SYNTHESIS, RADIOLABELLING AND BIOLOGICAL EVALUATION OF *SYN*-[^{18}F]FACBC AS A POTENTIAL TUMOR IMAGING AGENT

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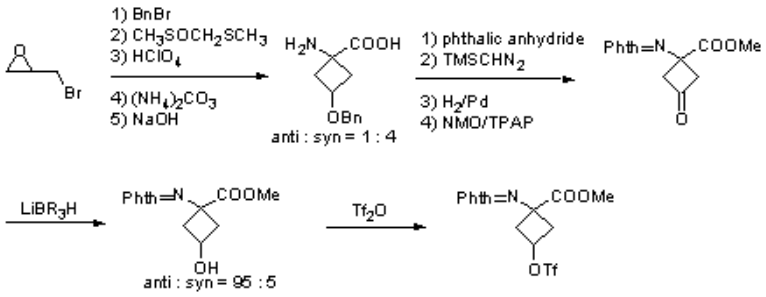
Amino acid *syn*-1-amino-3-[^{18}F]fluorocyclobutyl-1-carboxylic acid (*syn*-FACBC) was synthesized, [^{18}F] radiolabeled and biologically evaluated as a potential PET tumor imaging agent in several human cancer cell lines.

The FACBC triflate precursor for radiolabeling was prepared in a series of synthetic steps, which is shown below.



Radiofluorination was carried out with [^{18}F]KF/K_{222} and hydrolysis followed by chromatographic purification. The radiochemical purity of s*yn*-[^{18}F]FACBC was over 99% as measured by radiometric TLC.

The cell study was performed in A549 (lung), DU145 (prostate), SKOV3 (ovary), MDA MB468 (breast) and U87 (brain) human cancer cell lines in amino acid-free Dulbecco's Modified Eagle's Medium incubated for 30 minutes at 37 °C to evaluate the compound tumor cell uptake and transport mechanism. 10 mM 2-Amino-bicyclo[2.2.1]-heptane-2-carboxylic acid (BCH), 10 mM N-methyl-α-aminoisobutyric acid (MeAIB) and 10 mM alanine-cysteine-serine (ACS) were used as L-, A- and all-type amino acid transporter inhibitors, respectively. The results of cell assays were reported in the table1, with high cell uptake from 12 to 34 % CPM/1e6 cells. The average inhibition of uptake of *syn*-FACBC by BCH was 64%, *vs.* 24% by MeAIB. These findings suggested that *syn*-FACBC entered these cells *in vitro* primarily *via* L-type amino acid transport. These results support the candidacy of *syn*-[^{18}F]FACBC as a PET tumor imaging agent. Research supported by Nihon Medi-Physics Co., Ltd., and NIH.

Table 1: Cell uptake of *syn*-FACBC (mean % CPM/1e6 cells)

	DU145	SKOV3	U87	MB468	A549
Control	20.27	11.67	24.77	33.53	11.91
BCH	9.11	5.87	5.00	10.93	3.85
MeAIB	17.16	8.48	14.80	28.25	9.61
ACS	3.52	1.98	4.40	2.65	2.41

Acknowledgement: NMP has the license of syn-FACBC. Emory University may have the loyalty.

Keywords: syn-FACBC, Fluorine-18, PET Tumor Imaging

C-11 LABELLING AND BIODISTRIBUTION OF AG1478

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The chemical compound AG1478 is an inhibitor of the Epidermal Growth Factor (EGF) receptor. A phase 1 clinical trial using AG1478 as an anticancer agent in patients with advanced or metastatic solid brain tumours has recently commenced at the Ludwig Institute for Cancer Research in Melbourne. We have labelled AG1478 with the PET isotope C-11 at the 7 position of the dimethylquinazoline ring in order to investigate the potential of this compound to measure the EGF receptor expression in tumors.

Radiolabelling

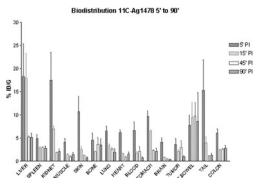
The labelling was achieved by reacting 2 mg of the desmethyl precursor with C-11 methyl iodide in DMF at 80 °C using 10 µL of 1M $NaHCO_3$. The product was purified on a reversed phase HPLC and formulated in 10% ethanol. Decay corrected radiochemical yields of 43-56% and an average specific activity of 21 MBq/µmol could be obtained with this method.

In vitro experiments

We studied the binding of AG1478 to the EGF receptor in solubilised A431 cell membrane fractions and found that 5% of the total radioactivity was bound to these fractions. The binding of C-11 AG1478 to whole A431 cells reaches a maximum of 45% at a cell concentration of 4×10^7 cells.

In vivo biodistribution studies

100 µCi of C-11 AG1478 was injected into the tail vein of nude mice bearing A431 tumors. Mice were sacrificed at various time points after injection, major organs were excised and the radioactivity was measured in a gamma counter. Our results show high uptake in the liver and kidneys after 5 minutes. Uptake in A431 tumors was 5% at 5 minutes and 4.5% at 45 minutes post injection and decreased to 2.5% at 90 minutes post injection.



Comparison with other PET labelled analogues of AG1478

The compound PD153035 has been labelled with C-11 and evaluated in rats with and without neuroblastoma implants[1,2]. The biodistribution data of C-11 PD153035 is similar to C-11 AG1478 with fast clearance fom the blood stream and uptake of the tracer peaked in less than 10 minutes post injection. An acrylamide quinazoline derivative (ML03) has also been labelled with C-11 and evaluated as a potential biomarker of EGF receptor positive tumors[3]. The in vivo studies of A431 tumor bearing rats did not indicate high accumulation of [^{11}C]ML03 in the tumor. The authors suggested that the low bioavailability of this irreversible inhibitor is the main reason for the low uptake. A series of F-18 labelled derivatives of AG1478 has also been investigated in A431 tumor bearing rats[4]. These tracers showed high non-specific binding and the authors concluded that these compounds might not be suitable as imaging agents.

Conclusion

We have been able to demonstrate that [^{11}C]AG1478 rapidly clears from the bloodstream and shows uptake in A431 tumors. Imaging studies need to be performed in order to establish whether this tracer can be used as an imaging agent for EGF receptor expression.

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Keywords: C-11 Labelling, EGF Receptor, AG1478

ONE-POT SYNTHESIS OF [^{11}C]-UREAS *VIA* TRIPHENYLPHOSPHINIMINES

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The demand for new radiolabeling methods is expanding with the increasing use of PET (Positron Emission Tomography) throughout drug discovery processes and medical imaging. Because of the increased molecular complexity and diversity of biologically active compounds, there is a need for general, new, and simple methodologies which give access to ^{11}C-labeled pharmaceuticals in short time, high yield and are reliable and suitable for automation as well.

A structural element that can often be found in biologically active compounds is the urea group. So far, ^{11}C-labeling of ureas in the carbonyl function has been performed mainly starting from the secondary precursor [^{11}C]phosgene.[1] Although effective chemically, [^{11}C]phosgene is very volatile and is obtained in low radiochemical yields. Synthetic routes to isocyanates and ureas with phosgene substitutes have been described (i.e. diphosgene,[2] [^{11}C]CO_2 / dehydration[3] or [^{11}C]CO[4]), all having their own disadvantages.

Here, an efficient, one-pot synthesis of [^{11}C]ureas via triphenylphosphinimines[5] and [^{11}C]CO_2 is described. This appeared to be a powerful tool to circumvent disadvantages of the other phosgene substitutes (i.e. the relatively large number of reaction steps, the need of particular catalysts or reagents, or specific automation). A number of compounds were synthesized using this method in a decay corrected yield of 7 – 49 % from [^{11}C]CO_2. The scope of the reaction is demonstrated with a diversity of amines.

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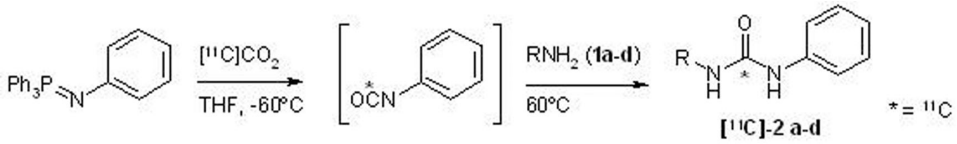
Keywords: [11C]-Ureas, Triphenylphosphinimines, One-Pot Synthesis



A NOVEL, HIGHLY EFFICIENT WAY TO PREPARE [^{18}F]FLUOROMETHYL BROMIDE

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[^{18}F] fluoromethylation is a main route for labelling PET ligands[1]. But, compared with [^{11}C] methyl iodide, only a few examples have been reported using [^{18}F] fluoromethylating agents[2, 3]. The main reasons for this are poor radiochemical yields and difficulties in handling and automation [3-5].

To solve these problems, a novel method has been developed based on nucleophilic substitution of trimethylsilylmethyl triflate with [^{18}F] fluoride followed by bromination of the resulting trimethylsilylmethyl [^{18}F] fluoride, to give ^{18}F] fluoromethyl bromide.

In short, commercially available trimethylsilylmethyl triflate was reacted with [^{18}F] fluoride in acetonitrile at 50^{o}C for 5 minutes to give trimethylsilylmethyl [^{18}F] fluoride. N-bromosuccinimide (NBS) and caesium fluoride was added and the resulting mixture was heated to 105^{o}C for 8 minutes. The resulting [^{18}F] fluoromethyl bromide was distilled using a stream of helium and trapped in a suitable solvent, e.g. acetonitrile or DMF. When needed, [^{18}F] fluoromethyl bromide was obtained free from acetonitrile by passing the distillate over a heated silica gel column and trapping the reagent in the required solvent.

The method was found to give [^{18}F] fluoromethyl bromide in 60-80% isolated decay corrected yield in approximately 35-40 minute after EOB. The radiochemical purity for [^{18}F] fluoromethyl bromide was > 95% as determined by radio-GC. Optimization and characterization of specific activity is in progress.

In conclusion, a novel method for preparing [^{18}F]fluoromethyl bromide is described that provides consistently high isolated yields in short time. The simplicity of the method should facilitate automation.

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Keywords: ^{18}F-Fluoromethylation, PET Radioligands, ^{18}F-Fluoromethyl Halides

ONE STEP SYNTHESIS OF [*carbonyl*-^{11}C]WAY-100635

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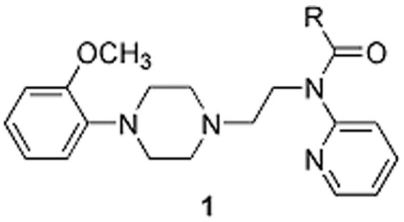
^{11}C-Labeled WAY-100635 (**1, R=**cyclohexyl) has been recognized as a useful probe for the in vivo imaging of 5-HT_{1A} receptors.[1] Despite the favorable binding properties of the tracer, the quantification of PET data is restricted due to its fast metabolism. The search for a tracer with better metabolic stability brought up an issue of developing a reliable labeling synthesis for an array of analogous compounds. Using the palladium mediated carbonylation with ^{11}CO gave a convenient access to a number of analogues (**1**). However the scope of feasibility was limited mostly to compounds having an aryl moiety **R** due to the β-hydride elimination.



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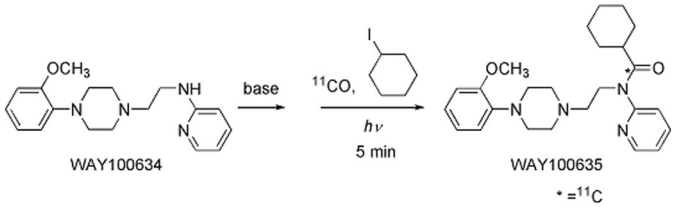
Recently, an alternative method for the labeling of amides using [^{11}C]carbon monoxide, based on synergetic radical/ionic mechanism, was introduced.[2] This method makes possible to label amides possessing alkylcarboxamide moiety, such as WAY-100635. While many aliphatic amines and aniline provided high radiochemical yield in the radical-mediated reaction, WAY-100634 showed low reactivity. The synthesis was improved by the pretreatment of the precursor amine with a strong base before reacting it with the alkyl iodide in the presence of [^{11}C]carbon monoxide at photoirradiation conditions. [*carbonyl*-^{11}C]WAY-100635 was obtained in 40-50 % isolated radiochemical yield in 30 min synthesis time. The conversion of [^{11}C]carbon monoxide exceeded 85 %.



WAY100634 WAY100635
* =^{11}C

The free radical mediated carbonylation of WAY-100635 illustrates the use of the new method for the preparation of PET tracers. Provided the technique for handling of [^{11}C]carbon monoxide is available, this approach is an attractive alternative to multistep Grignard synthesis regarding convenience and the scope of applicability.

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Keywords: C-11 Carbon Monoxide, Radical Carbonylation, WAY-100635

SYNTHESIS, RADIOSYNTHESIS AND *IN VIVO* IMAGING STUDIES COMPARING THREE C-11 LABELED PET LIGANDS FOR MAPPING THE SEROTONIN TRANSPORTER (SERT)

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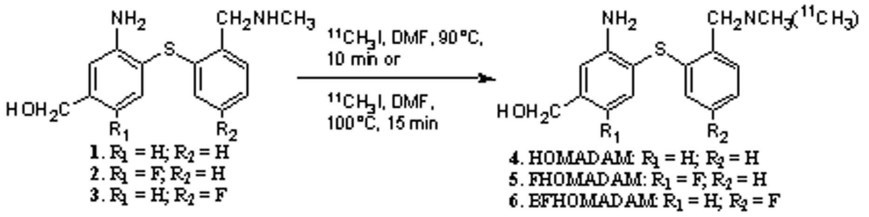
Recently, considerable effort has been spent on the development of PET radiotracers for the *in vivo* imaging of the SERT in the human brain. We have previously reported the synthesis and pharmacological characterization of C-11 *N,N*-dimethyl-2-(2′-amino-4′-hydroxymethylphenylthio)benzylamine ([^{11}C]HOMADAM), highly specific and selective PET radioligand showing fast kinetics for SERT[1]. In our continued effort to develop appropriate SERT PET radiotracer that can be labeled with either C-11 or F-18, two additional C-11 labeled SERT ligands, *N,N*-dimethyl-2-(2′-amino-5′-fluoro-4′-hydroxymethylphenylthio)benzylamine ([^{11}C]FHOMADAM) and *N,N*-dimethyl-2-(2′-amino-4′-hydroxymethylphenylthio)-4-fluoro-benzylamine ([^{11}C]BFHOMADAM) have been synthesized. The characterization as well as the comparison of [^{11}C]HOMADAM (^{11}C-**4**), [^{11}C]FHOMADAM (^{11}C-**5**) and [^{11}C]BFHOMADAM (^{11}C-**6**) in the same monkey will be presented.

The *in vitro* binding studies of **4, 5 and 6** in cells transfected to express human DAT, NET and SERT showed that HOMADAM exhibited the highest SERT affinity with Ki (SERT) = 0.57, 5.45 and 1.10 nM for HOMADAM, FHOMADAM and BFHOMADAM, respectively. ^{11}C-**4**, ^{11}C-**5** and ^{11}C-**6** were prepared by C-11 methylation of their *N*-desmethyl precursors **1, 2 and 3** with $^{11}CH_3I$ in DMF at 90°C for 10 min to give ^{11}C-**4** and ^{11}C-**5** in 23% radiochemical yield (RCY) and in DMF at 100°C for 15 min to give ^{11}C-**6** in 9% RCY (E.O.B). The total synthesis time was 65 min.

The lipophilicity of ^{11}C-**4**, ^{11}C-**5** and ^{11}C-**6** was similar with log $Ps_{7.4}$ of 1.60, 1.77 and 1.91, respectively. The regional brain uptake in monkeys of **^{11}C-HOMADAM**, **^{11}C-FHOMADAM** and



^{11}C-BFHOMADAM was studied with microPET and showed that all three ligands displayed the highest uptake in the midbrain followed by the thalamus, in a pattern consistent with the rank order of SERT distribution in the monkey brain. A quasi-equilibrium was established at 20 min, 30 min and 55 min post injection of **4, 5 and 6**, respectively. Peak midbrain to cerebellum activity ratio was 3.60, 1.58 and 2.62 at 45 min post injection of **4, 5 and 6**, respectively. *In vivo* bindings of ^{11}C-**4** and ^{11}C-**6** were shown to be specific to the SERT as, displacement with citalopram (a potent SERT ligand) reduced radioactivity in SERT-rich regions such as midbrain and thalamus to the level of cerebellum.

These results demonstrate that the two radiotracers **^{11}C-HOMADAM** and **^{11}C-BFHOMADAM** were suitable candidates for the *in vivo* SERT imaging in monkeys with **^{11}C-HOMADAM** showing the highest specific binding in all monkey brain regions. Research supported by NIMH and DOE.

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Keywords: HOMADAM, Benzylamine, Carbon-11

RADIOSYNTHESIS AND *IN VIVO* EVALUATION OF *N*- [^{11}C]-METHYLATED IMIDAZOPYRIDINE DERIVATIVES AS POSITRON EMISSION TOMOGRAPHY TRACER FOR PERIPHERAL BENZODIAZEPINE RECEPTORS

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Peripheral benzodiazepine receptor (PBR) ligands with positron-emitting radionuclide and positron emission tomography (PET) have shown to be useful tools for in vivo imaging of neurodegenerative diseases such as Alzheimer's disease[1]. Imidazopyridineacetoamide **1-4**, novel potential and selective PBR ligands, whose affinities are comparable with that of known PBR ligands[2], were investigated for potency as a PET radioligand (Chart1).

The radiosynthesis of **[^{11}C] 1-4** were examined using *N*- [^{11}C] methylation on the corresponding desmethyl precursors **1-4** with 11C methyl iodide or 11C methyl triflate in the presence of NaH or NaOH in DMF or DMSO (table1). The radiochemical yields of **[^{11}C] 2** from 11C methyl iodide was higher than that from 11C methyl triflate. Since the use of 11C methyl iodide with NaH produced dimethyl-substitued compound, the radiosynthesis of **[^{11}C] 1-4** were optimized by limiting the amount of NaH. After HPLC purification, each of the labeled compounds was obtained with specific activities of 28-146 MBq/μmol.

Each of the labeled compounds was injected to ddY mice and radioactivity and weight of dissected peripheral organs and brain regions were measured. The distribution of **[^{11}C] 3** was consistent with the known PBR distribution[3,4]. Moreover, **[^{11}C] 3** possesses the best combination of brain uptake and PBR biding leading to its high retention in olfactory bulb and cerebellum, the areas whose PBR distribution are relatively high. Co-injection of PK1115 or unlabeled **3** significantly reduced the brain uptake of **[^{11}C] 3**.

These results suggest **[^{11}C] 3** should be a potential PET radioligand for imaging of PBR.

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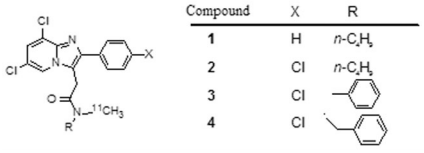
Chart 1. Chemical structure of [^{11}C] 1-4



Compound	X	R
1	H	*n*-C$_4$H$_9$
2	Cl	*n*-C$_4$H$_9$
3	Cl	
4	Cl	

Table 1. Radiolabeling of [11C] 1-4

Compound	reagent	base (mg)	solvent	radiochemical yields (%)	specific activity (MBq/umol)
1	[11C] CH3I	NaH (0.3)	DMF	31	114
2	[11C] CH3I	NaH (0.35)	DMF	77	146
2	[11C] CH3OTf	NaOH (Saturated)	DMSO	2.5	27
3	[11C] CH3I	NaH (0.3)	DMF	36	60
4	[11C] CH3I	NaH (0.5)	DMF	37	28

Keywords: Carbon-11, Peripheral Benzodiazepine Receptor, Imidazopyridineacetoamide

SYNTHESIS AND INITIAL EVALUATION OF 2-[^{18}F]FLUORO-5-[2-(2-METHYLTHIAZOL-4-YL)ETHYNYL]PYRIDINE, A POTENTIAL PET LIGAND FOR THE METABOTROPIC GLUTAMATE SUBTYPE 5 RECEPTOR

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The metabotropic glutamate receptor subtype 5 (mGluR5) is involved in the regulation of synaptic transmission in the central nervous system. This receptor is a member of the Group I family, which initiates cellular responses through G protein coupling to phospholipase C and stimulation of phosphoinositide hydrolysis. mGluR5 is present in many brain areas, including the olfactory bulb, striatum, hippocampus and frontal cortex. To research the physiological roles of mGluRs, we have synthesized 2-[^{18}F]fluoro-5-[2-(2-methylthiazol-4-yl)ethynyl]pyridine ([^{18}F]FMEP), and conducted initial evaluation studies.

The precursor, 2-bromo-5-[2-(2-methylthiazol-4-yl)ethynyl]pyridine, was synthesized using the Sonogashira coupling reaction between 2-methyl-4-[(trimethylsilyl)ethynyl]thiazole (ref. 1) and 2-bromo-5-iodopyridine (ref. 2) in the presence of triethylamine, copper(I) iodide, tetrakis(triphenylphosphine)palladium(0), and anhydrous 1,2-dimethoxyethane. The "cold" standard, 2-fluoro-5-[2-(2-methylthiazol-4-yl)ethynyl]pyridine (FMEP), was obtained by coupling of 4-ethynyl-2-methylthiazole with 2-fluoro-5-iodopyridine in the presence of copper(I) iodide, $PdCl_2(PPh_3)_2$, and ethyldiisopropylamine. [^{18}F]FMEP was synthesized by the routine K_{222}/K_2CO_3 method in DMSO as the solvent for 10 min at 130 ^{0}C, followed by HPLC purification on a μBondapak® C-18 column (7.8 × 300 mm, Waters) with a flow of 6 ml/min using methanol and phosphate buffer (pH 7.4, 0.15 mM) as the mobile phase (40/60 v/v). The total synthesis time was 90 minutes, and the product is obtained with high radiochemical purity and high specific radioactivity.

PET imaging studies were conducted with a microPET, P4 system (Concorde Microsystems Inc, Knoxville, TN). For imaging studies, 1.0-1.5 mCi [^{18}F]FMEP was injected into the rats tail vein. To evaluate the specific binding of this new ligand, the standard mGluR5 antagonist 2-methyl-6-(2-phenylethynyl)pyridine (MPEP) was injected (10 mg/kg) at 5 min before the administration of [^{18}F]FMEP. The accumulation of [^{18}F]FMEP was reversible in all areas of the body. The highest accumulation was obtained 1-2 min after administration of the labeled ligand and was localized in the olfactory area. The ratio of radioligand binding in the olfactory bulb vs. that in the cerebellum had the maximum value of 5.0 at 10 minutes. With pre-administration of unlabeled MPEP, an average binding decrease of 53.7 % in the olfactory bulb was observed at 10 min.

Conclusion: 2-[^{18}F]Fluoro-5-[2-(2-methylthiazol-4-yl)ethynyl]pyridine is a potential PET ligand for the imaging of mGluR5 in the olfactory system.

This work was supported by NIH/NIBIB grant EB01850 to A-LB.

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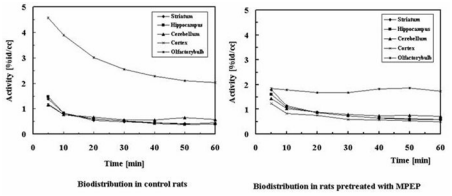
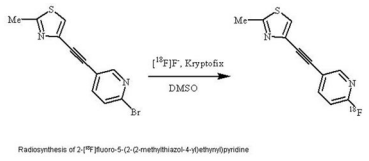
Keywords: Metabotropic Glutamate Receptor, PET, Fluorine-18





HPLC UV DETECTOR RESPONSE TO RADIOACTIVITY IN THE ANALYSIS OF PET RADIOTRACERS

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Introduction.

PET radiotracers are usually analysed by radio HPLC prior to their use on the PET camera. A well established technique is to use a UV detector to determine the amount of non-radioactive parent compound present with the radio-labelled tracer. This technique depends on the injection of standard solutions of 'cold' non-radioactive compound at known concentrations. This is followed by an injection of the radiolabelled tracer, where the unknown concentration is to be determined. The presumption has always been that the UV response will be the same in the radioactive sample as in the standard solutions.

Our recent work has shown that the radioactivity present in the sample has a significant effect on the UV detector, causing an underestimate of the stable content in the radioactive product.

Method.

[11C]GB67 was prepared at a radioactive concentration of 4788 MBq / ml in 10 ml saline + 10% ethanol. Stable GB 67 0.21 µg / ml. Specific activity 1005 GBq /µmol.

100µl aliquots of the [11C]GB67 solution were withdrawn and injected onto an analytical HPLC system. The HPLC system was equipped with a radioactive and UV detector. The peak corresponding to GB 67 was integrated and the area compared with the area for a standard GB 67 solution. Repeat injections were made at times 0, 10, 20, 30, 60, 90 and 120 minutes.

Results.

Peak areas corresponding to GB 67 decreased as the radioactivity decayed away, Figure 1.

There was a good correlation between the radioactivity injected onto the HPLC system and the loss of HPLC peak area (fig 2)

Conclusions

This reduction in UV peak area is greatest when the specific activity and radioactivity are high and has been demonstrated in the analysis of 11C-GB67. This effect is presumably due to the detector photo diodes being sensitive to radioactivity and interpreting this signal as light, which corresponds to a lack of UV absorbing material in the flow cell. The effect of radioactivity on UV HPLC detectors is dependant on the detector manufacture, one type having a large response to radioactivity, another no response.

All analysis of high specific activity radiotracers provided at high yield should be examined to assess that the UV HPLC detector is not responding to radioactivity. This may be achieved by repeat injections of samples of the radioactive product over several half lives.

Keywords: Radiotracers, UV-Detection, Analysis

Figure 1 HPLC Peak area V Time of HPLC injection

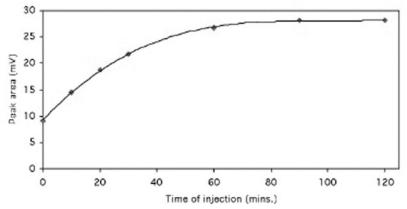
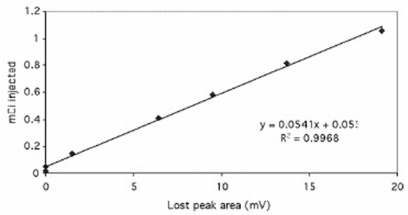


Figure 2 mCi injected onto HPLC V "lost" HPLC peak area



APPLICATION OF RADICAL REACTIONS IN ^{11}C-LABELING CHEMISTRY

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Development of techniques for handling [^{11}C]carbon monoxide has led to considerable advances in synthetic methods for the preparation of ^{11}C-labeled PET tracers. So far focus has been on palladium mediated carbonylation reactions, which proved to be a versatile route to [*carbonyl*-^{11}C]carboxylic acids and acid derivatives starting from aryl halides[1] and triflates[2]. The use of alkyl halides, however, was out of the scope of palladium chemistry due to facile β-hydride elimination.

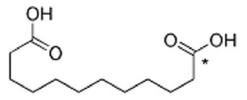
Here is presented a simple method for one-step radiosynthesis of saturated [^{11}C]carboxylic acids and their derivatives using [^{11}C]carbon monoxide, alkyl iodides and appropriate nucleophiles.

In brief, alkyl radicals generated by photolysis of an alkyl iodide add to carbon monoxide, subsequently forming transient acyl iodide, which, in turn, is trapped by a nucleophile producing corresponding carbonyl compound.[3] Since this approach circumvents the problem with β-elimination, a new range of labeled compounds becomes accessible.

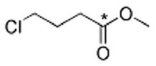
The radiolabeling reactions were carried out in a 270 μl stainless steel reaction vessel equipped with a sapphire window. First, a solution of an alkyl iodide and an appropriate nucleophile was transferred the reactor pre-filled with [^{11}C]carbon monoxide in helium. Then the reaction mixture was pressurized to 35-40 MPa and irradiated with a focused light (280-400 nm) from a xenon-mercury lamp with stirring at 35°C for 6-7 min. The crude mixture was then evacuated and purified using semipreparative LC.

In the following table selected examples are presented:

compound	**reaction time (min)**	**convn of ^{11}CO (%)**	**yield (%)[a]**
1	7	84	58
2	6	82	61



1



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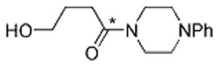

3

* = ^{11}C

3	6.5	78	44

[a] isolated decay-corrected radiochemical yield

As the examples illustrate, the radical-mediated carbonylation with [^{11}C]carbon monoxide has a wider scope then the multistep Grignard synthesis. An advantage may be the specific radioactivity of labeled products, because the atmospheric carbon dioxide does not contribute to the isotopic dilution. For example the compound (**1**) was synthesized with specific radioactivity of 188 GBq/μmol.

In conclusion, one-pot free radical mediated carbonylation was employed in the labeling of fatty acids and their derivatives using [^{11}C]carbon monoxide. This approach complements palladium-mediated carbonylations and widens the scope of available PET tracers.

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Keywords: C-11carbon Monoxide, Radical Carbonylation, Amides, Acids, Esters

RADIOSYNTHESIS OF [^{11}C]GI181771, A NEW SELECTIVE CCK-A AGONIST

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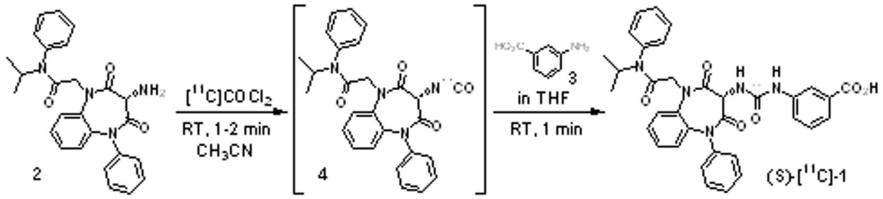
Cholecystokinin (CCK) is an important mammalian hormone binding to specific receptors named CCK-A and CCK-B predominately located in the gastrointestinal system and in the CNS, respectively. CCK receptors have been investigated in relation to a variety of disorders. In particular, studies demonstrating that exogenous CCK can diminish meal duration and size have triggered the development of selective and potent synthetic CCK-A ligands as satiety agents for the treatment of obesity. Among a series of 1,5-benzodiazepine selective CCK-A receptor agonists, GI181771, namely (S)-3-(3-{1-[(isopropylphenylcarbamoyl)methyl]-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[*b*][1,4]diazepin-3-yl}ureido)benzoic acid ((S)-**1**), has been identified as an orally active satiety agent (1) and considered for positron emission tomography studies. GI181771 ((S)-**1**) was therefore isotopically labelled with carbon-11 ($t_{1/2}$: 20.38 min). Among others, the urea site was chosen, requesting on-site routinely used [^{11}C]phosgene and using the two amines, **2 and 3**, available to us off shelf, as non radioactive labelling precursors.



[^{11}C]phosgene was radiosynthesized from cyclotron-produced [^{11}C]methane via [^{11}C]carbon tetrachloride using minor modifications of published processes (2,3). Optimized conditions for the one-pot two-step process preparation of (S)-[^{11}C]-**1** were the following: (a) Trapping of [^{11}C]phosgene at room temperature for 1 to 2 minutes in 300 microlitres of acetonitrile containing 0.6 micromole of the structurally-complex chiral-amine **2**, giving the corresponding [^{11}C]isocyanate **4**, followed by (b) Addition of an excess of 3-aminobenzoic acid (**3**, 40 micromoles in 100 microlitres of THF) as the second amine giving the desired urea derivative (S)-[^{11}C]-**1** and (c) HPLC purification on a semi-preparative Waters Symmetry C18. Starting from a typical 1.2 Ci (44.4 GBq) batch of [^{11}C]methane, 25 to 35 mCi (0.92-1.29 GBq, 6.8-9.6% decay-corrected yield based on starting [^{11}C]methane, n = 5) of > 99% chemically, radiochemically and enantiomerically pure (S)-[^{11}C]-**1** could be obtained within 35 minutes of radiosynthesis (HPLC purification and formulation as an i.v. injectable solution using a home-made Sep-pak Plus C18 device included) with specific radioactivities ranging from 500-1500 mCi/micromole (18.5-55.5 GBq/micromole).

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Keywords: Carbon-11, Phosgene, Isocyanate

PRODUCTION OF THE P-GLYCOPROTEIN MARKER, [^{11}C]LOPERAMIDE, IN CLINICALLY USEFUL QUANTITIES

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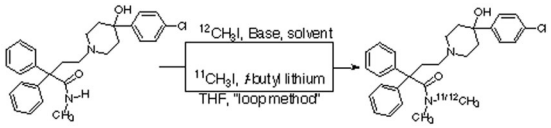
[^{11}C]Loperamide has been described as a novel radiotracer for P-glycoprotein studies. Animal studies have demonstrated that [^{11}C]loperamide has higher sensitivity and specificity for P-glycoprotein compared to existing tracers and could be useful for measuring small changes in P-glycoprotein function (1,2,3) and linking these changes to disease states. However both the chemical yield for the production of the desmethyl precursor (5% overall yield, 4) and the radiochemical yield in the reported synthesis of [^{11}C]loperamide were both modest and variable. Here we report our efforts to increase both yields in order to improve our ability to perform clinical studies with [^{11}C]loperamide.

To improve the manufacture of normethylloperamide, we studied the feasibility of a one-step fusion reaction between 3,3-diphenyl-dihydro-furan-2-one and 4-(4-chloro-phenyl)-piperidin-4-ol and preparing the desired product by converting the resulting carboxylic acid into its N-methyl amide via in-situ formation of the mixed anhydride by reaction with trifluoroacetic anhydride, followed by treatment with methylamine. The use of the method described above led to a much improved yield (56% overall).

To explore the reactivity of the secondary amide, a series of model reactions were performed to investigate the effects of solvents, bases, and adjuncts in the methylation of normethylloperamide with iodomethane. Assorted bases (K_2CO_3, KOH, KH, NaOH, NaH, TBAOH), solvents (DMF, DMSO, THF) and adjuncts e.g. Kryptofix 2.2.2, were permutated but to no avail. Yields of loperamide were consistently low (0-5%) while side-products were produced in abundance. However the use of t-butyl lithium as base in THF produced a much cleaner reaction mixture with >30% yields of loperamide.

These conditions were successfully applied to the radiosynthesis of [^{11}C]loperamide. Thus reaction of 1 mg of normethylloperamide with [^{11}C]iodomethane in the presence of t-butyl lithium, using our reported "loop" method (5) produced moderate yields of [^{11}C[loperamide as a formulated, sterile pyrogen-free solution, suitable for human studies. Results (based on a 20 min, 40 μA bombardment, n=7): radiochemical yield (EOS) – 65 ±16 mCi; specific activity (EOS) – 3070 ±1120 mCi/μmole, radiochemical purity — >99%, time of synthesis – 30 min. It is possible that protective hard: hard interactions between the lithium cation from t-butyl lithium and the tertiary alcohol anion produced from normethylloperamide are partially responsible for the improved results.



Conclusions: The methods of preparation for both normethylloperamide through a fusion reaction and for [^{11}C]loperamide using t-butyl lithium as base in THF have greatly improved the feasibility of performing clinical studies with this promising radiotracer. The first study in man with [^{11}C]loperamide is currently being initiated to investigate its research and clinical utility.

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Keywords: P-Glycoprotein, Loperamide, Loop Methylation

DOUBLE-TRACER TECHNIQUE FOR QUANTITATIVE AUTORADIOGRAPHY WITH SHORT-LIVED POSITRON EMITTERS USING A BIO IMAGING ANALYZER SYSTEM

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Ex vivo autoradiography (ARG) coupled with Imaging Plate (IP) is a useful technique for investigating the distribution of radiotracers in the brain as well as the whole body. To obtain more information in one experiment and avoid errors caused by individual specificity, a double-tracer method combining the use of shorter- and longer-lived radioisotopes (e.g., ^{18}F and ^{3}H) has been performed on the same slice prepared from the same subject. However, the method requires a relatively long time (e.g. 1 week) to obtain the respective images corresponding to each radioisotope. Recently, L-Process, an analyzing software of Bio Imaging Analyzer System (BAS5000), for subtraction between two different images was commercialized. In this study, we applied the double tracer method with two short-lived radioisotopes (^{18}F and ^{11}C) to perform quantitative analysis of two radioisotopes and evaluated the usefulness.

Three groups of 20 μm-thick gelatin samples containing ^{11}C (6.7 MBq), ^{18}F (1.7 MBq) and ^{11}C (6.7 MBq) +^{18}F (1.7 MBq) were prepared using a Cryotome and placed in contact with IP for 60min. After exposure, the IP was analyzed by using BAS 5000 (Image-1). One hundred twenty min after the first contact, the IP was exposed again to the same samples for another 60 min and analyzed (Image-2, where the residual ^{11}C radioactivity is only 0.2 % compared to that of Image-1). A subtraction image for ^{11}C was obtained by subtracting $2^{t/T}$ x Image-2 from Image-1 with the software L-Process attributed to BAS5000 (t; time from the start of the first contact, 180 min., T; half-life of ^{18}F, 110 min). The subtraction image and Image 2 were compared with corresponding images obtained separately with ^{11}C and ^{18}F after correction for the amount of radioactivity.

An *ex vivo* ARG study with rat using double tracer of central benzodiazepine receptor ligand [^{11}C]Ro15-1788 and a peripheral benzodiazepine receptor ligand [^{18}F]FEtDAA was carried out by intravenously injecting the mixture into rats, then sacrificing the rat 30 min later. Twenty μm thick-sagittal brain slices were prepared using a Cryotome and imaging was carried out as described above.

The value of PSL/mm^{2}corresponding to ^{11}C on Image-1 was 508±43 PSL/mm^2, which was about 10 % lower than that by the single tracer method with ^{11}C (557±24 PSL/mm^2). The PSL/ mm^2 value of Image-2 with mixed sample (460±25 PSL/mm^2) was similar to that (486±28 PSL/mm^2) obtained with ^{18}F. The background level of the PSL/mm^2 was 1.4±0.13 under the same condition. In *ex vivo*ARG, the accumulation ratio of [^{11}C]Ro15-1788 in the cortex versus cerebellum was 2.4±0.1 by the double-tracer method and 2.5±0.06 by the single-tracer method. Accumulation ratio of [^{18}F]FEtDAA in the cortex versus striatum was 1.2±0.2 by the double-tracer method and 1.6±0.3 by the single-tracer method. From these observations, the double tracer method with two short lived radioisotopes might be applied to the practical evaluation of biodistribution of radiotracers in *ex vivo* or *in vitro* experiments although a slight difference was observed between the single-tracer method and the double-tracer method. This method has the advantages of allowing the experiment to be performed with two radiotracers under the same conditions within a short time.

Keywords: Double-Tracer, Autoradiography, Short-Lived Positron Emitter

NOVEL F-18 LABELLED AMINOALKYLINDOLE WITH POTENTIAL FOR IMAGING NEURONAL CANNABINOID RECEPTOR BY POSITRON EMISSION TOMOGRAPHY

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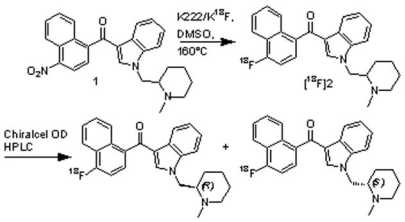
Efforts to develop PET and SPECT radioligands for the CB_1 receptor have focused on finding new ligands with improved binding affinity and reduced lipophilicity. A new series of aminoalkylindoles with high binding affinity (K_i = 0.7-100 nM) at cerebral cannabinoid receptor (CB_1) and lipophilicity ($cLogD_{7.4}$) in the range of 2.1 - 4.5 were synthesized. The binding affinities of the series for CB_1 receptors was determined by measuring the ability of each ligand to compete with [^{3}H]CP55,940 binding in membrane fractions prepared from rat cerebellum.

The racemic ligand **2** with highest affinity within the series (0.7 nM) was radiolabeled with 10% radiochemical yield and high specific radioactivity by radiofluorination of **1** (Fig 1). A chiral HPLC separation of racemic [^{18}F]**2** revealed radiolabeled enantiomers, [^{18}F]**2-1** and [^{18}F]**2-2**.

Figure 1. Radiosynthesis of [^{18}F]**2-1**, [^{18}F]**2-2**

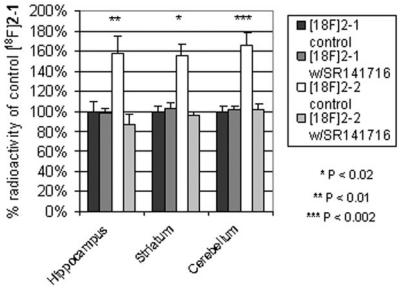
Racemic [^{18}F]**2** and its enantiomers have been studied in vivo. [^{18}F]**2** specifically labeled CB_1 receptors in mouse brain. The patterns of regional distribution of [^{18}F]**2** matched the known distribution of the CB_1 receptor. Thus, the highest accumulation of radioactivity was consistently observed in the hippocampus and the lowest was seen in the brain stem. The ratio of these two tissues (the target-to-non-target ratio) reached the value of 1.6 at 80 min after injection. Preadministration of SR141716 (1 mg/kg), a selective antagonist of CB_1, significantly reduced the radioactivity of [^{18}F]**2** in the brain demonstrating specific binding to the cannabinoid receptor *in vivo*.



The fraction of [^{18}F]**2-2** total brain radioactivity decreased by pre-administration of SR141716 was nearly double that obtained with the racemate [^{18}F]**2**. In contrast, there was no significant effect of SR141716 pre-administration on accumulation radioactivity from [^{18}F]**2-1.** These results suggested the [^{18}F]**2-2** enantiomer was active and [^{18}F]**2-1** was inactive.

Radioactivity accumulation was compared after administration of [^{18}F]**2-1** and [^{18}F]**2-2** in control animals and animals pretreated with SR141716. The data presented in Figure 2 suggests that [^{18}F]**2-2** is a promising PET radioligand for studying CB_1, whereas [^{18}F]**2-1** is a useful indicator of the non-displaceable accumulation of radioactivity for [^{18}F]**2-2**.

