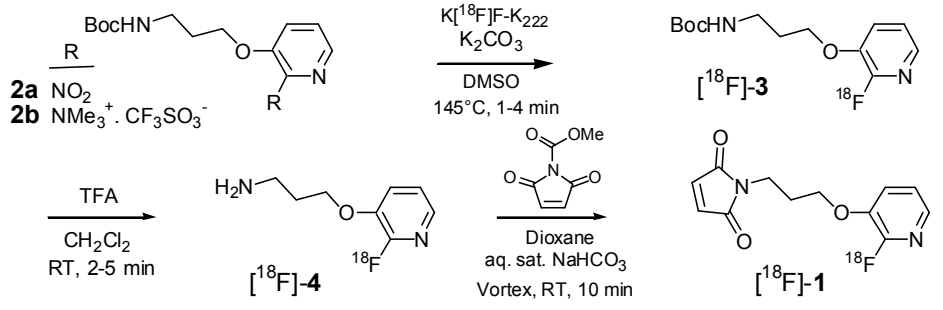
RADIOSYNTHESIS OF A [^{18}F]FLUOROPYRIDINE-BASED MALEIMIDE REAGENT FOR PROTEIN LABELLING

F. Dollé[1], F. Hinnen[1], B. Lagnel[1], R. Boisgard[2], A. Sanson[3], F. Russo-Marie[3], B. Tavitian[2]

[1] Service hospitalier Frédéric Joliot, Département de Recherche Médicale, CEA/DSV, 4 Place du Général Leclerc, F-91401 Orsay, France. [2] ERM103, Laboratoire d'Imagerie de l'Expression des Gènes, same address. [3] Bionexis, CEA-Saclay, F-91191 Gif-sur-Yvette, France. E-mail contact address: dolle@dsvidf.cea.fr.

Keywords : Fluorine-18, maleimide reagent, protein labelling

Positron Emission Tomography (PET) is a high-resolution, sensitive and non-invasive imaging technique that can be used in humans. It is the most advanced technology currently available for studying *in vivo* molecular interactions and also represents the method of choice to assess the distribution and pharmacokinetics of proteins *in vivo*. The most widely used positron-emitting radiohalogen is fluorine-18 (half-life : 109.8 minutes). Among the positron emitters, it is likely to be the radionuclide of choice for its physical and nuclear characteristics. However, apart from a few exceptions, its use precludes the "direct labelling" of macromolecules such as proteins and requires the design and development of dedicated fluorine-18 labelled prosthetic groups (1,2). Carboxylic acids and their succinimidyl derivatives are the most common reagents developped and dedicated to the labelling of amine functions (Lys-( )NH_2 essentially) via acylation reactions. In the present work, we have designed and synthesized a [^{18}F]fluoropyridinyne-based maleimide reagent, [^{18}F]-**1**, for specific radioactive alkylation of thiol functions (Cys-SH).



[^{18}F]-**1**, namely 1-[3-(2-[^{18}F]fluoro-pyridin-3-yloxy)-propyl]-pyrrole-2,5-dione was synthesized in 3 radiochemical steps. No-carrier-added nucleophilic heteroaromatic substitution was performed in DMSO with the activated K[^{18}F]F-Kryptofix®222 complex and either the nitro- (**2a**) or the trimethylammonium trifluoromethanesulfonate (**2b**) precursors by conventional heating at 145°C ([^{18}F]-**3** : 45-85 % incorporation yields). TFA removal of the *tert*-butoxycarbonyl protective group was quantitative (in dichloromethane at room temperature) and afforded the desired amine [^{18}F]-**4**. Maleimide formation was performed using *N*-methoxycarbonylmaleimide in a mixture of dioxane and aq. sat. $NaHCO_3$ (45-65% radiochemical yield). Final HPLC purification gave pure [^{18}F]-**1** in 105-110 minutes synthesis time. Typically, 50-70 mCi (1.85-2.59 GBq) of [^{18}F]-**1** (1-2 Ci/µmol or 37-72 GBq/µmol) can be obtained starting from a 500 mCi (1.85 GBq) aliquot of a batch of cyclotron-produced [^{18}F]fluoride. This fluorine-18 reagent has been recently used in the labelling of AFIM™, a 8 kDa mini-protein derived from the specific binding domain of annexins for phosphatidylserine and showing high thermodynamic stability, reversible folding and a high affinity for phosphatidylserine-containing biological membranes.

