AUTOMATED PRODUCTION OF [^{18}F]FECh AND [^{18}F]FCH: PREPARATION AND USE OF [^{18}F]FLUOROALKANE SULFONATES AS FLUOROALKYLATION AGENTS

J. L. Lim, E. T. Dorman, C. L. Cabral

The Queen's Medical Center, Cyclotron Lab, 1301 Punchbowl Street, Honolulu, HI 96813
E-mail: jlim@queens.org

Keywords: F-18, choline, fluoroalkane sulfonates, fluoroalkylation

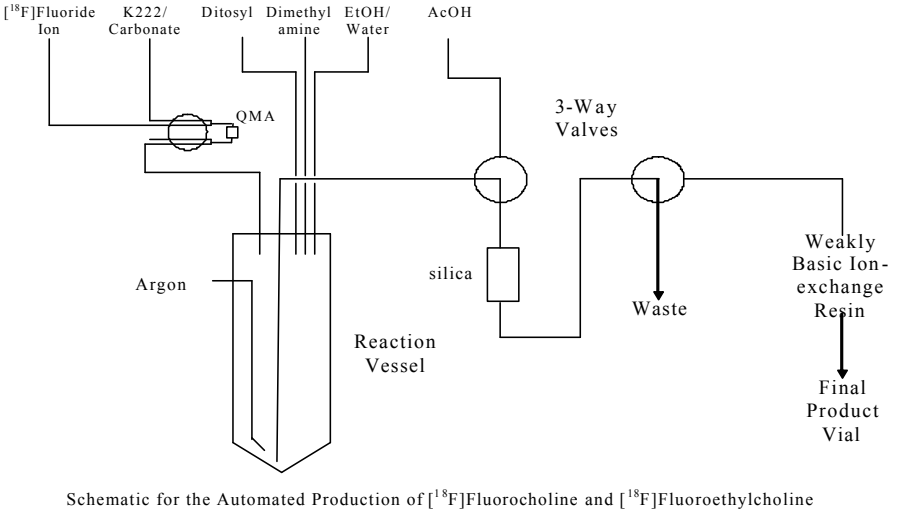
Recently, there is much interest to label choline with C-11 and F-18 for PET imaging of various tumors. Hara et al. reported the preparation of [^{18}F]fluoroethylcholine (FECh) and DeGrado et al. the synthesis of [^{18}F]fluorocholine (FCH). Both syntheses require sophisticated purification step (preparative purification using ion-pair reagent for FECh and GC purification for FCH).

We have designed an approach to prepare FECh and FCH that allows us to use the same equipment that produces FDG. Coenen et al. systematically studied the [^{18}F]fluorination of di-substituted alkanes and found that [^{18}F]fluoroethyl tosylate can be prepared in high yield from di-tosylethane, while the corresponding [^{18}F]fluoromethyl tosylate can not prepared in useful yield.

The 1-pot 2-step reaction started with [^{18}F]labeling of disulfonate alkane followed by fluorosulfonate intermediate's alkylation with dimethylethanolamine. [^{18}F]fluorination of di-tosylethane proceeded efficiently (80%) while the alkylation step was surprisingly average. Thus, FECh overall yield was only 50%, decay corrected. [^{18}F]Fluorination of di-tosylmethane was more delicate. This step, when driven to completion, only yielded [^{18}F]di-fluoromethane, not the mono-fluorosulfonate intermediate needed for the alkylation step. Under mild conditions, sufficient fluorotosylmethane was formed for the alkylation (quantitatively) with dimethylethanolamine to produce FCH in 25% yield.

The purification of the final product relies on the quaternary amine ability to hold on strongly to the silica, whereas all undesirable impurities are washed out to waste. Mulholland et al. reported this simple purification method to isolate [^{11}C]quaternary amines. After the reaction completion, the mixture was diluted with EtOH and passed through a SiO_2 Sep-Pak followed by washings with EtOH and water. The purified [^{18}F]quaternary amines were eluted with 2% AcOH which was subsequently removed using a weakly basic ion-exchange resin (Scheme).