Figure 2. Inhibition of radioactivity accumulation of ^{18}F labeled enantiomers, **2-1 and 2-2**, by SR141716 in brain regions enriched with CB_1 receptors in mice. For each enantiomer SR141716 was administered 15 min before the radiotracer in one study, and not administered in another control study. Radioactivity accumulation is expressed as a percent of the control **2-1** radioactivity. Measurements were taken 70 min after injection of the radiotracer. Bars represent mean ± SEM (n=3). P values indicate the significance of the difference of **2-2** control radioactivity from the other measurements in the same tissue.



Keywords: Cannabinoid, Radiotracer, PET

HYPERVALENT IODINE REAGENTS AS PRECURSORS FOR RADIOLABELLING PYRIMIDINES USING N.C.A. [^{18}F]FLUORIDE

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The use of hypervalent iodine complexes as precursors offers a novel method for introducing n.c.a. [^{18}F]fluoride into organic molecules. Despite encouraging results reported with model compounds, the use of hypervalent iodine complexes in PET chemistry has been limited.[1] The purpose of this study was to investigate the efficacy of using hypervalent iodine salts for the preparation of [^{18}F]fluorinated pyrimidines.

Phenyl(*N,N*-dibenzyluracil)iodonium tosylate (**1**) and phenyl(*N,N*-diethylmethylether)uracil iodonium tosylate (**2**) were synthesized in 3 steps from commercially available 5-iodouracil. Treatment of 5-iodouracil with potassium carbonate in DMF followed by benzyl chloride or chloromethylethylether afforded the *N1, N*3-diprotected uracils **3 and 4** (60% and 44% yield). The corresponding 5-boronic acid derivatives were prepared by Mg/I exchange followed by reaction of the uracil Grignard reagent with trimethylborate. After work-up with dilute acid the 5-boronic acid derivatives **5** and **6** were isolated in 66 and 47% yields. Subsequent reactions of **5 and 6** with Koser's reagent, hydroxyl(tosyloxy)iodobenzene, in dichloromethane gave the desired uracil iodonium salts **1 and 2** in 64% and 60% isolated yields, respectively.

[^{18}F]Fluorination was achieved by heating a solution of iodonium salts **1 and 2** in acetonitrile in the presence of [^{18}F]fluoride at 90°C for 40 minutes. [^{18}F]Radiofluorination of **1** afforded [^{18}F]*N,N*-dibenzyl-5-fluorouracil (**7**) in 40% decay corrected yield as determined by HPLC. However, [^{18}F]radiofluorination of **2** was poor, with <5% formation of the desired [^{18}F]*N,N*-diethylmethylether-5-fluorouracil (**8**). In both cases the labelling chemistry is regioselective for the pyrimidine moiety with <5% formation of [^{18}F]fluorobenzene. Deprotection of **7** using boron tribromide was achieved in 10 minutes at 90°C and provided [^{18}F]-5-fluorouracil in near quantitative yield.

In conclusion, we have demonstrated that pyrimidines can be labelled regioselectively with [^{18}F]fluoride in good yields using iodonium salt precursors. The simplicity of this method makes it a highly attractive alternative to electophilic labelling of pyrimidines with [^{18}F]fluorine.

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Keywords: Iodonium Salt, ^{18}F Fluoride, PET

METHODS FOR LABELING THE 5-HT$_{1A}$ RECEPTOR AGONIST, S14506, WITH A POSITRON-EMITTER AT ANY OF THREE ALTERNATIVE POSITIONS

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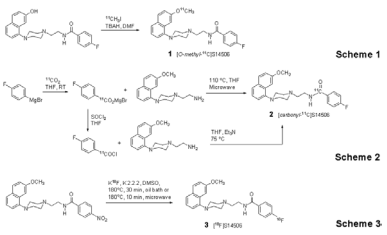
Introduction: S14506 is one of the most potent and selective agonists at 5-HT$_{1A}$ receptors (K_i = 0.98 nM). Lima et al[1] noticed that, as an agonist, S14506 binds to both the G-protein coupled and uncoupled forms of the 5-HT$_{1A}$ receptor with a nanomolar affinity, in contrast with the prototypical 5-HT$_{1A}$ agonist, 8-OH-DPAT, which has a nanomolar affinity for the G-protein coupled form of the receptor only. The similarity between the specific binding of the agonist radioligand [^{3}H]S14506 and that of the antagonist radioligand [^{3}H]WAY-100635 under *in vitro* conditions implies that S14506 could be a potential agonist radioligand for PET imaging of brain 5-HT$_{1A}$ receptors. The aim of this work was to develop methods for labeling S14506 at alternative positions so that [*O-methyl*-^{11}C]S14506 (**1**), [*carbonyl*-^{11}C]S14506 (**2**) and [^{18}F]S14506 (**3**) are readily available for PET imaging and the investigation of radioligand metabolic pathways.

Methods: **1** was obtained by treating its *O-desmethyl* precursor with [^{11}C]iodomethane and TBAH in DMF (Scheme 1) using a 'loop' method[2]. **2** was synthesized through either classical ^{11}C-carboxylation of a Grignard reagent followed by conversion into the [^{11}C]acid chloride for reaction with amine precursor[3] or a microwave-enhanced direct coupling of *in situ* generated [^{11}C]organocarboxymagnesium halide with amine precursor[4] (Scheme 2). **3** was prepared by nucleophilic aromatic fluoridation of the nitro precursor (Scheme 3). The labeled compounds were purified with reverse phase HPLC on a luna C-18 semi-preparative size column using acetonitrile and aqueous ammonium formate as mobile phase.

Results and Discussion: The [^{11}C]*O*-methylation to produce **1** was highly selective; No significant *N*-methylation occurred when reaction was carried out at RT in DMF for 4 min. The total synthesis time was ~35 min. The overall (decay-corrected) radiochemical yields (RCY) of **1** range between 6 and 24% and specific activity (SA) between 1343 and 3101 Ci/mmol (average 2390, n = 30) at EOS. **2** was made in 20% RCY (estimated from radio-HPLC) using the [^{11}C]acid chloride route, and in similar RCY through direct coupling of [^{11}C]organocarboxymagnesium bromide with amine precursor under microwave-enhanced conditions. The latter avoids the need to introduce thionyl chloride into the procedure. Further work to improve radiochemical yields and SA are in progress. In production mode, **3** was prepared with 20% RCY (decay-corrected) and SA at 1735 Ci/mmol (n = 2) for a total synthesis time of 130 min. Heating the reaction mixture for 10 min at 180 °C in a single mode microwave cavity gave a similar RCY to heating for 30 min in oil bath at the same temperature. The compound is chemically stable up to 200 °C in DMSO.


Scheme 1

Scheme 2

Scheme 3

Conclusion: S14506 can be labelled with carbon-11 or fluorine-18 at any one of three different positions in acceptable RCY and SA for PET imaging.

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Keywords: Carbon-11, Fluorine-18, Radiosynthesis

SYNTHESIS AND REACTIVITY OF NOVEL ^{18}F-LABELED ARYLSULFONATE ESTERS OF 2-FLUOROETHANOL

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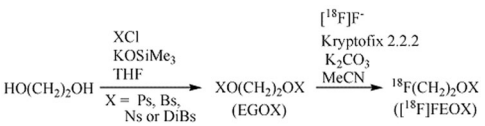
Introduction. Of interest to our group are radioligands that possess an [^{18}F]2-fluoroethyl group such as the dopamine uptake tracer, [^{18}F]FECNT,[1] and the β-amyloid probe, [^{18}F]FEM-IMPY[2]. [^{18}F]2-Fluoroethyl tosylate (FEOTs), due to its ease of synthesis via a commercially available precursor and its stability toward purification, has featured as a labeling agent in this work. However, little work has been reported on the synthesis of other [^{18}F]2-fluoroethyl arylsulfonates that bear a less electron-rich aryl group, even though these might offer enhanced reactivities towards weak nucleophiles (Nuc). Here we set out to prepare a series of such labeling agents and to compare their abilities to alkylate various Nuc (2β-carbomethoxy-3β-(4-chlorophenyl)-nortropane (CNT); 6-iodo-2-(4′-*N*-methylamino)phenylimidazo[1,2-*a*]pyridine (HM-IMPY); *p*-nitrophenol).

Experimental. *Bis*-ethylene glycol arylsulfonates (EGOX; X = benzenesulfonyl, Ps; brosyl, Bs; nosyl, Ns; 3,4-dibromobenzensulfonyl, DiBs) and 2-fluoroethyl arylsulfonates (FEOX) were prepared by treating the appropriate alcohol with arylsulfonyl chloride and $KOSiMe_3$ in THF (**Scheme**).

[^{18}F]2-Fluoroethyl arylsulfonates ([^{18}F]FEOX) were prepared from cyclotron-produced [^{18}F]fluoride (**Scheme**) in an adapted Synthia radiochemistry module that enabled fine control of reaction parameters. Thus, EGOX (8.1 μmol) was reacted with dry [^{18}F]fluoride kryptofix 2.2.2/K_2CO_3 in acetonitrile (MeCN) at 110 °C for 5-10 min. Product [^{18}F]FEOX was separated by reverse phase HPLC, isolated by solid phase extraction (SPE) and reacted for 10 min at 130 °C with Nuc (2.7 μmol) in 1 mL of MeCN or MeCN-DMF (5:1 v/v) for *p*-nitrophenol (1 equiv. TBAH). Radioalkylations were carried out in an open tube with He bubbling. In an efficient synthesis of [^{18}F]FECNT, [^{18}F]FEODiBs (t_R = 5.3 min) was well separated from EGODiBs (t_R = 7.7 min) on an Adsorbosphere UHS C-18 column using MeCN-water (9:1 v/v) as eluent and collected in a tube containing CNT (2.7 μmol) for reaction as described above. Each labeled product ($n \geq 2$) was separated by HPLC and measured to calculate isolated decay-corrected radiochemical yield (RCY).



Scheme. Synthesis of [^{18}F]2-fluoroethyl arylsulfonates

Results. EGOX and FEOX were obtained in 32-65% yield. Regardless of substitution pattern, [^{18}F]FEOX were obtained in similar RCYs (51-57%). Reaction of [^{18}F]FEOPs, FEOTs, FEOBs or FEONs with CNT gave [^{18}F]FECNT in 64-75% RCY (**Table 1**). The highest RCYs of [^{18}F]FECNT were obtained using HPLC-purified [^{18}F]FEODiBs either directly or after SPE isolation. When HM-IMPY or *p*-nitrophenol was treated with [^{18}F]FEOX, RCYs were higher for X = Bs or Ns than for X = Ts, while [^{18}F]FEODiBs again gave the highest RCY for these weaker Nucs.

Table 1. RCYs of reaction of [F-18]FEOX with Nuc.

[F-18]FEOX (X)	Nuc	Product	RCY (%)
Ps, Ts, Bs or Ns	CNT	[F-18]FECNT	64-75
DiBs*	"	"	84
DiBs	"	"	87
Ts	HM-IMPY	[F-18]FEM-IMPY	3
Bs	"	"	17
Ns	"	"	29
DiBs	"	"	38
Ps	4-nitrophenol	[F-18]FE 4-nitrophenyl ether	23
Ts	"	"	19
Bs	"	"	34
Ns	"	"	50
DiBs	"	"	63

*No SPE isolation of [F-18]FEODiBs.

Conclusion. Owing to the stability of EGOX containing electron-withdrawing groups and the high reactivity of the resulting [^{18}F]FEOX, such agents should be considered as alternatives to [^{18}F]FEOTs. In particular, [^{18}F]FEODiBs shows excellent utility owing to its high reactivity and ease of purification.

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Keywords: Fluorine-18, Sulfonate Ester, Labeling Agent

[^{11}C]AR-A014418: A RADIOLABELLED GLYCOGEN SYNTHASE KINASE 3-SPECIFIC INHIBITOR FOR PET STUDIES

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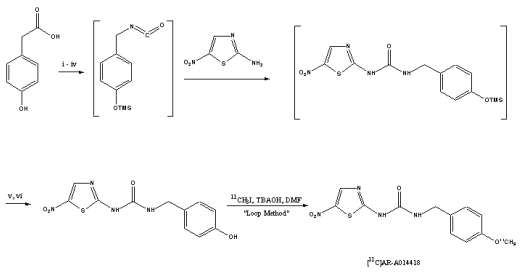
Glycogen synthase kinase 3 (GSK-3) is a serine/threonine kinase that is highly abundant in brain tissues and involved with signal transduction cascades of multiple cellular processes. GSK-3 is well known to promote cell death, thus, the realization that excessive levels of GSK-3 are associated with the onset of several neurological diseases has prompted efforts to develop GSK-3 inhibitors as therapeutics.[1] Small molecule inhibitors of GSK-3 are being developed for a broad range of illnesses including depression, diabetes, Alzheimer's disease, stroke and bipolar disorder and malignancy. Thus, there is a great need to measure the localization, concentration and affinity of GSK-3 in the living human brain of both healthy and clinical populations.

The synthesis and in vitro evaluation of AR-A014418 was recently reported and is the most potent and selective small-molecule inhibitor of GSK-3.[2] This is a major advance because previously developed ligands lack selectivity for GSK-3. The goal of the present work is to synthesize [^{11}C]AR-A014418 for PET studies.

While demethylations of anisoles to phenols are standard organic transformations, it is a difficult procedure in the presence of a nitro-group.[3] In addition to containing a nitro group, AR-A014418 presents a further complication as 2-thiazolamines are highly susceptible to substitution reactions. A variety of demethylation conditions were attempted in the present work;not surprisingly all resulted in very low yields and/or halogenation at the thiazole moiety. In light of these results, a one-pot synthesis of desmethyl AR-A014418 was developed by the reaction of in situ generated TMS-protected 4-hydroxybenzylisocyanate[4] with excess 2-amino-5-nitrothiazole (Scheme 1). The desired desmethyl AR-A014418 was obtained in 23% yield by this route. This synthetic route will be extended to other derivatives of AR-A014418.

Scheme 1. Synthesis of desmethyl AR-A014418 and [^{11}C]AR-A014418. Reagents: (i) TMS-Cl, Et_3N, Toluene, reflux; (ii) $SOCl_2$, CH_2Cl_2, reflux; (iii) TMS-N_3, dioxane, r.t.; (iv) reflux (Curtius rearrangement); (v) 2N HCl; (vi) Silica gel purification.

Desmethyl AR-A014418 was reacted with [^{11}C]CH_3I, in the presence of tetrabutylammonium hydroxide (TBAOH), to produce [^{11}C]AR-A014418 using the "loop method" developed by our group.[5] Following the methylation reaction, [^{11}C]AR-A014418 was purified by preparative HPLC and formulated in saline with an average decay-corrected radiochemical yield of 16%, based on [^{11}C]CO_2. The development of [^{11}C]AR-A014418 as a radiopharmaceutical has been successful (n = 3; 22 mCi produced) with >98% radiochemical purity and >850 Ci/mmol specific activity after a 30 min synthesis time. The radiochemistry is presently being optimized and in vivo biodistribution studies with [^{11}C]AR-A014418 are underway in our laboratory.



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Keywords: Glycogen Synthase Kinase 3, Carbon-11, AR-A014418

MICROPET IMAGING OF THE BRAIN SEROTONIN TRANSPORTER WITH [^{11}C]*m*ZIENT

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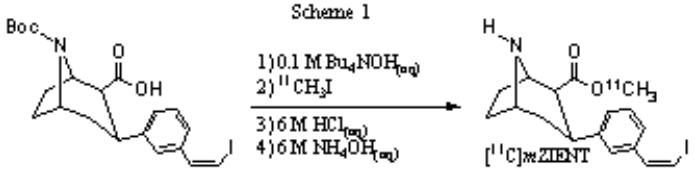
The serotonin transporter (SERT) resides on presynaptic serotonergic neurons and functions to remove serotonin from the synapse. A reduction in SERT density has been attributed to neuropsychiatric disorders such as depression and suicide and, therefore, the SERT is the target of antidepressants (SSRI's). Because CNS SERT serves as a marker for serotonergic neuronal anatomy and integrity the ability to image CNS SERT with PET may aid in the diagnosis and maintenance of depression by allowing *in vivo* measurement of SERT density in specific brain regions. As part of an ongoing research project in our group to develop tropane-based SERT imaging agents we have been investigating the monoamine transporter binding of 2β-carbomethoxy-3β-(3′-((*Z*)-2-iodoethenyl)phenyl)nortropane (*m*ZIENT) and the microPET imaging properties of [^{11}C]*m*ZIENT.

*m*ZIENT was synthesized by Pd^0-catalyzed coupling of 2β-carbomethoxy-3β-(3′-bromophenyl)nortropane with (*Z*)-1,2-bis(trimethylstannyl)ethylene followed by iododestannylation with ICl. *In vitro* competition binding assays with HEK cells stably expressing the SERT, dopamine transporter (DAT), or norepinephrine transporter (NET) afforded the following inhibition constants (K_i): SERT 0.3 nM, DAT 35 nM, NET 102 nM. The radiolabeling precursor was prepared by hydrolysis of *m*ZIENT in refluxing H_2O/1,4-dioxane followed by *N*-Boc protection. Radiolabeling (Scheme 1) was achieved by *O*-alkylation of the carboxylate salt with $^{11}CH_3I$ in DMF, acid-catalyzed cleavage of the *N*-Boc group, neutralization, and HPLC purification to afford [^{11}C]*m*ZIENT with an average decay corrected yield of 18 % (from end of $^{11}CH_3I$ synthesis) and an average radiochemical purity of 99 %. The octanol/H_2O partition coefficient of [^{11}C]*m*ZIENT was determined to be $\log P_{7.4}$ = 1.75.

Brain imaging was performed in anesthetized monkeys with a Concorde microPET P4. Analysis of the images indicated a high uptake in SERT-rich brain regions with the following ratios to cerebellum

Scheme 1



uptake at 65 min post-injection: caudate = 1.32, putamen = 1.62, thalamus = 1.36, midbrain = 1.50, pons = 1.11, occipital lobe = 1.09, and frontal lobe = 0.99. A chase study with the SERT-selective ligand (*R,S*)-citalopram HBr (1.5 mg/kg) at 60 min p.i. displaced [^{11}C]*m*ZIENT from the SERT-rich regions of the brain whereas a chase study with the DAT-selective ligand methylphenidate (0.3 mg/kg) showed no displacement of [^{11}C]*m*ZIENT.

In conclusion, *m*ZIENT is a high affinity ligand for the SERT and is selective for the SERT over the DAT and NET. MicroPET imaging with [^{11}C]*m*ZIENT showed high uptake in the SERT-rich regions of the brain at 65 min p.i. This uptake was displaceable with citalopram but not methylphenidate.
Supported by NIMH.

Keywords: Tropane, Brain Imaging, Serotonin Transporter

SYNTHESIS OF [^{18}F]-FLUOROQUINOLINES FOR THE LABELLING OF A TALNETANT ANALOGUE AS NK-3 RECEPTOR LIGAND

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Human NK-3 receptor (h-NK-3-r) belongs to the superfamily of G-protein-coupled seven transmembrane-spanning receptors and mediates the action of neurokinin B. It is characterized by a predominent expression in the brain[1] and numerous data suggests its involvment in the modulation of central monoaminergic systems, and in various disorders such as psychosis, anxiety, and depression[2]. No radioligand for PET or SPECT studies have been developed so far.

Talnetant is a highly potent and selective NK-3 receptor antagonist (Scheme 1).[3] It has been showed metabolically stable and able to cross the blood brain barrier. Recently, we synthesized several Talnetant analogues bearing a fluorine atom onto the quinoline ring and we found that the presence of fluorine at the 8-position did not altered affinity at NK-3 receptor.[4] With the aim of studying h-NK3 receptor by PET, we envisaged the labelling with fluorine-18 of this analogue. To our knowledge, the synthesis of 5-[^{18}F]-fluoro-6-nitroquipazine was the sole example of introduction of a fluorine-18 atom onto a quinoline ring.[5] This latter occurred by displacement of a chlorine atom of the chloronitroquinoline precursor by no carrier-added [^{18}F]fluoride ion as its activated K[^{18}F]F-K_{222} complex. We turned to the nucleophilic aromatic substitution of a nitro group, and we examined the radiofluorination of nitroquinolines **1-6** bearing or not in alpha a carboxaldehyde function as activating electron withdrawing group. All reactions were performed in DMSO. Parameters including the quantity of the precursor used (5-20 mg in 0.4-1 mL of DMSO), the type of activation (conventional heating or microwave irradiation), the temperature (110-180 °C), the microwave power (40-300 W) and the reaction time (2-60 min), were studied.

Starting from nitroquinolinecarboxaldehydes **1b-6b**, no incorporation of fluorine-18 could be detected whatever the conditions used. A rapid decomposition of the starting material was observed even at 110 °C. Using conventional heating at 180 °C for 2-5 min, quinolines **1a-3a** were converted into the corresponding [^{18}F]-fluoroquinolines in radiochemical yields ranging from 55 to 65%. Under the same conditions, Talnetant precursor **4a** bearing the secondary amide function, was found unreactive towards the fluoro exchange (radiochemical yields < 10%). Protection of this latter with a Boc group did not allow to improve yields. Again, degradation of the starting material **5a** was observed. The use of the benzylated compound **6a** made the fluorination successful, yields reaching 20-35%. Full details will be reported and total synthesis of [^{18}F]-fluoroanalogue of Talnetant will be described.

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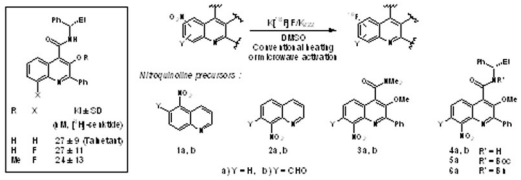

Keywords: Fluorine-18, NK3 Receptor, Fluoroquinoline

STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF IMPY DERIVATIVES AS CANDIDATE RADIOLIGANDS FOR β-AMYLOID

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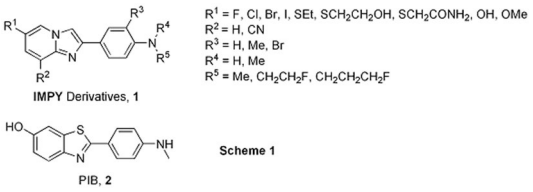
Introduction: We have evaluated the tertiary amines, [^{18}F]FEM-IMPY [*N*-(2-fluoroethyl)-4-(6-iodo-*H*-imidazo[1,2-*a*]pyridin-2-yl)-*N*-methylbenzeneamine] and its 3-fluoropropyl analog, [^{18}F]FPM-IMPY, as b-amyloid radioligands [1]. After *i.v.* injection of either radioligand into rodent or monkey there is a rapid and adequate uptake of radioactivity into brain followed by biphasic clearance. Metabolism is rapid *via* de-alkylation of the tertiary aromatic amino group, culminating in defluoridation and high uptake of radioactivity in bone. With a view to avoiding rapid defluoridation and residual brain radioactivity, reducing lipophilicity and increasing binding affinity, we decided to make use of 'isosteric' and 'isoelectronic' effects in the design of further analogs of IMPY. Here, we report the syntheses and SAR relationship of these derivatives in our effort to develop useful radioligands for detecting brain A-beta amyloid aggregates.

Results & Discussion: The binding affinities for the different IMPY derivatives (Scheme 1) are summarized in Table 1. Comparison of the Ki values of **1a, 1k** and **1l** shows that the "isosteric effect" works partially. If we consider Br and Me are similar in size, it appears that a polar group increases binding. Similar analysis of the binding affinities of **1a-1d** shows that the thiol ether group provides optimal electronic and steric effects for binding, but the effect disappears as soon as a hydrophobic group is attached (*c.f.* results for **1e-1g)**. Increasing the size of substituents on the aromatic amino group decreases binding affinity, as reflected in **1a, 1n** and **1c**. However, there is substantial requirement for size in the 6-position (*c.f.* **1c, 1h** and **1i**). For **1h**, the combination of small and polar properties for the 6-substituent abolishes all binding affinity. Interestingly, introduction of a CN group into 8-position also completely removes the binding affinity (as in **1j**), reflecting minimal tolerance for any substituents at the periphery of the IMPY skeleton.

Conclusion: We have identified strategies to overcome problems encountered in our initial investigation with IMPY derivatives as radioligands for b-amyloid. Through this *in vitro* evaluation, a promising candidate, **1d,** appears for evaluation as a potential PET radioligand. The SAR study identifies that the binding site typical of 6-OH-BTA-1 (PIB) is a relatively small site, with only the 6-position in IMPY derivatives tolerating substantial structural change.



Scheme 1

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Table 1. IMPY derivatives

Ligand	R1	R2	R3	R4	R5	cLogD7.4	Ki (nM)	
1a (IMPY	I	H	H	Me	Me	4.53±0.66	73±11	
1b	Cl	H	H	Me	Me	4.13±0.62	23±4	
1c	F	H	H	Me	Me	3.55±0.66	49±7	
1d	SEt	H	H	Me	Me	4.37±1.17	17±2	
1e	SCH2CH2OH		H	H	Me	Me	3.10±1.22	>1000
1f	SCH2CONH2		H	H	Me	Me	2.14±1.20	>1000
1g	SCH2CONH2		H	Me	H	Me	2.27±1.20	>1000
1h	OH	H	H	Me	Me	1.53±1.02	>1000	
1i	OMe	H	H	Me	Me	3.34±0.93	154±18	
1j	Br	CN	H	Me	Me	3.27±1.09	>1000	
1k	Br	H	Br	H	Me	4.42±0.72	31±3	
1l	Br	H	Me	H	Me	4.29±0.66	>1000	
lm	Br	H	Me	H	H	3.40±0.66	>1000	
1n	I	H	H	Me	CH2CH2F	4.68±0.71	55±6	
1o	I	H	H	Me	CH2CH2CH2F		5.07±0.71	420±158

Keywords: beta-Amyloid, Structure Activity Relationship, IMPY-Derivatives

SYNTHESIS AND *IN VIVO* EVALUATION OF C-11 LABELED MAZINDOL ANALOGS FOR IMAGING THE NOREPINEPHRINE TRANSPORTER WITH PET

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Objectives: We have synthesized and evaluated several new ligands for imaging the norepinephrine transporter (NET) system in baboons with PET [1,2]. 5-(4-Chlorophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]isoindole (mazindol) is one of the most potent NET ligands. The fact that mazindol has not been considered as a candidate for the development of a NET selective PET ligand is perhaps due to its poor binding selectivity (high affinity towards the dopamine transporter (DAT) as well as the serotonin transporter (SERT)), and the lack of suitable positions for labeling with C-11. A recent report by Houlihan et al. [3] showed: (1) the deschloro analog of mazindol (deschloromazindol) preserves high affinity towards NET, though with a substantially decreased affinity towards DAT and SERT; (2) 6-methoxy substituted mazindol (6-OCH_3-mazindol) increases its affinity towards NET, but with a much decreased affinity towards SERT. Based on these results, we selected two deschloro- and 6-methoxy-substituted analogs of mazindol (6-OCH_3-DCM and 6-OCH_3-t-OCH_3-DCM) and evaluated their potentials as NET ligands for PET studies in baboons.

Methods: 6-OCH_3-DCM, 6-OCH_3-t-OCH_3-DCM, and their corresponding normethyl precursors were synthesized via multi-step synthetic approaches. The radiosynthesis of [^{11}C]6-OCH_3-DCM and 6-[^{11}C]OCH_3-t-OCH_3-DCM were performed by simple alkylation of the corresponding normethyl precursors with no-carrier-added [^{11}C]CH_3I in DMF. The logP values were measured by a conventional extraction method. In vivo evaluation of both radiotracers was carried out with PET in baboons.

Results: [^{11}C]6-OCH_3-DCM and 6-[^{11}C]OCH_3-t-OCH_3-DCM were isolated by semipreparative HPLC in 68-76 % and 80-88 % radiochemical yields, respectively, in a synthesis time of 36 min from the end of bombardment (EOB) with radiochemical purities of > 97 %. The specific activities for both tracers were 2.0-9.3 Ci/μmole (EOB). PET studies showed that the uptake of [^{11}C]6-OCH_3-DCM in baboon brain was low, possibly due to its low logP value (0.84 ± 0.02). Transforming the tertiary hydroxyl group of 6-OCH_3-DCM into a methyl ether significantly increased its lipophilicity, which was evidenced by an increased logP value of the resulting 6-OCH_3-t-OCH_3-DCM (2.25 ± 0.03). As a result, higher uptake of 6-[^{11}C]OCH_3-t-OCH_3-DCM was observed in NET-rich tissues including thalamus and cerebellum. The specific binding of 6-[^{11}C]OCH_3-t-OCH_3-DCM to NET, and its selectivity over dopamine transporter and serotonin transporter, are currently under investigation.

Conclusions: We have prepared [^{11}C]6-OCH_3-DCM and 6-[^{11}C]OCH_3-t-OCH_3-DCM in high radiochemical yields with high radiochemical purity and specific activity. With higher logP value and high uptake in NET-rich tissues in baboon brain, 6-[^{11}C]OCH_3-t-OCH_3-DCM may have potential as a PET tracer for imaging NET.

Supported by DOE-OBER and NIH.

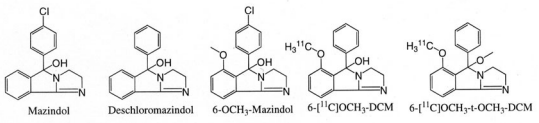

Figure 1. Chemical structures of mazindol analogs.

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Table 1. Affinity (Ki, nM) and selectivity of mazindol analogs for DAT, SERT and NET.

NET Inhibitors	DAT	SERT	NET	DAT/NET	SERT/NET
Mazindol	43	94	4.9	8.8	19
Deschloromazindol	730	2140	2.8	261	764
6-OCH3-Mazindol	60	3600	1.7	35	2118

Keywords: Norepinephrine Transporter, Mazindol, PET

N.C.A. ^{18}F-FLUORINATION OF VARIOUS ARENES *VIA* ARYL(2-THIENYL)IODONIUM SALTS

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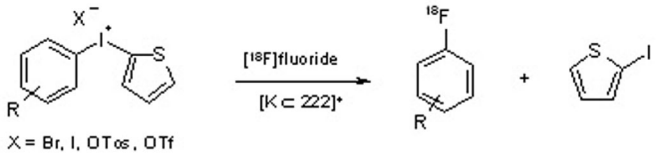
Diaryliodonium salts are known as suitable precursors for single-step nucleophilic ^{18}F-fluorination of arenes [1-4]. Especially their ability to introduce n.c.a. [^{18}F]fluoride in electron rich arenes without further activating groups induced a growing interest in these precursors [1, 2].

The nucleophilic substitution with the n.c.a. [^{18}F]fluoride proceeds via the S_NAr-mechanism and yields [^{18}F]fluoroarenes and the corresponding iodoarenes. The ^{18}F-introduction is influenced by electronic effects due to the fact that the electron deficient ring of the diaryliodonium salt is preferred for the nucleophilic attack. Also, *ortho*-substituents show a directing steric effect (so-called *ortho*-effect) and enhance the ^{18}F-labelling of the *ortho*-substituted ring [5]. Iodonium salts containing the 2-thienyl group as highly electron rich group were earlier found to lead to highly regioselective fluorination with non-radioactive nucleophiles [6, 7] and were synthesised here as precursors for ^{18}F-labelling of various arenes (Scheme).

The electron rich precursors *ortho*-, *meta*- and *para*-methoxyphenyl-2-thienyl-iodonium salts were synthesised as their bromides, iodides, tosylates and triflates. An additional set of iodonium salts (R = 4-CH_3, 4-OBn, H, 4-I, 4-Br, 4-Cl) was prepared as their bromides to form a series of precursors with decreasing electron density in the target ring. The best results for electron rich [^{18}F]fluoroarenes were obtained with freshly prepared 2-methoxyphenyl(2-thienyl)iodonium bromide.



X = Br, I, OTos, OTf
R = 2-OMe, 3-OMe, 4-OMe, 4-CH_3, 4-OBn, H, 4-I, 4-Br, 4-Cl

Under optimised conditions a RCY of up to 60 % was achieved in DMF at 130 °C and 20-25 min reaction time. Here the strong influence of the *ortho*-substituent is observable. Although it is a deactivated arene, the RCY almost approximates that of the highly activated electron poor arenes 4-Br (68 %) and 4-Cl (64 %). All ^{18}F-labelling reactions showed a strong dependence on the general reaction conditions. In case of the temperature only in the range of 125 to 135 °C satisfactory yields are attained. Beyond this range the RCY decrease rapidly due to thermal instability of the iodonium salts, which competes with the ^{18}F-introduction.

The series of precursors with decreasing electron density in the target ring showed that the electronic character of the substituents strongly influences the kinetics and leads to higher reaction rates, according to the series 4-Cl > 4-Br > 4-I > H > 2-OMe > 4-CH_3 > 4-OBn > 4-OMe > 3-OMe. Only the 2-OMe derivative stands out, which is probably due to the *ortho*-effect. All precursors confirmed a very high regioselectivity up to regiospecificity and led to the desired n.c.a. [^{18}F]fluoroarenes without any radioactive side-products.

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Keywords: Iodonium Salts, Radiofluorination, Fluoroarenes

RADIOSYNTHESIS OF 1-[^{11}C]-D- AND L-LACTIC ACID AS POTENTIAL MARKERS OF NEURONAL LACTATE METABOLISM

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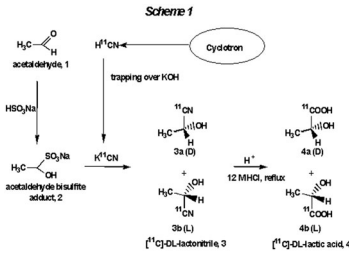
Introduction: Lactate may be a (preferential) energy substrate for neuronal function. While L-lactate as the physiological enantiomer is fully metabolized to CO_2, D-lactate may be transported into neurons by the same mechanism but its further metabolism could be slowed down.

Radiochemistry: Racemic [^{11}C]lactic acid (**4**) (*Scheme 1*) was made using the *GE PETtrace* to produce [^{11}C]HCN. [^{11}C]HCN was collected as [^{11}C]KCN on a trap coated with 0.5 M KOH. [^{11}C]KCN was washed from the trap with a solution of acetaldehyde bisulfite adduct (**2**) and after 5 min at room temp. [^{11}C]-dl-lactonitrile (**3**) was hydrolyzed by boiling with conc. HCl for 5 min.

To remove HCl and salts the reaction mixture was injected onto a polymeric HPLC column (Phenomenex, Polymerx 10µ, 250x10) and eluted with 0.03% H_3PO_4. [^{11}C]-dl-lactic acid (**4**) was collected for separation of the enantiomers (**4a** and **4b**) by ligand exchange chromatography. The large volume (3–4ml) of [^{11}C]lactic acid (**4**) required a high capacity chiral ligand exchange HPLC column, which was not commercially available. We developed a new chiral selector for ligand exchange HPLC separation of a-hydroxy acids and used it to coat a preparative RP C_{18} column. Thus, we achieved an excellent resolution of the [^{11}C]lactic acid (**4**) enantiomers (**4a** and **4b**, Figure 1).



The eluate with [^{11}C]-D- or [^{11}C]-L-lactic acid, was passed through a Sep-Pak® anion exchanger cartridge (Accell™ Plus QMA, in CO_3^{2-} form) where the Cu^{2+} ions (as insoluble carbonate) and the [^{11}C]lactate are retained. The latter was selectively washed from the cartridge with phosphate buffer pH 7.4 to obtain an injectable solution of enantiomerically pure Na-[^{11}C]lactate. The quality control of the final product was done by chiral ligand exchange HPLC.

Biology: [^{11}C]-D-lactate total cerebral ^{11}C activity in the cortex of 5 male Sprague-Dawley rats was measured (TAC) along with the arterial input function. A one-tissue compartment model (K1, k2) was fitted. HPLC showed that the only metabolite was [^{11}C]CO_2. The fraction of [^{11}C]CO_2 increased from 24 to 77%. K1 and k2 were 0.08±0.01 [ml/min/ml] and 0.07±0.02 [min^{-1}]. At a flow F of 0.5 ml/min/g the extraction fraction K1/F was 16%.

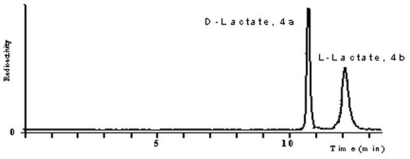


Figure 1. Preparative chiral ligand exchange radio-HPLC separation of 1-[^{11}C]-D-lactic acid (4a, t_R 10.8 min, 2.4 GBq) and 1-[^{11}C]-L-lactic acid (4b, t_R 12.2 min, 2.4 GBq) on a preparative column Luna (C_{18}, 250x21 mm, 5µ, Phenomenex) coated with the L-penicillamine derived chiral selector, 1 mM $CuSO_4$, 9 ml/min.

Conclusion: 1-[^{11}C] labeled D(*R*)- and L(*S*)-2-lactic acid) can be synthesised in about 45 min and leads to nearly 50% decay corrected overall radiochemical yields of each enantiomer with > 99% radiochemical and enantiomeric purity. The specific activity at the end of the synthesis is about 40 GBq/mmol.

[^{11}C]-D-lactate is a new tracer for neuronal lactate metabolism. The occurrence of only one metabolite facilitates the tracer kinetic modeling. It is not yet clear whether the [^{11}C]CO_2 is produced in the brain. The EF of [^{11}C]-D-lactate is big enough to perform *in vivo* experiments.

Keywords: D- L- Lactic Acid, 11C-Labeling, Neuronal Metabolism

SYNTHESIS AND *IN VIVO* CHARACTERIZATION OF ^{76}Br-LABELED FLUTAMIDE AND BICALUTAMIDE ANALOGS

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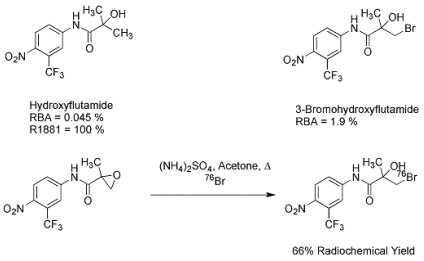
Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in American men. The role of androgens in prostate tumor growth has been well established and androgen receptors are expressed in most primary and metastatic prostate cancers.[1] Androgen receptor ligands capable of external diagnostic imaging of tumor sites using positron emission tomography (PET) have been developed.[2] To date however, most of these studies have focused on using fluorinated steroid ligands with few studies utilizing known non steroidal anti-androgens as the basis for the diagnostic agent.[3]

Flutamide and Bicalutamide are the leading anti-androgens used for the treatment of prostate cancer. Hydroxyflutamide is a potent anti-androgen although it has fallen out of favor because the androgen receptor protein can readily mutate into a form that recognizes this antagonist as an agonist.[4]

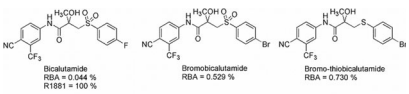
Binding affinity and Synthesis of [^{76}Br] 3-Bromohydroxyflutamide

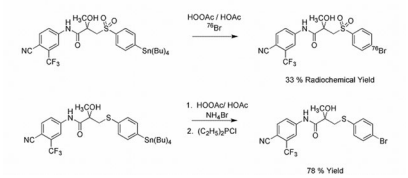
Bicalutamide on the other hand, while it does not bind as tightly to the androgen receptor as testotsterone and dihydrotestosterone, has little to no agonist activity.[5] Resistance to this agent develops more slowly. Additionally it binds prefentially to receptors located outside of the central nervous system, and thus causes little increase in testosterone levels. It is also well tolerated with few side effects. We have prepared one of these analogs in ^{76}Br-labeled form, by a highly efficient electrophilic bromination of a trialkyltin precursor. The tissue distribution of the compound has been studied in androgen-depleted rats.



Binding affinity and Syntheses of [^{76}Br] -Bromobicalutamide and Bromo-thiobicalutamide Analogs.

We are grateful for support of this research through grants from the Department of Energy (DE FG02 86ER60401 to J.A.K.) and (DE FG02 84ER60218 to M.J.W.) and the National Institutes of Health (PHS 1R24 CA86307 to M.J.W.).





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Keywords: Non-Steroidal Androgens, 76-Br, Prostate Cancer

EVALUATION OF [^{11}C]FORMYL CHLORIDE AS A CARBON-11 FORMYLATION AGENT: SYNTHESIS OF *N*-(4-TOLYL)-[^{11}C]FORMAMIDE AS A MODEL REACTION

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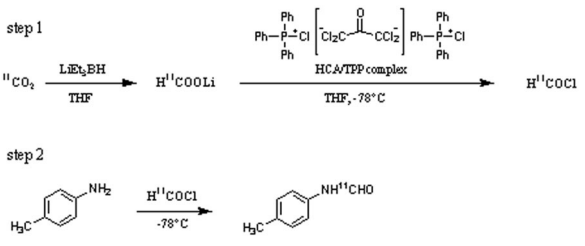
Formyl chloride was generated for the first time in 1963 (1). This acid chloride of formic acid is stable below -60°C. At higher temperatures it dissociates spontaneously into carbon monoxide and hydrochloric acid. Recently, a new, highly efficient method for the preparation of formyl chloride at -78°C appeared in the literature (2): A complex made of hexachloroacetone (HCA) and two equivalents of triphenylphosphine (TPP) reacted at -78°C with formic acid to give formyl chloride, which was subsequently used in *N*-formylation reactions.

As part of a project to evaluate [^{11}C]formic acid as a precursor for labelling, we have already employed this reaction successfully to produce [^{11}C]carbon monoxide via [^{11}C]formyl chloride at ambient temperature (3). Here we present the preliminary results of our efforts to use [^{11}C]formyl chloride at low temperature in an *N*-formylation reaction.