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NEW FLUORINE-18 SYNTHONS FOR PEPTIDE LABELLING

M. Glaser,[1] M. Solbakken,[2] H. Karlsen,[2] J. Arukwe,[2] A. Cuthbertson,[2] F. Brady,[1] S. K. Luthra [1]

[1] Imaging Research Solutions Ltd., Hammersmith Hospital London, W12 0NN,UK
[2] Amersham Health AS, Dept. of Medicinal Chemistry P.O. Box 4220 Nydalen, N-0401 Oslo, Norway
m.glaser@csc.mrc.ac.uk

Keywords: fluorine-18, de–mesylation, indirect labelling, peptides

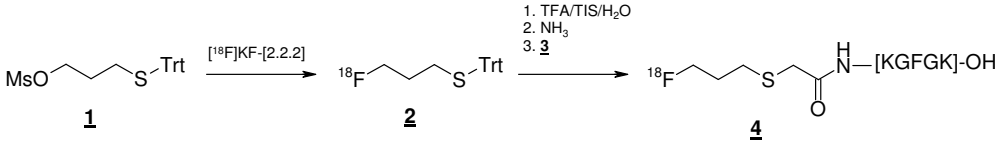
Introduction

Peptides labelled with fluorine-18 have many potential applications as probes for PET. On the other hand, the possibilities for introducing ^{18}F regioselectively into peptides are rather limited. Usually, pre–labelled synthons are the method of choice. However, these reagents normally require a number of preparative and purification steps leading to a decrease of radiochemical yields. For example, the popular reagent succinimidyl *p*-[^{18}F]fluorobenzoate (^{18}F-SFB) is synthesised via three steps of hot chemistry [1].

In this paper we present a new approach to conjugate ^{18}F containing synthons site–specifically with peptide precursors.

Methods

Potassium [^{18}F]fluoride Kryptofix® complex was dried azeotropically using acetonitrile and reacted with methanesulphonic acid 3-tritylsulphanylpropyl ester **1** in DMSO at 80 °C for 5 minutes. Crude product **2** was loaded onto a Sep-Pak C18 cartridge, eluted with acetonitrile, and the trityl protecting group removed by heating with mixture of trifluoroacetic acid, triisopropylsilane and water (5:1:1 v/v/v). After adding a solution of ammonia at 0 °C the mixture was incubated with chloroacetyl peptide **3** at 80 °C for 30 minutes.



1 **2** **4**

3 = Cl-CH_2C(O)NH-[KGFGK]-OH

Results

The overall r.c.y. of peptide **4** was 74 % as estimated by HPLC (not decay–corrected). Two other similar ^{18}F synthons with thiol function have been prepared. The syntheses, peptide conjugations and optimisation of reaction conditions will be presented.

Conclusion

A new type of ^{18}F reagents suitable for labelling chloroacetylated peptides has been established. After promising model labelling reactions, the scope of this chemistry can now be extended to peptides of medical interest.

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BREAKTHROUGH IN ^{18}F-LABELLING OF PEPTIDES?

T. Poethko[1], G. Thumshirn[2], U. Hersel[2], M. Schottelius[1], G. Henriksen[1], M. Herz[1], R. Haubner[1], H. Kessler[2], M. Schwaiger[1], H.-J. Wester[1]

[1]Department of Nuclear Medicine, Technische Universität München, Ismaninger Str. 22, 81675 Munich, Germany and [2]Institute of Organic Chemistry and Biochemistry, Technische Universität München, Lichtenbergstr. 4, 85747 Garching, Germany. email: H.J.Wester@lrz.tum.de

Keywords: radiohalogenation, peptide, Fluorine-18, chemoselective conjugation, octreotide, RGD

The methodologies currently used for ^{18}F-labeling of peptides and proteins are complicated and time consuming multi step procedures. Acylation reagents, which are often used as labeling reagents, have to been prepared in 3-steps, followed by peptide conjugation and -in most cases- a final deprotection step. Thus, only a few ^{18}F-peptides were used in clinical studies so far. To overcome this major drawback, a methodology for radiolabeling by chemoselective conjugation of peptides, i.e. ^{18}F-labeling using an easy to prepare prosthetic group was developed and evaluated.

