochemical yield (RCY) and in shorter time after filtration with a short silica column. With the successful result from naphthalene model compound, we applied this method to the synthesis of [$^{18}$F]FLT and [$^{18}$F]FMISO.

**Conclusions:** We used click reactions for the purpose of simple purification. Both two-step synthesis and one-step synthesis produced the corresponding [$^{18}$F]fluorinated products in excellent RCY with small amount of byproducts.



## P112 SYNTHESIS AND AROMATIC [F-18]FLUORINATION OF meta-POSITION ON PHENYL RING OF DONEPEZIL USING IODONIUM SALTS

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**Objectives:** Donepezil (DP) is well known as a centrally reversible acetylcholinesterase (AChE) inhibitor. Among of various donepezil analogs, 1-(3-fluorobenzyl)-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine (3-FDP) showed the highest anti-acetylcholinesterase activity ($IC_{50}$ = 3.1 nM). The radiofluorination of 3-position on phenyl ring of donepezil, however, was difficult to the general nucleophillic aromatic substitution. We designed two synthetic routes to 3-[$^{18}$F]FDP, the first for direct fluorine-18 labeling with precursor and the second designed to proceed through labeled prosthetic group (3-[$^{18}$F]fluorobenzaldehyde) for the eventual radiolabeling of 3-[$^{18}$F]FDP.

**Methods:** To direct label F-18 at 3-position on phenyl ring of DP, we prepared diaryliodonium salt as a precursor, which was prepared by the reaction of tributylstannyl-DP with hydroxyl(tosyloxy)iodobenzene (Koser's reagent). Fluorine-18 labeling of precursor was achieved under microwave irradiation (100~150 W, 9 min) in different solvents depend on radical scavenger. In addition, we also synthesized another diaryliodonium salt precursor for the 3-[$^{18}$F]fluorobenzaldehyde (3-[$^{18}$F]FB) as an alternative method to prepare the 3-[$^{18}$F]FDP. In case of prosthetic pathway, 3-[$^{18}$F]FB was optimized in various condition including microwave irradiation, followed by reductive-alkylation.

**Results:** The direct radiosynthesis of 3-[$^{18}$F]FDP showed in 1-15% radiochemical yields (decay-corrected) with high radiochemical purity over 95% and total elapsed time was approximately 50 min. Reproducibility of radiofluorination, however, was unstable by unknown reason. On the other hand, 3-[$^{18}$F]FB was obtained in 20-25% radiochemical yield in prosthetic radiosynthesis of 3-[$^{18}$F]FDP.

**Conclusions:** We evaluated the synthesis of aromatic fluorination of 3-[$^{18}$F]FDP with unstable reproducibility. To overcome this disadvantage, 3-[$^{18}$F]FB was obtained in reasonable radiochemical yield which can be effectively labeling of 3-[$^{18}$F]FDP as a prosthetic pathway. Biological evaluation of 3-[$^{18}$F]FDP and reductive-alkylation with 3-[$^{18}$F]FB are going to optimization.

## P113 SYNTHESIS OF F-18 LABELED RGD DIMER USING CLICK CHEMISTRY

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**Objectives:** Tumor growth requires the formation of new blood vessels. RGD peptides, $\alpha_v\beta_3$ integrin antagonists, showed specific binding to $\alpha_v\beta_3$ expressing tumors in vivo. Therefore, radiolabeled RGD peptides have been extensively used for tumor angiogenesis studies. We have previously synthesized a radiolabeled synthon, 4-[F-18]fluoro-1-butyne, for click chemistry with azido compounds. In the present study, we applied this method to preparation of F-18 labeled RGD dimer containing tri-PEG moiety ([F-18]1).

**Methods:** A linker for click chemistry, 16-azido-4-oxo-8,11,14-trioxa-5-azahexadecanoic acid was synthesized from 11-azido-3,6,9-trioxaundecan-1-amine and succinic anhydride. The resulting azido hexadecanoic acid was further converted to the N-succinimidyl ester using TSTU in the presence of DIPEA (rt, 30 min), which was then conjugated to E[c(RGDyK)]$_2$. 4-[F-18]Fluoro-1-butyne was synthesized from 4-tosyloxy-1-butyne and K[F-18]F in the presence of K2.2.2. in acetonitrile (100 °C, 20 min), during which the resulting 4-[ F-18]fluoro-1-butyne was distilled with $CH_3CN$ into another vial containing the azido tri-PEG-RGD peptide and CuI at -50 °C (dry ice in an acetone bath). After completion of the distillation, the reaction mixture was warmed to rt, and to it were added DIPEA, 2,6-lutidine, and water. After stirring at rt for 20 min, the resulting mixture was purified by reverse phase HPLC (a linear gradient from 95:5 to 40:60 mixture of 0.1% TFA-water and 0.1% TFA-$CH_3CN$ over 60 min at a flow rate of 3.5 mL/min). The desired product ([F-18]1), which eluted at 24-25 min, was collected and solvents were removed using a rotary evaporator. An aliquot of [F-18]1 was co-injected with non-radiolabeled compound 1 into a HPLC system to confirm its identity.