We chose 4-tolylamine as a model compound which, in the original paper on this reaction (2) gave N-formylation in 88% yield. [^{11}C]Carbon dioxide (~20 mCi) was converted into [^{11}C]formic acid (3) by bubbling it into 0.12M $LiEt_3BH$ in THF (~60 µL) at -10°C and radioactivity in the reaction vial was measured. The reaction mixture was then cooled in a dry-ice/acetone bath (inert atmosphere) and the HCA/TPP complex in THF (0.33M, 100 µL (3)) was added slowly. After one minute, 4-tolylamine in THF (1M, 100 µL) was added slowly and the mixture was left for 5 minutes at -78°C. Finally, the temperature was allowed to rise to ambient in 5 minutes and the reaction mixture was analyzed by HPLC (C18 XTerra; eluent: acetonitrile/water 1/1).



The technical difficulty of keeping the temperature below -60°C while adding reagents to a small volume had not yet been overcome during these preliminary experiments and important amounts of [^{11}C]carbon monoxide were released (70-90% of total radioactivity) by thermal decomposition of [^{11}C]formyl chloride (3). The best yield of *N*-(4-tolyl)-[^{11}C]formamide, which we express here relative to the non-volatile radioactivity, was 47%. In other instances, when the yield was lower, a major unknown labelled side product was observed (20-70%) which possibly arose either from direct coupling of [^{11}C]formyl chloride with the HCA/TPP complex, which necessarily was present in excess, or via N-chlorination of the 4-tolylamine by this complex. A possible remedy to this problem could be the addition of another, non-labelled, acid, e.g. benzoic acid, to take care of the excess of the HCA/TPP complex.

Our results show that [^{11}C]formyl chloride potentially is a new carbon-11 labelling reagent that could be used in various formylation reactions. Work on fine-tuning and control of the reaction conditions are currently underway.

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2 Villeneuve GB, Chan TH. *Tetrahedron Lett 1997*; **38**:64
3 Roeda D, Crouzel C, Dolle F. *Radiochim Acta* 2004; **92**:329-332

Keywords: [^{11}C]formic Acid, [^{11}C]formyl Chloride, N-Formylation

EFFICIENT [^{18}F]FLUORINATION OF HYNIC-[TYR^3]-OCTREOTATE (HYNICTATE) *VIA* HYDRAZONE FORMATION

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Routine application of [^{18}F]-labeled ligands for in vivo receptor imaging with PET is limited by the current lack of rapid and high-yield [^{18}F]-labeling strategies. Here we present a two-step [^{18}F]-labeling methodology based on the linkage of an aldehyde, p[^{18}F]fluorobenzaldehyde ([^{18}F]FB), to a hydrazinonicotinamide (HYNIC) derivatized ligand by hydrazone formation. [^{18}F]-fluorination via hydrazone formation was optimized using a model peptide and the optimized conditions were applied to the [^{18}F]-fluorination of HYNIC-[Tyr^3]-octreotate (HYNICTATE) for PET imaging of neuroendocrine tumors.

Methods: HYNIC was linked to a model peptide of 14 amino acids (p12A). [^{18}F]FB was prepared from p-trimethylammonium-benzaldehyde triflate by reacting with dried [^{18}F]-fluoride cryptate for 10 minutes at 120 °C. The reaction of [^{18}F]FB with the HYNIC-derivatized model peptide was investigated as a function of time, peptide concentration, temperature and pH. Subsequently, HYNICTATE was [^{18}F]-fluorinated under optimized conditions and purified using RP-HPLC. The biodistribution of [^{18}F]-octreotate was investigated in rats with or without coadministration of an excess of unlabeled octreotide.

Results: The overall synthesis time of [^{18}F]FB including purification was 30 min with an overall decay corrected radiochemical yield between 65% and 85% and a radiochemical purity exceeding 95%. Formation of [^{18}F]FB-HYNIC-p12A proceeded to its equilibrium within 30 minutes. Highest radiochemical yields were obtained at peptide concentrations of 0.5 mM or higher and at a moderate reaction temperature of 50 °C. The optimal pH was between 3.0 and 4.2. Consequently, [^{18}F]FB was conjugated to HYNICTATE at these optimized conditions. Reversed phase HPLC conditions were chosen to separate [^{18}F]-octreotate from both unlabeled HYNICTATE and unconjugated [^{18}F]FB in a single run. In rats, counting of the dissected tissues at 1.5 h p.i. revealed highest uptake of [^{18}F]-octreotate in SSTR2 receptor-positive tissues: pancreas (0.87 ± 0.03 %ID/g) and adrenals (0.35 ± 0.05 %ID/g). Receptor blocking revealed decreased uptake in the pancreas (0.10 %ID/g) and the adrenals (0.054 %ID/g), demonstrating specific uptake in these organs. The radiolabel cleared rapidly from the blood (0.020 ± 0.003 % ID/g at 1.5 h p.i.), resulting in high tissue-to-blood ratios in receptor-positve organs: pancreas-to-blood = 45 ± 8 and adrenals-to-blood = 18 ± 1. Accumulation in the kidneys was low (0.10 ± 0.01 %ID/g).

Conclusion: The present study showed the feasibility of a new [^{18}F]-labeling methodology based on the linkage of p[^{18}F]fluorobenzaldehyde to HYNIC-functionalized compounds. This method offers several advantages over existing [^{18}F]-fluorination methodologies with respect to general applicability, efficiency and simplicity of its chemistry. Hydrazone formation between HYNIC-functionalized compounds and [^{18}F]FB is a fast, one-step [^{18}F]-fluorination methodology. The [^{18}F]-labeling methodology was used to label an octreotide analog with [^{18}F]. It was shown that [^{18}F]-octreotate accumulated in SSTR2 receptor expressing tissues. Therefore, [^{18}F]-labeling of HYNIC-octreotate will allow PET imaging of SSTR2 receptor-positive tumors.

Keywords: [F-18]fluorination, New Methodology, HYNIC-[Tyr3-Thr8]-Octreotide (HYNICTATE)

RADIOSYNTHESES OF 2-[^{18}F]FLUORO-1,3-THIAZOLES

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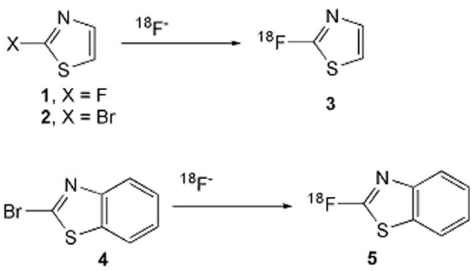
Introduction. In medicinal chemistry, the 1,3-thiazole ring is a classical replacement for a pyridinyl ring, since in some cases this replacement may improve properties of the ligands, especially lipophilicity and/or affinity to the target receptor. The nucleophilic radiofluorination of homoaromatics has been studied extensively. The similar labeling of pyridines has been investigated only quite recently (1), but has led already to useful radiotracers for imaging with positron emission tomography (PET) (2). However, to our knowledge, there is no report of the direct radiofluoridation of a 1,3-thiazole ring. Here we report on feasibility for the direct radiofluoridation of 1,3-thiazole rings, entities which now feature in some promising radioligands for PET (*e.g.* radioligands for mGluR5 receptors) (3).

Experimental. A solution (100 µL) containing potassium carbonate (0.5 mg) and kryptofix 2.2.2 (5 mg) was added to aqueous cyclotron-produced [^{18}F]fluoride (150-250 µL), and dried by three cycles of acetonitrile evaporation-addition under a nitrogen stream (taking about 20 min). Each substrate **1, 2 or 4**, 1 mmol was heated with the generated [^{18}F]F$^-$-K$^+$-kryptofix $_{2.2.2}$ complex for different times and temperatures in the chosen solvent (200 µL), either in a sealed V-vial in an oil bath or in a sealed Pyrex tube under argon at 150°C using a single mode microwave cavity at 200 W. The radioactive products were detected and measured by radio-TLC or reverse phase analytical HPLC on a Luna C-18 column.

Results and Discussion. Initially, it was found that treatment of **1** with [^{18}F]fluoride in acetonitrile under thermal conditions (35 min, 110°C) resulted in exchange of fluorine to produce carrier-added [^{18}F]2-fluoro-1,3-thiazole (**3**) in 29% decay-corrected radiochemical yield (RCY). In a further set of reactions, it was found that treatment of the bromo compound (**2**) in DMSO with [^{18}F]fluoride under thermal conditions (30 min, 150°C) gives no-carrier-added **3** in 34% RCY. Under microwave irradiation conditions (10 min at 150°C; 200 W) in DMSO under argon, the RCY of **3** from **2** was higher at 84%. Since **2** slowly degrades in the presence of air and light at high temperature, the more stable benzothiazole (**4**) was chosen as a model for more extensive study of the substitution reaction. Experiments were conducted at 80°C or 150°C for 10 or 30 min with acetonitrile, DMF or DMSO as solvent. The highest RCY (40%) of [^{18}F]2-fluoro-1,3-benzothiazole (**5**) was obtained in DMSO at 150°C for 30 min. Under argon, in DMSO using a 10 min microwave irradiation (200 W, 150°C), up to 51% RCY of **5** was produced. Under much milder conditions (acetonitrile, 80°C, 30 min) still 20% RCY of **5** was obtained.



Conclusion. The susceptibility of the 1,3-thiazole ring for no-carrier-added radiofluoridation by direct nucleophilic substitution of a bromo leaving group is demonstrated and this opens a new path for radiotracer/radioligand development. Further work is in progress to improve the conditions for these reactions and to apply this mode of labeling to candidate radioligands.

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Keywords: Fluorine-18, Nucleophilic Radiofluoridation, 1,3-Thiazole

SYNTHESIS AND BIODISTRIBUTION OF 2-[^{34m}Cl]CHLORO-2-DEOXY-GLUCOSE IN RAT

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Introduction: Chlorine radioisotopes have not been available for medical imaging despite their importance. However, our previous report [1] demonstrated a new production method for ^{34m}Cl ($T_{1/2}$ = 32 min). To expand the possibility of ^{34m}Cl for PET radiopharmaceuticals, development of rapid and high yield ^{34m}Cl chlorination reactions is necessary due to the short half-life and low yield of $^{34m}Cl^-$.

A chlorinated glucose analog of 2-deoxy-2-chloro-D-glucose (ClDG) was recognized as a major impurity in [^{18}F]FDG injections [2]. Although the detailed metabolic fate of ClDG is not yet clear, its biological behavior is assumed to be similar with that of ^{18}FDG; intercellular formation of ClDG-6-P and ClDM-6-P. In the present study, [^{34m}Cl]ClDG was prepared from $^{34m}Cl^-$ using K[^{34m}Cl]Cl / Kryptofix 222 complex as nucleophilic chlorinating agent. Additionally, in vivo biodistribution of [^{34m}Cl]ClDG was evaluated by animal PET.

Methods: Synthesis of [^{34m}Cl]ClDG was performed using an [^{18}F]FDG-like procedure. An aqueous [^{34m}Cl]Cl^- solution was obtained as previously reported [1]. [^{34m}Cl]Cl^- was trapped on a Sep-Pak light QMA cartridge, then eluted with K_2CO_3 solution into a vial containing Kryptofix 222. The mixture was dried under a stream of N_2 at 100°C to yield a [^{34m}Cl]chlorination reagent of the K[^{34m}Cl]Cl / Kryptofix 222 complex. Reaction of the [^{34m}Cl]chlorinating agent with 1, 3, 4, 6-tetra-O-acetyl-2-trifluoromethansulfonyl mannose in dry CH_3CN was carried out at 100°C for 5 min. Acetyl groups on the chlorinated compounds were removed by treatment with 2N H_2SO_4 at 140°C for 10 min. [^{34m}Cl]ClDG was purified by passing through an ion retardation resin column and a Sep-Pak C-18 cartridge. Biodistrbution of [^{34m}Cl]ClDG was evaluated by PET using rats. The obtained [^{34m}Cl]ClDG was injected intravenously into a rat under anesthesia. PET data was acquired for 90 min.

Results: ^{34m}Cl-chlorination reaction using K[^{34m}Cl]Cl / kryptofix 222 complex proceeded with almost quantitative radiochemical yield within 5 min. Hydrolysis of the [^{34m}Cl]chlorinated compound with 2N H_2SO_4 proceeded with high yield (>95%) in a short reaction time. [^{34m}Cl]ClDG was obtained with a radiochemical yield of 79% within a total synthesis time of 45 min from the start of synthesis. The radiochemical purity of [^{34m}Cl]ClDG was greater than 99%. Animal PET study using rats showed that higher uptake was observed in the kidney and liver than in heart and brain (Fig.1). Additionally, most of ^{34m}Cl activity was found in urine. TLC analysis of urine sample showed more than 90% of activity existed as [^{34m}Cl]ClDG. Consequently, it was suggested that [^{34m}Cl]ClDG was rapidly excreted from blood without accumulation in the brain or heart.

Conclusion: $^{34m}Cl^-$ was rapidly incorporated into [^{34m}Cl]ClDG with high radiochemical yield using K[^{34m}Cl]Cl/kryptofix 222 complex as chlorinationg reagent via nucleophilic reaction. Animal PET study showed that behavior of [^{34m}Cl]ClDG differed from that of [^{18}F]FDG. [^{34m}Cl]ClDG showed low accumulation in the heart and brain and was excreted into urine as [^{34m}Cl]ClDG.

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Keywords: Chlorine-34m, ClDG, Biodistribution

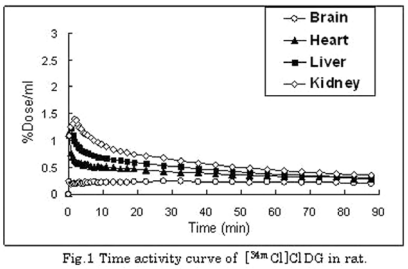

Fig.1 Time activity curve of [^{34m}Cl]ClDG in rat.

SYNTHESIS AND EVALUATION OF CANDIDATE RADIOLIGANDS FOR THE BRAIN CANNABINOID TYPE-1 (CB_1) RECEPTOR BASED ON 1,5-DIARYLPYRAZOLES

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Introduction. Ligands based on 1,5-diarylpyrazoles are recognized as potent CB_1 receptor antagonists [1]. Here we further explore this class of ligand for the possible development of a PET radioligand for the brain cannabinoid type-1 (CB_1) receptor.

Methods. Ligands **NIDA 41087** [2] and **1-9** were synthesized in 5 or 6 steps from an appropriate propiophenone and diethyl oxalate. An agonist-stimulated [^{35}S]GTP-γS binding assay was used to assess candidate ligands *in vitro*. cLogP values were calculated with Pallas 3.0 (Table 1). **NIDA 41087** and **7** were each selected for labeling with carbon-11 and evaluation in *Cynomolgus* monkey with PET. Addition of BBr_3 to **NIDA 41087** or **7** gave the *O-desmethyl* precursors. [^{11}C]**NIDA 41087** or [^{11}C]**7** were prepared by reacting precursor (0.5 mg) with [^{11}C]methyl triflate in acetone (400 μL) containing of 5M NaOH (10 μL) and purified with reverse phase HPLC. [^{11}C]**NIDA 41087** or [^{11}C]**7** was intravenously injected into a *Cynomolgus* monkey and examined with PET. Regions of interest were drawn on pons, striatum, caudate, putamen and mesencephalon. Pons was used as a reference region for free and non-specifically bound radioactivity. Autoradiography was performed with [^{11}C]**7** on thick (100 μm) post-mortem human brain slices**.** Non-specific binding was determined by co-incubation with the known CB_1 receptor antagonist, SR 141726A (1 μM).

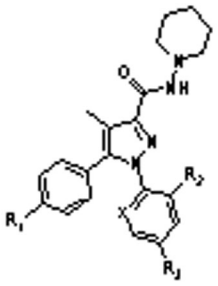

Table 1. Ligands based on 1,5-diarylpyrazoles

Ligand	R_1	R_2	R_3	x	CB1 Kb (nM)	clog P
NIDA 41087	MeO	Cl	H	CH	25	5.69
1	FCH_2O	Cl	H	CH	38	5.68
2	MeO	H	Cl	CH	154	5.69
3	MeO	Me	Me	CH	85	6.15
4	MeO	H	H	N	>4254	4.39
5	MeO	Me	H	CH	56	5.61
6	MeO	CF_3	H	CH	52	6.12
7	MeO	Br	H	CH	11	5.85
8	MeS	Cl	H	CH	>4224	6.11
9	$MeSO_2$	Cl	H	CH	302	4.17

Results. Kb[/sub] values for the ligands ranged from 11 to > 4254 nM and cLogPs ranged from 4.17 to 6.15. [^{11}C]**NIDA 41087** and [^{11}C]**7** were prepared in 40% incorporation yield (non-decay corrected). The specific activity was 184-360 GBq/mmol and radiochemical purity >99%. Radiosynthesis time was 27 min. In *cynomolgus* monkey [^{11}C]**NIDA 41087** and [^{11}C]**7** entered brain adequately and gave similar brain region time-activity curves. Brain radioactivity reached 3.5% ID at 7 min and declined to 1.9% ID at 70 min. The ratio of radioactivity concentration in the receptor-rich regions to that in pons reached 1.6 at 4.5 min and was subsequently maintained. Autoradiography showed no regional differences in bound radioactivity and no displacement of radioactivity in the co-incubation experiment.

Discussion. In a previous post-mortem human autoradiography study, [^{11}C]**NIDA 41087** was reported to show specific binding to the CB_1 receptor [3]. In this study, the higher affinity [^{11}C]**7** was selected for a post-mortem autoradiography experiments. However, [^{11}C]**7** showed only non-specific binding. This result was unexpected, since both compounds have similar lipophilicity. The thicker brain slices in this set of experiments may explain the apparent discrepancy.

Conclusion. Due to their high non-specific binding, radioligands [^{11}C]**NIDA 41087** and [^{11}C]**7** appear unsuitable for effective imaging of brain CB_1 receptors *in vivo*.

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Keywords: CB1 Receptor, 1,5-Diarylpyrazoles, PET

[11C]-METHYLATED LY2181308, AN ANTISENSE OLIGONUCLEOTIDE TO SURVIVIN: RADIOCHEMICAL SYNTHESIS AND PRELIMINARY RODENT BIODISTRIBUTION STUDIES

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Survivin is involved in the control of cell division and in apoptosis by modulation or inhibition of caspases; it is expressed in many human neoplasms and appears to be involved in tumor cell resistance to some anti-cancer agents and ionizing radiation. Survivin has been described to be selectively expressed in the most common human neoplasms and to be associated with clinical tumor progression. Because of all these properties, survivin has been proposed as a promising target for new anticancer treatments. LY2181308 is an 18 mer oligonucleotide with a molecular weight of 6,404 Da designed against survivin mRNA. A number of structural modifications to the sugar and phosphodiester backbone performed to increase its *in vivo* stability are: introduction of a 2-methoxyethyl ether (into the sugar moiety) and incorporation of phosphothiorioate (into the phosphodiester backbone) respectively.

Purpose: of this study was to develop a rapid and reproducible method to synthesize [11C]methylated LY2181308 for PET studies and to determine its *in vivo* biodistribution in rats and its uptake in a solid tumor xenograft in mice.

Method: 0.5 mg of LY2181308 (Eli Lilly & Co) was dissolved in 150 µL DMF/0.033 M borate buffer pH 8.2 (7/1 v/v), heated at 90ºC with [11C]MeI (GE box) for 15 minutes and purified on Sephadex G-25 (3.5 mL suspension in a 6 mL syringe). The radioactive product was eluted with saline. Quality control analysis was performed by FPLC on a Superdex 75™ gel filtration column, eluted with an isocratic mixture of 20 mM Hepes and 150 mM NaCl (pH 7.3) buffer at 1.0 mL/min and monitored with a UV detector at 280 and a radio-detector. The column was calibrated with known molecular weight standards. The retention time of a molecule with 6,000 Da was around 13 minutes. The retention time of [11C]methylated LY2181308 was 13.59 minutes.

Biodistribution: was performed in normal SD male rats and in BALB/C mice with implanted EMT-6 tumors at 5, 30, 60 min (n=4). Rodent studies at one hour were conducted at a "low" and "high" concentration of unlabeled surviving in order to observe the effect of mass in the biodistribution of the labeled compound.

Results: [11C]methylated LY2181308 was obtained with a radiochemical purity of 98%, and overall radiochemical yield of 48-65% (EOB) in a total synthesis time of 30 minutes. Rat biodistribution showed that at 1 hour the highest %ID/g was found in the kidneys (19.35) and liver (3.93); all other organs were below 0.2. In the tumor implanted mice the tumor/blood and tumor/muscle ratios were 4.4 and 1.9 ("low") and 6.0 and 2.26 ("high"), respectively, at one hour post injection. As before, the highest accumulation (%ID/g) in tumor-bearing mice ("low") was in the kidneys (87.8) followed by the liver (30.9) and spleen (6.9).

Conclusion: a fast and reproducible method to label surviving with [11C] was developed. The compound was obtained with a very high radiochemical purity and relatively good yield. The only mass "contaminant" was the non-radioactive starting substrate which is not separated from the [11C]methylated product. Preliminary *in vivo* data and tumor uptake in rodents warrants further studies with this radiopharmaceutical for clinical PET.

This work was supported by Eli Lilly and Company, Indianapolis, IN.

Keywords: Carbon-11, Oligonucleotide, SURVIVIN

A RADIOLABELED ALLOSTERIC POTENTIATOR FOR THE METABOTROPIC GLUTAMATE 2 RECEPTOR

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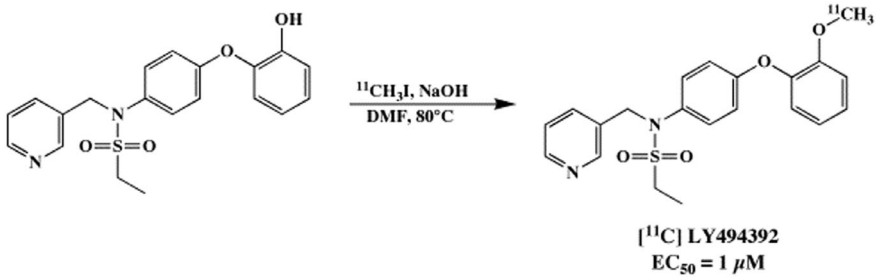
Imbalances in the glutamatergic system have been implicated in anxiety disorders, neurodegenerative disorders, and psychoses. The development of metabotropic glutamate (mGlu) radiotracers would provide essential tools for examining the role of these sites, thus providing critical information about certain disease processes involving the glutamaterigic system and its regulation in humans. In order to better understand the role of the recently discovered mGlu2 allosteric potentiators, *N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulfonyl)pyridin-3-ylmethyl amine (LY487379) was synthesized by literature procedure (1). While attempts to radiolabel this ligand were unsuccessful, the ethanesulfonamide analog (LY494392) was labeled by reacting [^{11}C]methyl iodide with a desmethyl phenolic precursor. The time for synthesis, purification, and formulation was 26 minutes (n=8) with an average radiochemical yield of 22% and an average specific activity of 7,768 mCi/μmol at end-of-synthesis.

An initial *ex vivo* biodistribution study in mice using [^{11}C]LY494392 showed ~ 0.5% of the



injected dose getting into the brain. The distribution of [^{11}C]LY494392 within the brain showed no significant differentiation between regions. A blocking study was performed in mice using the mGlu2/3 agonist LY379268 and the allosteric potentiator LY487379. Both blocking drugs were administered intravenously at a dose of 1 mg/kg 5 minutes before injection of the radiotracer. At 30 minutes, overall brain uptake was inhibited 30% by the potentiator but not by the agonist LY379268. No inhibition was observed at 60 minutes. *In vivo* PET imaging in mice confirmed the results of the *ex vivo* studies with inhibition of binding observed out to 35 minutes post-injection. Further evaluation of [^{11}C]LY494392 with kinetic modeling is warranted in larger animals.

This work was supported by NIH grants DA016182 and CA092871.

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Keywords: Allosteric Potentiator, Metabotropic Glutamate, PET

RADIOSYNTHESIS OF NOVEL COPPER (II) BIS(THIOSEMICARBAZONES) PET TRACERS

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Introduction

To derive PET tracers optimised for cerebral hypoxia imaging in stroke and cell labelling for inflammation imaging we are interested in developing novel bis(thiosemicarbazone) ligands for radiolabelling with the copper-64 radionuclide ($t_{1/2}$ = 12.7h). As part of these investigations we have synthesised a range of ligands in which the backbone of the bis(thiosemicarbazone) structure has been modified. Our objective is then to synthesise the radiolabelled complexes and determine the effects of the structural changes to their biological properties.

Methods

Synthesis of bis(thiosemicarbazone ligands: These were prepared by literature methods from the reaction of methylthiosemicarbazide with the appropriate 1,2- or 1,3-diketone [1].

Production of copper-64 radioisotope: This was produced through the nuclear reaction $^{64}Ni(p,n)^{64}Cu$ [2], involving the irradiation of an enriched nickel layer electrodeposited on a gold disc. This layer is then dissolved in hydrochloric acid and then subjected to ion exchange chromatography to obtain the copper-64 radionuclide in 0.1N hydrochloric acid as $^{64}Cu(II)Cl_2$ (0.7-2.5GBq).

Radiosynthesis of Copper-64 complexes: $^{64}Cu(CH_3CO_2)_2$ was prepared by neutralising a solution of $^{64}CuCl_2$ in 0.1N hydrochloric acid (0.2 ml, 20-50 MBq) with 0.1M Sodium acetate (pH 5.5, 1.8 ml). To $^{64}Cu(CH_3CO_2)_2$ (100 µl) was added water (400 µl) and the ligand in dimethyl sulfoxide (50 µl, 1mg/1ml). This was then stirred at RT for 10 mins then subjected to analysis by radio-HPLC.

Radio-HPLC Analysis methods: A Primesphere C18-HC column (250 x 4.6 mm) was eluted at 1ml/min for 30min with a solvent gradient of water/acetonitrile/trifluoroacetic acid: initially 95/5/0.1 (v/v), then 65/35/0.1 at 20 min and finally 95/5/0.1 at 25 min. UV monitoring was at 254 nm.

Results and Conclusion.

Based on radio-HPLC analysis (e.g. figure 2) the radiochemical yield of the copper-64 tracers (figure 1. **A-D**) were determined, with verification of product identity by comparison of retentions times with their non-radioactive copper (II) complexes. Ligand **A** a conformational restrained lipophilic derivative of ATSM was successfully labelled in high radiochemical yield and purity (>95%). The ligands **B, C and D** were also shown to radiolabel in high radiochemical and purity with formation of complexes which incorporate a keto group in the bis(thiosemicarbazone) backbone. Their increasing lipophilicity from **B to D** was reflected in their significantly longer HPLC retention times. With the radiosynthesis of these tracers established we are now evaluating their biological properties with regard to their application as cell labelling tracers or hypoxia markers.

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Keywords: Copper-64, bis(thiosemicarbazones), Hypoxia

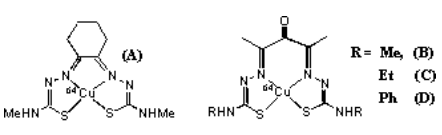

Figure 1. ^{64}Copper(II) bis(thiosemicarbazones) complexes

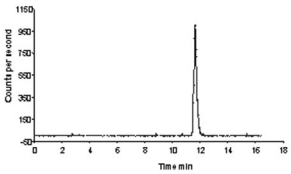

Figure 2: Radio-HPLC of the radiosynthesis of (A)

EVALUATION OF Cu-ETS FOR USE AS A PET TRACER OF REGIONAL RENAL PERFUSION

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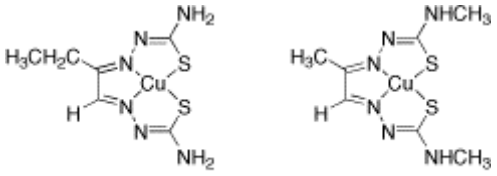
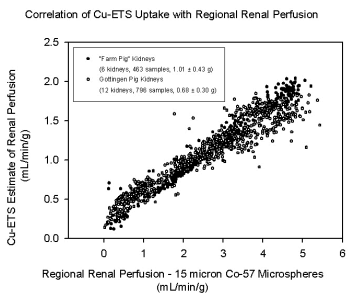
The copper-62 complex of ethylglyoxal bis(thiosemicarbazone) (Cu-ETS, Figure 1) has potential utility as a generator-based PET radiopharmaceutical for evaluation of myocardial and renal perfusion. The Cu-ETS complex does not exhibit the strong binding to human serum albumin that is observed with the related Cu-PTSM radiopharmaceutical, making Cu-ETS more suitable as a clinical agent for perfusion measurements in high-flow tissues like the renal cortex and/or hyperemic myocardium. Like Cu-PTSM, following i.v. administration Cu-ETS rapidly clears from the blood, affording prolonged tissue retention of the Cu-radiolabel via intracellular reductive decomposition of the chelate. However, unlike Cu-PTSM, the albumin-binding of Cu-ETS is not strongly species-dependent, providing reasonable confidence that animal models can reliably predict the suitability of Cu-ETS for PET quantification of perfusion in humans. Figure 1.



Cu-ETS Cu-PTSM

Three immature pigs from a local farm and six Gottingen mini-pigs were studied, comparing regional renal uptake of ^{64}Cu-ETS to renal perfusion measured using 15 micron ^{57}Co-microspheres. The ^{64}Cu-ETS radiopharmaceutical was administered as an i.v. bolus, while the ^{57}Co-microspheres were administered into the left ventricle via a multi-sideport pigtail catheter. Arterial blood was sampled at a constant rate for 2-minutes following each radiotracer injection. After animal sacrifice, the kidneys were dissected and counted to quantify ^{64}Cu and ^{57}Co.

Figure 2 shows the results obtained by treating total arterial blood ^{64}Cu-counts as though they simply represent a material behaving like ^{64}Cu-microspheres. In both swine models, this produces a linear correlation between ^{64}Cu-ETS flow estimates and microsphere flow values (Figure 2). Cu-ETS does afford the presumed "microsphere-like" tissue trapping of ^{64}Cu; however, this data handling inaccurately assumes all arterial ^{64}Cu to be available to tissue as "Cu-ETS." By neglecting the partial decomposition of ^{64}Cu-ETS by red blood cells, this modeling approach significantly overestimates the concentration of ^{64}Cu-ETS in blood. Accurate flow quantification with $^{62/64}$Cu-ETS will require correction of the radiocopper arterial input function for the fraction of $^{62/64}$Cu remaining present as Cu-ETS. Figure 2. Renal flow estimates from immature farm swine and mature Gottingen minipig models. The ^{64}Cu-ETS flow estimates were calculated assuming all arterial ^{64}Cu was present as ^{64}Cu-ETS following i.v. ^{64}Cu-ETS administration, and that that ^{64}Cu exhibits the properties of radiolabeled microspheres (i.e., complete first-pass extraction and retention in tissue). The farm pig data are fit by the line: $y = 0.36x + 0.16$, $R^2 = 0.96$. The Gottingen Pig data are fit by the line: $y = 0.29x + 0.34$, $R^2 = 0.91$. In aggregate, these data are fit by the line: $y = 0.31x + 0.29$, $R^2 = 0.92$.



Acknowledgement

This work was supported by NIH Grant #R44 DK58466. The ^{64}Cu production at Washington University was supported by NCI grant R24 CA86307.

Keywords: Copper-62/64 Radiopharmaceuticals, Cu-ETS, Renal Perfusion

SYNTHESIS AND RADIOSYNTHESIS OF [^{18}F]FPhEP AND [^{18}F]F$_2$PhEP, TWO NOVEL HIGH-AFFINITY AND α-4-β-2-SELECTIVE, EPIBATIDINE-BASED RADIOLIGANDS FOR PET IMAGING OF NICOTINIC ACETYLCHOLINE RECEPTORS

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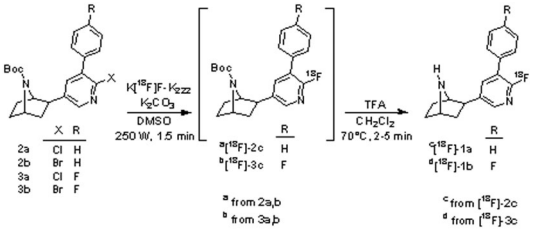
Central nicotinic acetylcholine receptors (nAChRs) have been implicated in learning-memory processes and neuropsychiatric disorders. Over the last few years, several agonist radioligands labeled with fluorine-18 (half-life: 109.8 minutes) have been developed, as for example 2-[^{18}F]F-A-85380, to image the nAChR alpha-4-beta-2 subtype using Positron Emission Tomography (PET). Recently, two novel high-affinity and selective nAChR alpha-4-beta-2 antagonists, namely 2-*exo*-(2′-fluoro-3′-phenyl-pyridin-5′-yl)-7-azabicyclo[2.2.1]heptane (FPhEP, **1a**) and 2-*exo*-(2′-fluoro-3′-(4-fluorophenyl)-pyridin-5′-yl)-7-azabicyclo[2.2.1]heptane (F$_2$PhEP, **1b**) have been described (1,2). Both derivatives are chemically and structurally closely related to epibatidine, another high-affinity nAChR alpha-4-beta-2 ligand, but less toxic than the latter. They both show a fluoropyridinyl moiety, allowing their labeling with fluorine-18. The goal of the present project was the preparation of [^{18}F]-**1a** and [^{18}F]-**1b** from the corresponding *N*-Boc-protected chloro- and bromo precursors.

1a and **1b** as well as the corresponding *N*-Boc-protected chloro and bromo derivatives (**2a, 2b**, **3a, 3b**) were synthesized in 8 steps from commercially available *N*-Boc-Pyrrole (overall yields = 17% and 6% for **1a** and **1b** respectively; 4-9% for **2a, 2b**, **3a** and **3b**). The key-steps in these syntheses were: (1) the *exo*-selective Heck cross-coupling reaction involving *N*-Boc-azanorbornene and 2-amino-5-iodopyridine giving the corresponding pyridinyl-7-azabicyclo[2.2.1]heptane; (2) the Suzuki cross-coupling reaction, involving *N*-Boc-2-*exo*-(2′-amino-3′-bromo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane and phenylboronic acid or 4-fluorophenylboronic acid giving the corresponding 2′-amino-3′-phenylpyridines. The 2'-fluoro derivatives (**1a** and **1b**) were then obtained by diazotation using sodium nitrite and hydrofluoric acid. The *N*-Boc-protected 2′-chloro and 2′-bromo analogues (**2a, 2b**, **3a, 3b**) were also obtained using similar conditions, involving copper chloride or bromide followed by amine protection.



1a and **1b** have been labeled with fluorine-18 using the following two-step radiochemical process: (a) n.c.a nucleophilic heteroaromatic ortho-radiofluorination from the corresponding *N*-Boc-protected chloro- or bromo-derivatives (**2a, 2b**, **3a, 3b** - 1 mg) and the activated K[^{18}F]F-Kryptofix$_{222}$ complex in DMSO using microwave activation at 250W for 1.5 min, followed by (b) removal of the *N*-Boc protective group. 1.11-2.22 GBq of radiochemically pure (> 99%) [^{18}F]-**1a** or [^{18}F]-**1b** (111-185 GBq/micromol) were obtained after semiprep. HPLC in 78-85 min starting from 18.5 GBq of [^{18}F]fluoride. Noteworthy, radiochemical yields for the fluorine-18-incorporation step were only slightly higher when **2b** and **3b** were used compared to **2a** and **3a**. Also, chemical purities of [^{18}F]-**1a** and [^{18}F]-**1b** were only 90% using the chloro derivatives **2a** and **3a** and systematically greater than 97% when using the bromo derivatives **2b** and **3b**.

Dynamic PET studies in baboons are currently underway to evaluate the potential of [^{18}F]-**1a** and [^{18}F]-**1b** to image nAChRs alpha-4-beta-2 *in vivo*.

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2. Carroll F Ivy *et al. J. Med. Chem.* 2004; **47**: 4588-4594.

Keywords: Fluorine-18, Nicotinic Receptors, Imaging

SYNTHESIS AND RADIOLABELING OF N-[4-[4-(2-[^{11}C]METHOXYPHENYL)PIPERAZIN-1-YL]BUTYL] BENZO[*b*]THIOPHENE-2-CARBOXAMIDE, A POTENTIAL RADIOTRACER FOR D3 RECEPTOR IMAGING WITH PET

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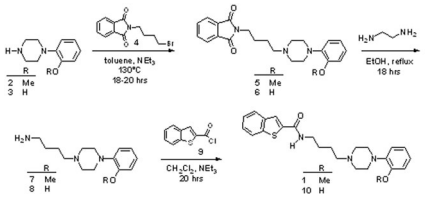
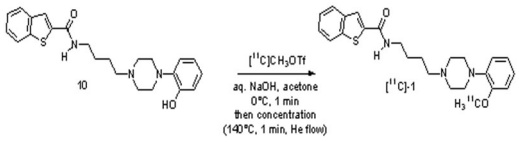
Recent pharmacological developments have underlined the role of D3 receptors in several CNS disorders and addictive behaviours. Today very few selective D3 radiotracers are available. In the present study, we report (a) the synthesis of *N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]benzo[*b*]thiophene-2-carboxamide (**1**), a selective D3 receptor partial agonist (1) and the nor-derivative **10** as precursor for labeling with carbon-11, (b) the radiosynthesis of **1** using [^{11}C]methyl triflate and (c) its preliminary in vivo pharmacological characterisation.

Derivatives **1** (as reference) and **10** were both synthesized in three chemical steps from commercially available 1-(2-methoxyphenyl)piperazine (**2**) and 1-(2-hydroxyphenyl)piperazine (**3**), respectively. Piperazine **2** was reacted with *N*-(4-bromobutyl)phtalimide (**4**) in refluxing toluene for 18-20 hrs to give **5** in 91% yield that was hydrolyzed to the corresponding amine **7** with hydrazine in refluxing ethanol for 18 hrs. Derivative **7** was finally acylated using benzo[*b*]thiophene-2-carbonyl chloride (**9**) in dichloromethane, containing triethylamine, for 20 hrs to give the expected derivative **1** in 60% yield. Derivative **10** (as precursor for labeling) was prepared analogously starting form 2-hydroxyphenylpiperazine (**3**) in 35% overall yield.



Derivative **1** was labeled from the corresponding nor-analogue **10** using [^{11}C]methyl triflate in acetone containing aq. NaOH (5 equiv) at 0°C for 1 minute and purified on semi-preparative RP-HPLC. Up to 5.5 GBq (at EOS) of [^{11}C]- **1** with a specific radioactivity ranging from 46.2 GBq/micromole to 55.5 GBq/micromole (at EOS) starting from 44.4 GBq of [^{11}C]CO_2 could be obtained in 35 to 40 minutes including HPLC purification and formulation. Biodistribution studies in rat were performed by injection of [^{11}C]- **1** (1.85 MBq) via the tail vein. Animals were sacrificed at different times after injection, the brain dissected and samples were assessed for radioactivity. Another group of rats was pretreated either with several D3 or D2/D3 competitors (haloperidol, GR-103691, BP 897 and non-labeled derivative **1**) or a 5HT1A competitor (WAY100135) or a alpha-1 competitor (prazosin). Brain uptake of [^{11}C]- **1** was rapid and the regional distribution of radioactivity was rather homogeneous (0.5-0.8 %ID/mg tissue at 30 minutes). The uptake of [^{11}C]- **1** was strongly inhibited by all D3 and D2/D3 competitors but not affected by either the 5HT1A- or the alpha-1 competitors. Brain radioactivity monitoring was also performed using beta-microprobes implanted in two brain regions (nucleus accubens and cerebellum). Similar time-activity curves were observed in these two tissues. Metabolism studies in the brain show a total recovery of all the HPLC injected radioactivity as the parent compound [^{11}C]- **1**, at 30 minutes after tracer injection.



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Keywords: Carbon-11, D3 Receptor, Imaging

SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIP OF 2,5-DISUBSTITUTED THIOPHENE DERIVATIVES FOR ß-AMYLOID PLAQUE IMAGING

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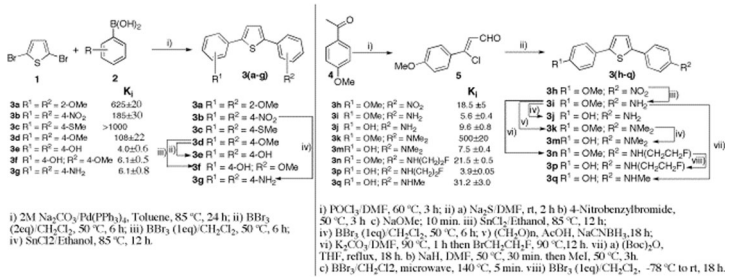
It is known that accumulation of ß-amyloid (Aß) plaques in the brain is implicated in the development of Alzheimer's disease (AD)[1]. Many imaging agents, labeled with C-11, F-18 and I-123 targeting amyloid plaques, for positron emission tomography (PET) and single photon emission tomography (SPECT) have been reported[2-5]. These tracers will be useful for diagnosis and monitoring of disease progression. We have recently developed a novel series of ^{18}F imaging agents targeting Aß plaques for PET. Reported herein are the syntheses and initial structure activity relationship (SAR) studies of a series of 2,5-disubstituted thiophene derivatives designed as novel Aß plaque imaging agents. Symmetrically substituted thiophene derivatives **3(a-g)** were prepared by the Suzuki coupling of 2,5-dibromothiophene with corresponding phenylboronic acid as explained in the above scheme. The sequential one pot treatment of the ß-chloroacrolein - prepared by reacting Vilsmeier-Hack reagent with 4-methoxyacetophenone- with $Na_2S.9H_2O$, 4-nitrobenzyl bromide and NaOMe afforded the unsymmetrically substituted thiophene **3h**[6]. Subsequent transformations of NO_2 and/or OMe groups in **3h** as depicted in the above scheme afforded compounds **3(i-q)**. The initial *in vitro* competitive binding studies of compounds **3(a-q)** with [I-125]-IMPY in AD brain homogenates established the necessary structural features present in order for these compounds to have desirable Ki values (in nanomolar range) as tabulated above. In conclusion, the preliminary studies established the structure activity relationship (SAR) of 2,5-disubstituted thiophene derivatives and the initial data clearly show these fluorinated compounds, i.e. **3p** and **3n**, are suitable candidates for the development of novel Aß plaque imaging agents. Further studies along this direction are currently underway.