All peptides were prepared by SPPS using Fmoc-protocols. Introduction of the amino-oxy functionality (OAE) was carried out by using Boc-protected amino-oxyacetic acid and HOAt/DIC/DIPEA activation. 4-[^{18}F]Fluorobenzaldehyde ([^{18}F]BA) was prepared by ^{18}F-fluoination of 4- trimethylammoniumbenzaldehyde triflate (DMSO, 15 min, [K/2.2.2]$^{+}$ ^{18}F^{-}, 60°C). The reaction mixture was diluted with 2ml H_2O, passed through a SCX cartridge and trapped on a RP-18 cartridge. After washing with 4-5 ml H_2O/MeCN (10/90) and 1 ml H_2O, 4-[^{18}F]BA was eluted with 0.5-1ml MeOH (20 min, RCY 50-70%). For peptide conjugation, 11 µl of a peptide solution (25 nmol peptide in 10µl H_2O/1µl 2.5% TFA) were added to 40 µl of 4-[^{18}F]BA in MeOH and reacted for up to 60 min. For large scale conjugation, 1ml of 4-[^{18}F]BA in MeOH was reacted with 1mg peptide (0.5mM).

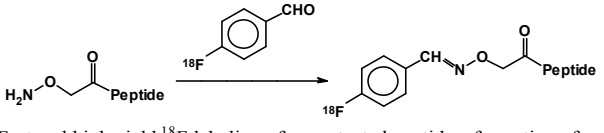

Fig 1: Fast and high yield ^{18}F-labeling of unprotected peptides: formation of oximes by chemoselective conjugation

Tab 1: ^{18}F- and ^{123}I-labeling of unprotected peptides

	RCY* [%]	Metabol. Analysis [1]		RCY* [%]	Metabol. Analysis [1]
OAE-*LEF*-NH_2	88 %	n.d.	(c(*RGDfE*)PEG)$_2$-*K*-Dpr-AOE	85 % [3]	n.d.
c(*RGDfE*)AH-Dpr-AOE	85 %	stable [2]	((c(*RGDfE*)PEG)$_2$-*K*)$_2$-*K*-Dpr-AOE	79 %	unstable
c(*RGDfE*)-PEG-Dpr-AOE	81 %	stable	OAE-minigastrin	86 % [3]	n.d.
(c(*RGDfE*)AH)$_2$-*K*-Dpr-AOE	78 %	n.d.	Gluc-merc-Dpr(OAE)-TOCA	60 %	n.d.
((c(*RGDfE*)AH)$_2$-*K*)$_2$-*K*-Dpr-AOE	83 % [3]	unstable	Cel-merc-Dpr(OAE)-TOCA	86 %	n.d.

* conjugation yields; reaction conditions: 15min, 60°C, 0.5 mM pept., MeOH/H_2O/TFA (s.o.), 1) blood, kidney, liver, tumor; 2) only urine; n.d.: not determined; 3) 0.5h; PEG: polyethylene glycol, Dpr: diamino prop. acid; merc=mercapto propionyl, TOCA=Tyr^3-octreotate; AH: 6-amino hexanoic acid

Unprotected model peptides, mono-, di- and tetrameric-RDG peptides, octreotates and minigastrin were labelled in high yields (60-88%, 15 min) on the GBq level with 4-[^{18}F]- and 4-[^{125}I]benzaldehyde. Synthesis of ^{18}F-peptides are completed within <1h (based on ^{18}F-fluoride). Final purification by a cartridge system is under investigation. The monomeric compounds investigated show high in vivo stability (1h and 2h p.i.). Similar coupling yields were obtained in initial experiments with other ^{18}F-carbonyl compounds. This methodology is well suited not only for the simple, fast and easy to automate high yield synthesis of ^{18}F- (and ^{123,131}I-, ^{211}At-...) labelled peptides with high in vivo stability, but is also currently investigated for fast and easy synthesis of the corresponding chelator peptides.

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[^{18}F]-FLUOROALKYLATION USING THE "LOOP" SYSTEM

V. Tadino[1] , D. Le Bars[2], S. Shulman[1], J. Mather[1], M. Nilsson[1],M. Kiselev[3]
[1]Bioscan Inc, 4590 MacArthur Blvd, Washington DC 20007 USA, vtadino@bioscan.com
[2]CERMEP, 59 Blvd Pinel, 69003 Lyon, France, lebars@univ-lyon1.fr
[3]Eastern Isotopes Inc., 100 Executive Dr, Sterling, VA 20166 USA, mkiselev@iba.com

Keywords : Bromofluoromethane, Fluorocholine, Fluroalkylation, Loop, Automation

There is increasing interest in [^{18}F]-fluoroalkyl halides in PET tracer development (Coenen[1], DeGrado[2]). The alkylation step used to prepare these tracers is similar to the labeling step with [^{11}C]-methyl iodide. We have used this similarity to develop a simple labeling system which adapts the "loop" ^{11}C-methylation method of Wilson et al.[3] to the production of [^{18}F]-fluorocholine.