In effect, this approach allows anyone with a FDG module to prepare easily [^{18}F]FECh and [^{18}F]FCH in 45 and 40 minutes with 50% and 25% yields respectively.



Schematic for the Automated Production of [^{18}F]Fluorocholine and [^{18}F]Fluoroethylcholine

^{18}F-GLYCOSYLATION OF FMOC-PROTECTED AMINO ACIDS USING TETRA-ACETYLATED FDG

O. Prante, S. Maschauer, T. Kuwert

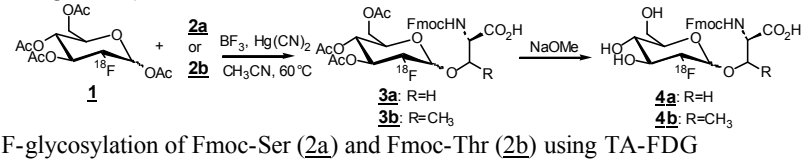
Friedrich-Alexander University Erlangen-Nuernberg, Clinic of Nuclear Medicine, Laboratory of Molecular Imaging, Schwabachanlage 6, D-91054 Erlangen, Germany. E-mail: olaf.prante@nuklear.imed.uni-erlangen.de

Keywords: F-18, FDG, ^{18}F-glycosylation

The development of ^{18}F-labelling methods adopted to bioactive peptides for diagnostic PET-imaging has gained enormous interest in the field of molecular imaging. Since now, ^{18}F-acylation is the commonly used strategy for the radiosyntheses of ^{18}F-labelled peptide-based imaging agents that show remarkable advantages over large proteins or antibodies due to their higher uptake in target tissue and improved blood clearance. Moreover, glycosylation of peptides and subsequent ^{18}F-acylation has clearly shown to be an effective method to further improve the biokinetics of radiolabelled peptides (1).

Consequently, the aim of this study was to develop a one step ^{18}F-glycosylation method based on the ^{18}F-labelled prosthetic group 1,3,4,6-tetra-O-acetyl-2-[^{18}F]fluoro-2-deoxyglucose (TA-FDG, 1), which occurs as an intermediate product in the [^{18}F]FDG synthesis (2).

The reaction parameters for the ^{18}F-glycosylation of Fmoc-protected amino acids (Ser, Thr) using 1 as a glycosyl donor were optimized with respect to reaction time, solvent, amount of precursor amino acid, choice of Lewis acid and reaction temperature (scheme 1). 1 was obtained by acetylation of [^{18}F]FDG using Ac_2O/pyridine and solid phase extraction (SepPak C-18) or by HPLC purification of the intermediate reaction solution of the [^{18}F]FDG synthesis (MeOH:H_2O 40:60, LiChrosorb RP-18, 125x8 mm, 4ml/min). Deacetylation of the ^{18}F-glycosylated acid 3a was carried out using 7.5mM NaOMe in dry MeOH. Radio-thin layer chromatography was used to identify radiolabelled by-products (SiO_2, $CHCl_3$:MeOH:AcOH 80:10:1). The final products, N -Fmoc-3-O-(3,4,6-tri-O-acetyl-2-[^{18}F]fluoro-2-deoxyglucopyranosyl)-L-serine (4a) and the corresponding threonine derivative (4b), were identified by gradient radio-HPLC (20-100% CH_3CN in H_2O (0.1% CF_3COOH) during 60min).



Scheme 1: ^{18}F-glycosylation of Fmoc-Ser (2a) and Fmoc-Thr (2b) using TA-FDG

The radiochemical yield (RCY) of 3a was 52% after 30min using CH_3CN (T=60°C) as a solvent and boron trifluoride (0.2M) and $Hg(CN)_2$ (0.1M) as promoters. Without adding $Hg(CN)_2$, 3b was obtained in 30% RCY. The reaction was found to be severely temperature-dependent. Increasing reaction temperature above 80°C led to rapid degradation of 2. CH_3CN in combination with BF_3 as a promoter turned out to be the optimal reaction solvent. The use of alternative Lewis acids such as $SnCl_4$, $SnBr_4$, BBr_3 or copper triflate was ineffective (RCY<10%). The amount of precursor (2) was varied (4-22µmol), which did not affect RCY. After deacetylation, the overall RCY of 4a and 4b was determined to be 46% and 24%, respectively (total synthesis time: 70min).