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Keywords: PET Imaging, Alzheimer's Disease, Thiophenes

SYNTHESIS OF 5′-(2-[^{18}F]FLUOROPHENYL)-SPIRO[1-AZABICYCLO [2.2.2.]OCTANE]-3,2′(3′H)-FURO[2,3-b]PYRIDINE ([^{18}F]FPS), AN AGONIST AT THE α7 NICOTINIC ACETYLCHOLINE RECEPTOR

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The α4β2- and α7-nicotinic acetylcholine receptor (nAChR) subtypes are the most abundant nAChRs in the central nervous system (CNS). The α7-nAChR is a pentameric, cationic, ligand-gated calcium channel (1). The α7-nAChR signaling cascade has been implicated in a variety of pathologies within and external to the CNS. Imaging α7-nAChR *in vivo* could provide a direct and quantitative method to study entities as varied as schizophrenia and inflammation (2).

As part of our program to develop radiotracers for α7-nAChR (3), we have synthesized the α7-nAChR agonist 5′-(2-[^{18}F]fluorophenyl)-spiro[1-azabicyclo[2.2.2]octane]-3,2′ (3′H)-furo[2,3-b]pyridine ([^{18}F]FPS), K_d = 350 p*M*, a fluorine-18-labeled analog of PSAB-OFP (4). The synthesis from the non-radioactive *p*-nitrobenzaldehyde precursor **1** required two steps, namely nucleophilic [^{18}F]fluorination ([^{18}F]KF/$K_{2.2.2}$, 130ºC, DMSO, 10 min) followed by decarbonylation with $RhCl(PPh_3)_3$ (120ºC, anhydrous dioxane, 10 min) (Scheme 1). Subsequent semi-preparative reverse-phase HPLC provided [^{18}F]FPS in a radiochemical yield approaching 5% at end-of-synthesis within a total synthesis time of approx. 85 minutes. Radiochemical purity was > 90% with calculated specific radioactivities of up to 259 GBq/mmol (7,000 Ci/mmol) achieved. The early steps of the synthesis have been automated (GE TracerLab Fx) to reduce radiation dose. Preliminary biodistribution studies in rodent and baboon brain are underway.

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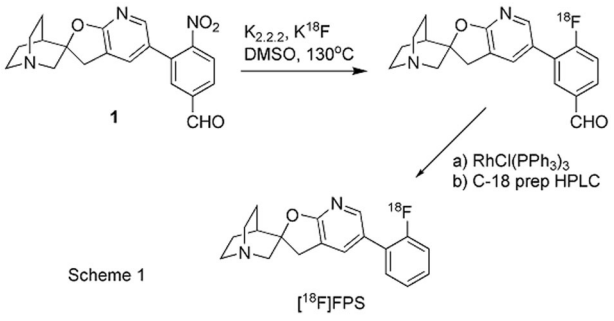
Keywords: alpha-7 Nicotinic Acetylcholine Receptor, Fluorine-18



Scheme 1

SYNTHESIS, RESOLUTION, AND STABILITY OF OPTICALLY ACTIVE Ga(III) AND Co(III) COMPLEXES WITH LINEAR HEXADENTATE $N_4O_2^{2-}$ LIGANDS

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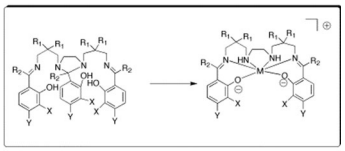
Lipophilic monocationic gallium complexes of linear hexadentate salicylaldimine ligands, such as $[Ga(3\text{-}EtOsal)_2Me_4BAPEN]^+$ and $[Ga(3\text{-}MeOsal)_2Me_4BAPEN]^+$, have potential utility in PET imaging of myocardium (1-5), as well as in tumor imaging to assess MDR1 Pgp transport function (6-9). Chelates of this type, in which the oxygen donor atoms occupy trans coordination sites, exhibit chirality due to the clockwise, or anticlockwise, arrangement of the hexadentate ligand about the metal center (10). Since chelate stereochemistry may influence radiopharmaceutical interactions with biological molecules, the present study was undertaken to examine the feasibility of resolving the racemic bis(salicylaldimine) complexes produced in radiopharmaceutical (^{66}Ga, ^{67}Ga, ^{68}Ga) synthesis using such hexadentate ligands.

Figure 1. Ligand precursors and the resulting metal salicylaldimine complexes. The ligand precursors were prepared by reaction of a salicylaldehyde or 2-hydroxyacetophenone derivative with either N,N'-bis(3-aminopropyl)ethylenediamine (BAPEN) or tetramethyl-BAPEN (bis(3-amino-2,2-dimethy-propyl)ethylenediamine) (1-5).



$R_1 = R_2 = Y = H$, $X = OMe$, $M = Co$; $[Co(3\text{-}OMeSal)_2BAPEN]^{1+}$
$R_1 = R_2 = Y = H$, $X = OMe$, $M = Ga$; $[Ga(3\text{-}OMeSal)_2BAPEN]^{1+}$
$R_1 = CH_3$, $R_2 = Y = H$, $X = OMe$, $M = Co$; $[Co(3\text{-}OMeSal)_2Me_4BAPEN]^{1+}$
$R_1 = CH_3$, $R_2 = Y = H$, $X = OMe$, $M = Ga$; $[Ga(3\text{-}OMeSal)_2Me_4BAPEN]^{1+}$
$R_1 = CH_3$, $R_2 = Y = H$, $X = OEt$, $M = Ga$; $[Ga(3\text{-}OEtSal)_2Me_4BAPEN]^{1+}$
$R_1 = R_2 = CH_3$, $X = H$, $Y = OMe$, $M = Co$; $[Co(4\text{-}OMeAcph)_2Me_4BAPEN]^{1+}$
$R_1 = R_2 = CH_3$, $X = H$, $Y = OMe$, $M = Ga$; $[Ga(4\text{-}OMeAcph)_2Me_4BAPEN]^{1+}$

We have synthesized a series of both known (1-9) and novel hexadentate Schiff-base ligands, and their Ga(III) and Co(III) complexes (Figure 1). The cobalt complexes adopt structural geometries analogous to the gallium compounds, and were of interest because, once resolved, their kinetic stability was expected to minimize the rate of stereoisomer interconversion.

Resolution of the chelate stereoisomers has been achieved by HPLC using a Chiralcel OD-R column (Table 1). A Chiralyzer HPLC detector immediately downstream of the photodiode array (PDA) detector determined the optical rotation of the enantiomers. The identity of the resolved enantiomers was confirmed by the matching UV-visible spectra obtained for the resolved peaks using the PDA. With both Co(III) and Ga(III), the HPLC-resolved and isolated stereoisomers were found to be stable for at least 2 weeks on storage at room temperature. The stability of the resolved chelate stereoisomers will allow comparison of their performance as probes of cellular MDR1 Pgp and /or MXR1 transport function.

Table 1. Chiralcel OD-R HPLC Results for the Ga(III) and Co(III) Bis(salicylaldimine) complexes.

Chelate Cation	Retention time for (+) isomer (min)	Retention time for (-) isomer (min)
$Co(3\text{-}MeOsal)_2BAPEN$	25.4	21.5
$Ga(3\text{-}MeOsal)_2BAPEN$	14.8	19.6
$Co(3\text{-}MeOsal)_2Me_4BAPEN$	13.2	20.6
$Ga(3\text{-}MeOsal)_2Me_4BAPEN$	10.0	18.0
$Ga(3\text{-}EtOsal)_2Me_4BAPEN$	12.9	17.6
$Co(4\text{-}MeOAcph)_2Me_4BAPEN$	13.0	17.5
$Ga(4\text{-}MeOAcph)_2Me_4BAPEN$	11.3	16.7

Acknowledgement

This project was supported by grants from the U.S. Public Health Service (R01-CA092403 and Research Facilities Improvement Program Grant #C06-14499).

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Keywords: Gallium Radiopharmaceuticals, Stereoisomer Resolution, Multidrug Resistance

SIGMA-2 SELECTIVE FLUORINATED LIGANDS: SYNTHETIC METHOD AND OPTIMIZATION OF DECARBONYLATION FOR RADIOLABELING

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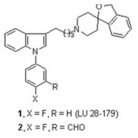
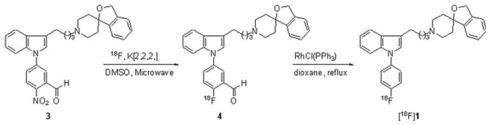
Sigma receptors are membrane-bound proteins having high affinities for a variety psychotropic drugs with opiate-type structures. The sigma receptor subtypes, sigma-1 and sigma-2, have different molecular weights and pharmacological roles.[1] Many cancer cell lines (breast, melanoma, prostate cancer) express high levels of sigma receptors,[2,3] and proliferative tumor cells express much higher levels of sigma-2 receptors than quiescent cells.[4,5] Thus, the sigma-2 receptor has been proposed as a suitable target for imaging proliferative tumor cells.

While many ligands are selective for the sigma-1 receptor or are nonselective, very few ligands are selective for the sigma-2 receptor. A radiopharmaceutical based on an azabicyclo[3.3.1]nonane framework was developed by Mach,[6] who demonstrated that the rhenium surrogate showed exceptional sigma-2 selectivity; later this agent was labeled with technetium-99m by Kung for tissue distribution studies.[7] In considering potential fluorine-18 labeled sigma-2 receptor ligands, our attention focused on members of an indole piperidine series, especially **1** (LU 28-179), which is reported to have a remarkably high sigma-2 binding affinity and selectivity.[8,9] Figure 1. The structure of indole piperidine ligands and their inhibition constants toward sigma receptors.



	Inhibition Constant K_i (nM)		Ratio (K_i)
	σ_1	σ_2	σ_1/σ_2
1	13	2.6	5.0
2	31.6	4.4	7.2



Target compounds **1 and 2** were prepared in several steps by a route based on a previously described method,[7] with modifications making it more efficient for the synthesis of the precursor molecule (**3**); compounds **1 and 2** showed high binding affinity and good selectivity (6 fold) for the sigma-2 receptor (Figure 1). The preparation of [^{18}F]-labeled indole piperidine [^{18}F]**1** was achieved in two steps from a o-nitroaldehyde precursor (**3**). Aromatic [^{18}F]fluorination was not reproducible when **3** was treated with F-18 fluoride in the presence of Bu_4NOH under microwave heating conditions. However, by using F-18, K_2CO_3 and kryptofix[2.2.2] under microwave heating, we obtained radiochemical yields of 5-50%. The final decarbonylation step with Wilkinson's catalyst was studied in various solvents. Decarbonylation using dioxane as solvent produced [^{18}F]**1,** but with toluene, extensive decomposition resulted. Herein, we present the synthesis of precursor **3**, optimization of [^{18}F]fluorination, and decarbonylation to produce [^{18}F]Lu 28-179 (Scheme 1). Future work to prepare [^{18}F]**1** will be done using an alternate synthetic route.[10]

Supported by DOE grants 86ER60401 (to JAK) and 84ER60218 (to MJW).

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Keywords: sigma-2 Receptor, Decarbonylatioluorinen, Fluorine-18

PALLADIUM-CATALYZED AMINOCARBONYLATION OF NITROGEN-CONTAINING ARYL HALIDES USING [^{11}C]BH_3CO AS SOURCE OF CARBON MONOXIDE

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Recent developmental work in PET radiochemistry has demonstrated the utility of [^{11}C]carbon monoxide as a building block for the synthesis of [^{11}C]carbonyl compounds despite reactivity and methodological limitations (1). An ongoing project focussed on new techniques to trap and react this versatile building block has established [^{11}C]BH_3CO as a good alternative to autoclaving methods with [^{11}C]CO for palladium-catalysed carbonylation reactions (2,3). Further developmental work has been performed in order to optimize the radiochemical yields and to show that *N*-containing heterocycles commonly used in medicinal chemistry programmes can be labelled using this method (scheme 1).

Depending on the nature of the amine, the carbonylation position and PPh_3/Pd ratios modest to good radiochemical yields were observed. As a representative example, the Pd-catalyzed aminocarbonylation of 3-iodopyridine **1a** with methylamine yielded to [^{11}C-carbonyl]*N*-methylnicotinamide **2a** with good radiochemical yield, routinely between 0.5 and 1.0GBq, and with good radiochemical purity (scheme 2).

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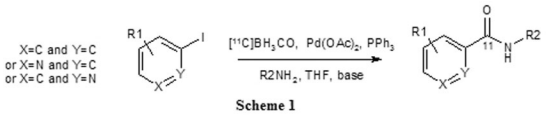
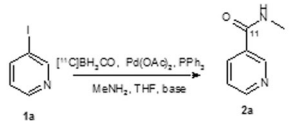
Keywords: PET, [11C]carbon Monoxide, Aminocarbonylation



Scheme 1



Scheme 2

SYNTHESIS AND C-11 LABELING OF THREE POTENT NOREPINEPHRINE TRANSPORTER SELECTIVE LIGANDS (LORTALAMINE, OXAPROTILINE AND (R)-NISOXETINE) FOR COMPARATIVE PET STUDIES IN BABOONS

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Objectives: Down regulation of the density of norepinephrine transporter (NET) in the brain has been associated with major depression. Potent radiotracers which selectively target NET could help (1) to understand the role of NET in depressive illness, (2) to facilitate the development of effective antidepressants, (3) to optimize the therapeutic dosage, and (4) to monitor the efficacy of treatment. Nisoxetine, a potent and selective norepinephrine reuptake inhibitor, has previously been labeled with C-11 at the amino nitrogen as an R/S racemic mixture to evaluate its potential as an NET ligand using a rodent model. The results of this ex vivo biodistribution study showed moderate specific uptake of (R/S)-[N-$^{11}CH_3$]nisoxetine in the brain. In our study we proposed to synthesize the more potent (R) enantiomer and label it with C-11 at different positions, namely (R)-[O-$^{11}CH_3$]nisoxetine and (R)-[N-$^{11}CH_3$]nisoxetine, as labeling at different position might provide different metabolism profile. Two other NET inhibitors, lortalamine and oxaprotiline, with high affinity, high selectiveity and reasonable calculated logP values were also selected and radiolabeled with C-11 for comparative PET studies in baboons.

Methods: The reference compounds and their corresponding normethyl precursors were synthesized via multi-step synthetic approaches. The radiosyntheses of [^{11}C]lortalamine, [^{11}C]oxaprotiline, (R)-[O-$^{11}CH_3$]nisoxetine and (R)-[N-$^{11}CH_3$]nisoxetine were performed by simple alkylation of the corresponding normethyl precursors with no-carrier-added [^{11}C]CH_3I in DMF.

Results: After HPLC purification, [^{11}C]lortalamine, [^{11}C]oxaprotiline and (R)-[N-$^{11}CH_3$]nisoxetine were obtained in 63-97 % radiochemical yields, whereas (R)-[O-$^{11}CH_3$]nisoxetine was obtained in 23-29 % radiochemical yields due to substantial formation of the undesired N-[^{11}C]methylated byproduct, (R)-[^{11}C]-N,N-dimethyl-O-nornisoxetine (64-70 %). The radiosynthesis times for these tracers ranged from 36 to 42 min from the end of bombardment (EOB), and the final C-11 labeled products were obtained with > 99 % radiochemical purity and 1.7-3.7 Ci/μmole (EOB) specific activity. Comparative PET studies in baboons were then carried out.

Conclusions: C-11 labeled NET-selective ligands including [^{11}C]lortalamine, [^{11}C]oxaprotiline, (R)-[O-$^{11}CH_3$]nisoxetine and (R)-[N-$^{11}CH_3$]nisoxetine were prepared in moderate to high radiochemical yields with high radiochemical purity and specific activity. These NET-selective radiotracers allow us to carry out comparative PET studies, and evaluate their potential as PET radiotracers for imaging brain NET using a nonhuman primate model. Supported by DOE-OBER and NIH.

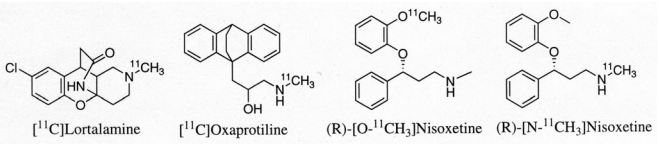

Figure 1. Chemical structures of potent and selective NET ligands.

Table 1. NET inhibitors: lipophilicity and their affinity (nM) and selectivity for DAT, SERT and NET.

NET Inhibitors	DAT	SERT	NET	DAT/NET	SERT/NET	CSLogP*
Lortalamine (IC50)	> 10000	>100000	< 1	> 10000	> 1000000	2.08
Oxaprotiline (Kd)	4340 ± 30	3900 ± 100	4.9 ± 0.2	890	800	3.50
Nisoxetine (Ki)	360	1000	1	360	1000	1.74

*Lipophilicity was calculated as CSLogP using the ChemSilico LLC (Tewksbury, MA) family of property prediction software (CSPridict).

Keywords: Norepinephrine Transporter, Antidepressant, PET

TETRA- OR MONO- ORGANOTIN REAGENTS IN THE STILLE REACTION FOR THE LABELLING OF POTENTIAL RADIOTRACERS WITH CARBON-11: A COMPARATIVE STUDY

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The Stille reaction remains the method of choice for the radiosynthesis of PET tracers by introducing a methyl group labelled with carbon-11 onto an aromatic moiety. It usually proceeds by a pallado-catalyzed cross-coupling reaction between an aryltrialkyltin precursor and [^{11}C]-iodomethane[1], and efforts are made for searching non toxic alternative approaches[2,3]. Recently, we have developed a new methodology based on the transfer reaction of a [^{11}C]-methyl group from a ^{11}C-labelled monoorganotin reagent onto an aryl halide[4]. This method was found very efficient starting from various bromoquinolines and bromonaphtalene as model compounds. Here, we compared both methods, using either a tetra- or a monoorganotin derivative, for the radiosynthesis of polyfunctional structures **1 and 2-4**, ligands of delta opioid[5] and NK-3 receptors[6] respectively (Scheme 1).

According to the classical method (route A), [^{11}C]-methyl iodide was distilled into a DMF solution containing Pd_2dba_3 and P(*o*-tolyl)$_3$. After addition of a mixture of K_2CO_3, CuCl and the tetraorganotin precursor in DMF, the reaction medium was heated at 90 °C for 5 min. In the recent method (route B), [^{11}C]-methyl iodide was reacted with Lappert's stannylene ($Sn[N(TMS)_2]_2$) in solution in THF, leading to the ^{11}C-labelled monoorganotin intermediate. After addition of TBAF and evaporation of THF, the aryl halide and Pd_2dba_3 in dioxane were added, and the mixture was heated for 5 min at 150 °C. Yields for the incorporation of the labeled methyl group were determined by radioTLC and HPLC. Results will be presented and discussed.

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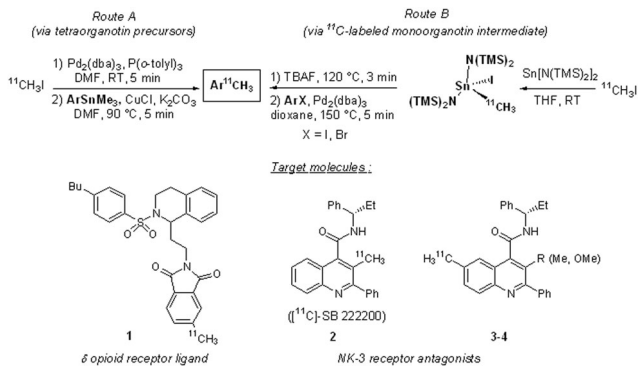
Keywords: Carbon-11, Stille Reaction, Monoorganotin



RADIOSYNTHESIS OF 2-[^{11}C]METHOXY-3-METHOXY-5-(2′-PROPENYL)-*N*-[[(2*S*)-1-(2-PROPENYL)-2-PYRROLIDINYL]METHYL]BENZAMIDE AS A NEW RADIOTRACER FOR DOPAMINE D-2/D-3 RECEPTORS

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^{18}F-Fallypride is currently being used as a striatal and extrastriatal dopamine D2/D3 receptor imaging agent. Amphetamine-induced dopamine release is able to compete with the binding of ^{18}F-fallypride in extrastriatal regions such as the thalamus. In order to carry out same-day repeat PET studies, we have previously reported the preparation of ^{11}C-fallypride. However, ^{11}C-fallypride requires greater time for equilibration than permitted by a ^{11}C-radiolabel (Mukherjee et al., *Bioorg. & Med. Chem.* 2004, 12, 95). In an effort to explore a more rapidly equilibrating tracer, we have prepared a *C*-allyl analog of fallypride. Here we wish to report the radiosynthesis of 2-^{11}C-Methoxy-3-dimethoxy-5-(2′-propenyl)-*N*-[[(2*S*)-1-(2-propenyl)-2-pyrrolidinyl]methyl]benzamide (^{11}C-alipride).

The synthesis of the precursor, 2-hydroxy-3-methoxy-5-(2′-propenyl)-*N*-[[(2*S*)-1-(2-propenyl)-2-pyrrolidinyl]methyl]benzamide (desmethylalipride) was accomplished using modifications of reported methods in 5 steps and was characterized by mass spectra, NMR and elemental analysis (Mukherjee et al., *Nucl. Med. Biol.* 1995, 22, 283).

Radiotracer was prepared by adapting the reported method for radiosynthesis of ^{11}C- fallypride (Mukherjee et al., *Bioorg. & Med. Chem.* 2004, 12, 95). ^{11}C-CO_2 from a RDS-112 cyclotron was converted to ^{11}C-CH_3I in a GE methyl iodide unit. This ^{11}C-CH_3I was then transferred to a NI (Nuclear Interface) methylation unit where the precursor was dissolved in 100 mL DMF in the presence of 1.0 mL of tetrabutylammonium hydroxide maintained at -10°C. The reaction was carried out at 50°C for 2 min and 55°C for 4 min.

Scheme1. Radiochemical Synthesis of ^{11}C-Alipride

The residual was taken up in the HPLC solvent and injected onto a Waters µ-BondaPak Prep column eluted with a mobile phase containing 25% ethanol buffered with 10 mM NaH_2PO_4 (pH 3.5). The modified purification procedure simplified the dose preparation by avoiding the subsequent evaporation of the toxic acetonitrile used previously. The injectable dose could then be prepared by sterilely filtering the collected radioactive fraction followed by diluting it with saline. The ^{11}C-alipride was prepared in 20% radiochemical yield (decay corrected) with a total reaction time of approximately 30 min from the end of ^{11}C-methyl iodide trapping with the specific activity exceeded 300 Ci/mmol. PET imaging studies of ^{11}C-alipride on rhesus monkeys demonstrate an ability to visualize both striatal and extrastriatal receptors. Comparison of the *in vivo* binding properties of ^{11}C-alipride with ^{11}C-fallypride is currently underway.

Keywords: Carbon-11, Fallypride, Dopamine D-2/D-3 Receptors

PREPARATION OF N.C.A. [^{18}F]FLUOROPHENYLUREAS

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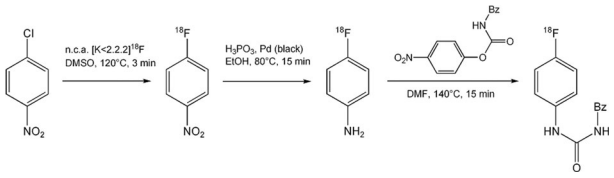
Modifications at a phenylurea moiety is often a useful tool to improve the pharmacological properties of drugs. The introduction of a fluorine-atom is widely used to modify the pharmacologically-active site of a molecule. In this regard there is a need for devolpment of new radiolabelling methods to prepare no-carrier-added fluorine-18 labelled fluorophenyl urea moieties.

Urea derivatives have been labelled earlier only by insertion of prosthetic groups in an already prepared molecule [1, 2]. As a related compound, the synthesis of labelled guanidines was described [3]. The pathway presented in this work provides the posibility to synthesise of a large number of urea derivatives starting from a labelled aniline or amino compound. As a model compound n.c.a. N-4-[^{18}F]fluorophenyl-N'-benzyl urea was prepared in a three step synthesis.



First, the preparation of n.c.a. 4-[^{18}F]fluoroaniline was modified and improved compared to former syntheses [4]. Starting from 1-chloro-4-nitrobenzene the labelling was performed as a standard procedure in DMSO at 120°C in the presence of the dried n.c.a. [K<2.2.2]^{18}F complex. The product and precursor were fixed on a Waters C18^{+}™ cartridge and eluted with methanol. Reduction of the nitro group using phosphorous acid and palladium black provides n.c.a. 4-[^{18}F]fluoroaniline after 15 min at 80°C which was fixed on a Merck EN™ cartridge. The radiochemical yield was about 50 % after 35 min of synthesis.

The 4-[^{18}F]fluoroaniline is eluted with DMF and after addition of benzoyl-carbamic acid 4-nitro-phenyl ester, the mixture was heated to 140°C for 15 min according to the preparation of heteroaromatic urea derivatives [5]. Analysis via HPL-chromatography shows the desired product with about 95 % radiochemical purity.

Thus, the method developed opens the possibility of preparing a wide variety of [^{18}F]fluorophenyl-ureas.

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Keywords: Radiofluorination, Fluoroaniline, Fluorophenyl-Ureas

SYNTHESIS OF CARBON-11 LABELED CAMPTOTHECIN DERIVATIVES AS NOVEL PET TRACERS FOR IMAGING OF TOPOISOMERASE IN CANCERS

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Plant antitumor agent camptothecin is a cytotoxic pentacyclic ring alkaloid first isolated from the Chinese tree *Camptotheca acuminata* in 1966. Camptothecin and its derivatives such as 9-nitrocamptothecin have been used as chemotherapeutic drugs to treat various cancers like breast cancer, prostate cancer and lung cancer due to their inhibition activity to topoisomerase. Carbon-11 labeled camptothecin derivatives may serve as novel radiotracers for positron emission tomography (PET) to image topoisomerase in cancers. Here we present the synthesis of carbon-11 labeled camptothecin derivatives, 9-[^{11}C]methoxy-20(*S*)-camptothecin ([^{11}C]**1**), 10-[^{11}C]methoxy-20(*S*)-camptothecin ([^{11}C]**2**), 9-nitro-10-[^{11}C]methoxy-20(*S*)-camptothecin ([^{11}C]**3**) and 9-[([^{11}C]trimethylamino)methyl]-10-hydroxy-20(*S*)-camptothecin ([^{11}C]**4**) (Figure 1).

The precursors, 9-hydroxy-20(*S*)-camptothecin (**5**), 10-hydroxy-20(*S*)-camptothecin (**6**), 9-nitro-10-hydroxy-20(*S*)-camptothecin (**7**) and 9-[(dimethylamino)methyl]-10-hydroxy-20(*S*)-camptothecin (**8**), and their corresponding reference standards, 9-methoxy-20(*S*)-camptothecin (**1**), 10-methoxy-20(*S*)-camptothecin (**2**), 9-nitro-10-methoxy-20(*S*)-camptothecin (**3**) and 9-[(trimethylamino)methyl]-10-hydroxy-20(*S*)-camptothecin triflate (**4**) were synthesized in multiple steps starting from the parent compound camptothecin in moderate to excellent chemical yields.

The target tracers [^{11}C]**1-3** were prepared by the *O*-[^{11}C]methylation of their corresponding precursors **5-7** using [^{11}C]methyl triflate and isolated by C_{18} solid-phase extraction (SPE) purification method in 30-50% radiochemical yield based on [^{11}C]CO_2, 15-20 min overall synthesis time from end of bombardment (EOB), >95% radiochemical purity and >1.0 Ci/μmol specific activity at end of synthesis (EOS). The target tracer [^{11}C]**4** was prepared by the *N*-[^{11}C]methylation reaction of its corresponding precursor **8** with [^{11}C]methyl triflate and isolated by SiO_2 SPE purification procedure in 40-65% radiochemical yield based on [^{11}C]CO_2, 10-15 min overall synthesis time from EOB, >99% radiochemical purity and >1.0 Ci/μmol specific activity at EOS.

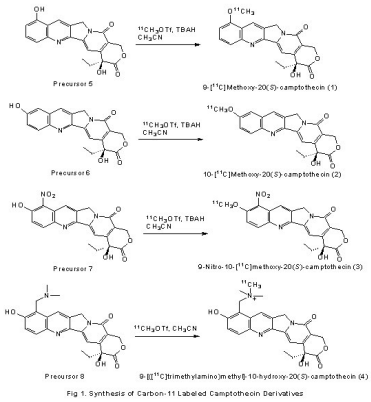

Fig 1. Synthesis of Carbon-11 Labeled Camptothecin Derivatives

Keywords: Carbon-11 Labeled Camptothecin Derivatives, Synthesis, PET Cancer Tracers

FAST AND MILD AQUEOUS-PHASE REDUCTION OF [^{11}C]FORMIC ACID WITH SAMARIUM DIIODIDE TO [^{11}C]METHANOL

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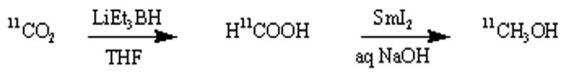
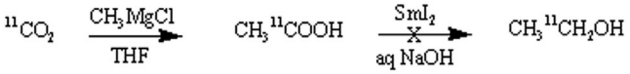
Radiopharmaceutical chemistry with carbon-11 (T1/2: 20.4 minutes) rests for an important part on the methylating agent [^{11}C]methyl iodide. This radioactive building block is usually made by reduction of [^{11}C]carbon dioxide with lithium aluminium hydride in tetrahydrofuran (THF) to [^{11}C]methanol, which, after hydrolysis, is then converted with hot aqueous hydriodic acid into [^{11}C]methyl iodide. This method is robust and reliable and has been successfully in practice for many years. However, in our experience non-negligible quantities of radioactivity, on average about 28% of total starting amount, regularly stay behind in the reduction vial when distilling out the [^{11}C]methanol. Also, on average 12% of total radioactivity remains in the hydriodic acid solution after [^{11}C]methyl iodide distillation. Having identified these residues as [^{11}C]formate, we then showed that [^{11}C]formate could be produced almost quantitatively from [^{11}C]carbon dioxide using the milder reducing agent lithium triethylhydroborate ($LiEt_3BH$) (1).

Samarium diiodide is a versatile one-electron reducing agent in organic chemistry. It has recently been shown to be able to reduce, in the presence of water and a base, a range of carboxylic acids to the corresponding alcohols in high yields under very mild conditions in extremely short reaction times (2). For example, benzoic acid was converted into benzyl alcohol in 92% by samarium diiodide in aqueous sodium hydroxide at room temperature in one minute. We reasoned that if [^{11}C]formic acid could be reduced under the same conditions to [^{11}C]methanol we might have a way to circumvent the above-mentioned problems in the classical method.

[^{11}C]formic acid was made almost quantitatively by bubbling no-carrier-added [^{11}C]carbon dioxide, contained in nitrogen vector gas, into $LiEt_3BH$ in THF (0.12M, 60 µL) at -10°C (2). At this stage we tried two altenative sequences for treating the $LiEt_3B$-[^{11}C]formate complex: 1) Addition of samarium diiodide in THF (0.1M, 300 µL) followed by addition of aqueous NaOH (0.2N, 300 µL) or 2) Hydrolysis with aqueous NaOH (0.2N, 300 µL) followed by addition of samarium diiodide in THF. After one minute of reaction time, HPLC analysis (Aminex HPX-87H (BioRad), 300 x 7.8 mm; temperature: 44°C; mobile phase: 1 mM sulphuric acid in water; flow rate: 0.6 mL/min) of the mixture revealed in both cases a very high conversion into [^{11}C]methanol of more than 90% and sometimes up to 100%.



Remarkably, analogous treatment of both the adduct of [^{11}C]carbon dioxide and methylmagnesium chloride and purified [^{11}C]acetate, led to only a few percent of [^{11}C]ethanol. In this connection it is worth mentioning that in the original paper on this reaction (2) aliphatic acids were said to be less reactive than aromatic acids and no straight-chain aliphatic acids of less than five carbon atoms were mentioned. If this was to imply that the reaction works less well for the shorter chains, our results converge with this, formic acid (zero-carbon chain) being perhaps the only exception.



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Keywords: [^{11}C]formic Acid, [^{11}C]methanol, Samarium Diiodide

VIOXX: A SECOND CHANCE AS PET TRACER FOR CYCLOOXYGENASE EXPRESSION?

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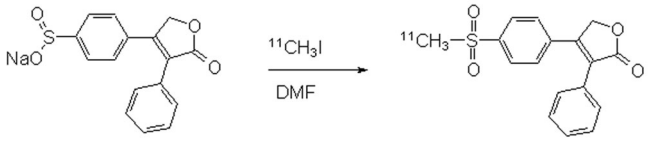
Objective: Cyclooxygenase (COX) catalyzes the first steps in the biosynthesis of prostanoids. The inducible isoform of the enzyme, COX-2, is expressed during inflammation. Increasing evidence also suggests a role of COX-2 in the initiation and progression of cancer and a neurotoxic role of COX-2 in neurodegenerative diseases like Alzheimer's disease, Parkinson's disease and ischemia. VIOXX (Rofecoxib) is a potent and selective inhibitor of COX-2 and has been successfully used as an anti-inflammatory drug. However, VIOXX was recently withdrawn from the market, because prolonged use of the drug (>12 months, 25 mg/day) was accompanied by an increase in the incidence of heart attacks and strokes. These side-effects, however, are not expected to occur after a single tracer dose of the drug. Therefore, we investigated the potential of radiolabeled VIOXX as a PET tracer for COX-2 imaging.

Methods: VIOXX was labeled with carbon-11 via methylation of the sulfinate precursor with [^{11}C]methyl iodide (DMF, 4 min, 90 °C) and purified by reversed phase HPLC. The in-vitro stability of [^{11}C]VIOXX in rat and human plasma was determined by HPLC analysis. In Wistar rats, a sterile inflammation was induced by injection of 50 µl turpentine into the thigh muscle of the right hind leg.



After 24 h, 55±16 MBq [^{11}C]VIOXX was i.v. administered and biodistribution studies were performed 60 min after injection of the tracer. Specific binding was assessed by blocking studies using 1.5 mg/kg of the COX inhibitors NS398 (COX-2 selective), SC560 (COX-1 selective) and indomethacin (non-selective).

Results: [^{11}C]VIOXX was prepared from [^{11}C]methyl iodide in 60±8% radiochemical yield (n=15) and had a specific activity of 14±8 MBq/nmol (EOS). The total synthesis time (EOB - EOS) was 35-40 min. After the in-vitro incubation of [^{11}C]VIOXX in rat plasma at 37 °C for 30 min, 45% of the radioactivity consisted of polar degradation products, probably due to hydrolysis of the furanone ring. In human plasma, on the other hand, no degradation products were observed. Biodistribution studies in Wistar rats (n=4) at 60 min after injection of [^{11}C]VIOXX showed highest levels of radioactivity in excretory organs, such as ileum, duodenum, liver and kidney (SUV: 5.3±5.6, 3.3±1.3, 3.2±0.3 and 1.3±0.1, respectively). Tracer uptake was not higher in the inflamed muscle than in control muscle (SUV: 0.42±0.05 vs. 0.45±0.05). Blocking studies with the COX-2 selective inhibitor NS398 (n=3) did not reveal specific binding in any of the tissues tested. Interestingly, administration of the COX-1 selective inhibitor SC560 (n=4) prior to tracer injection caused a significant reduction in tracer uptake in cerebral cortex, whereas the non-selective inhibitor indomethacin (n=4) significantly reduced tracer uptake in both cerebral cortex and midbrain (Student's t-test, $P<0.01$).

Conclusion: In spite of the fact that VIOXX was reported to be a potent and highly selective inhibitor of COX-2, [^{11}C]VIOXX did not show any specific binding to the COX-2 enzyme and therefore is not suitable for imaging of COX-2 expression. Remarkably, the blocking studies with (non-)selective COX-1 inhibitors indomethacin and SC560 appear to suggest that [^{11}C]VIOXX does exhibit specific binding to the COX-1 isoform in the brain.

Keywords: [^{11}C]VIOXX, COX-2, Inflammation

RADIOSYNTHESIS OF (*E*)-*N*-(2-[^{11}C]METHOXYBENZYL)-3-PHENYL-ACRYLAMIDINE, A NOVEL SUBNANOMOLAR NR2B-SUBTYPE SELECTIVE NMDA RECEPTOR LIGAND

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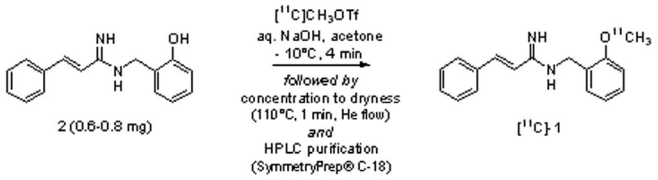
Recently, a novel series of amidines has been described, exhibiting high NR2B-subtype selective NMDA antagonist activity with nanomolar or subnanomolar affinity (1,2). Within the subclass of benzamidines, *N*-(3,5-dichlorobenzyl)-4-(fluoromethoxy)benzenecarboximidamide-d_2 has recently been labelled with the positron-emitter fluorine-18 ($T_{1/2}$: 109.8 min) and is currently under investigation (3). Within the styrylamidine subclass, *N*-(2-methoxybenzyl)cinnamamidine **1** (or (*E*)-*N*-(2-methoxybenzyl)-3-phenyl-acrylamidine), displayed the highest affinity (Ki = 0.7 nM versus [^{3}H]ifenprodil) and was considered an appropriate candidate for isotopic labelling with carbon-11 ($T_{1/2}$: 20.38 min) at its methoxy moiety for imaging of NMDA receptors with PET.

Derivative **1** and its *nor*-analogue were synthesised in two chemical steps. Commercially available 3-phenyl-acrylonitrile was converted to the corresponding imidate salt, (*E*)-3-phenyl-acrylimidic acid methyl ester hydrochloride, via the Pinner synthesis. Coupling with 2-methoxybenzylamine and 2-hydroxybenzylamine gave **1 and 2** respectively in moderate and not optimised yields (41% for **1** and 23% for **2**).



Reaction of **2** with [^{11}C]MeOTf as the alkylating agent, employing the standard conditions that have so far been used in our laboratory for the routine radiosynthesis of several radiotracers, gave satisfactory yields for the preparation of [^{11}C]-**1**. The conditions used were the following: (1) trapping at -10°C of [^{11}C]MeOTf in acetone (0.3 mL) containing 0.6 to 0.8 mg of **2** (2.4 to 3.2 micromoles) and 5 microlitres of aq. 3M NaOH (5 eq.); (2) concentration to dryness; (3) taking up the residue with 0.5 mL of the HPLC mobile phase and (4) purification using semi-preparative HPLC. Typically, starting from 1.5 Ci (55.5 GBq) of a [^{11}C]CO_2 production batch, 120 to 240 mCi (4.44-8.88 GBq) of [^{11}C]-**1** (20-40% decay-corrected radiochemical yield, n=5) was obtained within 25 to 30 min of radiosynthesis with specific radioactivities ranging from 0.8 to 1.2 Ci/micromole (29.6-44.4 GBq/micromole). As demonstrated by HPLC, the radiotracer preparation was found to be > 99% chemically and radiochemically pure and the preparation was shown to be free of non-radioactive precursor **2** and was radiochemically stable for at least 30 min. No further optimisation was performed as yields produced sufficient quantities for pharmacological evaluation.

The *in vivo* pharmacological profile of [^{11}C]-**1** has been evaluated in rodent using biodistribution studies and microPET imaging studies (on Focus 220, CTI Concorde) as well as brain radioactivity monitoring with intracerebral radiosensitive beta-microprobes. The data obtained illustrates an extremely poor brain penetration and clearly indicates that [^{11}C]-**1** does not have the required properties for further development as a PET tracer for imaging NMDA receptors.

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Keywords: Carbon-11, NMDA Receptor, Methyl Triflate

RADIOSYNTHESIS OF [^{18}F]LBT-999, A SELECTIVE RADIOLIGAND FOR THE VISUALISATION OF THE DOPAMINE TRANSPORTER WITH PET

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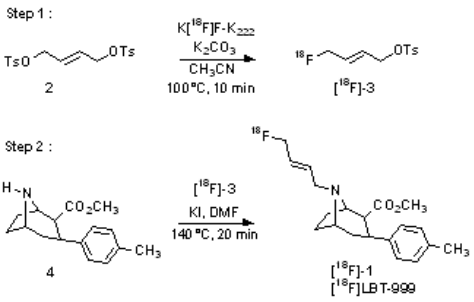
LBT-999 (**1**, (E)-N-(4-fluorobut-2-enyl)-2-beta-carbomethoxy-3-beta-(4-tolyl) nortropane) is a cocaine derivative belonging to a new generation of high-selective dopamine transporter (DAT) inhibitors (K_D: 9 nM and IC_{50} > 1000 nM for SERT and NET). LBT-999 (**1**) has already been labeled with carbon-11 (half-life: 20.38 min), at its methyl ester function from the corresponding carboxylic acid precursor and the efficient methylation reagent [^{11}C]methyl triflate (1). Routine production batches of 150 to 250 mCi of [^{11}C]LBT-999 (specific radioactivities = 0.8-1.2 Ci/mmol) have been obtained, permitting preliminary in vivo pharmacological evaluation of this radiotracer by both biodistributions in rodents and brain imaging in non-human primates with positron emission tomography (PET) (2,3). The *in vivo* results obtained clearly confirm that LBT-999 is an excellent candidate for quantification of DAT using PET in humans. Due to its chemical structure, LBT-999 can also be labeled with fluorine-18 (half-life: 109.8 min), the most widely used positron-emitting radiohalogen, which procedure we describe herein.