Using a Coincidence FDG Synthesis System (G.E. Medical Systems, Liege), we have developed a new sequence to prepare [^{18}F]-bromofluoromethane. The standard Coincidence FDG disposable kit was adapted with the addition of silica Sep-Pak cartridges to include the simple purification method developed by Iwata et al.[4] The "loop" system (based on a standard 2mL stainless steel HPLC injection loop) was prepared for the labeling reaction by injecting 100µL of the precursor, dimethylaminoethanol in dimethylformamide (10µL/100µL).

The [^{18}F]-bromofluoromethane was distilled from the Coincidence reactor and transferred into the "loop" in a 10mL/min flow of nitrogen. Because the [^{18}F]-bromofluoromethane distillation time is longer than that for [^{11}C]-methyl iodide, the "loop" was left open until the distillation was complete (about 12min). During the distillation, untrapped [^{18}F]-bromofluoromethane was collected, and the trapping efficiency of the "loop" was found to be greater than 80%. At the end of the distillation, the reaction mixture was rinsed from the "loop" into a vial with 5mL of water. The reaction mixture was analyzed on a C-18 HPLC column, and the only radioactive peak was the [^{18}F]-fluorocholine (purity > 99%). The total synthesis time, including production of the [^{18}F]-bromofluoromethane labeling agent, was 30 minutes. In a routine production, the bulk solution could be purified using either preparative HPLC or a Sep-Pak Light Accel CM Cartridge[4].

This work demonstrates the following: first, it is possible to adapt a classical FDG synthesizer to produce [^{18}F]-fluoroalkyl halides, thus providing a new source of F-18 labeling agents. Second, the "loop" method in general, and its commercial embodiment in the Bioscan AutoLoop System, can be used to produce a wide range of [^{18}F]-fluoroalkylated PET radiotracers. The alkylation efficiency in the loop has already been proved through the production of a wide range of C-11 labeled compounds. This approach can be now used in F-18 chemistry both for routine production and for the development of new tracers. Additional tests are planned with [^{18}F]-bromofluoroethane and with [^{18}F]-fluoromethyl- and [^{18}F]-fluoroethyl triflate.

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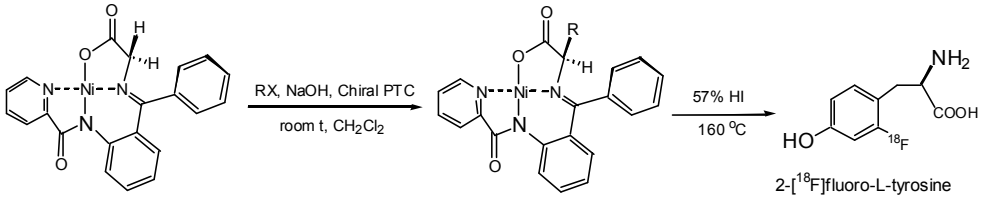
SYNTHESIS OF ^{18}F-FLUORINATED α-AMINO ACIDS VIA STEREOSELECTIVE ALKYLATION CATALYZED BY (*S*)-NOBIN

P.A.Schubiger[2], R. Krasikova[1], V. Zaitsev[1], O. Kuznetsova[1], O. Fedorova[1], I. Mosevich[1], S.M. Ametamey[2], Y. Belokon[3], Š. Vyskocil[4]

[1]Institute of Human Brain RAS, 9, Pavlov str, 197376, Saint-Petersburg, Russia; [2]Center for Radiopharmaceutical Science of ETH, PSI and USZ, CH-5232, Villigen PSI, Switzerland; [3]A.N. Nesmeyanov Institute of Organo-Element Compounds RAS, Vavilov 28, 119991, Moscow; [4]Charles University, Faculty of Science, Dept.of Organic Chemistry, Hlavova 2030,12840, Prague 2, Czech Republic. E-mail contact address: raisa@ihb.spb.ru