In summary, this study shows that tetraacetylated [^{18}F]FDG is a promising ^{18}F-labelled prosthetic hydrophilic group suitable for ^{18}F-glycosylation of amino acid side chains. This approach might offer the opportunity to realize ^{18}F-glycosylation of bioactive peptides.

References

1. Haubner R, Wester HJ, Burkhart F et al. *J Nucl Med* 2001; 42: 326-336.
2. Hamacher K, Coenen HH, Stöcklin G. *J Nucl Med* 1986; 27: 235-238.

A COMBINATORIAL STRATEGY FOR THE DESIGN AND SYNTHESIS OF 18F-LABELED QUINOLINE DERIVATIVES AS KINASE IMAGING AGENTS

J. C. Walsh, D. Maclean, J. P. Northrop, H. C. Padgett, T. Ysaguirre

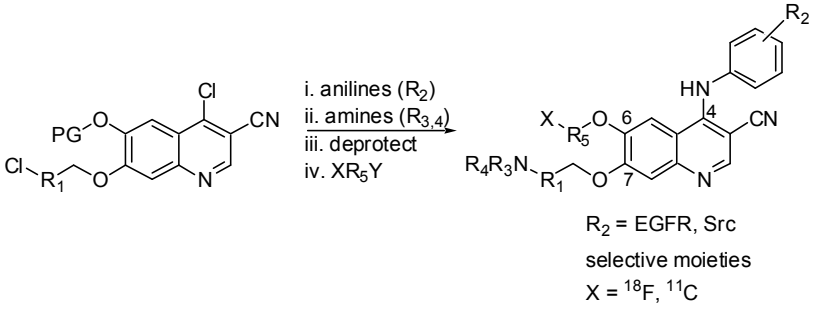
PETNET Pharmaceuticals, Inc., 6140 Bristol Parkway, Culver City, CA 90230 USA joseph.walsh@ctimi.com

Keywords: Combinatorial, tyrosine kinase, cancer, MicroPET

Tyrosine phosphorylation in proteins, mediated by tyrosine kinases, is a crucial link in cell signaling pathways. Overexpression and overactivation of tyrosine kinases may cause normal cells to develop cancer phenotypes. Labeled tyrosine kinases inhibitors are potential imaging agents for determining the location and type of kinase-expressing tumors in humans.

A number of compounds discovered by pharmaceutical companies are both potent and selective tyrosine kinase inhibitors. 18F-labeled kinase inhibitors, to date, have been relatively unsuccessful as biomarkers for imaging kinase-expressing tumors. We sought to solve this problem using combinatorial chemistry as a means of exploring how changes in the inhibitors' structure affects their pharmacokinetic profile using in vitro biochemical assays and MicroPET imaging data.

Our goal was to design and build inhibitors around a generic scaffold amenable to diversification yet capable of generic radiolabeling with a variety of positron emitting isotopes. We based our library of kinase inhibitors on a known quinoline scaffold (1,2) which allows for diversification at several positions. As a result based on known SAR, libraries can be designed for high inhibition potency and selectivity, as well as metabolic stability. The scaffold was elaborated with various commercially available amines and anilines. Different amino groups were substituted off the 7-position for the purposes of exploring changes in inhibition potency, and different aniline moieties were substituted at the 4-position for the purposes of exploring changes in kinase selectivity. The synthesis of the inhibitors was accomplished in eleven steps from commercially available starting materials. Starting from the dichloroquinoline intermediate, a large number of radiolabeled materials can be prepared in only four steps.