Chemistry: Briefly, LBT-999 (**1**), as well as the amine **4** as precursor for labeling, were synthesized from ecgonidine methyl ester (4). The key-step in this synthesis was the 1-4 addition involving 4-tolyl magnesium bromide and affording, after purification, the 2-beta-3-beta nortropane isomer. *N*-demethylation cleanly gave derivative **4**, which was coupled with (*E*)-1-fluoro-4-tosyloxybut-2-ene (**3**) to give the target compound LBT-999 (**1**) in 50% overall yield. Derivative **3** was prepared from dimethylfumarate following a three-step sequence: (a) reduction of the ester functions using DIBAL-H; (b) conversion of the two alcohol functions into their corresponding tosylates; (c) controlled mono-substitution of one tosylate function into fluoride.

Radiochemistry: LBT-999 (**1**) was labeled with fluorine-18 at its fluoromethylvinyl moiety using the following two-step radiochemical process: (a) no-carrier-added nucleophilic aliphatic radiofluorination from derivative **2** and the activated K[^{18}F]F-Kryptofix®$_{222}$ complex in acetonitrile using conventional heating at 95°C for 10 min giving [^{18}F]-**3**, followed by (b) condensation of [^{18}F]-**3** with the tropanamine **4** in dimethylformamide containing potassium iodide for 20 min at 145°C. Non-optimised and preliminary radiosyntheses cleanly gave 1.11-1.85 GBq of radiochemically pure (> 99%) [^{18}F]LBT-999 ([^{18}F]-**1a**) after semi-preparative HPLC (Zorbax C18, eluent CH_3CN/H_2O/TEA: 50/50/0.1 (v:v:v)) in 105-115 min starting from a 33.3 GBq aliquot of a cyclotron-produced [^{18}F]fluoride production batch (3-6% non decay-corrected overall yield).



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Keywords: Fluorine-18, LBT-999, Dopamine Transporter

A NEW MILD METHOD FOR THE ONE-POT SYNTHESIS OF [2-^{11}C]THYMINE USING [^{11}C]PHOSGENE

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Summary: β-(*N*-Benzoylamino)methacrylamide, a key intermediate for [2-^{11}C]thymine was synthesized in three steps from ethyl α-formylpropionate and NH_3. Reaction of the alkali metal salts of β-(*N*-Benzoylamino)methacrylamide with [^{11}C]$COCl_2$ gave [2-^{11}C]thymine. The yield of [2-^{11}C]thymine is 362 ± 53 MBq at EOS (n=3) (18 MeV proton beam; 10 μA, 10 min). The total synthesis takes 16 minutes from the end of bombardment.

Introduction: Uracil derivatives are of considerable interest because of their wide array of pharmacological properties, and many radiopharmaceuticals are developed. [^{11}C]thymidine was synthesized to evaluate cellular proliferation by PET. However, complexity of its synthetic route and length of the synthesis time limited its clinical application. Recently we have developed a simple and improved synthesis of [^{11}C]$COCl_2$ in high yield with high specific activity [1]. We envisaged that employment of the [^{11}C]$COCl_2$ in a final process would provide an efficient synthesis of such a tracer for PET as [2-^{11}C] thymine. Our strategy for the synthesis of [2-^{11}C]thymine is depicted in Scheme 1, wherein the key intermediate is compound **1**.

[Scheme 1] Experimental: ***Synthesis of precursor***: Treatment of ethyl α-formylpropionate (**5**) with NH_3 gave β-aminomethacrylate (**6**). After benzoylation, resulting **7** was re-treated with NH_3 afforded β-(*N*-benzoylamino)methacrylamide (**1**) quantitatively (Scheme 2).

[Scheme 2] ***Synthesis of [2-^{11}C]thymine*** [^{11}C]$COCl_2$ was synthesized according to the method reported previously[1]. The generated [^{11}C]$COCl_2$ was bubbled with helium flow into a solution of **1** containing a base at 30°C for 1 minute (Scheme 1). The solvent was removed from the reaction mixture by heating the reaction vial. Treatment of the residual **3** with 1.5 M ammonia-methanol for 1 minute at room temperature underwent debenzoylation to yield desired [2-^{11}C]thymine (**4**).

Results and Discussion: We synthesized bi-functional β-(*N*-Benzoylamino)methacrylamide (**1**) that serves as the key precursor to combine with [^{11}C]$COCl_2$ in the final step to give [2-^{11}C]thymine (**4**). Although the direct reaction of **1** with [^{11}C]$COCl_2$, however, failed to give **4, 1** activated as the alkali metal salt (**2**) reacted with [^{11}C]$COCl_2$ to give [2-^{11}C] thymine. The best result was obtained when the reaction was performed as the potassium salt (**2b**) in DME as shown in Table 1. It took 16 min from the end of bombardment to isolate **4**. Table 1

Conclusions: Because of a simple (one-pot reaction) and rapid (16 min from bombardment to isolation) procedure [2], the present work represents an substantially useful method for the synthesis of [2-^{11}C]thymine.

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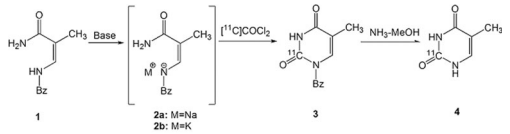
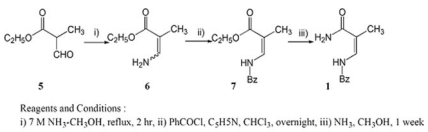
Yields of [2-^{11}C]Thymine (MBq)

Solvent	Base	Mol. eq.	Yield (MBq, EOS)
DME	—	—	ND
DME	NaH	2	140
DME	(CH3)3COK	1	362 ± 53 (n=3)

DME; dimethoxyethane. EOS; end of synthesis. ND; not detected.



Scheme 1



Scheme 2. Synthesis of β-(N-benzoylamino) methacrylamide (**1**)

Keywords: Synthesis, [^{11}C]thymine, [^{11}C]phosgene

A PREPARATIVE HPLC METHOD FOR ^{11}C-METHYLATED COMPOUNDS: CHANGING THE RETENTION ORDER OF PRODUCTS AND THEIR PRECURSORS

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By far the most prevelant method for labelling compounds with carbon-11 is methylation with [^{11}C]methyl iodide or triflate. Isolation of the labelled product is often achieved using preparative reversed phase HPLC.

The introduction of a methyl group as a rule increases the lipophility of a compound, which normally results in elution of the precursor prior to the radioactive product. The large precursor peak invariably tails in to the product peak, thus contaminating the final product. For neuroreceptor ligands this can be a problem if the precusor also has affinity for the receptor system being studied. Thus, a very good separation of the precursor and product by HPLC is required, which increases the overall synthesis time and thus reduces the yield and the specific radioactivity of the product accordingly.

In order to elute the radiolabelled product prior to the precursor, normal phase HPLC can be used but is not so desirable due to the use of toxic organic solvents. In addition, most pharmaceutically relevant compounds are strongly hydrophilic molecules having basic (or acidic) groups and are often insoluble in pure organic eluents.

In reverese phase HPLC there are three types of coulmn-sample interactions: hydrophobic, steric and polar (or silanol) interactions. We have developed two chromatographic systems for separation of a number of PET tracers and their nor-methyl precursors. Method A, using a Zorbax SB C8 column, utilises mainly the hydrophobic interactions, and separates the methylated product from its precursor according to the difference in lipophilicity. As a result, precursor elutes prior to the methylated product with relatively low selectivity. However, both precursor and product have narrow peaks with high theoretical plate numbers that ensures adequate resolution. Method A is therefore applicable as an analytical HPLC method. Method B, uning a Luna CN column, utilises mainly the silanol and steric interactions and separates the precursor and the product according to their polarities and shapes. As a result, the precursor in many cases elutes after the methylated product, i.e. a reversal of retention order is achieved. As typical of silanophilic interactions, the peaks were found to be rather broad with relatively low theoretical plate numbers, however, the high selectivity ensures the required resolution. Method B is, therefore highly suitable as a preparative HPLC method in the manufacturing of a number of ^{11}C-labelled compounds (Table 1). In addition to the reverse of retention order, method B uses only ethanol and a suitable buffer solution in the mobile phase. Use of toxic solvents such as acetonitrile is thus avoided. The serotonin transporter ligand [^{11}C]DASB is an excellent example of this new preparative HPLC method.

Table 1. Retention data of the precursor/methylated product pairs in chromatographic system A and B

Compound		**Method A**			**Method B**	
	logD (pH=2.6)	**Rt (min)**	**Selectivity**	**logD (pH=5.0)**	**Rt (min)**	**Selectivity**
DASB	-0.54	6.92		0.67	4.11	
nor-DASB	-0.84	6.40	1.11	-0.55	5.81	1.53
2-F-APO	0.43	3.27		2.55	3.90	
2-F-nor-APO	-0.45	3.13	1.11	0.77	6.97	2.02
2-PhOH-APO	1.49	3.75		3.85	5.10	
2-PhOH-nor-APO	0.64	3.55	1.11	1.92	7.21	1.51

In conclusion, we have developed a preparative HPLC method which can enable elution of a number of ^{11}C-methylated compounds prior to their precursors, thus reducing the overall sythesis time, avoiding contamination of the final product with precusor and avoiding the use of toxic solvents which would require residual solvent testing prior to human use.

Keywords: [11C]methylation, HPLC, Retention Order

PREPARATION OF A REVERSIBLE EGFR INHIBITOR, [^{11}C]CIRC, AS A PET RADIOTRACER FOR TUMOR IMAGING

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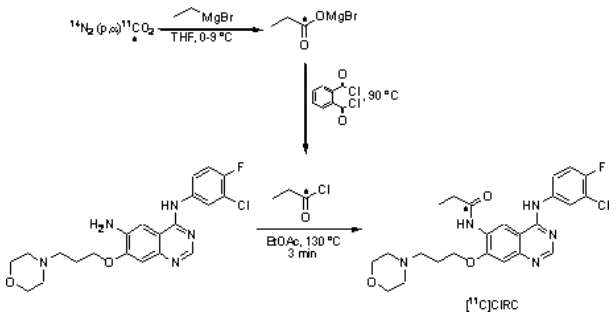
Objectives: The epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases are present in high numbers on many tumor types, are encoded by genes frequently mutated in cancer, and have direct correlation to cellular proliferation and tumor sensitivity to various therapies. Novel cancer therapies involving specific small molecules EGFR inhibitors or anti-EGFR antibodies are currently being investigation by several groups. One such EGFR inhibitor, Canertinib (CI-1033) is currently under clinical investigation. The expression of the EGFR-TK in a wide variety of human tumors and its potential prognostic value have also identified it as a target for diagnostic imaging. We report here the radiolabeling with carbon-11 of the reduced congener of Canertinib, CIRC, for use in imaging EGFR overexpressing tumors.

Methods: Using a modification of the method of Mishani and Ben-David (J Labelled Comp Radiopharm (2001) 44:S475-S476), [^{11}C]propionic acid derivative was prepared by the reaction of ethylmagnesium bromide with [^{11}C]carbon dioxide in tetrahydrofuran (THF) at 0-9 °C. Conversion of this labeled acid to [^{11}C]propionyl chloride was accomplished by heating the reaction mixture to 90 °C in the presence of excess phthaloyl dichloride and 2,6,-di-*tert*-butylpyridine in THF. The labeled acyl chloride formed was concurrently distilled into a second reaction vial containing a mixture of 1.5 ug of triethylamine and 1.5 mg of the appropriate 6-aminoquinazoline precursor (PD-0183803) in 0.1 mL of ethyl acetate. This reaction mixture was the sealed and heated at 130 °C for 3 minutes (Fig. 1). The reaction mixture was purified by reverse phase HPLC using Ultremex C8 column (10x250 mm) eluted at 5 mL per minute with 50 mM ammonium acetate in 75 % aqueous acetonitrile. After isolation of the product, the solvent was removed and the product formulated in sterile isotonic saline.

Results: This unoptimized synthesis provided [^{11}C]CIRC in a consistent 1-5% yield radiochemical yield in >97% radiochemical purity. Total synthesis time was 40 min., which equates to ~5-10 mCi of purified [^{11}C]CIRC for a 10 min. irradiation. The final formulation for these initial studies contained a cold mass of CIRC of 5 ug/mL and only traces of unreacted precursor (~0.65 ug/mL).



Conclusion: Although optimatization of this process is still in progress, sufficient amount of [^{11}C]CIRC (5-10 mCi) can be produced to begin *in vivo* biological evaluation of this radiotracer in tumor bearing mice. These biological studies are on going.

Acknowledgement: This work was supported by a grant from Pfizer Global Research & Development.

Keywords: Oncology, Tyrosine Kinase, Carbon-11

SELECTIVE SYNTHESIS OF [2-^{11}C]PROPYL IODIDE AND [1-^{11}C]ETHYL IODIDE USING GRIGNARD REACTION FROM METHYLMAGNESIUM BROMIDE

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Introduction of secondary [^{11}C]alkyl group into nucleophilic substrates using [2-^{11}C]PrI is an alternative route for developing promising PET ligands. To date, [2-^{11}C]PrI was reported as a by-product during the preparation of [1-^{11}C]EtI using Grignard reaction.[1-3)] MeMgBr reacted with [^{11}C]CO_2 to form [1-^{11}C]AcOMgBr which further reacted with the excess MeMgBr to yield [2-^{11}C]$Me_2C(OMgBr)_2$. This intermediate was reduced and iodinated to form [2-^{11}C]PrI mixed with the [1-^{11}C]EtI produced simultaneously in a low ratio. Here, we report the efficient synthesis of [2-^{11}C]PrI and [1-^{11}C]EtI by promoting or controlling the "side reaction". This is the first report of synthesizing [2-^{11}C]PrI as an available reagent for production of radiopharmaceuticals. An automated system was constructed for the synthesis of [2-^{11}C]PrI and/or [1-^{11}C]EtI using Grignard reaction. This system includes the following sequences: 1) concentrating [^{11}C]CO_2 into a loop; 2) transferring [^{11}C]CO_2 into a coil loaded with MeMgBr for the Grignard reaction; 3) producing [2-^{11}C]PrI and/or [1-^{11}C]EtI by reduction and iodination 4) purifying these substances by gas chromatography. Using this system, we determined the optimal conditions of forming [2-^{11}C]PrI and/or [1-^{11}C]EtI and then produced the two radiochemically pure agents.

After MeMgBr/THF (1.0 M) was loaded into a polypropylene coil and the excess reagent was wasted, [^{11}C]CO_2 was transferred into this coil with N_2 gas (2 mL/min) for the reaction at -5-30°C for 1.5-10 min. By this reaction, reduction and iodination, [^{11}C]alkyl iodide was yielded with 30-40% (EOB) incorporation rate of the total [^{11}C]CO_2 radioactivity. In the product, the ratio of [^{11}C]EtI and [2-^{11}C]PrI changed largely with the reaction conditions although [^{11}C]MeI was always <10%. When the reaction of MeMgBr with [^{11}C]CO_2 in the coil was maintained for 1.5 min at -5°C, [1-^{11}C]EtI reached the highest ratio of 91% while [2-^{11}C]PrI comprised <2%. Warming the reaction coil to 30°C and keeping it for 1.5 min decreased [1-^{11}C]EtI to 50% and increased [2-^{11}C]PrI to 41%. Prolonging the reaction time for 2.5 min, 5.0 min and 10 min at 30°C decreased [1-^{11}C]EtI from 32% to 8%. In contrast to [1-^{11}C]EtI, [2-^{11}C]PrI was increased to 45% at 2.5 min and 80% (the highest ratio) at 5 min. These results demonstrated that increasing the reaction temperature and time could promote the reaction of [1-^{11}C]AcOMgBr with the secondary MeMgBr, which is favorable for the formation of [2-^{11}C]PrI. At 10 min, 2-Me-[2-^{11}C]PrI, the reaction product of [2-^{11}C]$Me_2C(OMgBr)_2$ with the third MeMgBr, yielded a 5% ratio along with [2-^{11}C]PrI (67%).

Using the above conditions, [^{11}C]alkyl iodide was produced, distilled and directly introduced into the inlet of a Porapak column at room temperature without using a pre-column for trapping. Flowing N_2 gas through the heated Porapak column gave [^{11}C]EtI and/or [2-^{11}C]PrI with a radiochemical purity of >96%. Starting from about 1 Ci of [^{11}C]CO_2, 110-150 mCi of [^{11}C]EtI or 100-120 mCi of [2-^{11}C]PrI was obtained at EOS (n=3) with a specific activity of 1.0-2.7 Ci/μmol. This amount of radioactivity is sufficient for the synthesis of [^{11}C]radioligands.

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Keywords: [2-^{11}C]propyl Iodide, [1-^{11}C]ethyl Iodide, Grignard Reaction

SYNTHESIS, RADIOSYNTHESIS AND INITIAL BIOLOGICAL EVALUATION OF A NEW CLASS OF CANDIDATE BRAIN CANNABINOID TYPE-1 (CB_1) RECEPTOR RADIOLIGAND

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Introduction.

Positron emission tomography (PET) coupled to an effective radioligand for cannabinoid type-1 (CB_1) receptors might provide a further means for understanding the many neurodegenerative disorders linked to these receptors. Recently, diarylpyrazolines have been reported as a new class of potent and selective CB_1 receptor antagonist [1]. We have started to explore this class of CB_1 ligand for the possible development of a PET radioligand for CB_1 receptors.

Methods. Ligands **SLV** and **1-3** (Table 1) were synthesized in 5 steps from the commercially available benzyl 4-chlorophenyl ketone plus benzenesulfonamide or benzenesulfonyl chloride, based on published general methodology [1]. **SLV** and **1-3** were evaluated for K_b value at CB_1 receptors *in vitro* using an agonist-stimulated GTP-γS functional binding assay (Table 1). cLogP values were calculated with Pallas 3.0. Ligand **1** was first selected for labeling with carbon-11 and for evaluation in rhesus monkey *in vivo* with PET. The *O-desmethyl* precursor was prepared by treating **1** with BBr_3. [^{11}C]**1** was prepared by treating the precursor (0.5 mg) with [^{11}C]iodomethane in DMF —(80 μL) containing methanolic TBAOH (0.17 M; 6 μL) for 5 min at room temperature and purified with reverse phase HPLC. [^{11}C]**1** was injected intravenously into a rhesus monkey and evaluated with PET under baseline and pretreatment conditions (*i.v.* administration of 0.4 mg/kg of the CB_1 receptor antagonist, SR 141716A). Regions of interest in the PET images were drawn on cerebellum, thalamus, striatum, frontal cortex, and pons. Pons was used as a reference region for the free and non-specifically bound radioactivity.

Results. The K_b values of ligands **SLV** and **1-3** ranged from 8 to 26 nM and cLogPs from 4.20 to 4.56 (Table 1). [^{11}C]**1** was produced in about 15% overall decay-corrected radiochemical yield from cyclotron-produced [^{11}C]carbon dioxide. The final product was > 99% pure and the specific radioactivity ranged from 56 to 82 GBq/μmol at end of synthesis. The total synthesis time was 34 min. The imaging experiments with [^{11}C]**1** in a rhesus monkey revealed adequate brain penetration. Maximal peak radioactivity reached 240% SUV at 2 min which declined to 83% SUV at 70 min. However, the concentration of radioactivity in the various ROIs remained undifferentiated throughout the scan time. In the pretreatment experiment, the time-activity curves were very similar to those of the baseline study.

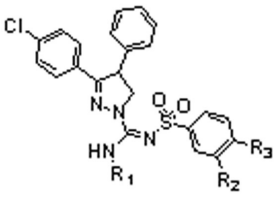

Table 1. Candidate radioligands for the CB1 receptor

Ligand	R_1	R_2	R_3	Kb (nM)	clog P
SLV	Me	H	MeO	24	4.50
1	Me	MeO	H	8	4.56
2	H	MeO	H	18	4.20
3	Me	H	CN	26	4.21

Conclusion. This study failed to reveal any CB_1 receptor-specific binding in rhesus monkey brain from the selected candidate radioligand. However, the acceptable brain uptake of radioactivity and fast washout of the radioligand suggests that higher affinity ligands from this platform (*e.g.* eutomer of ligand **1**) might result in an effective radioligand for the CB_1 receptor. We are continuing to explore this possibility.

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Keywords: CB1 Recptor, PET, Diarylpyrazolines

16α,17β-DIOXOLANE BROMINE- AND IODINE- SUBSTITUTED PROGESTIN FOR BREAST TUMOR IMAGING AND RADIOTHERAPY: SYNTHESIS

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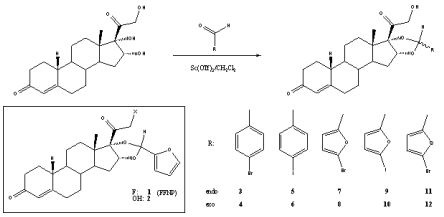
The presence of receptors for estrogens and progestins in breast tumors provides the possibility for diagnostic imaging and radiotherapy of those tumors. Administration and accumulation of an appropriately radiolabeled ligand for the appropriate hormone receptor allows for receptor quantification, and therefore diagnosis, of a given tumor. In the same manner, a differentially radiolabeled hormone receptor ligand that accumulates in a tumor can deliver a cytotoxic dose of high LET radiation selectively to the tumor cells, ablating the tumor while limiting widespread radiation toxicity. Therefore, the development of such a ligand allowing both diagnostic imaging and radiotherapy appears promising and is our current interest.

Several groups have studied the use of ER ligands labeled with high LET radionuclides and shown them to be effective in killing ER-positive cells. Also, it was shown by us that bromine-77 has a similar lethality to I-123. Because of the decay characteristics of bromine-76, 77 and iodine-124, they may allow both PET imaging for dosimetry and therapy studies.

A PgR-based ligand has some advantage over an ER-based one: (1) there is a better correlation between PgR status and hormonal responsiveness than there is with ER status; and, (2) a PgR-based ligand could be used after the initiation of anti-estrogen hormonal therapy, whereas an ER-based one would not be useful when tumor ER is saturated by the hormonal agent. Based on progestin ligands reported, the best candidate for labeling with bromine or iodine should have the skeleton of compound **1**, fluoro furanyl norprogesterone (FFNP). Compound **1** has a high relative binding affinity to PR (190% relative to R5020 = 100%), low nonspecific binding (log $P_{o/w}$ = 3.87) and high binding selectivity indices (the ratio of PR binding affinity to nonspecific binding). In tissue biodistribution studies in estrogen-primed immature female Sprague-Dawley rats, **1** demonstrated high PR-selective uptake in the principal target tissues, the uterus and the ovaries, and relatively low uptake in fat and bone. Also, the metabolism at the 21-position in **1** appears to be less than that in other 21-fluoroprogestins.

To follow up on this work and to adapt this steroidal system for labeling with other radiohalogens, we have synthesized a series of 16α,17β-dioxolane bromine- and iodine-substituted progestins, **3-12**, according to the scheme below. Starting from the triol and the corresponding aldehyde in large excess, the target compound was prepared in 1~3 hours in ~80% yield, with the ratio of endo/exo = 1:3 to 1:1, and the two stereoisomers were separated by reverse phase C-18 preparative HPLC using gradient solvent system. The bromine-76, 77 and iodine-124 labeled ligands could be made from the corresponding tin precursor by electrophilic radiohalogenation methods. Also, the C-21 fluorine substituted compounds of **3-12** could be converted from **3-12** easily. Relative bonding affinities of **3-12** are being determined.

This work was supported by DOE grants (DE FG02 86ER60401 to J.A.K, and DE FG02 84ER60218 to M. J. W).



Keywords: Synthesis of Bromine- and Iodine- Substituted Progestin, PET Imaging and Radiotherapy, Relative Bonding Affinity

RADIOCHEMICAL PURITY AND SPECIFIC ACTIVITY MEASUREMENTS OF [^{11}C]METHYL TRIFLATE USING ONLY A FLAME IONIZATION DETECTOR

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[^{11}C]Methyl triflate (CH_3OTf) has become a widely used synthon for PET radiopharmaceuticals. However, a practical method has yet to be developed to monitor its purity and specific activity. Rather, these parameters are commonly inferred from radiochemical yields and/or analytical measurements of [^{11}C]methylated end-products. This study investigates the utility of a flame ionization detector (FID) of a gas chromatography (GC) system to determine the radiochemical purity and specific activity of [^{11}C]CH_3OTf and its intermediate precursors [^{11}C]CH_4 and [^{11}C]CH_3Br. The FID is a very sensitive device which responds to virtually any molecule with a carbon-hydrogen bond. By directing the output of the GC column into a hydrogen flame, the analyte molecules become ionized. A collector electrode attracts the negative ions and, with appropriate signal amplification, as little as 100 picograms of hydrocarbon molecules can be detected. The FID also responds to ionizing radiation, although with much lower sensitivity than a sodium iodide crystal or Geiger-Müller detector. This can be demonstrated by the injection of [^{11}C]CO_2 onto the column. The FID does not respond to carbon dioxide, since it is not a hydrocarbon, but a sharp peak is clearly visible at the retention time for CO_2 (Figure 1). A similar response is seen with the injection of [^{11}C]CH_4, [^{11}C]CH_3OH, [^{11}C]CH_3Br and [^{11}C]CH_3OTf. However, these methyl compounds are hydrocarbons and also induce a detector response proportional to their mass. Separating and quantifying these two different responses is the ultimate goal of this work. Once accomplished, one should be able to calculate the specific activity of [^{11}C]CH_4 merely by performing two equal injections of the same gas sample several minutes apart.

Unwanted carrier ^{12}C-carbon (CO_2 and CH_4) is introduced to our [^{11}C]methyl triflate production system from both the target and processing gasses. However, once the [^{11}C]CH_4 has been produced and isolated, further conversion to [^{11}C]methyl triflate (and the subsequent ^{11}C-methylation) does not alter the specific activity of the radioligand. Accordingly, we present data documenting the mass response of the FID to a series of methane standards as well as the radioactivity-only response of the FID to known activities of [^{11}C]CO_2.

In addition to specific activity determinations, the FID can be used to make a rapid estimation of radiochemical purity of the [^{11}C]methyl triflate reagent delivered to our ^{11}C-methylation system. By diverting and injecting an aliquot of the [^{11}C]methyl triflate output to the GC column, the relative abundance of [^{11}C]CH_4, [^{11}C]CH_3Br and [^{11}C]CH_3OTf can be documented, along with any [^{11}C]CH_3OH decomposition product (Figure 2). In this manner, the performance of the heated silver triflate column can be rapidly and routinely evaluated independent of the preparation of the [^{11}C]methylated radiopharmaceutical product. Another practical advantage of this GC-FID method is that it employs the same capillary column (OVI-G43, 30 m x .53 mm) used for the analysis of residual organic solvents.

Figure 1

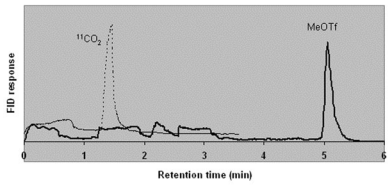
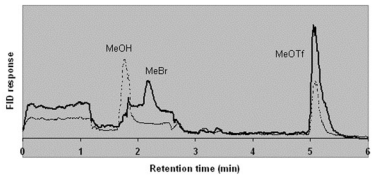


Figure 2



Keywords: Specific Activity, C-11 Methyl Triflate, Gas Chromatography Flame Ionization Detector

PROCESSING OF $^{68}Ge/^{68}Ga$ GENERATOR ELUATES FOR LABELING OF BIOMOLECULES *VIA* BIFUNCTIONAL CHELATORS

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Purpose: The $^{68}Ge/^{68}Ga$ generator (^{68}Ge, $T_{1/2}$ = 270.8 d) provides a cyclotron-independent source of positron-emitting ^{68}Ga ($T_{1/2}$ = 68 min, β^+ branching = 89%), which can be used for labelling of various biomolecules. Commercially available $^{68}Ge/^{68}Ga$ generators based on TiO_2 (Cyclotron Co., Obninsk, Russia) allow to elute > 50% of ^{68}Ga in 5-7 ml 0.1 M HCl solution. However, for labelling of biomolecules via bifunctional chelators (BFC) ^{68}Ga(III) must be pre-concentrated and purified from ^{68}Ge, Zn(II), Ti(IV) and Fe(III).

Methods: We have developed a system for simple and efficient handling of the $^{68}Ge/^{68}Ga$ generator eluate for labeling of nanomolar amounts of peptides. The main component of the system is a micro-chromatography column filled with about 50 mg of a cation exchange resin (Bio-Rad AG 50W-X8). Purification and concentration of ^{68}Ga(III) are carried out in hydrochloric acid-acetone systems. For labeling with ^{68}Ga(III) DOTA-octreotide (DOTATOC) and Desferrioxamine-B-succinyl-octreotide (DFOOC) were used.

Results: More than 97% of ^{68}Ga could be obtained finally in 400 µl of a 97.6% acetone/0.05 M HCl solution and used directly for labeling. The initial ^{68}Ge contamination was reduced by a factor 1000. Additional purification step with 80% acetone/0.15 M HCl solution allows to significantly reduce amounts of Zn(II) and Fe(III) (see Tab. 1). Within 25 min, an injectable radiopharmaceutical, e. g. ^{68}Ga-DOTATOC can be prepared with specific activities (SA) up to 40 MBq/nmol. SA of ^{68}Ga-DFOOC was about 200 MBq/nmol.

Conclusion: The developed system represents a simple and efficient way for labelling of DOTA-conjugated biomolecules with generator-produced ^{68}Ga(III). Using of acyclic BFC such as DFO simplifies labelling procedure and increases SA. ^{68}Ga-DOTATOC was successfully used in a series of human somatostatin receptor-expressing tumours diagnosis with PET/CT. Cation exchangers and hydrochloric acid-acetone media seem to be useful tools for preparation of gallium isotopes $^{66,67,68}Ga$. *Table 1. Relative distribution [%] of ^{68}Ga(III), ^{68}Ge(IV), Zn(II), Ti(IV), Fe(III) and Mn(II) on micro-chromatography column (53 mg Bio-Rad AG 50W-X8 cation exchanger) using **a)** 0.6 ml and **b)** 5 ml of 80% acetone, 0.15M HCl solution for purification*

Volume	Step / concentration	**68Ga(III)**	Ge(IV)	Zn(II)	Ti(IV)	Fe(III)	Mn(II)
a)							
7 ml	Generator elution / 0.1 M HCl	0.16	97.08	0.77	7.30	0.13	4.41
0.6 ml	Purification / 80% acetone/ 0.15 M HCl	1.43	2.92	98.15	0.68	37.86	0.49
0.4 ml	**Ga(III) elution / 97.6 % acetone/0.05 M HCl**	**97.82**	**0.03**	**1.08**	**0.07**	**49.78**	**11.10**
1 ml	Washing / 4 M HCl	0.41	0.005	0.005	72.15	11.61	69.38
1 ml	Washing / H2O	0.18	0.003	⟨0.001	19.80	0.62	14.62
b)							
7 ml	Generator elution / 0.1 M HCl	0.11	97.68	0.43	7.18	0.73	5.20
5 ml	Purification / 80% acetone, 0.15 M HCl	6.29	2.32	99.57	3.99	87.37	1.71
0.4 ml	**Ga(III) elution / 97.6 % acetone/0.05 M HCl**	**92.73**	**0.005**	**⟨0.001**	**0.11**	**11.10**	**9.79**
1 ml	Washing / 4 M HCl	0.77	0.002	⟨0.001	86.54	0.71	83.09
1ml	Washing / H2O	0.10	0.002	⟨0.001	2.18	0.09	0.21

Keywords: 68Ge/68Ga, Generator, DOTATOC

FACILE SYNTHESIS OF L-[1-^{11}C]LEUCINE AS A PET RADIOTRACER FOR THE MEASUREMENT OF CEREBRAL PROTEIN SYNTHESIS

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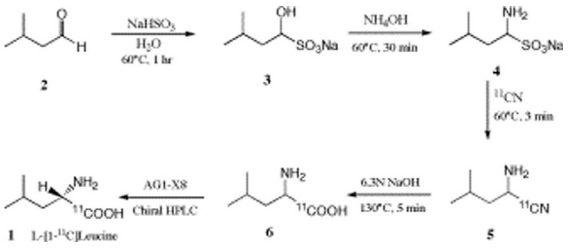
L-[1-^{11}C]Leucine (**1**) has been validated as an ideal PET radiotracer for the measurement of protein synthesis rates, especially in the brain (1). It has high incorporation into proteins, insignificant metabolism to nonproteins and high affinity for transport systems across the blood brain barrier. An early synthesis (2) of **1** was very labor intensive, involved harsh reaction conditions, and relied on enzymatic degradation of the unwanted D-enantiomer, which was also produced during the synthesis, for enantiomeric purification. We have used a modified Bucherer-Strecker synthesis with chiral-HPLC purification for the routine production of **1** by a method modeled after the recently reported synthesis of L-[1-^{11}C]phenylalanine and L-[1-^{11}C]tyrosine (3).

The starting material is the bisulfite adduct (**3**) of isovaleraldehyde (**2**). It is converted *in situo* to the aminosulfonate **4** just prior to EOB. Carbon-11 CO_2 is trapped and cleared of residual target gas oxygen using a Carbosphere™ trap (4). It is then converted to [^{11}C]HCN (5), which is bubbled into an aqueous solution of the aminosulfonate **4** at 0°C. After a 3 minute reaction at 60°C, the resulting aminonitrile **5** is hydrolyzed with 6.3N NaOH at 130°C for 5 minutes to the racemic [1-^{11}C]leucine (**6**). After cooling, the reaction mixture is neutralized with aqueous acetic acid and prepurified by passage through an anion exchange column (AG1-X8) to remove any free [^{11}C]CN^-. The L-[1-^{11}C]leucine is then isolated from the crude product solution by chiral-HPLC using a Chirobiotic-T column (250 x 10 mm) eluted with 5% ethanol, which provides clean enantiomeric separation. The isolated product fraction (~3 mL) is diluted with normal saline and sterilized by filtration.

The entire synthesis is performed remotely in a closed, lead-lined hot cell using a module for [^{11}C]CO_2 trapping and conversion to [^{11}C]HCN coupled to a module for the synthesis and purification of **1**. Both modules were fabricated in-house. The decay corrected radiochemical yield of **1** was 31.6 ± 2.4% (n = 31) based on the [^{11}C]CO_2 activity trapped in the Carbosphere trap (following oxygen removal) and 37.0 ± 2.7% based on the [^{11}C]HCN activity trapped in the reaction vial (conversion of [^{11}C]CO_2 to [^{11}C]HCN averaged ~85%). Starting with ~1200 mCi (44 GBq) of [^{11}C]CO_2, more than 140 mCi (5.2 GBq) of the radioligand was routinely obtained and ready for human application in 32 min from EOB (including 6 minutes for collection of the [^{11}C]CO_2 and conversion to [^{11}C]HCN) with chemical, radiochemical and enantiomeric purities of >98%. The clinical evaluation of **1** as a PET radiotracer for the measurement of cerebral protein synthesis is currently underway. An additional feature of this method is that D-[1-^{11}C]leucine is also isolated and can be used to determine the stereoselectivity of biochemical processes when compared to **1** in various *in vivo* and *in vitro* systems.



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Keywords: L-[1-11C]Leucine, PET, Protein Synthesis

DEVELOPMENT OF [^{13}N]CISPLATIN FOR CHEMOTHERAPY EVALUATION

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In order to obtain a better insight in the pharmacokinetics of cisplatin during chemotherapy and resistance of tumors towards cisplatin treatment, we started a program in which [^{13}N]cisplatin was prepared as PET-probe. A few publications on the synthesis of [^{13}N]cisplatin have appeared but could not be applied in our laboratory, because in these publications (De Spiegeleer, Holschbach) preparations are started with [^{13}N]nitrate, that was reduced to [^{13}N]ammonia with alkaline Devarda's alloy. We wanted to perform the production with in-target produced [^{13}N]ammonia, because this [^{13}N]ammonia production method is being used in our regular routine production for clinical studies. In addition, we wanted to automate the synthetic procedure using our Zymark robotic system.

One major challenge was to concentrate [^{13}N]ammonia from 30 ml (originating from a circulating water target) to 1 ml water (pH=10) within a few minutes without affecting the reactivity in the next complexation steps. Therefore, we investigated whether ammonia could be trapped on a cation exchange cartridge followed by an elution with an appropiate ionic solution. [^{13}N]Ammonia could almost quantitatively trapped. The elution was tested with solutions of NaCl, KOH and KCl. Unfortunately, the excess of chloride anions resulted in low radiochemical yields in the further reaction steps, whereas by using KOH it was difficult to control the pH. Using buffers for this purpose also resulted in low radiochemical yields.

Therefore, we moved to a procedure to distill the in-target produced [^{13}N]ammonia by collecting the irradiated water on KOH-pellets. Using additional external heating, distillation of [^{13}N]ammonia into 1 ml of water was completed in a few minutes. Co-distillation of basic aerosols were avoided by inserting quartz wool. The resulting distillate was transferred to the hotcell with the Zymark robot, that carried out the next steps, i.e. complexation to K_2PtI_4 to form $Pt([^{13}N]NH_3)_2I_2$, followed by conversion to $Pt([^{13}N]NH_3)_2(H_2O)_2$ and finally to [^{13}N]cisplatin, $Pt([^{13}N]NH_3)_2Cl_2$. Based on LC-MS estimations, about 30% of [^{13}N]ammonia was incorporated into the platinum complex. A molar ratio of ammonia to K_2PtI_4 of 2 was found to give the optimal yield. LC-MS demonstrated that also substantial amounts of $K_2Pt[^{13}N]NH_3I_3$ and $K_2Pt([^{13}N]NH_3)_3I$ were formed. The subsequent steps proceeded almost quantitatively.

Final purification was performed by HPLC using a Lichrosphere 100 RP-column and 0.1 mM hexadecyltrimethylammoniumbromide in 10 mM citrate buffer (pH=7) as mobile phase. [^{13}N]Cisplatin was eluted with a retention time of 10 min. For biological studies, the collected HPLC-fraction was diluted with 10 ml water, and passed through a C18 SepPAK. After washing with water, [^{13}N]cisplatin was eluted with ethanol and diluted with saline. Quantities of 50-100 MBq [^{13}N]cisplatin were reliably obtained. Radiochemical yields were 10-20% (EOB).

Biodistribution studies in rats bearing a GK4 tumor and the cisplatin-resistant counterpart GK4-CDDP demonstrated high uptake in kidney and urine after 30 min. No difference was found in [^{13}N]cisplatin uptake between the cisplatin-sensitive and resistant tumor. Tumor uptake was very low. Probably the half-life of ^{13}N is too short to measure differences in the uptake mechanisms.

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Keywords: Cisplatin, Chemotherapy, PET

SYNTHESES AND BIOLOGICAL EVALUATION OF F-18 AND I-123 LABELED PORPHYRINS AS POTENTIAL TUMOR IMAGING AGENTS

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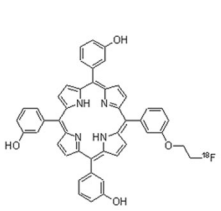
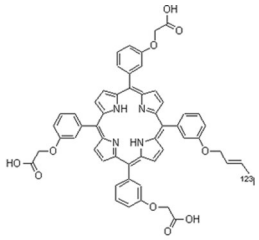
Photofrin has currently been approved for general use by licensing authorities to treatment for solid tumor and cancer using photodynamic therapy (PDT) [1] that treat to photochemical effect induced by light. Recently, meso-tetra(3-hydroxyphenyl)porphyrin has been developed as one of best tumor localizer and also shown a favorable tissue distribution. We have studied to develop fluorine-18 labeled meso-tetra(3-methoxyphenyl)porphyrins for tumor imaging [2].

We also studied another target compounds, F-18 or I-123 labeled porphyrin derivatives, as potential tumor imaging agents for positron emission tomography (PET). F-18 labeled porphyrin analogue **1** was obtained by using the activated K[^{18}F]F-K_{222} complex. After radiofluorination by heating at 90 °C for 20 min, the F-18 labeled porphyrin analogue was removed three MOM protecting groups in hydroxyl groups at phenols by 1 N HCl at 90 °C for 20 min. The radiochemical yield was obtained in 30-40% yield by HPLC. The desired target compound was purified by HPLC at 2.5 mL/min (isopropanol:dichloromethane:hexane = 1:2:7). Cellular uptake was tested for 9L and MCF7 cell, shown 0.15% ID/g at 240 min for 9L and 0.44% ID/g at 240 min for MCF7, respectively. The biodistribution of normal mice and PET image of 9L bearing rat were carried out. The PET image of rat with 9L was shown accumulation at tumor.

To increase accumulation of labeled porphyrin derivative, we have needed porphyrine labeled with longer half-life radioisotope. We have synthesized I-123 labeled porphyrin analogue **2**. The radioiodonated porphyrin compound was obtained by the reaction of iodinating porphyrin precursor with Na-^{123}I in the presence of peracetic acid in ethanol for 20 min. Iodine-123 labeled tert-butyl ester porphyrin derivative was obtained in 30-40% radiochemical yield by HPLC. The desired compound was purified by HPLC at 3 mL/min and the fraction at 10-11 min was collected and confirmed to desired compound by coinjection with cold *tert*-butyl ester porphyrin analogue. The *in vitro* and *in vivo* of I-123 labeled porphyrin derivative is under studying.