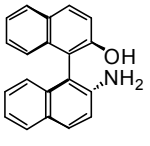
Key words: fluorine-18, amino acids, phase transfer catalysis

A growing application of [^{18}F]fluorinated α-amino acids (FAA) in PET has stimulated an interest to the new synthetic strategies. Recent advances in asymmetric synthesis belong to chiral phase transfer catalytic (PTC) reactions where a key alkylation step proceeds under mild conditions without necessity to use strong bases and low temperatures obligatory for "classical" chiral glycine enolates. Two PTC reagents, C2-symmetric chiral quaternary ammonium salt (**I**), and 0-(9)-benzyl-N-9-antracenylmethylcinchonidinium (**II**) were evaluated in a synthesis of FAA showing high asymmetric efficiency at 0°C alkylation [1]. New chiral catalyst NOBIN has provided high enantiomeric excess (e.e.) of amino acids via room temperature alkylation of achiral Ni^{II} complex of Schiff base of 2-benzoylphenylamide of pyridine-2-carboxylic acid (PBP) and glycine [2]. In contrast to (**I**) and (**II**), NOBIN can be readily prepared and available in *R*- and *S*- forms [3].



RX = 2-[^{18}F]fluoro-4-methoxy-benzyl bromide

Chiral PTC = (*S*)-NOBIN



Here we applied the (*S*)-NOBIN and defined an optimal PTC reactions conditions for a synthesis of two important FAA, 2-[^{18}F]fluoro-L-tyrosine (2-FTYR) and 6-[^{18}F]fluoro-L-3,4-dihydroxy phenylalanine (6-FDOPA). Alkylating agents, [^{18}F]fluorobenzyl bromides (RX), were prepared via three steps procedure from the corresponding nitrobenzaldehydes. The PTC alkylation was investigated in a wide range of conditions. The highest e.e. (97 % for 2-FTYR and 92 % for 6-FDOPA) was obtained in CH_2Cl_2 under 5 min stirring at room t. Radiochemical yield (EOB) was 25-28% for 2-FTYR and 15-20% for 6-FDOPA within 110-120 min synthesis time.

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POLYMER-SUPPORTED RADIOPHARMACEUTICAL PRECURSORS: BENZAMIDES AND N-SUCCINIMIDYLBENZOATES

M. Janabi[1], A.M Chacko[1], D.H. Hunter[1], J.W. Babich[2] and M.P. Smith[2]

1. Department of Chemistry, *The* University *of* Western Ontario, London, ON, N6A 5B7, Canada, dhunter@uwo.ca; 2. Biostream Inc., 160 Second Street, Cambridge, MA, 02142 USA, jbabich@biostream.net

Keywords: Aryl stannanes, Solid phase synthesis, Polymer-supported stannanes

N-Succinimidyl-3- and 4-halobenzoates (**A**) have been successfully developed as radiolabels for peptides, antibodies and small molecules using the full range of radiohalogens (^{18}F, ^{76}Br, ^{123}I, ^{125}I, ^{131}I and ^{211}At). The usual precursor for these species is the N-succinimidyl 3- or 4-tributylstannylbenzoate since it provides no-carrier-added material under mild conditions and generally in good radiochemical yield. One drawback to this precursor is the toxicity associated with organotin species which require careful purification. We have been attempting to solve the toxicity problem but keep the other advantages of tin precursors by developing insoluble polymer-supported tin precursors to radiohalopharmaceuticals.

This polymer-supported approach provides other potential advantages worth exploring. Besides acting as a source of N-succinimidyl-3- and 4-radiohalobenzoates, which are subsequently reacted with amine bearing species such as peptides, antibodies and small molecules, it is possible to react the polymer-supported N-succinimidylbenzoates (**B**) directly with the amine bearing species to prepare polymer-supported benzamides (**C**). This provides the possibility of preparing libraries of radiopharmaceutical precursors. It also means that, upon radiolabelling, all species in solution will bear the radiolabel.

We will report on the successful preparation of the 3- and 4-polymer –supported N-succinimidylbenzoates (**B**). A small library of polymer-supported benzamides (**C**) has been prepared both through the N-succinimidylbenzoate and from the corresponding acid. These polymeric materials have been characterized through a combination of MAS ^{119}Sn NMR spectroscopy, IR using a DRIFT attachment and by HPLC of iodinolysis products.