1. Wissner A, et al. *J Med Chem* 2000; 43: 3244-3256
2. Boschelli D H, et al. *J Med Chem* 2001; 44: 3965-3977.

REMOTE SEPARATION PROCEDURE FOR HALOGEN PRODUCTION

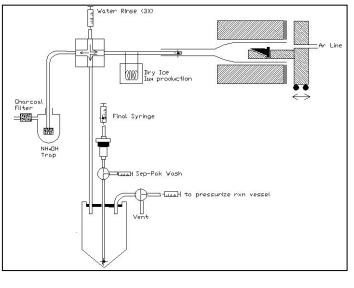
L. Tang, D.J. Rowland, D.H. Sultan, M.J. Welch

Mallinckrodt Institute of Radiology, Washington University School of Medicine, Division of Radiological Sciences, St. Louis, MO 63110, USA, *Contact*: Lucie Tang, Washington University School of Medicine, Campus Box 8225, 510 S. Kingshighway Blvd. Saint Louis, MO 63110, USA. Email: tangl@mir.wustl.edu

Keywords: Bromine-76, Iodine-124, Automation, Furnace, Separation

Halogens isotopes are of great interest for use in PET imaging and as therapeutic agents. Bromine isotopes have been successfully produced at Washington University School of Medicine for the past few years (1,2). Limited quantities of ^{76}Br were shipped for the first time during the end of year 2001. The production process is clearly defined for Br isotopes and since ^{124}I production is similar to the method for producing ^{76,77}Br, it is under investigation for routine production. These radionuclides are produced via the (p,n) reaction on enriched target material: Copper Selenide (^{76,77}Se)Cu_2Se to produce Br isotopes and Copper Telluride (^{124}Te)Cu_2Te to produce ^{124}I. The combined stoichiometric amounts of copper and selenium, or tellurium are pressed and melted on solid targets and irradiated on our small biomedical cyclotron using a 14.5MeV proton beam.

To optimise the production rate for ^{124}I, a slanted target is used to allow larger currents to be applied to the target (increasing the beam current from ~5μA to 20μA). However, this type of target does not permit pellet formation through the method of pressing the target material during the preparation process. Consequently a 1300ºC tubular furnace has been purchased to melt the target material onto the slanted target. The melting point of Cu_2Se is reported to be 1113ºC. Testing of Cu_2Te with the high temperature furnace showed that a physical change was noticed at 1133ºC. The prepared Cu_2Te target was irradiated for 10min at 5, 10, 15, and 20 μA and a good stability of the target material was observed. The 1300ºC tubular furnace will serve dual purposes, preparation of target material on the slanted target and thermal distillation of the irradiated target.



At the moment, ^{76}Br is produced from the distillation from a disk target using a conventional oven (3). The target is placed in a quartz tube inside the oven under argon atmosphere and the oven is heated ~1100ºC for approximately 1 hour. The activity is deposited outside of the oven on ~4cm at one end of the quartz tube and is recovered with several 0.01N sodium hydroxide (NaOH) rinses. The NaOH rinse solution is transferred manually using tubing and 3-way stopcock valves. A remote procedure has been set up to routinely make ^{76,77}Br and minimize absorbed radiation doses to personnel. This remote system uses electronic valves to distribute gas and liquid at different locations during distillation and recovery of ^{76}Br. The activity always remains inside a hotcell reducing exposure to the operator. In the final version, a device will be designed to slide the irradiated target in the quartz tube and seal the system during the distillation step (4). The separation will follow using the same procedure than the existing remote system with the valves. The 1300ºC tubular furnace will be used for the automation and everything will be placed in a hotcell of dimensions 71cm L x 53cm H x 46cm W. This work is supported by the US DOE grant DEFG02-84ER-60218 and the NCI grant R24 CA86307.

(1) Rowland DJ, et al. *Ninth International Worshop on Targetry and Target Chemistry* Turku, Finland 2002; C07.
(2) McCarthy DW et al. *J Label Compd Radiopharm* 2001; 44: S817-S819.
(3) Tolmachev V., et al. *Appl Radiat Isot* 1998; 49:1537.
(4) Blessing G, et al. *Int J Appl Radiat Isot* 1982; 33: 333-339.