5-(3-(2-[^{18}F]Fluoroethoxy)phenyl)-10,15,20-*tris*-(3-hdroxyphenyl)porphyrin, 1



5-(3-(3-[^{123}I]iodoallyloxy)phenyl)-10,15,20-*tris*-(3-carboxymethoxyphenyl)porphyrin, 2

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Keywords: Porphyrin, Tumor Imaging, Fluorine-18, I-123

[^{18}F] FESB: AUTOMATED RADIOSYNTHESIS, IMAGING AND β-AMYLOID PLAQUE STUDIES OF A NOVEL ^{18}F-LABELED PET TRACER IN TRANSGENIC MICE WITH ALZHEIMER'S DISEASE

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The significant roles of amyloid cascades and neurofibrillary tangles (NFTs) in the pathogenesis of Alzheimer's disease (AD) necessitate the development of a receptor specific biomarker that facilitates early diagnosis of the disease. A recent study suggests that anti-amyloid therapies, when co-investigated in combination with PET or SPECT amyloid imaging tracers, could facilitate in vivo evaluation of the efficacy of therapy in the aging human brain. In spite of recent advances in new PET radiopharmaceuticals for the clinical evaluation of (AD), mostly [^{18}F]Fluorodeoxyglucose, a PET marker for evaluating metabolic disorders, is being used clinically but serves as an indirect tool to detect the presence and progression of AD. The studies with [^{11}C]-labelled Congo Red and [^{11}C]-Methoxy-XO4 (2.3-6.7% RCY) related earlier PET tracers of β-amyloid plaques indicated their high specific binding to β-amyloid plaques but had only marginal entry in the brain leading to poor detection of the disease. Recently, Pittsburgh Compound-B (PIB), an ^{11}C-labeled PET marker, has been studied in human AD patients and has demonstrated marked retention in the areas known to contain large amounts of amyloid deposits that further supports the role of a PET AD diagnostic radiotracer in AD therapy management. However, the short half-life of a C-11 labeled tracer requires its use within the local area in which it is produced, leading to its less than optimal acceptance for wide-spread use. This led us to develop new, longer-lived PET tracers that possess similar binding efficacy to the β-amyloid plaques.

This work describes the development of a ^{18}F-labeled analog of 1-(2′-fluoroethoxy)-2,5-bis(4′-methoxystyryl)benzene (^{18}F-FESB) using an automated synthesizer. This radiofluorination was assisted by 'ionic fluids' that significantly enhanced the labeling efficiency of the product. ^{18}F-FESB, being a fluorinated analog, has much longer half life than other ^{11}C-labelled members of this class and, therefore, will have a direct impact on the patients radiation doses and delineation of β-amyloid plaques from other regions in the brain. The biological properties of ^{18}F-FESB will be discussed.

Keywords: ^{18}F-FESB, Beta Amyloid, PET Imaging

IODONIUM CHEMISTRY: SCOPE AND SELECTIVITY IN AROMATIC NUCLEOPHILIC LABELLING REACTIONS

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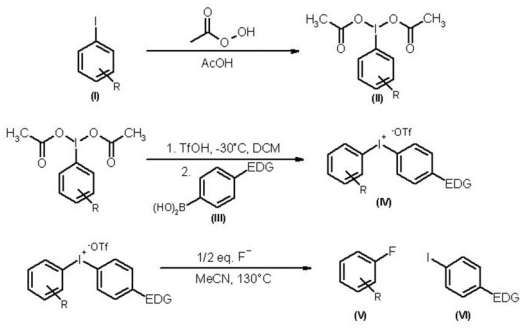
The success of PET is based on the ability of chemists to incorporate positron emitting isotopes into molecules of interest. However, the PET radiochemist still faces quite a number of challenges. One such challenge is the introduction of fluorine-18 onto an electron rich aromatic ring. If we focus on so-called high specific activity chemistry, fluorine-18 has typically been incorporated onto aromatic rings with reasonable amounts of success on the position *para* to the electron withdrawing group (EWG). This reaction also works on the position *ortho* to the EWG but yields tend to be lower (1-5% radiochemical yield). However, for aromatic ring systems in which the EWG is substituted by an electron donating group (EDG) or where the reaction is attempted on the *meta* position, the synthesis does not normally produce sufficient amounts of radiolabelled product for *in vivo* studies. As many new drug substances do have a fluorine attached to an electron rich aromatic ring or in the meta position, a strategy towards labelling these compounds is highly desirable. It has been demonstrated that the use of iodonium salts has the potential to greatly increase the yields in systems or positions that are normally not reactive enough to give sufficient yields. The idea behind the iodonium chemistry is that nucleophilic attack occurs on the more electron deficient ring. As a result, good counter rings need to contain an electron donating group(s). Unfortunately, little has been reported on the use of appropriate counter rings, the influence of various side chains, solvent systems, temperature, steric hindrance, varying sidegroups, electron density, etc. and the general scope of the reaction. Here we present a methodological approach towards characterising the reactivity and selectivity of iodonium chemistry as a means for introducing *e.g.* fluoride or cyanide onto deactivated aromatic ring systems.

Typically, the aryliodonium salt was prepared by reacting an aryl boronic acid (III) with the appropriate diacetoxyiodobenzene (II), scheme 1. Interestingly, in numerous cases it was possible to react the diacetoxybenzene directly with anisole, leading to improved yields and/or much reduced purification steps. Subsequently, the iodonium salt was reacted with CsF or KF and the ratios of desired products vs undesired products determined through HPLC. The following variables were investigated: solvent, temperature (conventional vs microwave), phase transfer agent, side chain on target ring, and electron donating group on the counter ring.

The results for the fluorination reaction varied quite extensively. Our work will assist in choosing the reaction conditions that are most likely to yield the desired product. The ability to react the diacetoxybenzene directly with anisole has greatly facilitated the production of iodonium salts. Further work is ongoing to investigate the use of *e.g.* veratrole as the electron rich aromatic ring and the use of cyanide as nucleophile. We will shortly investigate if the cold chemistry is a good predictor for radiochemical yields.



Keywords: Iodonium, Aromatic, Substitution

SYNTHESIS OF A PET RADIOTRACER FOR STUDYING DOPAMINE D_4 RECEPTORS: 3-(2-[^{11}C]METHOXYPHENYL-PIPERAZIN-1-YLMETHYL)-7-AZAINDOLE ([^{11}C]MPPMA)

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The dopamine D_4 receptor has received increasing attention since its possible involvement in schizophrenia, attention deficit hyperactivity disorder, drug addiction and alcoholism has been suggested. In the past, several D_4-specific and selective ligands have been developed. Among them L7545870 is one of the most highly D_4 selective antagonists developed to date[1)]. Its ^{18}F- and ^{123}I-labeled analogs have been synthesized and their distributions in animal brains have been investigated[2,3)]. We report here the radiosynthesis of a ^{11}C labeled analog of L7545870, [^{11}C]MPPMA (Figure). In our laboratory, the IC_{50} of MPPMA in displacement of [^{3}H]-N-methylspiperone binding to the recombinant human $D_{4.2}$ receptor has been determined to be 0.74 nM with the D_4 over D_2 selectivity of 570.

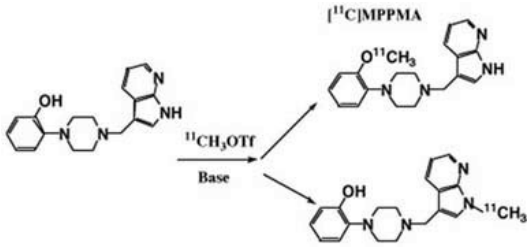
[^{11}C]MPPMA was synthesized by O-methylation of the desmethyl precursor with [^{11C}]methyltriflate (Figure). The O-[^{11}C]methylation reaction with [^{11}C]methyltriflate in DMF in the presence of tetrabutylammonium hydroxide completed almost instantaneously at room temperature giving [^{11}C]MPPMA in an average radiochemical yield of 62.5±3.5 % (n= 6, decay corrected) when the amount of the base was equivalent to or less than that of the precursor. A major radioactive by-product was from N-[^{11}C]methylation (Figure), and the ratio between the amount of the O-[^{11}C]methylation product and that of N-[^{11}C]methylation by-product depended upon the amount of the base present in the reaction: in the presence of the base in an amount equivalent to or less than that of the precursor, the ratio was approximately 9 to 1. With an excess amount of the base, N-[^{11}C]methylation predominated.

Using a C18 semi-preparative HPLC column and a mobile phase of acetonitrile/water (20/80) containing 0.1M ammonium formate, the product [^{11}C]MPPMA was well separated from the N-methylation by-product and the desmethyl precursor. The average radiochemical purity was 98.1±0.8 % (n=6), and the chemical purity was greater than 99.7%. The overall synthesis time including HPLC purification and formulation was approximately 20 minutes. The average specific activity at E.O.S. was 1413±790 mCi/μmol (n=6).

In conclusion, a highly potent dopamine D_4 receptor antagonist radiotracer [^{11}C]MPPMA, was synthesized with high radiochemical yield, high radiochemical and chemical purity and high specific activity using [^{11}C]methyltriflate as the alkylating agent and by controlling the occurrence of the N-methylation reaction.

References: 1) Kulagowski J et al. J Med Chem 39:1941-1942, 1996; 2) Eskola O et al. J Label Compd Radiopharm 45:687-696, 2002; 3) Staley JK et al. Nucl Med Biol 27:547-556, 2000

Keywords: Dopamine D4 Receptors, Methylation with ^{11}Cmethyltriflate, O-Methylation and N-Methylation



PRODUCTION OF [^{18}F]SPA-RQ AS A PET RADIOLIGAND FOR IMAGING HUMAN BRAIN NK_1 RECEPTORS

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Introduction. The neurokinin 1 (NK_1) receptor, a receptor for substance P, has been implicated in several neuropsychiatric and neurological disorders such as depression, anxiety, schizophrenia, Parkinson's disease and Alzheimer's disease. Although [^{18}F]SPA-RQ (**1**) has recently been developed and validated for imaging NK_1 receptors with PET in humans (1,2), improvements in the radiosynthesis of **1** (Figure 1) were needed to implement its production for regular clinical use in a USA setting. Four issues needed to be addressed before implementing a production method for PET experiments with **1** in human subjects: 1) optimization of [^{18}F]fluoromethyl bromide ([^{18}F]FCH_2Br) synthesis, 2) optimization of the subsequent ^{18}F-fluoromethylation reaction, 3) automation of the radiosynthesis, and 4) formulation of the final dose.

Experimental. Various bases (Cs_2CO_3, TBAOH, KOH, NaOH, K_2CO_3), solvents (DMF, DMSO, MeCN), labeling agents ([^{18}F]FCH_2Br, [^{18}F]FCH_2Cl, [^{18}F]FCH_2I, [^{18}F]FCH_2Br with NaI or KI, [^{18}F]FCH_2OTf, [^{18}F]FCH_2Br with Kryptofix 2.2.2 or 18-crown-6), different substrates (Boc-protected and unprotected precursors) and reactors (glassy carbon vessel or HPLC loop) were investigated to optimize the ^{18}F-fluoromethylation reaction. The optimized radiochemistry was incorporated into a commercial GE TracerLab FX_{FN} synthesis module linked to a specially constructed Peltier heating-cooling reactor (Figure 2). In production mode, [^{18}F]FCH_2Br is purified and trapped in a pre-cooled (0°C) vial (1 mL) containing *des-alkyl* Boc-protected precursor (0.3 mg), K_2CO_3 (2 mg), 18-crown-6 (5 mg) and DMF (750 mL). After [^{18}F]FCH_2Br trapping is complete, the secondary reactor expedites the ^{18}F-fluoromethylation reaction, subsequent deprotection of the ^{18}F-fluoromethylated precursor, and injection onto HPLC to give pure **1** for final formulation. Ethanol (0.95 mL) containing acetic acid (2 mL) was used to elute **1** from the C18 Sep-pak and the ethanolic solution of **1** was formulated with saline (0.9%; 9 mL) containing sodium bicarbonate solution (8.4%; 40 mL). The final formulation was filtered through a sterile Millipore MP filter (25 mm) into a sterile vial (10 mL) and analyzed.

Results and Discussion. All radiochemical yields (RCYs) are decay-corrected and reported as means ± SD. After efficient [^{18}F]FCH_2Br-trapping was demonstrated using an ice-cooled vial (RCY = 26 ± 7%; n = 55), non-automated radiosyntheses gave acceptable but variable RCYs (RCY = 8 ± 4%; n = 19) of formulated **1**. Although the Peltier heating-cooling reactor demonstrated similar [^{18}F]FCH_2Br-trapping efficiency (RCY = 26 ± 5%; n = 4), the improvement in RCY and greater consistency of the production of **1** were apparent in the ten most recent runs (RCY = 15 ± 2%). Chemical purity exceeded 99% when HPLC-grade acetone was eliminated as a cleaning agent and overheating of the reaction was avoided. The implementation of the Peltier heating-cooling reactor and modified reaction conditions has also reduced the radiation exposure to the production chemist.

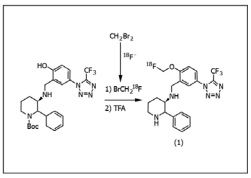

Figure 1. Production of 1.

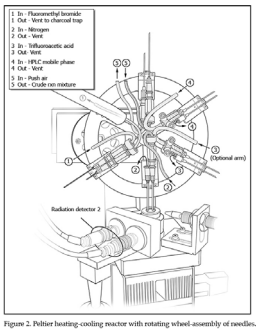

Figure 2. Peltier heating-cooling reactor with rotating wheel-assembly of needles.

Conclusion. The reproducibility of the production of **1** and the RCY are both improved for PET experiments in human subjects, with lower dose to the production chemist.

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Keywords: [18F]SPA-RQ, Neurokinin 1 Receptor, Automation

METABOLISM OF CORTICOTROPIN RELEASING HORMONE TYPE 1 RECEPTOR (CRHR1) PET LIGAND – [^{76}Br]MJL-1-109-2

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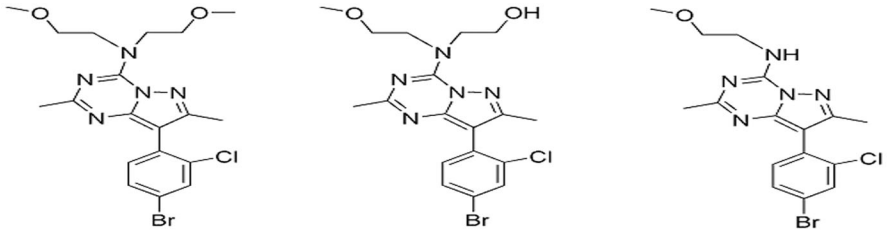
Over stimulation of CRHR1 in the brain has been associated with mental disorders such as anxiety, depression, and drug withdrawal syndromes. A specific PET CRHR1 ligand could be a useful tool for monitoring changes in CRHR1 density noninvasively. We have reported the design and radiosynthesis of [^{76}Br] MJL-1-109-2(**1**) that has potential for the in vivo study of the CRHR1 (Jagoda et al 2003).

LC/MS analysis of metabolites produced by commercially available hepatocytes has become an indispensable tool for identifying metabolites of novel radiotracers. In addition, these same i*n vitro* metabolism studies using hepatocytes from different species may reveal species differences. We studied the metabolism of **1** *in vitro* using rat, monkey, and human hepatocytes to produce metabolites and LC/MS/MS to identify these metabolites. *In vivo* studies in rats were used to evaluate metabolite uptake into the brain.

The metabolic profile of **1** was similar in rat, monkey, and human hepatocytes. The major metabolite, (**2**), resulted from *O*-dealkylation. The *N*-dealkylation metabolite, (**3**), was also detected. Following i.v injection of **1**, analysis of rat brain extract at 30 min showed **1** (80-85%) and **2** (15-20%); whereas the serum showed **1** (26-35%), **2** (7-8%), and the remainder very polar metabolites. These results suggest that **2** was efficiently taken up into the brain. The presence of metabolite **2** in the brain indicates that **1** is not optimal radiopharmaceutical because a metabolite in the brain will complicate the pharmacokinetics. Thus, the search has begun for a new chemical entity to serve as a CRHR1 radiotracer.



Keywords: PET, Corticotropin Releasing Hormone Type 1 Receptor (CRHR1) Ligand, LC/MS

MEASUREMENT OF BRAIN UPTAKE OF 2-[METHYL-^{11}C]METHOXYESTRADIOL BY MICROPET

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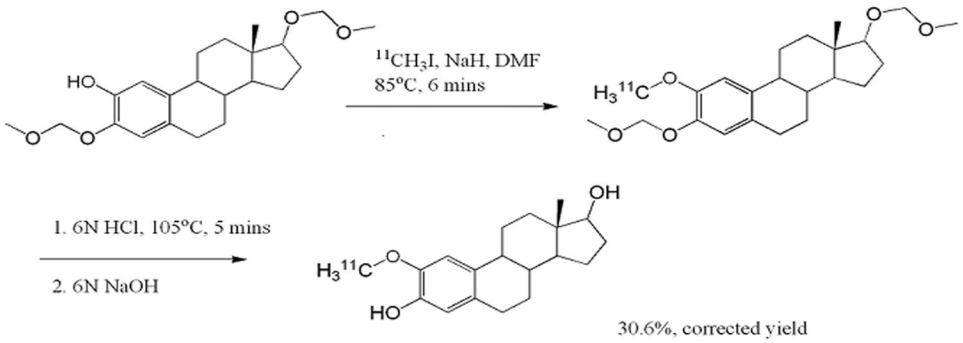
2-Methoxyestradiol binds to the colchicine binding site of tubulin, which results in inhibition of tublin polymerization. 2-Methoxyestradiol showed cytotoxicity in cancer cell cultures and inhibition of angiogenesis *in vitro* and *in vivo*. 2-Methoxyestradiol also showed inhibition of hypoxia-inducible factor-1(HIF) at the posttranscriptional level. We wanted to investigate the possibility to use 2-methoxyestradiol and its derivatives for brain tumor treatment, since these compounds showed no central nervous system (CNS) toxicity. The penetration of blood brain barrier (BBB) of 2-methoxyestradiol with γ-cyclodextrin carrier was examined by microPET with 2-[methyl-^{11}C]methoxyestradiol.

3,17-Bis(methoxymethoxy)-1,3,5-estratrien-2-ol was synthesized from estradiol in 3 steps with 62.1% overall yield from the synthetic method by Herbert E. Paaren and Steven R. Duff (Paaren, Herbert E; Duff, Steven R., U.S., 6448419, 10 Sep 2002). 3,17-Bis(methoxymethoxy)-1,3,5-estratrien-2-ol was labeled with $^{11}CH_3I$ by the modification of the method by B.-N. Park *et al* (Park, B.-N. *et al*, *J. Labelled Cpd. Radiopharm.* 42, Suppl. 1, 1999). 3,17-Bis(methoxymethoxy)-1,3,5-estratrien-2-ol was labeled with $^{11}CH_3I$ at 85°C for 6 min using NaH as a base in DMF. It was then deprotected with 6N HCl at 105°C for 5 min. The overall synthetic time, including HPLC purification, was 37 min. The isolated radiochemical yield at the end of synthesis was 31% with more than 98% radiochemical purity.



2-[Methyl-^{11}C]methoxyestradiol, dissolved in 5% ethanol, 40% γ-cyclodextrin aqueous solution, was injected to a nude rat intravenously on tail. Brain uptake of 2-[methyl-^{11}C]methoxyestradiol was observed for 60 min and showed maximum uptake around 13 min and followed by rapid washout of radioactivity.

Keywords: 2-Methoxyestradiol, Carbon-11, Brain Uptake

SYNTHESIS AND RADIOLABELLING OF A NOVEL IMAGING AGENT FOR HYPOXIC TISSUE IN STROKE AND TUMOURS

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Hypoxic tissue is of great significance in neurology and oncology. Until recently, efforts to investigate hypoxia *in vivo* have been hampered by difficulties in imaging the hypoxic fraction of tissue.

Positron Emmision Tomography (PET) has emerged as a powerful diagnostic tool in the characteriation of hypoxic tissues in cancers and stroke[i]. Nitroimidazoles labelled with Fluorine-18 such as ^{18}F-Fluoromisonidazole (FMISO), has been used with PET in humans for the identification of hypoxic tissues in Stroke and intracerebral hemorrhage[ii].[iii].

However, FMISO suffers from two disadvantages, low cellular uptake into hypoxic tissue and slow clearance from normoxic cells requiring long periods between injection and imaging. This period is not practical in regards to routine scanning of stroke patients; hence, the need to develop imaging agents with improved pharmacokinetics is necessary.

Sulfoxide containing nitrogen mustards are another class of compounds that have great affinity to hypoxic tissue.[iv] Derivatives of these compounds labelled with a Positron Emitting radionuclide, such as [^{18}F], may allow for the use of these agents to act as a diagnostic tool.

The preparation of nitrogen mustards was previously reported via a four-step synthesis[iv], beginning from the commercially available aniline derivative (**1**), shown in figure 1. The 4-(thiomethyl) aniline (**1**) is alkylated with chloroethanol in water to give the diol derivative (**2**). However this reaction step was very low yielding due to either the poor alkylating ability of chloroethanol, or alternatively the high volatility of the derived ethylene oxide.

figure 1: original synthesis.

We established that the reported method could be simplified by reducing the synthetic steps and increasing the overall yield to 63%. The proposed synthesis for the successful alkylation of the aniline derivative using the reactive triflate-leaving group is presented in Figure 2.

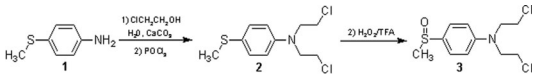
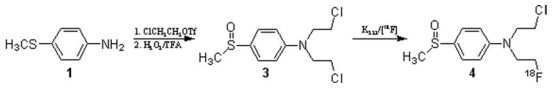


Figure 2: Overall synthesis and radiolabelling.

Compound **3** was successfully labeled with [^{18}F] (**4**) using a potassium fluoride kryptofix complex (**Figure 2**), giving the desired product in 40% radiochemical yield (10 min at 100°C). Preliminary *In vitro* analysis to determine the stability of **4** in plasma and saline indicated no defluorination.



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Keywords: Hypoxia, PET Tracer, F-18 Labelling

ROUTINE RADIOSYNTHESIS OF [^{11}C]METHYLPHENIDATE: CAN IT BE IMPROVED?

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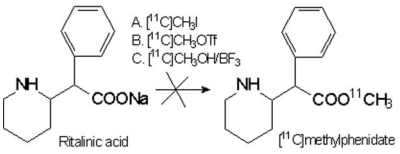
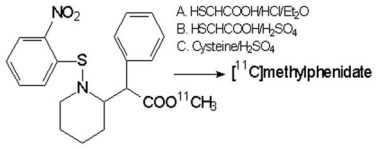
PET radiotracer for dopamine transporter d-threo[C-11]methylphenidate ([C-11]MP) has been routinely produced for over a decade following the originally developed procedure. We present here our effort to develop a modified radiosynthesis in the hope of improving the existing procedure.

The original reference (1) suggests that synthesis of [C-11]MP by direct C-11-iodomethylation of ritalinic acid is impracticable (Figure 1). Although ritalinic acid has two potential sites for methylation, it has been shown that regiospecificity of C-11-methylation can, in principle, be achieved by changing the reaction solvent (2,3) or C-11-methylating agent (4,5). Unfortunately, our attempts to regioselectively methylate ritalinic acid with [C-11]MeI or [C-11]MeOTf using DMF, acetonitrile or acetone as the reaction solvent were unsuccessful. Methylation of ritalinic acid with [C-11]methanol in the presence of boron trifluoride also could not be achieved.

An alternative improvement to the radiosynthesis could be development of a better method to remove the 2-nitrobenzenesulfenamide- (Nps-) protecting group (Figure 2) in the conventional radiosynthesis. These attempts have proven successful. In the original reference the Nps-group was cleaved using a mixture of the mercaptoacetic acid and HCl solution in diethyl ether. Both there reagents are volatile irritants. HCl is gradually depleted upon storage leading to an inefficient deprotection step and resulting in lower radiochemical yields. Because the addition of highly volatile liquid could not be reliably achieved through the add line from outside of the hotcell, the mixture of acids is injected directly into the reaction vessel resulting in undesirable radiation exposure to chemist. Occasionally, during the solid phase extraction workup in the radiosynthesis, [C-11]MP leaked through the C18 Sep-Pak. This could be either attributed to unbalanced acidity (due to difficulty of delivering a small amount of HCl-ether) or formation of a two-phase system (ether-aqueous solution) upon quenching with sodium borate buffer. We report here a new deprotection system using a solution of mercaptoacetic and sulfuric acids in acetonitrile. Preliminary experiment indicates that mercaptoacetic acid can be replaced with innocuous natural amino acid cysteine. Further investigation is underway.





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Keywords: [^{11}C]methylphenidate, Routine Production, Dopamine Transporter

BY-PRODUCT FORMATION DURING EVAPORATION WITH THE RADIOLYSIS SCAVENGER PROPYLENE GLYCOL

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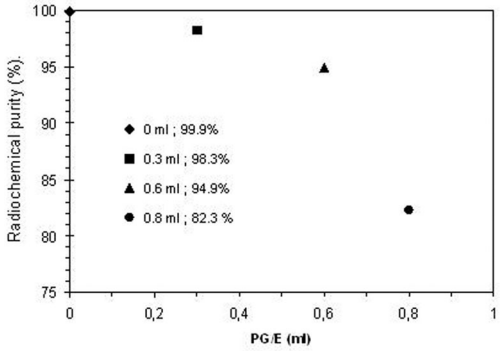
Radiolysis during evaporation of product solutions is often prevented by addition of alcohols as scavengers. We routinely use a sterile mixture of propylene glycol and ethanol, 7/3, (PG/E) originally used for low specific radioactivity [^{11}C]flumazenil formulations. Addition of small amounts (0.3-1.0 ml) of this mixture prevents radiolysis and assists in the re-solubilization of lipophilic products.

We have however recently found two radiopharmaceuticals where the use of this mixture during evaporation give rise to by-product formation. In the case of [^{11}C]WAY 100635 the by-products are formed by reaction with propylene glycol, as the use of ethanol do not give by-products.

The most striking example is [^{11}C]MHED. We have earlier reported the use of 0.3 ml of the PG/E mixture in the evaporation of [^{11}C]MHED HPLC purified product solutions. In a re-investigation of the production of [^{11}C]MHED we have systematically changed the amount of added PG/E and found an increased by-product formation with increased PG/E amounts. The results are illustrated below in Figure 1. There are at least two labelled by-products formed, one eluting earlier, and one eluting later than [^{11}C]MHED in reversed phase HPLC analysis.

Figure 1. Radiochemical purity of final [^{11}C]MHED product as a function of amount of PG/E added before evaporation of HPLC purified product.

By using 0.3 ml of PG/E the radiochemical purity of the product is higher than our limit for release, 95%. However, as we have found that omission of PG/E gives a higher radiochemical purity we subsequently do not use it in [^{11}C]MHED productions. As [^{11}C]MHED is hydrophilic there is also no need to add propylene glycol due to solubility problems. Obviously radiolysis is not an important issue for [^{11}C]MHED as we obtain higher radiochemical purity by omitting the radiolysis scavenger. In conclusion, one must thus be careful when using scavengers (radiolysis and/or oxidation) during evaporation of HPLC purified products and evaluate that these scavengers do not react with the product, creating by-product(s).



Keywords: By-Products, Radiolysis Scavenger, Propylene Glycol

SYNTHESES OF F-18 LABELED CAPECITABINE AND 5-FLUOROURACIL USING [^{18}F]F_2 GAS AS TUMOR IMAGING AGENTS

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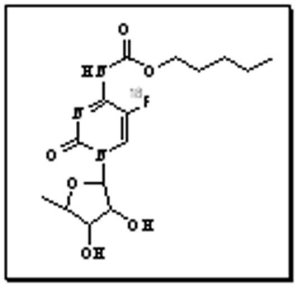
Capecitabine (N^4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine) is a novel fluoropyrimidine carbamate which is changed to 5-fluorouracil (5-FU) by three kinds of enzymes, such as carboxyesterase, cytidine deaminase, and thymidine phosphorylase, located in liver and tumors. Capecitabine is improved on selectivity and stability for tumor cell in comparison with 5-FU [1]. Thus we have prepared the title compounds as potential radiotracer for PET: Fluorine-18 was introduced at C5-position of capecitabine and 5-FU using F_2 gas by electrophilic fluorination. Precursor of capecitabine, 5′-deoxy-N^4-(pentyloxycarbonyl)cytidine was obtained from L-ribose in 8 steps [2,3]. Uracil was used as a precursor of 5-FU. [^{18}F]F_2 gas was generated by bombardment of a labeling source using 18.2 MeV proton beam: 1st irradiated to [^{18}O]O_2 gas with 30 mA beam for 30 min and 2nd irradiated 30 mA for 15 min to 1% F_2/Ar in aluminum target body. We synthesized F-18 labeled capecitabine from the mixture of saturated [^{18}F]F_2 gas passing through with NaOAc•3H_2O column and a solution of 10 mg of precursor of capecitabine in 15 mL of acetic acid for 30 min at rt. We checked each step for the preparation of the precursor of capecitabine using ^{1}H NMR and ^{13}C NMR and the overall yield of eight steps is about 8%. The average radiolabeling yield of electrophilic fluorination step using [^{18}F]F_2 was about 60% (n = 10). After extraction with dichloromethane, the desired compound was purified by HPLC system at 4 mL/min and the fraction at 13-14 min was collected and confirmed to desired compound by co-injection with authentic capecitabine analogue. Radiochemical yield and radiochemical purity were 10-15% and >95%, respectively. We have described an effective and convenient synthesis of F-18 labeled capecitabine. Further, this tracer will be characterized and tested with various cell line and bearing tumor rat model.



capecitabine

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Keywords: Capecitabine, 5-Fluorouracil, Tumor Imaging

REGIO- AND STEREOSELECTIVE ELECTROPHILIC FLUORINATION OF 3,4,6-TRI-*O*-ACETYL-D-GLUCAL (TAG) IN ACIDIC MEDIUM

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Introduction:

Fluorination of the carbon-carbon double bond in 3-,4-,6-tri-*O*-acetyl-D-glucal (TAG) using elemental fluorine, diluted with neon, was the first successful synthesis of 2-deoxy-2[^{18}F]-fluoro-D-glucose (FDG).[1] Stereoselectivity of direct fluorination of TAG in $CFCL_3$ is not ideal because 2-deoxy 2[^{18}F]-fluoro-D-mannose (FDM) is also formed in 50% yield. A number of studies have been carried out to improve the radiochemical yield and stereoselectivity of this reaction. We have studied the effect of the reaction medium acidity on the regioselective fluorination of aromatic amino acids. In this paper we report the efficiency and regio- and stereoselctivity of radiofluorination of TAG in different acid media.

Methods:

Fluorine-18 labelled F_2 was produced by the $^{18}O(p,n)^{18}F$ nuclear reaction in a Siemens 11 MeV cylotron (RDS112) using the double-shoot method.[2] Dilute [^{18}F]F_2 or F_2 (35 - 45 µmoles) was bubbled through a solution of TAG (70 - 75 µmoles) dissolved in anhydrous HF, HCOOH, TFA and $CFCl_3$. After the fluorination, the reaction mixture was diluted with 1 mL of a 95:5 CH_3CN:H_2O mixture and passed through a silica Sep-Pak® which was pretreated with ether. The solvent was evaporated and the dry residue was hyrolyzed in 1N HCl at 130 °C for 17 min. The final product was separated from the crude reaction mixture using LC consisting of ion retardation resin followed in sequence by alumina and C18 Sep-Paks®. The final product from each reaction was analised by radio-TLC, radio-PLC, LC-MS and muti-NMR spectroscopy.

Results:

In all cases, the radio-TLC and radio-HPLC of the product were very similar. The highest radiochemical yield (32 ± 3%, w.r.t [^{18}F]F_2 at the end of 55 min synthesis) and purity (95 ± 2%) were observed for the product resulting from the fluorination of TAG in HF. The molecular weight of the major product was determined by electrospray ionization MS in the negative ion mode. The ^{19}F NMR spectrum of the product resulting from the fluorination of TAG in HF consisted of a doublet of doublets of pseudotriplets. Further 1-D and 2-D NMR experiments confirmed that the compound was 2-deoxy-2[^{18}F]-fluoro-β-D-allose(2FβDA). Fluorination of TAG in TFA and formic acid produced 2FβDA as well as FDG, FDM and other unidentified products (Figure 1).

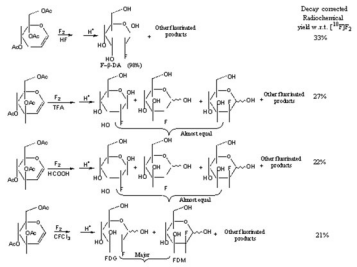

Figure 1. Fluorinated products resulting from the fluorination of TAG in $CFCl_3$, HF, TFA and HCOOH.

Conclusion:

A novel ^{18}F-labelled tracer for the rare sugar, D-allose, has been synthesized. Both radiochemical yield and purity are the highest reported to date for direct electrophilic fluorination of TAG. This is also the first reported case to date of regio- and stereoslective synthesis of a fluorinated sugar molecule by direct fluorination using F_2. Our two-step, reproducible synthesis is much more convenient and efficient than the seven step synthesis of 2FβDA reported in 1966.[3]

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Keywords: Regio and Stereoselective Fluorination, F-18 Labelled Allose, Rare Sugar

AN ALTERNATE SYNTHESIS OF [^{18}F]FAZA: A CLINICALLY USED PET TRACER FOR TISSUE HYPOXIA

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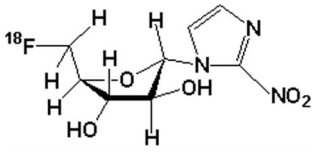
Hypoxic tumor cells are known to be more resistant to radiation therapy than normal cells and, therefore, an assessment of tumor tissue hypoxia may be of significant relevance for individual treatment planning, monitoring as well as predicting prognosis. Selective single electron reduction of radiolabeled nitroimidazoles in hypoxic tissues forms the basis for irreversible adduct formation and their clinical use in non-invasive (imaging) diagnosis of hypoxia in a variety of pathological disorders. Several 18F-labeled nitroimidazole derivatives, including 1-(5-fluoro-5-deoxy-α-D-arabinofuranosyl)-2-nitroimidazole ([^{18}F]FAZA)and fluoromisonidazole ([^{18}F]FMISO), have been reported to be useful for PET imaging of hypoxic tissues. Although both [^{18}F]FAZA and [^{18}F]FMISO appear to undergo quantitative binding to the same extent[1,2], [^{18}F]FAZA may be the preferred agent for routine PET because of superior contrast between target and non-target tissues[3] Furthermore, [^{18}F]FAZA has little or no uptake in normal brain, whereas [^{18}F]FMISO, a more lipophilic agent, shows substantial brain uptake.



[^{18}F]FAZA is normally prepared via nucleophilic radiofluorination of 1-(2,3-di-O-acetyl-5-O-tosyl-α-D-arabinofuranosyl)-2-nitroimidazole. This procedure has been optimized for automated radiosynthesis units (ASUs) to afford [^{18}F]FAZA in 5-15 % radiochemical yield at end of synthesis[4,5]. As with other nucleophilic radiofluorinations, like [^{18}F]FDG synthesis, that require hydrolytic deprotection to obtain the desired product, deprotection leads to the formation of 1-α-(D-arabinofuranosyl)-2-nitroimidazole (AZA) as a by-product. In an effort to eliminate the formation of this non-radioactive by-product, an alternate automated synthesis of [^{18}F]FAZA from the readily prepared, chemically stable iodinated precursor, 1-(5-iodo-5-deoxy-α-D-arabinofuranosyl)-2-nitroimidazole (IAZA) is now reported. Results of model halogen exchange radiofluorination reactions using both normal heating and microwave to promote exchange indicate comparable radiochemical yields but with the advantage of producing a cleaner product that may not require radiochromatographic purification.

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Keywords: 18F-FAZA, PET Tracer, Hypoxia

SYNTHESIS AND CHARACTERIZATION OF [^{18}F]MCL-322 AS A POTENTIAL PET RADIOTRACER FOR IMAGING THE DOPAMINE TRANSPORTER

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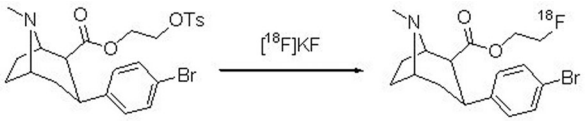
The fluoroethyl ester-containing phenyltropane derivative MCL-322 was shown to display high affinity to the dopamine transporter (DAT) (K_i = 2.3 nM). The binding affinities (K_i values) for the serotonin transporter and norepinephrine transporter were determined to be 5.1 nM and 280 nM, respectively [1]. The high binding affinities and selectivities of MCL-322 make the corresponding ^{18}F-labelled compound an attractive PET radioligand for imaging DAT in brain tissue. Here, we report on the radiosynthesis of [^{18}F]MCL-322 and its radiopharmacological characterization involving biodistribution, autoradiography and small animal PET studies. Fig. 1. Synthesis of [^{18}F]MCL-322

DAT ligand [^{18}F]MCL-322 was prepared in a single radiofluorination step starting from tosylate **1**. The reaction was performed in acetonitrile as the solvent at 80°C within 60 min including HPLC purification. [^{18}F]MCL-322 was obtained in radiochemical yields of 30-40 % (decay-corrected) at a specific radioactivity of >1.5 Ci/μmol at the end of synthesis. The radiochemical purity exceeded 95 %.



1 **[^{18}F]MCL-322**

Biodistribution studies in rats demonstrated a high uptake in the striatum (3.2 % ID/g) 5 min after injection, which increased to 4.2 % ID/g after 60 min. The uptake in the cerebellum was 1.8 % ID/g and 0.6 % ID/g after 5 min and 60 min post injection, respectively. Small animal PET images showed high accumulation of [^{18}F]MCL-322 in the striatum. Specific binding to DAT of [^{18}F]MCL-322 was confirmed by blocking experiments using the high affinity DAT ligand GBR 12909. The radiopharmacological characterization was completed with autoradiography studies confirming the highly selective uptake of [^{18}F]MCL-322 in the striatum.

In summary, the simple single-step radiosynthesis of [^{18}F]MCL-322 and the promising radiopharmacological data make [^{18}F]MCL-322 an attractive candidate for the further development of a potential PET radiotracer for clinical imaging DAT in human brain.

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Acknowledgement: The authors want to thank Dr. A. Kluge (ABX-CRO advanced pharmaceutical services, Dresden) for fruitful discussions.

Keywords: [18F]MCL-322, Dopamine Transporter (DAT), PET

POTENTIAL PITFALLS OF [^{11}C]METHYL TRIFLATE AS A METHYLATION REAGENT

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It is generally accepted that [^{11}C]methyl triflate (MeOTf) is a more potent and versatile methylation reagent than [^{11}C]methyl iodide. With a boiling point of 94-99 °C, methyl triflate is easily trapped in small reaction volumes at room temperature. Its higher reactivity allows for faster reaction kinetics at lower reaction temperatures. However, this reactivity may also result in the formation of additional side-products which must be removed from the desired end-product prior to formulation. Our experience with [^{11}C]MeOTf has revealed a number of pitfalls with the use of this labeling reagent.

First is the condensation or adsorption of the gas phase reagent in the valve bodies and transfer lines used to deliver the [^{11}C]MeOTf from the heated silver triflate column to the reaction vial. The longer the transfer line and the more valves, the greater is the potential loss of starting radioactivity for the radioligand synthesis. Fortunately, periodic rinsing of the transfer pathway with acetone is sufficient to restore complete delivery of the [^{11}C]MeOTf to the reaction vessel.

Second is the potential for incomplete conversion of the [^{11}C]CH_3I or [^{11}C]CH_3Br precursor by an improperly functioning silver triflate oven. [^{11}C]CH_3I requires nearly 200 °C temperature for quantitative conversion to [^{11}C]MeOTf, whereas [^{11}C]CH_3Br requires 300-325 °C. Neither of these [^{11}C]methyl halides is trapped by the reaction mixture as efficiently as the desired triflate. Coupled with their lower chemical reactivity, the overall radiochemical yield of methylated end-product is greatly reduced.

Another problem observed is the apparent on-column decomposition of [^{11}C]MeOTf into [^{11}C]CH_3OH as the silver triflate column ages. While easily delivered to and trapped by the reaction mixture, no incorporation of [^{11}C]tracer occurs. The precise mechanism for this decomposition is not clearly understood. Since we prepare [^{11}C]MeOTf from the [^{11}C]CH_3Br precursor, we suspect a gradual accumulation of bromine by the Graphpak™ (Alltech) carbon support used to bind the silver triflate reagent (Jewett, *Appl Radiat Isot* 43:1383-1385, 1992). In addition, back pressure increases as the carbon column ages, which could result in a prolonged transit of the [^{11}C]MeOTf through the column, increasing the possibility of decomposition to [^{11}C]CH_3OH. When we replaced the Graphpak support with Carbosphere™ (Alltech), back pressure was reduced but the transit time increased due to the significantly larger surface area of the Carbosphere matrix and [^{11}C]CH_3OH would still appear in the effluent. We have recently switched to a silicate support matrix (Vermiculite) for the silver triflate reagent which has greatly reduced the back pressure and decreased the transit time through the column. The effect of silicate support on [^{11}C]MeOTf decomposition will be discussed.

Lastly, the high reactivity of [^{11}C]MeOTf can be problematic if any trace of ethanol remains in any part of the pathway leading to the reaction vial. Since ethanol is commonly used as a sanitizing solvent, it is especially important that it is not the last solvent passed through the methylation system during the box cleaning procedure. Even at 0 °C, we have observed as much as 30-40% radiochemical yield of [^{11}C]$CH_3OCH_2CH_3$ which confounded our initial efforts to synthesize [^{11}C]raclopride in our newly designed automated methylation system. The volatile ether side-product exhibited a virtually identical retention time on the reverse-phase HPLC column as did raclopride. Upon mobile phase evaporation, the recovered radioactivity of [^{11}C]raclopride was far less than what appeared to have eluted from the semi-prep column. When the cleaning routine was changed to include a terminal rinse with acetone, the formation of [^{11}C]$CH_3OCH_2CH_3$ was eliminated.