**Results:** Distillation of reaction mixture of 4-tosyloxy-1-butyne and K[F-18]F gave purified 4-[F-18]fluoro-1-butyne, which did not require further purification for subsequent reaction. The resulting 4-[F-18]fluoro-1-butyne was reacted with azido tri-PEG-RGD peptide using click chemistry, in which CuI and two different bases were used. Radiotracer [F-18]1 was synthesized in 16-22% decay-corrected radiochemical yield and with specific activity of 41-43 GBq/μmol. Synthesis time including HPLC purification was 70 min. [F-18]1 was identified by co-elution with 1 on HPLC. Non-radiolabeled compound 1 was also synthesized and identified by MALDI-TOF MS.

**Conclusions:** F-18 Labeled RGD dimer containing tri-PEG moiety was synthesized from azido RGD peptide and 4-[F-18] fluoro-1-butyne using click chemistry. Biological evaluation of [F-18]1 is currently underway.

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## P114 EVALUATION OF 4-[F-18]FLUORO-1-BUTYNE AS A RADIOLABELED SYNTHON FOR CLICK CHEMISTRY WITH AZIDO COMPOUNDS

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**Objectives:** We have previously synthesized F-18 labeled compounds using click chemistry, because it is a useful approach for preparation of various radiotracers. 4-[F-18]Fluoro-1-butyne ([F-18]1) was selected as a radiolabeled synthon for click chemistry with azido compounds. In the present study, we evaluated [F-18]1 to find whether it is a suitable synthon for click chemistry.

**Methods:** [F-18]1 was prepared by nucleophilic [F-18]fluorination of 4-tosyloxy-1-butyne with [F-18]F in the presence of $K_2CO_3$ and K2.2.2. in either acetonitrile or t-butanol (95 °C, 15 min) and distilled into a vial containing acetonitrile at -50 °C. After completion of the distillation, an aliquot of the distilled fraction was analyzed by reverse phase HPLC, and the remainder was reacted with tetra-O-acetyl-1-azidoglucose in the presence of $CuSO_4$ and sodium ascorbate at 85 °C for 15 min. Non-radiolabeled compound (1) was synthesized from 4-tosyloxy-1-butyne and KF in the presence of K2.2.2. in either acetonitrile or t-butanol at 85 °C for 3 h. The rest of the procedure was the same as described above. Click reaction mixtures were purified by reverse phase HPLC (0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B); A:B=95:5 to 0:100 over 40 min; flow rate, 3.5 mL/min; 218 nm), and the non-radiolabeled products were collected and analyzed by $^1$H NMR spectroscopy and FAB mass spectrometry.

**Results:** HPLC analysis of the radiofluorinated mixture showed a non-radiolabeled impurity ($t_R$ = 20 min) along with 4-[F-18]fluoro-1-butyne ($t_R$ = 16 min). Subsequent click reaction of the radiofluorinated mixture with tetra-O-acetyl-1-azidoglucose resulted in the formation of a radiolabeled product ($t_R$ = 21 min) and non-radiolabeled product ($t_R$ = 23 min), which were co-eluted with 4-fluoroethyl-1-(tetra-O-acetyl-β-D-glucopyranosyl)-1,2,3-triazole and 4-vinyl-1-(tetra-O-acetyl-β-D-glucopyranosyl)-1,2,3-triazole, respectively. Cold reaction also gave the same products, even though the latter was the major product. In addition, the same result was obtained in the use of t-butanol as a reaction medium, which is known to have selectivity on alkyl fluoride formation over alkene by-product formation. This result clearly indicates that the radiofluorination of 4-tosyloxy-1-butyne with K[F-18]F produces not only 4-[F-18]fluoro-1-butyne (b.p. 45 °C) but also vinyl acetylene (b.p. 0-5 °C). Since both compounds contain terminal alkyne, they can participate in click reaction with azido compounds.

**Conclusions:** This result showed that nucleophilic substitution of 4-tosyloxy-1-butyne with K[F-18/F-19] produces 4-[F-18/F-19]fluoro-1-butyne and vinyl acetylene, probably due to the presence of a terminal alkynyl group close to the tosyloxy group at C4. Therefore, [F-18]fluoroalkyne longer than four carbon chain length may be a better radiolabeled synthon for click chemistry.