IN-TARGET PRODUCTION OF ^{18}F-LABELLED GASES: SPECIFIC RADIOACTIVITY OF [^{18}F]F_2

J. Bergman[1], S. Johansson[1], O. Eskola[1], N. Savisto[1] and O. Solin[1,2]

[1]Turku PET Centre, Radiopharmaceutical chemistry laboratory and Accelerator laboratory, Porthaninkatu 3, FIN-20500 Turku, Finland and [2]Radiochemistry Laboratory, Helsinki University, PO Box 55, FIN-00014 Helsinki University. Contact e-mail olof.solin@abo.fi

Keywords: [^{18}F]Fluoromethane, electrophilic fluorine, specific radioactivity.

Electrophilic fluorine-18, typically in the form of [^{18}F]F_2 or [^{18}F]CH_3COOF, is widely used in labelling of compounds containing aromatic groups. The main advantage is the facile chemistry for labelling. A major disadvantage is the relatively low specific radioactivity (SA) achievable with standard methods (1,2). The aim of this work is to develop electrophilic fluorine with high SA.

We have earlier developed a method where [^{18}F]F_2 is formed when applying an electrical discharge through a gas mixture containing [^{18}F]CH_3F and a small amount of carrier F_2 in neon (3). No carrier added [^{18}F]CH_3F is synthesised by reacting CH_3I with a Kryptofix 222/K[^{18}F]F complex (4). The amount and SA of the electrophilic fluorine formed in the discharge chamber are dependent on the amount and SA of [^{18}F]CH_3F, discharge efficiency (geometrical and physical aspects), impurities in the discharge chamber and the amount of carrier F_2.

We are now developing a route where the wet synthesis of [^{18}F] CH_3F from aqueous fluoride is replaced by in-target production of ^{18}F-fluoride from $^{18}O_2$ in a metallic target chamber. The radioactivity is extracted from the chamber walls in a post-irradiation process, and recovered as a ^{18}F-labelled gas (fluoromethane or other). This ^{18}F-labelled gas, after gas chromatographic purification is then used in the above mentioned discharge chamber for the synthesis of [^{18}F]F_2. By increasing SA and the initial amount of [^{18}F]CH_3F (or other fluorine labelled gas) we aim to increase the SA of [^{18}F]F_2.

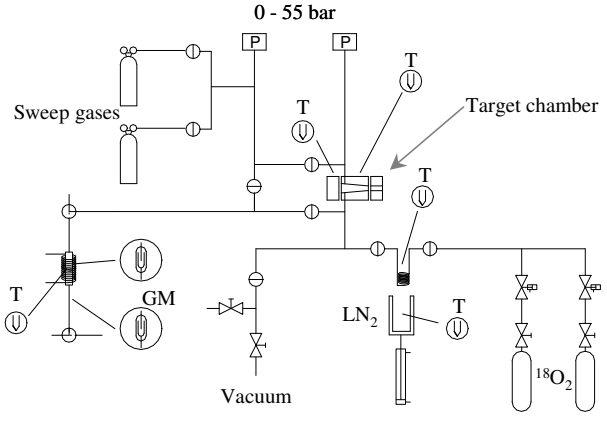


Figure 1. Schematic drawing of target system for production of fluorine-18. T = thermocouple, GM= geiger-muller tube, P=pressure transducer.

1. Bishop A, Satyamurthy N, Bida G, Phelps M, Barrio JR. *Nucl Med Biol* 1996;23:391-405
2. Nickles RJ, Gatley SJ, Votaw JR, Kornguth ML. *Int J Rad Appl Instrum [A]* 1986;37(8):649-61
3. Bergman J, Solin O. *Nucl Med Biol* 1997; 24: 677-683.
4. Bergman J, Solin O. *J Label Compd Radiopharm* 1994; 35: 174-175.