Keywords: C-11 Methyl Triflate, Radiolabeling Pitfalls, Silver Triflate Column

EFFICIENT *O*-[^{11}C]METHYLATION WITH [^{11}C]METHYL TRIFLATE: SYNTHESIS OF ^{11}C-LABELED DIARYL SUBSTITUTED PYRAZOLE AND IMIDAZOLE CYCLOOXYGENASE INHIBITORS

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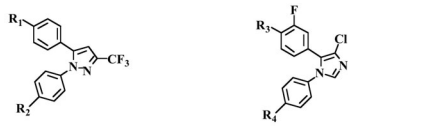
Cyclooxygenase (COX) is an important enzyme that converts arachidonic acid to prostaglandin H2, the precursor of all prostanoids. COX has recognized two isoforms, COX-1 and COX-2. COX-1 is a constitutive form, and presents in many tissues such as stomach, kidney and platelets. COX-2 is an inducible form, and presents mainly in brain and kidney. Furthermore, COX-2 expression can be induced by inflammatory stimuls. Development of selective probes for COX-1 and COX-2 may help us understand the involvement of the COX pathway in various inflammatory and neurodegenerative diseases. Therefore, many compounds with a central heterocyclic core having two vicinal aryl rings have been studied extensively for selective COX inhibitors. So far positron-emitting COX inhibitors have been prepared and evaluated *in vivo* [1,2]. We chose two diaryl substituted pyrazole derivatives, **1** (IC_{50} for COX-1 = 7.0 nM;, IC_{50} for COX-2 = 75 nM [3]) and **2** (IC_{50} for COX-1 = 2,600 nM;, IC_{50} for COX-2 = 8.0 nM [3]), and two diaryl substituted imidazole derivatives, **3** (IC_{50} for COX-1 = >10,000 nM;, IC_{50} for COX-2 = 4.0 nM [4]) and **4** (IC_{50} for COX-1 = 3,300 nM;, IC_{50} for COX-2 = 5.0 nM [4]), as a potent and selective COX inhibitors for labeling with ^{11}C (Fig. 1). ^{11}C-Labeled COX inhibitors were synthesized by [^{11}C]methylation of each phenol-precursor with for [^{11}C]CH_3I or [^{11}C]CH_3OTf. [^{11}C]CH_3I or [^{11}C]CH_3OTf was trapped in the 0.25 mL of DMF or acetone containing 0.25 mg (0.65-0.74 μmol) of each phenol-precursor in the presence of 1-2 mg of NaH or 5 μmol NaOH in 5 μL water or 0.7 μmol NaOH in 5 μL water as a base. The reaction was carried out at 120°C or room temperature for 1 minute. The methylation with [^{11}C]CH_3OTf in acetone containing 0.7 μmol of NaOH (one molar equivalent) produced higher radiochemical yields (RCYs) for each ^{11}C-labeled COX inhibitor than those with [^{11}C]CH_3I. By the use of excess amount of NaOH or by the use of DMF as a solvent, RCYs of [^{11}C]**1**, [^{11}C]**2** and [^{11}C]**4** were decreased. On the other hand, RCYs of [^{11}C]**3** with 5 μmol and 0.7 μmol NaOH were the nearly same (Table 1). In conclusion, ^{11}C-labeled COX inhibitors were synthesized in high RCYs by the methylation of each phenol-precursor with [^{11}C]CH_3OTf in acetone containing one molar equivalent of NaOH.



[^{11}C]**1** (R_1 = Cl, R_2 = O[^{11}C]CH_3) [^{11}C]**3** (R_3 = O[^{11}C]CH_3, R_4 = SO_2CH_3)
[^{11}C]**2** (R_1 = O[^{11}C]CH_3, R_2 = SO_2NH_2) [^{11}C]**4** (R_3 = O[^{11}C]CH_3, R_4 = SO_2NH_2)

Figure 1. Structure of ^{11}C-labeled COX inhibitors

[References]
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Synthesis of ^{11}C-labeled COX inhibitors.

	Agent	Solvent	Base	Radiochemical yields* (%)
[^{11}C]**1**	[^{11}C]CH_3OTf	Acetone	0.7 μmol NaOH	58.3-74.5 (n = 3)
		DMF	0.7 μmol NaOH	21.6 (n = 1)
	[^{11}C]CH_3I	DMF	1-2 mg NaH	19.9-40.0 (n = 3)
[^{11}C]**2**	[^{11}C]CH_3OTf	Acetone	0.7 μmol NaOH	44.3-51.5 (n = 3)
		Acetone	5 μmol NaOH	5.4 (n = 1)
		DMF	5 μmol NaOH	11.8 (n = 1)
	[^{11}C]CH_3I	DMF	1-2 mg NaH	20.6-22.2 (n = 3)
[^{11}C]**3**	[^{11}C]CH_3OTf	Acetone	0.7 μmol NaOH	75.1 (n = 1)
		Acetone	5 μmol NaOH	64.8-78.2 (n = 4)
	[^{11}C]CH_3I	DMF	1-2 mg NaH	9.9, 14.0 (n = 2)
[^{11}C]**4**	[^{11}C]CH_3OTf	Acetone	0.7 μmol NaOH	66.1-74.1 (n = 4)
		Acetone	5 μmol NaOH	1.1 (n = 1)
	[^{11}C]CH_3I	DMF	1-2 mg NaH	1.9-11.9 (n = 3)

*Decay-corrected radiochemical yields based on [^{11}C]CH_3OTf or [^{11}C]CH_3I.

Keywords: [11C]methyl Triflate, COX Inhibitors, O-[11C]methylation

OPTIMISATION OF THE SEPARATION OF [^{18}F]FLUOROBROMOMETHANE AND DIBROMOMETHANE USING DISTRIBUTION GAS CHROMATOGRAPHY

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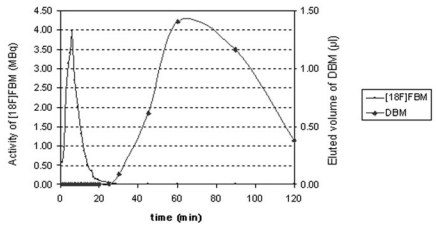
[^{18}F]Fluorobromomethane ([^{18}F]FBM) is a commonly used intermediate in the synthesis of fluorine-18 labelled molecules that contain the fluoromethyl moiety, and can easily be transformed to [^{18}F]fluoromethyl triflate, a more powerful [^{18}F]fluoromethylating agent. [^{18}F]FBM is usually produced by nucleophilic substitution of dibromomethane (DBM) with *n.c.a.* [^{18}F]fluoride. The critical part of the synthesis is the separation of [^{18}F]FBM from its precursor, DBM. For labelling reactions [^{18}F]FBM must be absolutely free from DBM, since this reacts in analogous way. If an efficient separation of the two can be achieved, the [^{18}F]fluoromethylated product will not contain its bromomethylated analogue, thus avoiding a second purification step. Although the difference in boiling points of FBM and DBM is considerable, a simple distillation has never resulted in precursor-free [^{18}F]FBM. Thus, preparative or semi-preparative gas chromatography has been applied to isolate [^{18}F]FBM which is free of contamination with DBM. This separation has been performed both by distribution (gas-liquid) gas chromatography applying 20% SF-96 on Chromosorb support and adsorption (gas-solid) gas chromatogtraphy using Porapack Q adsorbent as stationary phase. Recently, the separation has been performed on silica Sep Pac cartridges, which can also be regarded as adsorption gas chromatography.

We sought to develop a simple, efficient and reliable method for the separation of [^{18}F]FBM from DBM using gas-liquid chromatography on analytical scale column. A 4.0 x 200 mm straight glass column was packed with 6.6% Carbowax 20M on Porapack B (1.0 g), and tested in the preparation of [^{18}F]FBM. DMF was used instead of acetonitrile in the synthesis, which, according to our preliminary studies, provided on equal yield of [^{18}F]FBM and was a more suitable solvent for this reaction, which is performed at elevated temperature (110 ^{o}C). Thus, the system had three volatile compounds, FBM (b.p. 8-18^{o}C), DBM (b.p. 98^{o}C) and DMF (b.p. 152^{o}C). During the reaction, helium was bubbled through the reaction mixture (ca. 20 ml/min) and the gas flow led directly onto the packed column preheated to 35^{o}C. Samples of the column effluent were collected at various time points and the analysed for the amount of [^{18}F]FBM (Mbq) and DBM (µl). The results can be seen in Figure 1.

With this frontal chromatographic technique, [^{18}F]FBM had practically no retention and the collection rate was solely determined by the kinetics of the fluorination reaction. Within 20 min the vast majority (ca. 90%) of [^{18}F]FBM activity was collected. DBM (and DMF) remained on the column, until the break-through of DBM, which occurred after 25 min. The column can be easily regenerated while the [^{18}F]fluoromethylation reaction takes place by heating up to 190^{o}C with helium flow.



In conclusion, we have developed a new gas-liquid chromatographic method for separating *n.c.a.* [^{18}F]FBM from its precursor using an analytical scale packed GC column, which can simplify production of pure [^{18}F]fluorobromomethane and which can be easily automated. Further optimisation of this process is underway using a microwave reactor in order to increase the flourination reaction rate and thus reduce the overall synthesis time.

Keywords: [18F]fluorobromomethane, Dibromomethane, Gas Chromatography

A FULLY AUTOMATED ONE-POT SYNTHESIS OF [CARBONYL-11C]WAY-100635: VALIDATION IN ROUTINE PET STUDIES

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Increased research and clinical demands for [carbonyl-11C]WAY-100635 (I), a well-established PET radioligand for 5-HT1A receptors, has pursued our interest in a fully automated synthetic procedure suitable for routine application. As a first approach we attempted to use a "loop" technique [1] where cyclohexylmagnesium chloride was immobilized on the inner surface of polypropylene (or Teflon) tubing. A versatile synthetic apparatus operated via LabView program has been designed [2]. Despite we were able to produce usable quantities of (I) in high SA, this method was a subject of failures due to difficulty to control tracer amounts of water in the tubing.

An alternate "one-pot" synthesis of (I) is currently used by several groups. However radiochemical yield of the product is variable from run to run. To improve reproducibility of the method, an elimination of an excess of Grignard reagent by anhydrous HCl was suggested [3]. In addition, two SepPak purifications steps were introduced into synthetic procedure to get rid off amino precursor, WAY 100634, from the final preparation. This improved synthesis was realized in semi-automated modules, however an automation of such a complex process was hardly possible. We, therefore, reported a simplified "one-pot" procedure, which was fully automated using earlier developed LabView operated apparatus [2]. To simplify automation, the number of reaction steps was minimized.

The three steps reaction sequence was carried out in a conic 5 ml vial equipped with three Teflon delivery lines of 1/16" i.d. [11CO2] was trapped in solution of cyclohexylmagnesium chloride (0,05 mL, 2M solution in diethyl ether) and 0,35 ml of THF. A solution of SOCl2 (0.025 mL) in 0.3 mL of THF was delivered by nitrogen flow. The solvents were evaporated carefully to avoid over dryness of the resulting [carbonyl-11C]cyclohexane carbonyl chloride. The amino precursor, WAY 100634 (2-3 mg in 0.3 mL THF and 0.1 mL TEA) was then added by nitrogen flow. The [11C]acylation reaction was performed at 70oC within 5 min with continuous mixing of the reagents by nitrogen flow. The residual was dissolved in 1 ml of MeOH/0.1M ammonia formate mixture (60/40) and injected onto the C-18 HPLC column. The Rt of (I) was 7-8 min (flow 8 ml/min, eluent composition MeOH/HCO2NH4 (0.1M)/TEA (600/400/3) 270 nm). The appearance of an additional radioactive peak with Rt of 2-3 min. was observed when metal needles were installed in reactive vial.

To date, 29 successful routine preparations of (I) for human studies have been performed. The amount of the product was (973 ± 462) MBq, beam current was between 50-55μA and irradiation time 30-55 minutes. Four syntheses failed due to uncompleted injection into HPLC loop and one failed due to bad trapping in the Grignard reagent. The typical synthesis time for the procedure was 45 minutes. Auto cleaning procedure is started immediately after EOS without opening the hot cell. Next production can be started within 60 min.

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Keywords: Automation, One-Pot Synthesis, [carbonyl-^{11}C]WAY-100635

2′-[^{18}F]FLUORO-2′-DEOXYTHYMIDINE (2′-FTDR): A PET RADIOPHARMACEUTICAL TO MONITOR EXPRESSION OF HSV-1 TK

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Nuclear imaging techniques (PET and SPECT) are the only clinical monitoring methods with sufficient sensitivity for direct, whole-body assessment of gene expression following the administration of therapeutic genes. PET imaging additionally offers quantitative estimates of expression at the anatomical site of expression of the therapeutic gene.

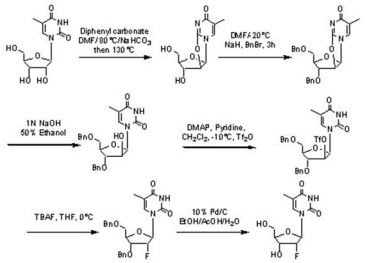
Expression of viral thymidine kinase (HSV-1 TK) by target tissues is one basis for suicide gene therapy (e.g., HSV-TK/ganciclovir). This protocol has been adapted to imaging, using the viral enzyme as a reporter (1). Radiopharmaceuticals used for the detection of viral TK are based on nucleosides that have antiviral properties due to their selective phosphorylation by viral TK but not by mammalian TK. Currently used radiopharmaceuticals, including the pyrimidine nucleosides [^{123}I]IVFRU, [^{123}I]FIRU and [^{124}I]FIAU, and the purine nucleosides [^{18}F]FHPG and ^{18}F-FACV, have been reviewed (2,3).

Fluoro-2′-deoxythymidine (2′-FTdR) was first developed as an antiviral agent (4), and later, as the tritiated nucleoside, investigated as a potential proliferation imaging agent (5). 2′-[^{18}F]FTdR is now under development to exploit its:

· selective phosphorylation by HSV-1 TK,

· low toxicity, which is attributable to its lack of incorporation into either nuclear or mitochondrial nucleic acids following phosphorylation, and

· chemical stability, which prevents catabolism and loss of radiolabel in vivo. In vitro cytotoxicity data are presented together with quantitative uptake by HSV-TK expressing cells and non-expressing cells in cell culture.



Previous syntheses of the 'cold' pyrimidine 2′-fluoro-2′-deoxyribosides required drastic conditions not appropriate for incorporation of F-18 (6). A new approach to the chemical synthesis of the radiolabelling precursor, described in Fig. 2, provides conditions suitable for incorporation of [^{18}F]fluoride. Initial radiolabeling data using a Coincidence^{TM} automated synthesis unit for 2′-[^{18}F]FTdR radiofluorination are presented.

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Acknowledgement: This work was supported by grants from CIHR and ACB.

Keywords: Gene Therapy Imaging, Radiofluorination, Fluoronucleosiides

SIMPLIFIED METHOD FOR SYNTHESIS OF ^{14}O- AND ^{15}O-LABELLED BUTANOL

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n-[^{15}O]Butanol, because of its optimal lipofilicity is an ideal tracer for measuring regional cerebral blood flow (rCBF) by PET. Labelled n-butanol, compared to [^{15}O]water, is a freely diffusible tracer over a wider range of rCBF. (The use of [^{15}O]water may underestimate cerebral blood flow in regions with high flow rate.) Despite its superior physiological properties, the n-[^{15}O]butanol is much less used than the [^{15}O]water, because of the inconveniences of its radiochemical synthesis.

Our aim was to find a simpler way of the synthesis of [^{15}O]butanol. On the other hand we had studied the possibility of the production of the ^{14}O isotope (T1/2=70,6 seconds) and the synthesis of ^{14}O labelled butanol as well.

The synthesis of [^{15}O]butanol is based on the reaction of molecular oxygen with tri-n-butilborane (TBB) loaded onto Alumina. Unlike the methods described earlier, we had not used cartridges previously loaded with TBB in inert atmosphere, but a glass column filled with alumina was applied. The TBB had been transferred onto the cartridge immediately before the synthesis by an automated syringe. The radiochemistry process itself took place on the usual way. After the instant reaction of [^{15}O]oxygen with TBB the reaction product was hydrolyzed with water, and the same time was transferred into a C-18 column, where the butanol became trapped. The latter was eluted with saline containing 10 % ethanol. After the synthesis both columns were washed with 10 ml water and 10 ml ethanol in succession, then was dried with helium for two minutes. After the drying process was ended, new portion of TBB could be loaded onto the column, and the synthesis could be started again. The 90-120 seconds long synthesis could be repeated in every 8-10 minutes.

The module was tested with ^{15}O produced by the $^{14}N(d,n)^{15}O$ and $^{15}N(p,n)^{15}O$ nuclear reactions. In the latter case the ^{15}N enrichment in the target gas was 25±5 %. In the first case (8 MeV, 10 μA deuterons) after 6-8 minutes irradiation 2,6-4 GBq, in the second case (11 MeV 25-30 μA protons) after 4 minutes irradiation 2,2-3,3 GBq [^{15}O]butanol was produced. The radiochemical purity was in each case >98%. The amount of [^{15}O]water was not more, than found using the traditional method.

The possibility of the production of the ^{14}O isotope with 11 MeV protons and the synthesis of ^{14}O labelled butanol had been studied as well. The saturation yield of ^{14}O at 11 MeV was found to be 90±2 MBq /μA. The [^{11}C]carbon dioxide by-product could be eliminated by tube filled with Ascarite. In one batch 440±90 MBq [^{15}O]butanol could be produced in 90 seconds. The radionuclidic purity of ^{14}O was found >99,9%.

Our method based on the finding, that the tri-n-butilborane can withstand a small amount of moisture, so the instant filling does not cause neither its degradation, nor decline in the quality of the product. It makes possible to perform multiple synthesises on a small size module, set up in the PET camera room. In such way the simplicity of the system is similar as the [^{15}O]water synthesis. The possibility to produce [^{14}O]Butanol may be attractive at the small, proton only cyclotrons, especially for small animal scanning.

This work was supported by the EC “Human Potential Programme: Access to Research Infrastructures”.

Keywords: O-15, O-14, Butanol

ASPECTS OF 6-[^{18}F]FLUORO-L-DOPA PREPARATION: EXPERIENCES WITH CHLOROFORM AS A SUBSTITUTE SOLVENT FOR FREON 11

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An increasing number of PET centers is using 6-[^{18}F]fluoro-L-DOPA ([^{18}F]FDOPA) to study presynaptic dopamine metabolism in vivo.

Currently the electrophilic radiofluoro-destannylation reaction of N-Boc-3,4-di(Boc-O)-6-trimethylstannyl-L-phenylalanine ethyl ester with [^{18}F]F_2 has emerged as the preparation method of choice for the reliable and reproducible radiosynthesis of [^{18}F]FDOPA (1-4).

Up to now the radiofluorination step of the trimethyltin precursor is carried out in trichlorofluoromethan ($CFCl_3$, Freon 11) as the solvent. De Vries et al. (3) have reported Freon 11 as the superior solvent to provide the highest radiochemical yields for the radiofluorination step. The authors also have investigated the possibility to substitute Freon 11 with other solvents. They observed considerable losses of radioactivity (61-71%) during the evaporation step of the solvents chloroform and acetonitrile. They discussed this finding with the formation of volatile radiofluorine compounds originating from the reaction of [^{18}F]F_2 gas with the solvents.

For Freon 11 is hardly available in Europe for environmental protection reasons today, we investigated the possibility of substituting Freon 11 with chloroform. In contrast to (4) we use 5 M HCl for protecting group hydrolysis. The acid is added directly after the radiofluorination step and the solvent was evaporated at 130°C. First results with chloroform also show increased losses of radioactivity in comparison to Freon 11. However, we found that the rate of radioactivity loss depends on the quality of the chloroform used. The radioactivity losses using chloroform stabilised with ethanol were in the range of 25%, whereas the amylene stabilised chloroform gave losses of 40%. In contrast, using deuterated chloroform ($CDCl_3$) for NMR analysis purposes stabilised with silver no significant losses of radioactivity were observed. We conclude that no competitive radiofluorination of the solvent chloroform, neither $CHCl_3$ nor $CDCl_3$, with [^{18}F]F_2 occures. Instead of that the observed radioactivity losses can be explained by the radiofluorination of stabilisers present in $CHCl_3$. The non-decay-corrected total yield for [^{18}F]FDOPA preparation using Freon 11 as the solvent is 17% (n = 26). Using $CDCl_3$ (silver stabilised) instead of $CFCl_3$ the average non-decay-corrected total yield is slightly higher, being 20% (n = 17).

The use of $CDCl_3$ has further advantages:

- easy handling (boiling point = 61°C) and unlimited availability,
- better environmental protection properties,
- the radiofluoro-destannylation reaction can be carried out at temperatures above freezing-point.

The results of our investigations indicate the possibility to substitute Freon 11 with $CDCl_3$ stabilised with silver.

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Keywords: [^{18}F]FDOPA Preparation, Substitution of Freon 11, Chloroform

MODIFICATIONS IN SYNTHESIS OF [^{18}F]-PEPTIDES AND APPLICATION TO SUBSTANCE P

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The most generally available synthetic route to ^{18}F-labeled peptides is the reaction of [^{18}F]succinimidyl fluorobenzoate (SFB) with the N-terminus or lysine moieties. Previously we reported the synthesis of ^{18}F-fluorobenzoyl substance P (SP)[1]. SP is een agonist for the NK_1-receptor and is considered to be a neurotransmitter or neuromodulator. Changes in SP have been implicated in diseases as Parkinson's disease, arthritis, inflammatory bowel disease and asthma.

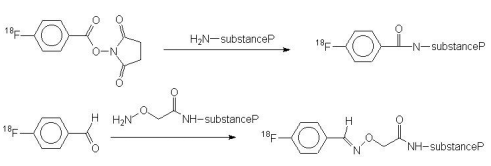
In the synthetic procedure of ^{18}F-fluorobenzoyl-SP, the production of SFB gave low radiochemical yields (<10%). The procedure has now been further optimized and is fully automated with a Zymark robotic system. The problematic step was the conversion of 4-[^{18}F]fluorobenzoic acid (FBA) to its N-succinimidyl ester using TSTU (O-[N-succinimidyl]-N,N,N,N-tetramethyluronium tetrafluoroborate). The radiochemical yield could be increased significantly by dissolving TSTU just before the addition to the azeotropically dried salt of FBA. Some other steps have been modified to make the process more reliable in the robot setting. Prior to the reaction of FBA with TSTU, FBA was dried in the presence of Kryptofix 2.2.2 and K_2CO_3 by azeotropical evaporation of acetonitrile as reported by Zijlstra[2], which prevented evaporation of FBA. The cartridge purification was replaced by a final HPLC-purification of SFB. The collected SFB was diluted with water and concentrated on a C18 cartridge. Subsequently SFB was eluted with acetonitrile. The overall radiochemical yield using the robot and including HPLC-purification was 24% (10-45%) (EOB).

The acylation of SP (at the e -Lys amino group) was carried out in a borate buffer (pH 8.5) in the presence of a catalytic amount of triethylamine at rt. The peptide was subsequently purified on a reversed phase HPLC column. Acylation yields are still low and variable 1-15%. Specific activities are in the range of 10-25 GBq/m mol.

During the HPLC-experiments, it was found that [^{18}F]fluorobenzoyl-SP was formed, when only SFB in acylation buffer was applied on the column. Probably, some SP from previous injections remained on the reversed phase column and reacted with SFB. This on-column chemistry is currently further investigated, whether this method can be applied on preparative scale.

As alternative, the reaction of a oxoamino derivative of SP (which was kindly donated by Dr. M Schottelius and Dr. H.J. Wester, Technical University, Munich, Germany) with [^{18}F]fluorobenzaldehyde to form an oxime, was investigated as described by Poethko[3]. In the production of [^{18}F]fluorobenzaldehyde, DMSO was replaced by DMI (1,3-dimethylimidazolidin-2-one). The reaction in DMSO led to the formation of substantial amounts of FBA. The amount of radioactive byproducts was reduced significantly using DMI.

After dilution of the reaction mixture with water, [^{18}F]fluorobenzaldehyde was purified by an Oasis HLB cartridge. Radioactive impurities were removed by elution with mixtures of water and organic solvent. Finally [^{18}F]fluorobenzaldehyde was eluted with methanol. This solution was added to the oxoamino derivative of SP (the oxoamino group was attached to the N-terminus) and heated at 60 °C for 20 min. The SP derivative (1 mg) was mixed with 0.5 ml phosphate buffer (pH 2.7). The radiochemical yield of this acylation was 35%, based upon HPLC.



Work is in progress to further optimize this reaction and to test other ^{11}C- and ^{18}F-labelled aldehydes and ketones.

Keywords: Peptides, Substance P, Fluorine-18

QUALITY CONTROL STUDIES OF [^{11}C]METHYL IODIDE ($^{11}CH_3I$) PRODUCED IN LIQUID AND GAS PHASE SYNTHESIS MODULES

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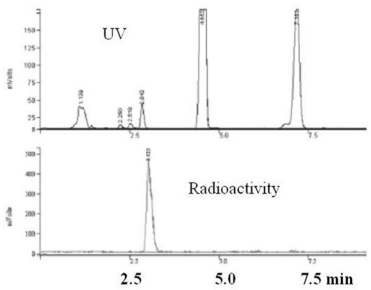
In the last two decades, a number of ^{11}C labeled radiotracers have been synthesized using $^{11}CH_3I$ as the reactive methylating agent. In a liquid phase reaction, the $^{11}CO_2$ produced in a cyclotron target is reduced and iodinated to generate $^{11}CH_3I$ by $LiAlH_4$/HI method. In a gas phase reaction, the $^{11}CO_2$ is first converted to $^{11}CH_4$, which reacts with iodine vapors at elevated temperature to generate $^{11}CH_3I$. Automated commercially available methyl iodide modules are based on these two reactions. The specific activities (SA) of ^{11}C labeled receptor binding radiotracers reported in the literature shows a wide range (1-15 Ci/micromole).The radiochemical yield, purity and SA of $^{11}CH_3I$ determines the final specific activity of ^{11}C labeled radiotracers. Therefore, we evaluated the quality of $^{11}CH_3I$ produced by automated synthesis modules.

[^{11}C]CO_2 was produced using EBCO TR-19 cyclotron. $^{11}CH_3I$ was synthesized in liquid phase manually (non-automatic mode) or using MeI-Plus Module (Bioscan Inc) and in gas phase using TracerLab FX_C (GE). $^{11}CH_3I$ was collected in DMF (0.1-0.3 mL). The chemical and radiochemical purity was determined by HPLC using analytical Novo-Pak C_{18} column and acetonitrile/water (2:3) containing ammonium formate (0.1M) at 2 mL/min. Using HPLC system and the cold methyl iodide (MeI), calibration curve was developed to determine the mass of $^{11}CH_3I$.

HPLC analyses of $^{11}CH_3I$ produced in liquid phase using the automated synthesis module showed relatively high amounts of chemical impurities as shown in Fig. 1. The solvent front shows at 1.1 min and the MeI has a retention time of 2.9 min. The two major impurities were seen at 4.6 and 7.4 min. The ratio of each impurity to MeI varied from batch to batch and was as high as 40 (average 13$\pm$12, n=30). The impurities were most likely unlabeled ethyl iodide (at 4.6 min) and isopropyl iodide (at 7.4 min) derived from ethanol and acetone, which were used to clean the system after each $^{11}CH_3I$ production. [^{11}C]isopropyl iodide also appeared some times as a radiochemical impurity (10-15%) and decreased the yield and radiochemical purity of $^{11}CH_3I$ production. Use of acetonitrile in place of ethanol and acetone for washing and cleaning the system decreased or eliminated the impurities. $^{11}CH_3I$ synthesized by manual liquid and gas phase methods showed minimal or no chemical impurities. Regardless of the amount of ^{11}C activity, the mass of MeI produced by automated liquid phase method (1.0-1.5 micromoles) was at least 10 times higher compared to that with gas phase method (0.02-0.1 micromoles).

Ideally, $^{11}CH_3I$ produced should have more than 90% radiochemical and chemical purity with a high SA (10-30 Ci/micromoles). Ethanol and acetone used for the cleaning procedure may contaminate the system and react with HI generating chemical impurities. Although reactivity of ethyl iodide or isopropyl iodide is significantly lower than methyl iodide, presence of high amounts of these competing alkylating agents may interfere with methylation reactions between desmethyl precursors and $^{11}CH_3I$.



Keywords: Methyl Iodide, Carbon-11 Chemistry, Synthesis Module

A PRACTICAL SYNTHESIS OF [^{11}C]ACETYLENE

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A practical synthesis of [^{11}C]acetylene is of interest for a number of reasons. First, [^{11}C]acetylene has been proposed as an inhaled radiotracer to measure regional cerebral blood flow agent [1]. Second, acetylene has been widely used in organic synthesis to produce a large variety of compounds. In radiochemistry, this important intermediate has yet to be fully exploited.Complication and difficulty of production have been major factors in the limitation of use.

Few studies have been made on the synthesis of of [^{11}C]acetylene [1-4]. These synthesis methods required several manual manipulations that prevented their automation. In this paper, we report a simple automated method for the synthesis of [^{11}C]acetylene that modifies the technique of Madsen et al.[1].

Barium was transferred to a quartz reaction tube under nitrogen gas at room temperature and was placed within a ceramic tube furnace. [^{11}C]CO_2 was produced by bombarding nitrogen containing 2.5% oxygen in a commercial aluminum gas. The synthesis apparatus and automation control electronics for the production of [^{11}C]acetylene were performed in our laboratory. A schematic of the synthesis apparatus is shown in Figure 1.

After trapping and release of [^{11}C]CO_2, the gas is transferred to the reaction tube at room temperature and accumulated on the barium. Heating of the tube is then initiated, and hydrogen (.26 cc/min.) with prescribed quantities of CO_2 are swept through the tube, resulting in evolution of [^{11}C]acetylene. Radiochemical identity and the Specific activity of the [^{11}C]acetylene was confirmed by GC/MS analysis.

Conversion of [^{11}C]CO_2 to [^{11}C]acetylene was as high as 75 percent with 99% radiochemical purity. Specific activity results ranged from 1.73-9.32 mCi/μmol. Total synthesis time was 15 min. The results of the study are summarized in Table 1. Reproducibility of the results was confirmed for more than three runs each. The temperature of heating was the major variable in these experiments; at 900°C maximal radiochemical yields were achieved.

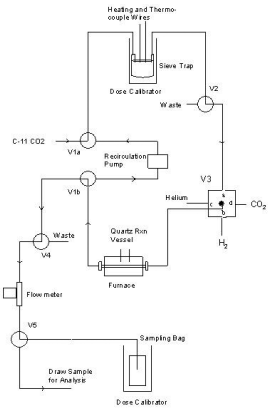

Figure 1: Synthesis apparatus schematic.

Table 1: Tabulated radiochemical yields and specific activities for [11C]acetylene.

Furnace Temperature. (°C)	Radiochemical Yield. (%)	Specific Activity. (mCi/μmol)
800	39.0 ± 5.72	1.73 ± 0.76
900	75.4 ± 3.04	9.32 ± 2.56
1000	58.9 ± 2.89	3.82 ± 2.35

Specific activity values obtained (Table 1) were low on the order of mCi/μmol. Improving the specific activity and keeping an acceptable radiochemical yield was our goal. After several runs, the maximum of 161 mCi/μmol in specific activity was reach with addition of 5 mL of [^{12}C]CO_2. However, the radiochemical yield was decreased to ~50%.

A facile and efficient synthesis procedure for the production of [^{11}C]acetylene has been developed that achieves moderate specific activities with favorable radiochemical yields. The simplicity of this method allows for easy automation and implementation.

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Keywords: [11C]acetylene, Barium, PET

SYNTHESIS OF FLUORINE-LABELED LIGANDS FOR PET IMAGING OF THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARγ)

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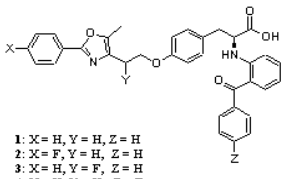
The peroxisome proliferator-activated receptor gamma (PPARγ) is expressed in adipose tissue, where it has been shown to be a primary regulator of lipid metabolism and to play a role in adipocyte differentiation [1]. Because PPARγ is capable of inducing differentiation and lipid storage in pre-adipocytes, it has also been proposed to play an intriguing role cancer. Therefore, PPARγ is a target for imaging that might be useful in assessing lipid metabolism disorders or identifying small metastatic tumors.

GlaxoSmithKline has developed (2S)-((2-benzoylphenyl)amino)-3-{4-[2-(5-methyl-2-phenyloxazol-4-yl)ethoxy]phenyl}propanoic acid (**1**, PPARγ pK_i = 8.94, PPARγ pEC_{50} = 9.47) as a potent and selective PPARγ agonist [2]. For the development of a fluorine-18 labeled analog of compound **1**, we designed and synthesized three fluorine-19 substituted derivatives, compounds **2, 3**, and **4**, to assess their potential as positron emission tomography (PET) imaging agents for PPARγ.

The structures of the three target molecules for imaging PPARγ are shown in **Table 1**, together with a summary of their binding affinities and transcriptional potencies for PPARγ, as well as for the two other receptor subtypes, PPARα and PPARδ. We anticipate that these three new ligands will prove to have an affinity and selectivity that is appropriate for imaging PPARγ. Optimization routes and conditions for labeling these compounds with fluorine-18 are underway.



1: X = H, Y = H, Z = H
2: X = F, Y = H, Z = H
3: X = H, Y = F, Z = H
4: X = H, Y = H, Z = F

	pKi (nM)			pEC$_{50}$
	PPARα	PPARγ	PPARδ	
2	7.28	9.46	6.27	>9.8
3	5.84	8.51	5.52	8.4
4	6.65	7.80	5.78	9.1

Table1. The binding affinities and potencies in transfection assays

Acknowledgements. This work was supported by a grant from the Department of Energy (DE FG02 86ER60401). We thank GlaxoSmithKline for determining the binding affinities and transcriptional potencies of these compounds.

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Keywords: The Peroxisome Proliferator-Activated Receptor gamma, Fluorine-18, PET

FLUORINE-18 LABELLED AROMATIC AMINO ACIDS FOR TUMOR IMAGING USING PET

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Introduction: Positron Emission Tomography (PET) is an important imaging modality for tumor imaging. Currently, FDG is the most widely used PET tracer for tumor imaging. However, FDG-PET is known to show non-specific uptake in inflammatory cells and granulation tissue.[2] Furthermore, the delineation of treated metastases against the surrounding tissue using FDG is inadequate particularly if the tumors have low growth rate resulting in slow nutrients consumption.[1] A number of ^{18}F-labelled amino acids have been suggested as PET tracers for imaging tumor cells.[3] We have developed a simple and reliable method for the production of clinically useful quantities of a variety of ^{18}F-labelled amino acids.

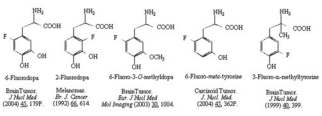

Figure 1. A few examples of ^{18}F-labelled amino acids for tumor imaging

Methods: Fluorine-18 labelled F_2 was produced by the $^{18}O(p,n)^{18}F$ nuclear reaction in a Siemens 11 MeV cyclotron using the double-shoot method. Fluorine-18 labelled amino acids were produced by the direct fluorination of commercially available precursors. The products were separated using reverse phase HPLC (Thermo-Hypersil Keystone, Fluophase™ PLP 5µ 250 x 10 mm) with 0.1% acetic acid containing ≈0.4 mg/mL ascorbic acid as the mobile phase at 4 mL/min.

Results: Radiochemical yields for different ^{18}F-labelled amino acids are given in Table 1

Table 1. Radiochemical yield of ^{18}F-labelled amino acids

F-18 Amino Acid	Reaction Medium	% Radiochemical yield w.r.t [18F]F_2
FDOPA	HF/BF_3	40 ± 2
3-*O*-methyl-FDOPA	HF/BF_3	40 ± 2
F-M-T	HCOOH/5%PFP	37 ± 3
F-a-MT	HCOOH/5%PFP	37 ± 2

Synthesis time = 60 min., PFP = pentafluoropropanol

Direct fluorination of DOPA and *meta*-tyrosine in anhydrous HF produced mainly 2- and 6-fluoro isomers of FDOPA and F-*meta*-tyrosine. To avoid the use of HF as solvent, our peers use fluorodestannylation, involving multistep syntheses of precursors and exhaustive deprotection steps, for regioselective fluorination. We have previously reported a method for the regioselective synthesis of 2- and 5-FDOPA by changing the acidity of the solvent.[4] We now report a method for the production of significant amounts of 2-, 4- and 6-fluoro-*meta*-tyrosine by the direct fluorination of *m*-tyrosine in formic acid containing 5% pentafluoropropanol (Figure 2)

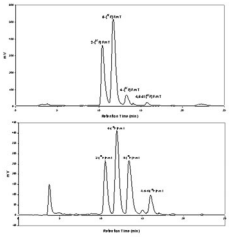

Figure 2: ^{19}F-HPLC trace (Thermo-Hypersil Keystone, Fluophase PLP 5 x 150 x 10 mm using 0.05% TFA in water containing 10% CH_3CN as mobile phase at 2.5 mL/min) of the reaction mixture after the fluorination of *m*-tyrosine in HF (Top) and Boric acid (Bottom).

Conclusions: Direct fluorination of commercially available precursors is an efficient method for the production of clinically useful quantities of FDOPA, FMT, 3-*O*-methyl-FDOPA and F-α-MT. We have developed an efficient isocratic preparative HPLC procedure for the separation of FDOPA and FMT isomers using an aqueous mobile phase suitable for *in vivo* use.

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Keywords: F-18 Aromatic Amino Acids, Tumor Imaging, Electrophilic Fluorination

NO-CARRIER-ADDED SPECIFIC ACTIVITY (NCA-SA) OF IODINE-124 RADIOPHARMACEUTICALS

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Iodine-124 (^{124}I) is an attractive isotope of iodine due to its complex radioactive decay scheme and convenient half-life (4.18 d). With the increasing use of positron emission tomography (PET) in nuclear medicine, medical oncology, pharmacokinetics and drug metabolism, ^{124}I-labeled radiopharmaceuticals could be most useful for PET imaging. Furthermore, the 4.18 d half-life would permit their use in PET facilities far away from the radionuclide production centers. Limited availability of this radionuclide so far has been a hindrance to its wider development and clinical use. Recently ^{124}I became commercially available, and this has accelerated the development of ^{124}I-based PET imaging agents. For example, sodium [^{124}I]iodide is potentially useful for diagnosis and dosimetry in thyroid disease. Another one, [^{124}I]*m*-iodobenzylguanidine ([^{124}I]MIBG) holds enormous potential for cardiovascular imaging as well as for diagnosis and dosimetry in malignant diseases such as neuroblastoma, paraganglioma, pheochromocytoma, and carcionois. The achievement of a radiopharmaceutical prepartion possessing the maximal specific activity and radiochemical purity always requires a radiohalogen starting material with no-carrier-added (NCA) specific activity as close to the theoretical carrier-free (CA) specific activity as possible. We have measured the NCA SA of different ^{124}I batches produced using the ^{124}Te(p,n)^{124}I reaction and are presenting the results herein.

Keywords: Iodine, No-Carrier-Added, Imaging

NEW APROACH TO THE PREPARATION OF ETHYL [^{18}F]FLUOROACETATE—A SIMPLE METHOD *VIA* DISTILLATION

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Introduction

Sodium [^{18}F]fluoroacetate is known as a PET tracer for prostate cancer, myocardial and cerebral oxidative metabolism. Ethyl [^{18}F]fluoroacetate, however, has never been investigated as a PET tracer, though the ester is an attractive brain imaging precursor because it is more lipophilic and facilely pass brain-blood barrier. Here we report a new approach to the synthesis of ethyl [^{18}F]fluoroacetate using distillation for purification, which made it possible to provide the ester in a pure and stable preparation for PET imaging study.

Experimental

The radiosynthesis was carried out with a simple module. No-carrier-added (NCA) aqueous [^{18}F]fluoride (3.7 GBq), prepared in a CTI RDS-eclipse cyclotron, was added into a Sep-Pak® QMA cartridge, which had been converted to CO_3^{2-} form by treatment with 0.1 M aqueous K_2CO_3 solution. 0.5 ml of 16.7 mM aqueous K_2CO_3 solution was employed to elute the trapped [^{18}F]fluoride from the cartridge. The eluate was introduced into the reaction vessel, and a solution of 16.4 mg kryptofix 2.2.2 dissolved in 1 ml acetonitrile was added. Water was azeotropically evaporated at 105°C under nitrogen gas flow adding anhydrous acetonitrile three times (0.5 ml × 3). After the drying step, 3 mg of ethyl *O*-mesyl-glycolate dissolved in 1 ml anhydrous acetonitrile was added to the dried residue in the reaction vessel. The labeling reaction proceeded at 105°C for 5 min. The reaction solvent was then removed at 95°C. After 5 min, the residue in the reaction vessel was distilled at 90°C under reduced pressure. The distillate was collected in 1 ml ethanol. Finally, the ethanol solution was purified by an Alumina N cartridge and filtered through a 0.22 mm sterile filter. The product was identified as ethyl [^{18}F]fluoroacetate by radioTLC with MeCN/H_2O (95/5) and by HPLC with EtOH/H_2O (10/90).

Results and Discussion

The reaction parameters of the labeling reaction including reaction temperature (75, 90 and 105°C), reaction time (5, 10, 15 and 20 min), and the amount of the precursor (3, 10 and 20mg/ml) were investigated in anhydrous acetonitrile. The condition found as the best was described in the "experimental" section. The labeling efficiency was higher than 70% at 105°C in 5 min with 3 mg precursor.

During the removal of the reaction solvent, part of the product was lost from the reaction vessel along with acetonitrile. Thus we tested several other organic solvents as the reaction media to replace acetonitrile. No product was detected when DMSO was used, even when the reaction time was lengthened to 30 min. Only a small amount of the product (ca. 5 %)was obtained using ethanol. In the case of aceton, the labeling efficiency was 23%. These results demonstrated that acetonitrile is so far the best media for this reaction.

For trapping the product during the distillation, we attempted to use water or saline. However, ethyl [^{18}F]fluoroacetate was unstable in them due to quick hydrolysis. Thereafter, ethanol was chosen, as ethanol could suppress hydrolysis of the ester and the diluted ethanol solution can be directly used for animal and human studies

The distillate contained a small amount of [^{18}F]fluorine (<2%). The application of a Sep-Pak alumina N cartridge could remove all free [^{18}F]fluorine. The final solution was analyzed by TLC and HPLC. The radiochemical purity was more than 98%. The total radiochemical yield was 28.6 ± 3.6 % (n=15) and the total radiosynthesis time was about 30 min.

Conclusion

We have successfully developed a novel method to prepare ethyl [^{18}F]fluoroacetate, with information on relevant reaction conditions, in a solution ready to be used for PET imaging study.

Keywords: Ethyl Fluoroacetate, PET Tracer, Synthesis via Distillation

AUTOMATED SYNTHESIS OF [^{18}F]FLUOROMETHYLCHOLINE (FCH)

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Introduction: [^{18}F]fluoromethylcholine (FCH) has shown very promising results for the staging and therapy monitoring of patients with prostate, brain and breast cancer [(1,2,3)]. An automated synthesis of FCH has been developed based on the method of DeGrado et al. [(1)]. This synthesiser is based on two distinct units: a FBM (^{18}F-fluorobromomethane) unit, producing pure FBM by Kryptofix 2.2.2/ K_2CO_3 assisted [^{18}F]fluorination of dibromomethane (DBM) followed by GC purification, and a fluoromethylation unit allowing the trapping of the FBM, the reaction with dimethylethanolamine (DME) and SEP-PAK purification.

Methods: Optimisation of FBM synthesis was conducted by varying the reaction temperature, the fluorination time, helium sweep flow rate during GC purification, and the volume of DBM precursor. The outlet of the GC column was connected to a conical vial containing 0.6 mL of a 1:1 mixture of DME/acetone, cooled to 5° C. The trapped FBM was allowed to react with DME at 80° C for 5 minutes before cooling and purification on a cation exchange SEP-PAK cartridge (Waters CM light).

Results-discussion: A reaction temperature of 100° C for two minutes was found to be optimal, resulting in uncorrected yields of 27.3± 4.7% of FBM. Reaction temperatures of 90° C and 110° C resulted in yields of 21.6± 2.5% and 22.2± 2.8% respectively. The helium gas flow rate through the GC column was found to be optimal at 20 cc.min^{-1} to allow the isolation of pure FBM. Variation of the amount of DBM did not have a bearing on the FBM yield, with 50 mL of DBM in 0.7 mL of anhydrous acetonitrile being routinely used. The GC purification resulted in a 17.2± 5.6% loss of FBM, which could be attributed to a poor trapping efficiency or to the decomposition of the dihalogenated intermediate on the GC column [(4)]. After trapping of the FBM in the FCH vial and the reaction with DME, the mixture was cooled and transferred to cation exchange Sep-Pak cartridge. To minimise the loss of the activity, the reaction FCH vial was rinsed twice with 1 mL of EtOH, and the EtOH solutions passed through the SEP-PAK cartridge. After rinsing with water, FCH was eluted with 7 mL of 0.9% saline. Most of the residual activity was found to be in the FBM reaction vial (48.8± 8.1%), while less than 3% was recovered on the QMA cartridge and the GC exhaust. A total average of 81.1± 6.2% of the initial activity was recovered, which correlates with the 17.2± 5.6% loss of activity on the GC column encountered during the FBM synthesis. FCH was successfully produced at a 17.8± 2.6% uncorrected yield with radiochemical purity >97%.

Conclusion: FCH was produced on a fully automated synthesiser with reasonable yields. Availability of this tracer on a routine basis will lead to further studies on patients with prostate cancer, breast cancer and brain tumours.

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Keywords: FCH, PET, Cancer

DETERMINATION OF THE CHEMICAL PURITY OF [C-11]CHOLINE

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The chemical purity of [C-11] choline focuses on quantitative determination of dimethylaminoethanol (DMAE), the precursor most commonly used for the synthesis. The registry of toxic effects of chemical substances lists DMAE toxicity for the 50% kill range as 234–1080 ppm for doses administered intraperitonealy. High performance liquid chromatography (HPLC) is traditionally used to determine the chemical purity of [C-11] choline. The lack of a good chromaphore in both choline and DMAE make ultraviolet absorbtion a poor choice of detector for these compounds. The use of refractometry to detect low levels of DMAE and choline was first reported by Hara et al however Mishani et al observed both poor sensitivity and poor resolution between the mass peaks of sodium chloride and DMAE using refractometry. We report here our data using conductivity detection for the ion-based separation of DMAE, sodium chloride and choline, as used by Mishani et al in the QA of [C-11] choline.

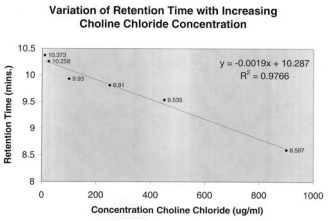
Equipment Dionex DX500 HPLC with IP25 isochratic pump (Dionex, CA), DS3 Conductivity cell (Dionex, CA), 4mm CSRS External chemical supressor (Dionex, CA), Cation exchange IC-PAK ™ 3.9 x 150mm analytical column (Waters Corporation, Ma), Flowcount radio-detector with PMT/ Scintillator (Bioscan).

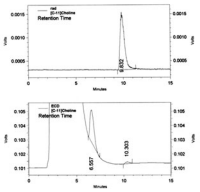
Chemicals 1M HCl (Aldrich), 25 mM TBAOH (Dionex, CA), DMAE > 99.5% (Aldrich), Choline chloride (Aldrich > 99.0%).

Chromatographic conditions 5 mM HCl at 1mL/minute using 25mM TBAOH external chemical suppressor (13 p.s.i. head-pressure). Choline chloride and DMAE reference standards were prepared in 0.9% sodium chloride by serial dilution.

Results The retention time (Rt) of choline chloride varied linearly according to concentration (see figure 2). DMAE did not exhibit any significant retention time variability.

Injection of the final product produced a single [C-11] choline radioactive peak (see figure 1), mean Rt of 10.2 minutes (n = 14, RSD = 2.4%). The conductivity chromatogram showed 3 mass peaks, sodium chloride, DMAE and choline. Specific activitites up to 3. 5 Ci/umole *c.f.d.* to EOB were observed however a choline mass peak was not always detectable. DMAE was present in all final product formulations. DMAE content was calculated by ratio analysis using a single-point calibration reference standard. DMAE eluted at mean Rt 6.5 minutes (n=14, RSD=1.9%), DMAE content ranged from <0.1 – 29.9 µg/mL (n = 13, mean = 14.4 µg/mL). The mean resolution between the mass peaks of sodium chloride and DMAE was 2.0 (n=13, range 1.29 – 2.51). 9 of 13 runs had a peak resolution > 2.0





RAD

Pk #	Retention Time	Area	Area Percent
1	9.832	38482	100.000
Totals		38482	100.000

ECD

Pk #	Retention Time	Area	Area Percent
1	6.557	65307	94.590
2	10.303	3735	5.410
Totals		69042	100.000

Conclusions Conductivity detection was sensitive for the quantitative analysis of both high specific activity [C-11] choline and detection of low levels of DMAE. The resolution between sodium chloride and DMAE peaks was sufficient for the determination of chemical purity. The mechanism by which retention time varies with choline concentration is being investigated; it is believed to be caused by interference of retention processes by sodium chloride. By using a 10 µg/mL choline chloride reference standard, the retention time drift was minimized allowing correspondence between the radioactive and mass paks of [C-11] choline.

Keywords: [C-11] Choline, Conductivity Detection, Quality Assurance

NOVEL FLUORINE-18 LABELED ESTROGEN RECEPTOR LIGAND: FLUOROALKYL CYCLOFENIL ANALOGUES

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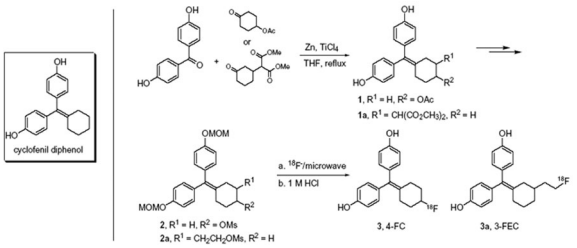
The estrogen receptor (ER) is a transcription factor that mediates the action of estrogens. There are two ER subtypes (ERa and ERb), and their tissue distribution and the gene regulatory activities of the two subtypes are different. Several ER subtype-selective ligands have been reported; among them, cyclofenil is well-known non-steroidal estrogen. Cyclofenil diphenol has a very high binding affinity for both ERs and a 2.3-fold selectivity for subtype of ERb (Table 1)[1] We have designed and prepared fluorine-substituted cyclofenil derivatives as potential imaging agents for ERb.

Relative Binding Affinity (RBA) values of cyclofenil diphenol, 4-FC and 3-FEC (RBA[estradiol] = 100)

	ERα	ERβ	ERβ/ERα
Cyclofenil diphenol	124±43	281±61	2.3
4-FC (3)	27.0±6.8	62.0±17	2.3
4-FEC (3a)	122±7.8	129±35	1.0

The fluoro and fluoroalkyl derivatives, 4-fluoro-[bis(4-hydroxyphenyl)methylene]cyclohexane (4-FC) and 3-(2-fluoroethyl)-[bis(4-hydroxyphenyl)methylene]cyclohexane (3-FEC), were synthesized as shown in Scheme 1, and their relative binding affinity (RBA) values were determined for each receptor subtype (Table 1). As did cyclofenil, 4-FC (**3**) showed 2.3-fold selectivity for ERb, but 3-FEC (**3a**) did not show ER subtype selectivity. A radiochemical synthesis of each of these compounds was carried out, as shown in Scheme 1. The F-18 fluorinations, which were carried out under microwave heating conditions, gave a 30% yield of **3** and an 85% yield of **3a**, and tissue-distribution studies of both labeled compounds were performed. Curiously, despite the good affinity of these compounds for the ERs, no target tissue-selective uptake was observed. In conclusion, 4-[^{18}F]FC and 3-[^{18}F]FEC were efficiently synthesized under microwave heating in high radiochemical yield and with high radiochemical purity; however, target tissue-selective uptake was not observed. We are searching for other fluoroalkylcyclofenil analogues that might be more effective for imaging ER_b.



Acknowledgment. This research was supported by grants from the National Institutes of Health (PHS 5R37 CA25836 [to J.A.K.] and PHS 1R24 CA86307 [to M.J.W.]) and the Department of Energy (DE FG02 84ER60218 to M.J.W.).

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Keywords: Estrogen Receptor, Cyclofenil, ER-beta Selective

SYNTHESIS AND *IN VIVO* CHARACTERIZATION OF ^{18}F LABELED NON-STEROIDAL ANDROGENS

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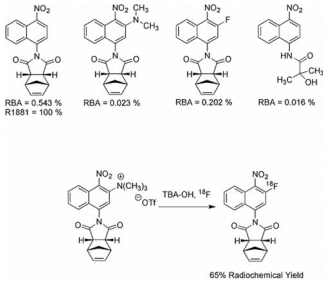
Prostate cancer is the second leading cause of cancer death in American men. The role of androgens in prostate tumor growth is well known, and significant levels of androgen receptor are frequently observed in primary and metastatic prostate cancer. For the past 50 years, androgen ablation via castration has been the most effective therapy for the treatment of advanced prostate cancer. Recently, a more modern treatment alternative of complete androgen blockade is used, in which an antiandrogen is administered along with chemical castration through high-dose treatment with a luteinizing hormone releasing agonist.[1]

In addition to the potential for clinical therapy, expression of androgen receptors on prostate tumors offers the potential for external diagnostic imaging of prostate tumor sites using position emission tomography (PET). To date however, these studies have principally used fluorinated steroid ligands, with few studies utilizing the therapeutic anti-androgens as a diagnostic agent. In addition to hydroxyflutamide, bicalutamide, and nilutamide, several groups have reported potent non-steroidal antiandrogens. Recently, Ishioka et al. have reported isoxazolone based compounds

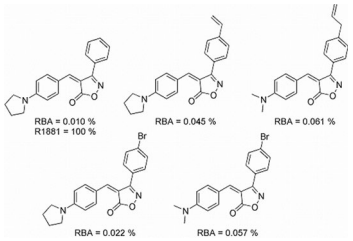
Binding Affinity of Isoxazolone Antiandrogens as high affinity antiandrogens.[2] Salvati et al. at BMS have also reported a number of high potency bicyclic isoindole-based androgen receptor antagonists.[3] We have synthesized a number of analogs of these compounds and evaluated their potential as PET imaging agents.



Despite literature reports to the contrary,[2] the isoxazolones proved to be poor ligands for the androgen receptor. A fluorine-substituted analog of the lead compound in the isoindole series was prepared. The nitronapthelene unit in this molecule enables labeleing with ^{18}F through a remarkably efficient nucleophilic aromatic substitutition reaction on a trimethylammonium-substiututed analog. *In vivo* uptake studies in androgen depleted rats, however, showed high bone uptake without significant accumulation in the prostate.

Binding affinity and Synthesis of [^{18}F] Bicyclo [3.2.2]nonane Antiandrogens

We are grateful for support of this research through grants from the Department of Energy (DE FG02 86ER60401 to J.A.K.) and (DE FG02 84ER60218to M.J.W.) and the National Institutes of Health (PHS 1R24 CA86307 to M.J.W.).



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Keywords: Fluorine-18, Non-Steroidal Androgens, Prostate Cancer

THE TRACERLAB MAXIMUM (Mx) IS ALSO A FLEXIBLE (Fx) TOOL

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In most PET centers, the main focus is [^{18}F]FDG preparation, but the demand for other labeled compounds is increasing. The growing number of either Micropet cameras users or physician requests for PET human protocols involving other ligands, prompted us to prepare and deliver other [^{18}F]radiotracers. Being a cGMP lab, working under Market Authorization for FDG production, we are using the TracerLab Mx (GE, Belgium). For this reason, decision has been taken to dedicate one of our synthesizers to the production of [^{18}F]labeled compounds. An HPLC pump and injection valve, a pin diode for radioactivity detection, and three ways valves are the only added components. They are all connected on the PLC interface and the software is flexible enough to control them.

[^{13}N]NH_3 and [^{13}N]N_2 The radiation-safety authorities require an exact calibration of the stack detectors to quantify the radioactivity released to the environment. Since our cyclotron is not yet equipped with a ^{11}C target, production of [^{13}N]N_2 has been used for calibration of chimney detectors. The irradiated [^{16}O]H_2O is collected in the reactor, mixed with NaOH and DeVarda's[1] catalyst to generate [^{13}N]NH_3, and was trapped in a HCl solution. By addition of NaOH and NaOCl, [^{13}N]N_2 was produced (up to 11GBq). The [^{13}N]N_2 was trapped in a syringe located in an ionization chamber, and high flow gas is used to pop up the plunger to release the nitrogen in the chimney. This method is safe and full automatic.

[^{18}F]NaF Simple modifications of kit and sequence allow us to get injectable [^{18}F]NaF in less than 10 minutes in quantitative yield. The $^{18}F^-$ is trapped on the QMA cartridge, rinsed with WFI, and finally eluted with saline.

[^{18}F]Fluorocholine The synthesis of [^{18}F]bromofluoromethane and [^{18}F]fluoromethyltriflate have already been reported[2].We developed three different ways to produce the F-choline, the first one, very simple uses the reaction of [^{18}F]BFM on a C-18 loaded with DMAE. The second, more complicated uses a triflate oven and the same reaction on the C-18. And the last one, uses a loop for the reaction of DMAE with [^{18}F]FMeOTf which requests an HPLC purification. That system allows also the production of [^{18}F]fluoromethyltyrosine. Even if the yield is lower with the first method (20% uncor. yield, n=48), we use it to produce routinely more than 18 GBq of [^{18}F]F-choline.

[^{18}F]FLT Starting with the publication of Oh et al.[3], and after different improvements of both sequence and chemistry, we are able to produce FLT with reliable yields higher than 30% uncorrected (>18.5GBq). The sequence is completely automated until the collection of the final product.

[^{18}F]MPPF The published synthesis of MPPF requested the use of either reformulation system[4] or new interface development[5]. This is now full automatic, using only our modified TracerLab Mx, from the [^{18}F] recovery to the formulation after HPLC purification, without additional computer or PLC. Using the same installation [^{18}F]FDDNP and [^{18}F]Fallypride have also been labeled.

Stainless steel manifold The limitation of the polysulfone manifold is its lack of resistance to solvent like DMSO. For that reason, the development of a stainless steel manifold prototype has been undertaken. The preliminary tests of flow, pressure and liquid transfer gave very promising results.

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Keywords: Automation, Fluorination, Synthesizer

AUTOMATED MEASUREMENT OF SPECIFIC ACTIVITY OF RADIOLABELED LIGANDS DURING SYNTHESIS

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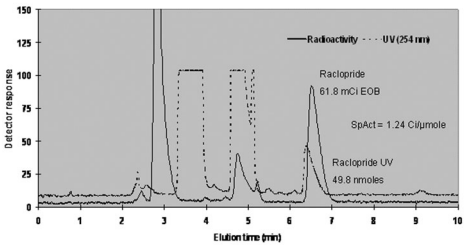
Accurate determination of specific activity is vital to the analysis of PET imaging data for saturable processes where unlabeled ligand will compete with the radioligand at the target receptor, transporter or enzyme. Accordingly, it would be helpful to know specific activity of the tracer prior to administration of the dose. This is particularly difficult for ^{11}C-tracers due to the short physical half-life and the time required to analyze an aliquot of the final formulation. While it is easy to measure the radioactivity concentration (Ci/mL) of the injectate, it can be problematic to measure the low chemical concentration ($_{\mu}$mol/mL) of the diluted analyte. For low yield reactions, it is often difficult to reserve sufficient volume of final product to perform all required quality control analyses. Also, chemical instability of the ligand can lead to over-estimation of specific activity when using delayed measurements. We present a method for simultaneously measuring the total radioactivity and mass of the labeled compound as it elutes from the semi-prep HPLC column. In this manner, specific activity can be rapidly calculated irrespective of the amount of tracer which might be lost during solvent evaporation, recovery and product formulation.

The new methodology required the development of a dedicated data acquisition system that performs automatic decay-correction of radiochromatographic data to a single point in time, e.g. end of bombardment (EOB). In this application, HPLC chromatography data was simultaneously acquired for a UV detector and a shielded silicon-diode radiation detector circumscribed by 3-4 loops of Teflon tubing. Data acquisition was integrated with automated control of an in-house developed ^{11}C-methylation module that includes a reaction vessel, solvent distribution system, automated injection loop filling, and evaporation-reformulation functionalities. Programming of control and data acquisition functions was done using SoftWire tools and Visual Basic, while analysis of chromatographic data utilized PeakSimple software. Immediately following elution of the product, chromatographic data are exported to PeakSimple readable files, and the area of the radioactivity peak is converted to mCi at EOB by comparison to a reference curve previously calibrated using the same detector, loop and flow rate. The mass peak from the UV chromatogram is similarly compared to a standard curve made at the same wavelength, mobile phase and flow rate. Simple division of the total EOB radioactivity peak (in mCi) by the total mass peak (in nmoles) gives specific activity in Ci/$_{\mu}$mol at EOB. This analysis is performed while the product is undergoing evaporation and formulation. The calculated specific activity value can then be decay-corrected to injection time or displayed in real-time at the PET scanner to enable investigators to time injections based on the specific activity information. The method has been successfully applied to the synthesis of [^{11}C]raclopride (Fig 1). The current method requires controlled flow elution from a semi-prep HPLC column to give reliable radioactivity and UV-absorption data. However, the method can be adapted to syntheses that normally do not utilize HPLC purification, e.g. [^{11}C]CFT. In these cases, the benefit of immediate specific activity determination is weighed against the added complexity, time and cost of the synthesis.

Figure 1. C-11 Raclopride elution



Keywords: Specific Activity, Automated Measurement, C-11 Radioligands

A MULTI-PURPOSE ^{11}C-RADIOSYNTHESIS SYSTEM

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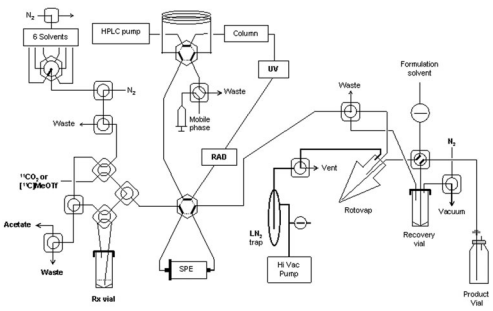
Computer controlled radiochemistry synthesis systems have become essential for routine radiopharmaceutical production in light of the regulatory emphasis on current good manufacturing practices. However, budgetary restraints of academic radiochemistry facilities preclude the purchase of multiple automated single product systems for the large number of radiotracers used on a routine basis. Therefore, we have focused our efforts to develop multi-use radiochemistry systems. Herein we describe a prototype device which performs ^{11}C-methylation reactions with [^{11}C]methyl triflate, as well as Grignard reactions with [^{11}C]CO_2. Product purification capabilities include semi-preparative HPLC, disposable solid phase extraction (SPE) cartridges, and direct distillation. Organic mobile phase solvents can be removed when necessary by conventional roto-evaporation, and the product recovered for formulation and direct aseptic delivery to the final product vial. In addition, tracer specific activity ($Ci/_{\mu}mole$ at EOB) can be automatically determined prior to product delivery for compounds purified by the HPLC-portion of the system.

Figure 1 illustrates the overall schematic design of the radiosynthesis, purification and formulation capabilities of the prototype system. Multiple solvents and/or reagents can be loaded as prompted by the operating software prior to isolation in the hot cell. Likewise, the software prompts the user to select the appropriate HPLC column and mobile phase or to install the required SPE cartridge. Pin-diode radiation detectors (Carroll and Ramsey Associates, Berkeley, CA) are easily relocated to method-specific sites for real-time monitoring of the radiosynthesis. Automated cleaning routines have also been developed to prepare the device for subsequent syntheses and to minimize radiation exposure to production personnel. To date, we have utilized this prototype device to prepare [^{11}C]raclopride, [^{11}C]β-CFT, [^{11}C]choline, [^{11}C]pyridostigmine and its ^{11}C-labeled analogs, [^{11}C]acetate and [^{11}C]palmitic acid.

The control program was developed using SoftWire and Visual Basic tools. Radiotracer specific routines can be programmed under manual operating control and the various steps automatically recorded for subsequent use as a single command. In addition, manual override capability is included for each computer controlled relay and for each step in the programmed method if unexpected events are observed during the automated synthesis procedure. Excel spreadsheets are used for both input of operational parameters and recording of data acquired during each run. Each step performed, whether automatic or manual, is recorded in a production batch record form. Output data from the UV detector and all of the radioactivity detectors are displayed graphically during the synthesis and within the output spreadsheet. Radioactivity data can be decay-corrected and exported during the run for immediate analysis, for example to measure specific activity from HPLC chromatograms. Data is also stored electronically in an abbreviated format to provide input of batch-specific dose information to an automated pneumatic tube transport system also developed at our institution.

Fig. 1 ^{11}C-System Schematic



Keywords: C-11 Radiosynthesis, Automated Radiochemistry System, Multiple Functionality

EVALUATION OF COMMERCIAL AUTOMATED [^{11}C]METHYL IODIDE (MEI-PLUS) SYNTHESIS MODULE

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Liquid phase synthesis of [^{11}C]methyl iodide ($^{11}CH_3I$) is widely used for the routine production of many ^{11}C radiotracers. The traditional manual synthesis set-ups for the production of $^{11}CH_3I$ are not ideal for multiple synthesis runs over a period of 6-8 hours. In 2004, Bioscan Inc, introduced an automated MeI synthesis module designed to produce $^{11}CH_3I$ or [^{11}C]-methyl triflate for labeling. The system uses pre-packaged reagents that enable up to ten consecutive runs to be achieved from a single reagent loading. We report here the evaluation and improvements of the first module installed in March 2004. The MeI-Plus module consisted of 4 major components; a molecular sieve column to trap $^{11}CO_2$ from the target, LAH dispenser vial, HI dispenser vial, a conical glass MeI production vial (1.0 mL capacity). In addition, a 1mL glass vial was used to collect the $^{11}CH_3I$. The synthesis was fully automated to deliver $^{11}CH_3I$ into the reaction vial in <9 minutes after EOB.

We have performed >400 $^{11}CH_3I$ runs using this system. [^{11}C]CO_2 was produced using EBCO TR-19 cyclotron. The trapping of $^{11}CO_2$ at RT and also release at 260°C were both efficient (90-95%) as indicated by sodalime/charcoal trap. The trapping and release time is short (<2 min each). The use of 316 stainless steel tubing (1/4" OD) to prepare the molecular sieve trap improved its performance. Reconditioning the trap daily at 250-260°C for15 min with nitrogen flow (30 mL/min) would prolong the life of trap for 60-70 runs. Preloading 0.1 M $LiAlH_4$ (5 mL) and HI (5 mL) in the dispenser vials allows one to use the module for multiple runs during the day. Initially, dispensing of LAH(0.1 mL) and HI (0.3 mL) were not reliable due to blockage of solenoid valve and/or the reagent transfer lines by fragments of rubber stoppers used for the vials. Use of gray rubber septa coated with fluoratec polymer prevented this problem. Commercially available unstabilized HI resulted in lower yields of $^{11}CH_3I$. The use of HI distilled over red phosphorous improved the yields (15-30%). Evaporation of THF in the MeI reaction vial (at 115°C) and subsequent distillation of $^{11}CH_3I$ (at 115°C) after the addition of HI are fairly efficient and of short duration. The production of $^{11}CH_3I$ is reproducible and efficient with 60-80% yields. With all the modifications and regardless of the amount of ^{11}C activity, the mass of MeI (both cold and hot) in the last several runs was 1.0-1.5 micromoles. The system, however, was not optimized yet for reducing the mass of MeI. The distillation of $^{11}CH_3I$ from the reactor vessel into methylation vial is carried out with nitrogen (10 mL/min). Because of the low flow rate, it is possible to trap the $^{11}CH_3I$ in 0.1 mL of DMF at RT.

After each run, an automated washing and cleaning (with ethanol, acetone and ether) program prepares the system for next synthesis in <20 min. HPLC analysis of $^{11}CH_3I$ preparation identified several chemical impurities such as ethyl iodide and isopropyl iodide. However, the effects of these impurities on the relative yields of ^{11}C labeled tracers have not yet been investigated. The clean up with acetonitrile in place of ethanol and acetone reduced these impurities. At the end of the day, it is recommended to replace the LAH vial with a vial of ethanol to remove residual LAH from transfer lines. Periodic use of acetonitrile containing 15-20% (v) of 1M nitric acid is suggested. Since the time of installation, several hardware and software upgrades were done and at the present time, the production of $^{11}CH_3I$ by MeI-Plus module is reliable and relatively efficient.

Keywords: Methyl Iodide, Carbon-11 Chemistry, Synthesis Module

SITE-SPECIFIC CYSTEINE-DIRECTED RADIOBROMINATION OF ANTI-HER2 AFFIBODY MOLECULE DIMER

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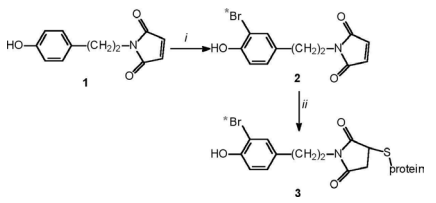
Affibody molecules are a new class of small phage-display selected affinity proteins. They can be selected for specific binding to a large variety of protein targets including tumor-associated antigens. The use of the scaffold domain of the bacterial receptor protein A enables stable folding, which is independent on disulfide bonds. Stable structure enabled Affibody molecules to retain binding capacity after labeling. Small size of Affibody molecules ensures quick blood clearance and good tumor penetration. High contrast of tumor imaging can be obtained for this reason.

An Affibody molecule binding with high affinity to a tumor antigen HER2 was recently developed. Expression of HER2 has established prognostic and predictive values in breast cancer. There are numerous evidences concerning importance of HER2 in other carcinomas, including tumors of ovary, urinary bladder and prostate. For this reason, radiolabeled anti-HER2 Affibody molecule can a valuable diagnostic tool. The use of the positron-emitting nuclide ^{76}Br ($T_{½}$ = 16.2 h) could improve the sensitivity of detection of HER2-expressing tumors. A site-specific radiobromination of a cysteine-containing variant of the anti-HER2 Affibody, $(Z_{HER2:4})_2$–Cys, using ((4-hydroxyphenyl)ethyl)maleimide (HPEM) was evaluated in this study.

HPEM was radiobrominated with an efficiency of 83 ± 0.4%. Coupling efficiency to freshly reduced Affibody was 65.3 ± 3.9%. Purification of the product on the disposable size-exclusion column provided conjugates with radiochemical purity of more than 97%. The label was stable against challenge with large excess of nonlabeled bromide, and in a high molar strength solution.



In vitro cell tests demonstrated that radiobrominated Affibody binds specifically to the HER2-expressing cell-line, SK-OV-3. Biodistribution studies in nude mice bearing SK-OV-3 xenografts have shown tumor accumulation of 4.8 ± 2.2% IA/g, and good tumor-to-normal tissue ratios (Table 1).

In conclusion, indirect radiobromination using HPEM enables to introduce positron-emitting label into $(Z_{HER2:4})_2$–Cys in a site-specific manner. Radiolabeled Affibody retains capacity to bind to HER2-expressing cells and provide selective accumulation of the label in HER2-expressing tumors.

Tumor-to-organ ratio 4 h after injection of radiobrominated Affibody molecule in Balb C nu/nu mice bearing HER2-expressing SK-OV-3 xenografts.

blood	9.1 ± 2.9
heart	11.8 ± 5.6
lung	8.8 ± 2.9
liver	3.7 ± 0.8
spleen	14.2 ± 6.1
pancreas	20.2 ± 9.9
kidney	4.9 ± 2.2
stomach	9.2 ± 5.5
muscle	31.1 ± 10.4
brain	35.2 ± 11.7

Data presented as an average from four animals ± standard deviation

Acknowledgement: This work was financed by Affibody AB, Bromma, Sweden, and by grants from Swedish Cancer Society

Keywords: Affibody Molecule, Bromine-76, ((4-Hydroxyphenyl)ethyl)maleimide

PEPTIDE CONJUGATES FOR IMAGING β-AMYLOID IN THE BRAIN

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Amyloid plaques consist of dystrophic neurites, altered astrocytes, and microglia surrounding an insoluble fibrillar core comprised primarily of a family of proteins known collectively as the amyloid β-proteins ($A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$)[1,2]. Aβ is derived from the ubiquitously expressed cell surface amyloid precursor protein (APP) [3,4]. Diagnostic probes that are capable of evaluating noninvasively the presence of brain amyloid aggregates *in vivo* would have wide applications for premortem diagnosis of AD and the efficient evaluation of candidate therapeutics.

Among various radionuclides, Tc-99m, the most commonly used isotope in the medical imaging, offers a versatile and inexpensive alternative. Initial efforts to design Tc-99m labeled chrysamine G (CG) and Congo Red (CR) derivatives[5,6] or mixed functionalities such as isonitriles[7] have been unsuccessful. Thus, an Aβ binding Tc-99m labeled radiopharmaceutical remains a highly desired diagnostic agent.

Candidate Aβ-targeted Tc-99m agents should demonstrate five critical characteristics: a) efficient synthesis for rapid formulation, b) binding to Aβ plaques; c) metabolic stability, d) permeability across the blood brain barrier (BBB); and e) amenable to kit formulation. While exploring peptide-based motifs for biomedical applications, we have synthesized a BBB permeation peptide **1** (Acetyl-KKLVFFAε-KGC-Amide).

Peptide **1** was obtained by standard Fmoc chemistry, cleaved from resin, and purified on a HPLC system using a Vydac C-18 column. The fraction eluting at R_t=13.6 min was collected, lyophilized, and analyzed. Amino acid analysis was consistent with the proposed formulation and electron spray mass spectrometry (ESMS) of the collected fractions confirmed the identity of the peptide **1** (ESMS: Calcd for $C_{57}H_{92}N_{14}O_{11}S_1$: 1181.5; Found (M+H): 1182.5). The purified peptide was labeled with Tc-99m (**Figure 1**) using glucoheptonate through a ligand exchange reaction at RT in saline. Radio-HPLC demonstrated the existence of two isomers of the N_3S chelation moiety in relation to the metal-oxygen bond, due to participation of the chiral α-C atom of lysine in the chelating ring. The formation of the two anticipated diastereoisomers (the apical oxygen being *syn* and *anti* relative to the side chain of lysine residue) were consistent with other ^{99m}Tc-labeled peptides using an identical chelation core[8]. The ^{99m}Tc-peptide remained nonmetabolized in human serum. Preliminary binding assays indicated that ^{99m}Tc-peptide binds specifically to Aβ in a concentration-dependent and saturable manner. In addition, competitive binding assays demonstrated that the radiolabeled peptide was displaceable to background conditions with 1,000-fold molar excess of unlabeled peptide, suggesting a receptor-like binding behavior of the ^{99m}Tc-peptide to Aβ aggregates. Thus, peptide **1** represents a promising lead for the development of a ^{99m}Tc-labeled Aβ imaging agent.

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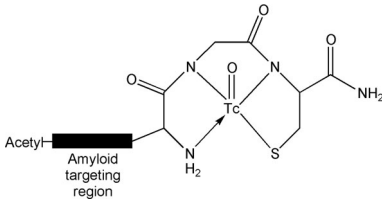

Proposed structure for technetium complex of amyloid targeted peptide **1**.

Keywords: Alzheimer's Disease, Amyloid Plaques, Tc-99m-Peptides

PET IMAGING OF PANCREATIC CANCER USING Cu-64 LABELLED DOTA-PNA-PEPTIDE CHIMERA

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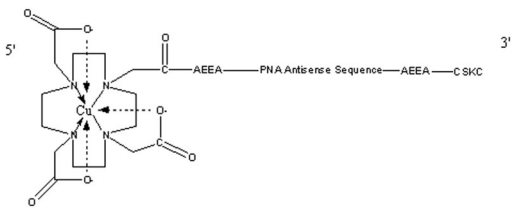
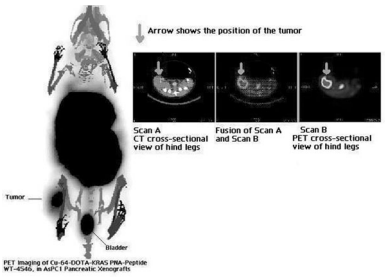
Objective: Early detection of the cancer continues to be a challenging task. Oncogene KRAS with mutation at the twelfth codon is overexpressed in ninety percent of pancreatic tumors, providing the most genetically clearcut indication for genetic diagnosis. Therefore, PET imaging of tumors using Cu-64-chelator-PNA-peptide probes at an early stage provide a powerful and promising technique for molecular detection of oncogene overexpression. Cu-67 will be used for therapeutic purpose.

Methods: A number of probes containing 12-base antisense PNA sequences flanked by DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) at the N-terminus as radionuclide chelator and a small cyclic IGF1 ligand peptide at the C-terminus as shown below in schematic diagram were synthesized using standard Fmoc coupling on a polymer bead support. PET imaging of the overexpression of *KRAS* mRNA using these Cu-64 probes and their mismatch controls were carried out in immunocompromised mice bearing in human AsPC1 and CAPAN2 cancer xenografts 4 hrs and 24 hrs after administration. Tissue distribution of Cu-64 probes was also determined.

Results: All synthetic probes were synthesized on Novasyn TG Sieber Resin at 100 micromole scale as a single continuous synthesis. Cyclization of the cysteine thiols were carried out on solid phase by iodine prior to their cleavage by trifluoroacetic acid and precipitation from ether. The probes were purified by reversed phase HPLC as a single peak and were characterized by electrospray mass spectroscopy, showing their expected masses, prior to radiolabeling. The Cu-64 chelating efficiency was 98% to 100%. Average ITLC elution times of the pure Cu-64 labeled probes were in the range of 8 to 12 minutes. The Cu-64 PET images displayed high resolution and clarity. Tumor uptake of the Cu-64 probes was 1.07%±0.33% ID/g. A representative scan of the probe WT4546 is shown in Figure 2 below.



Conclusion: Analysis of the scans and tissue distribution showed that the tumors could be imaged with high resolution, sensitivity, and specificity. The higher uptake of Cu-64 is attributable to the *in vivo* stability of that radionuclide. These results support the hypothesis that these hybridization probes could be used for molecular diagnosis and therapy. Supported by NCI CO27175



Keywords: PET Imaging, PNA Peptide Conjugate, KRAS

SYNTHESIS OF N-SUBSTITUTED 9-AZABICYCLO[3.3.1]NONAN-3α-YL PHENYLCARBAMATE ANALOGS AS SIGMA-2 RECEPTOR LIGANDS

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Sigma (σ) receptors are a distinct class of receptors that are present in the central nervous system as well as in peripheral tissues. It is now widely accepted that there are at least two classes of sigma binding sites, σ_1 and σ_2. Several studies have reported an overexpression of σ_2 receptors in both human and rodent tumors.[1] Additional studies have shown the expression of σ_2 to be a reliable biomarker for the proliferative status of solid tumors.[2] Therefore, radioligands having a high affinity and high selectivity for σ_2 receptors should be good tracers for the non-invasive assessment of the proliferative status of solid tumors using PET or SPECT.

We previously reported the synthesis and *in vitro* binding of a series of azabicyclo[3.3.1]nonan analogs having a modest affinity and selectivity for σ_2 receptors.[3] The goal of the current study was to extend the structure-activity relationship study of this class of compounds by: 1) increasing the aminoalkyl groups attached to the bridgehead nitrogen atom; and 2) varying the halogen atoms in the aromatic ring attached to the alkyl amino group (for future labeling).

A series of N-substituted 9-azabicyclo[3.3.1]nonan-3α-yl phenylcarbamate analogs was prepared, and their affinity for sigma (σ_1 and σ_2) receptors was measured in vitro (Table). The results of this study led to the identification of **1a, 1e** and **2a** as σ_2 selective ligands. An unexpected observation was the high affinity and high selectivity of compounds **2d,e,f** for σ_1 receptors. Compound **1a** and **2a** will be radiolabeled with Br-76 and compound **1e** will be labeled with I-125 and I-124 for in vivo and in vitro studies of σ_2 receptors of breast tumor cells.

Acknowledgment. This research was supported by NIH grant CA102869 and the US Army Medical Research and Material Command under DAMD 17-01-1-0446.

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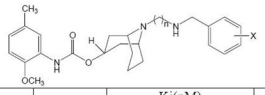
Table.



Compound	n	X	Ki(nM)		σ_1/σ_2
			σ_1	σ_2	
1a	4	3-Br	66.29	3.06	21.66
1b	4	4-Br	1.70	4.07	0.42
1c	4	3-F	0.30	4.13	0.07
1d	4	4-F	22.15	29.07	0.76
1e	4	3-I	63.87	1.21	52.77
1f	4	4-I	0.34	4.00	0.08
2a	6	3-Br	112.40	8.94	12.57
2b	6	4-Br	43.16	9.93	4.35
2c	6	3-F	0.46	4.85	0.09
2d	6	4-F	1.00	70.88	0.01
2e	6	3-I	0.56	32.68	0.02
2f	6	4-I	0.65	35.93	0.02

Keywords: sigma-2 Receptor, Structure-Activity Relationship, Azabicyclo[3.3.1]nonan Analogs

SYNTHESIS OF [^{18}F]FLUOROMETHYLSPIPERONE ([^{18}F]FMSP) *VIA* [^{18}F]FLUOROMETHYL TRIFLATE SYNTHESIS

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N-[^{11}C]Methylspiperone ([^{11}C]NMSP) and [^{18}F]fluoroethylspiperone ([^{18}F]FESP) have been reported as useful radiopharmaceuticals for D_2R imaging by PET [1,2,3]. We report a synthesis of [^{18}F]fluoromethylspiperone ([^{18}F]FMSP) using the reaction with [^{18}F]fluoromethyl triflate [4].

Preparation of [^{18}F]fluoromethyl triflate was carried out by a reported method [4]. [^{18}F]fluoromethyl triflate from a silver triflate column was transferred by Ar steam to a reaction vial containing a mixture of 1 mg (2.5 μmole) spiperone and 5 mg (125 μmole) sodium hydroxide (60% in mineral oil) in 0.8 mL acetonitrile. After transfer of activity, the reaction vial was heated for 5 min at 80°C. The mixture was mixed with ~0.09 mL of 1.5 M HCl and 1 mL of 0.1 M HCO_2NH_4. The mixture was injected into semi-preparative HPLC (Waters, Xterra RP8, 10 x 250 mm; 40% CH_3CN/0.1 M HCO_2NH_4; 4 mL/min). The aliquot eluted at 12-14 min was collected, diluted with 200 mL of 0.1 M NaH_2PO_4 and filtered through a tC18 SepPak cartridge. The activity captured on the SepPak cartridge was eluted with 1 mL EtOH and diluted with 10 mL water. The pooled activity was analyzed with analytical HPLC (BioRad, RSil C_{18}(?)HL, 4.6 x 150 mm; 50% acetonitrile/0.1 M HCO_2NH_4; 1 mL/min).

The analytical HPLC identified the radioactivity as [^{18}F]FMSP by comparing retention time (5.3 min) with cold authentic FMSP. Radiochemical yield and radiochemical purity of [^{18}F]FMSP were 1.8% (decay corrected) and >99%, respectively. Specific activity was 2.9 x 10^5 Ci/mole. Synthesis time was ~90 min.

We successfully synthesized [^{18}F]FMSP using [^{18}F]fluoromethyl triflate as a fluoromethylating agent.

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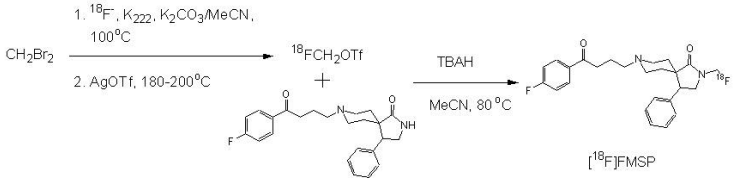

Keywords: [^{18}F]Fluoromethylation, Dopamine D2 Receptor, Fluoromethylspiperone