SYNTHESIS OF 4-[^{18}F]FLUOROIODOBENZENE AND ITS APPLICATION IN SONOGASHIRA CROSS-COUPLING REACTIONS

F. Wüst, T. Kniess

Institut für Bioanorganische und Radiopharmazeutische Chemie,
FZ-Rossendorf , PF 510119, 01314 Dresden, Germany; f.wuest@fz-rossendorf.de

Keywords: [^{18}F]fluoroiodobenzene, Sonogashira cross-coupling, steroids

Despite the success of using palladium-mediated cross-coupling reactions in ^{11}C-chemistry [1,2] only a few attempts have been made to extent this approach to ^{18}F-chemistry. The first few reports on the synthesis of ^{18}F-labelled radiotracers via palladium-mediated cross-coupling reactions have mainly exploited Stille [3] and Hartwig-Buchwald *N*-arylation reactions [4] with 4-[^{18}F]fluorohalobenzenes as the coupling partner. The copper-palladium catalysed Sonogashira reaction of terminal alkynes with vinyl- or arylhalides is an other example for a mild, functional group tolerating and high-yielding cross-coupling reaction. Herein we describe the first Sonogashira cross-coupling reaction in ^{18}F-chemistry, exemplified by coupling 4-[^{18}F]fluoroiodobenzene with 17 -ethynyl-3,17 -estradiol and 17 -ethynyl-3,17 -estradiol-3-methylether.

4-[^{18}F]fluoroiodobenzene was synthesised in 13-70 % radiochemical yield using the thermal decomposition of symmetrical 4,4'-diiododiaryliodonium salts in the presence of [^{18}F]fluoride in DMF at 140°C or by microwave activation. 4-[^{18}F]fluoriodobenzene was obtained in a radiochemical purity >95% after solid phase extraction (SPE). SPE-purified 4-[^{18}F]fluoriodobenzene (50-150 MBq) was subjected to palladium-copper catalysed Sonogashira cross-coupling with terminal alkynes **1** and **2** (Figure 1). The reactions were performed in a sealed vial at 110°C for 20 min using THF as the solvent and triethylamine as the base. The obtained radiochemical yields were determined by HPLC-monitoring the conversion of 4-[^{18}F]fluoroiodobenzene into the corresponding cross-coupled products. Hence, 65-88% of 4-[^{18}F]fluoroiodobenzene was converted into cross-coupled product **3** and 34-64% into **4**, respectively. Lowering the reaction temperature to 60°C did not lead to any product formation. The optimum amount of components in the cross-coupling reaction was found to be about 3 mg for each component. Higher amounts of catalyst (CuI and $Pd[PPh_3]_4$) and terminal alkynes did not further improve the radiochemical yields.

In conclusion, we have developed a novel approach for a transition-metal mediated carbon-carbon bond formation in ^{18}F-chemistry, being the Sonogashira cross-coupling of terminal alkynes with 4-[^{18}F]fluoroiodobenzene. The reaction proceeds in sufficient radiochemical yields in short reaction times, and the reaction is compatible with functional groups (e.g. OH).

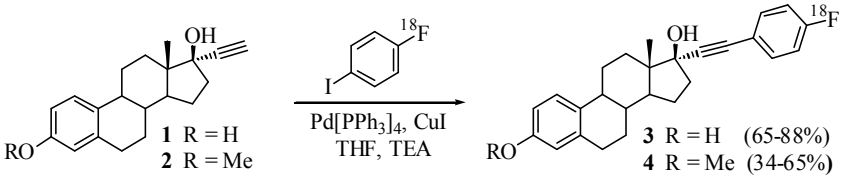


Figure 1: Sonogashira cross-coupling with 4-[^{18}F]fluoroiodobenzene

1.Långström B, Kihlberg T, Bergström M, Antoni G, Björkman M, Forngren BH, Forngren T, Hartvig P, Markides K, Yngve U, Ögren M. *Acta Chem Scand*. 1999; 53: 651-669.
2.Wüst F, Zessin J. *J Label Compd Radiopharm.* In press.
3.Allain-Barbier L, Lasne M-C, Perrio-Huard C, Moreau B, Barré L. *Acta Chem Scand*. 1998; 52: 480-489.
4. Marriére E, Chazalviel L, Dhilly M, Toutain J, Perrio C, Dauphin F, Lasne M-C. *J Label Compd Radiopharm.* 1999; 42: S69-S71.