CYTISINE TAGGED WITH A 2-[^{18}F]FLUOROPYRIDINYL-5-YL GROUP AS A CANDIDATE FOR BRAIN a 4b2 NICOTINIC RECEPTOR IMAGING WITH PET

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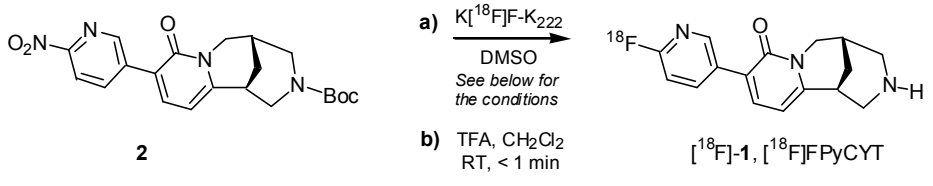
Keywords : Fluorine-18, Nicotinic Receptors, Cytisine

In recent years, considerable effort has been spent on the design, synthesis and pharmacological characterization of radiolabelled ligands for the *in vivo* imaging of nicotinic acetylcholine receptors (nAChRs) using Positron Emission Tomography (PET). We herein report the preparation of a fluoropyridinyl derivative of cytisine (FPyCYT, **1**) and its labelling with the positron emitter fluorine-18 ($T_{1/2}$: 110 minutes).

The synthesis of the nitropyridine precursor **2** and the non radioactive fluorinated reference **1** was based on a previously described strategy starting from (-)-cytisine (1). Protection of cytisine as its *N*-Boc derivative followed by iodination of the pyridone ring yielded the *N*-Boc-9-iodocytisine. Conversion into its trimethylstannyl analogue was performed using $(Me_3Sn)_2$ and $(Ph_3P)_4Pd$. Stille coupling with 5-bromo-2-nitropyridine or 2-fluoro-5-iodopyridine gave the *N*-Boc-protected 2-nitro- and 2-fluoropyridinyl-5-cytisines, respectively. Removal of the Boc protective group of the fluorinated derivative cleanly gave compound **1**.

Binding assays demonstrated that fluoropyridinylcytisine **1** only displays a moderate affinity for the 4 2 nAChR (Ki : 23.9 nM) but was highly selective relative to the 7 subtype (Ki : 3.4 µM).



2 [^{18}F]-**1**, [^{18}F]FPyCYT

Fluoropyridinylcytisine **1** has been labelled in two radiochemical steps by (a) nucleophilic heteroaromatic nitro-to-fluoro substitution (2) using the activated K[^{18}F]F-K222 complex as the no-carrier-added radiofluorinating reactant (conventional heating at 150°C for 7-10 min or microwave activation at 100W for 1 min ; (b) TFA removal of the Boc protective group. HPLC purification (semi-preparative C-18 Zorbax SB Hewlett Packard, 250 x 9.4 mm, 5 µm) cleanly gave chemically and radiochemically pure [^{18}F]-**1**. Typically, 20-30 mCi (0.74-1.11 GBq) of [^{18}F]-**1** were obtained in 70-75 minutes starting from a 100-150 mCi (3.7-5.5 GBq) aliquot of a batch of cyclotron-produced [^{18}F]fluoride.

The pharmacological profile of [^{18}F]-**1** is currently being evaluated *in vivo*. Biodistribution studies and brain radioactivity monitoring using intracerebral radiosensitive -microprobes in rodents are underway.

1. Marrière E, Rouden J, Tadino V, Lasne M-C. *Org Lett* 2000; 2: 1121-1124.
2. Dolci L, Dollé F, Jubeau S, Vaufrey F, Crouzel C. *J Label Compd Radiopharm* 1999; 42: 975-985.

SYNTHESIS AND RADIOSYNTHESIS OF A NOVEL NMDA LIGAND LABELLED WITH CARBON-11

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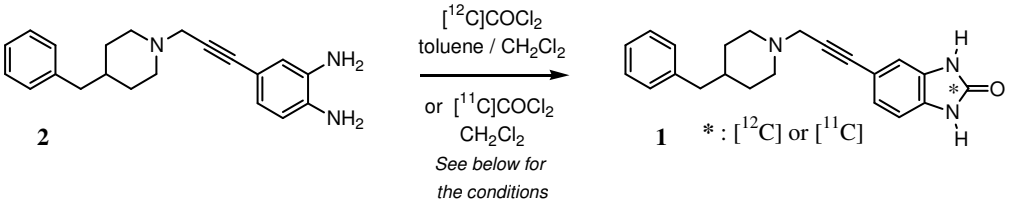
Keywords : Carbon-11, NMDA Receptor, Sonogashira

The development of positron-emitting radiotracers for the *in vivo* imaging of the N-Methyl-D-Aspartate (NMDA) receptors in brain with Positron Emission Tomography (PET) has been the goal of many researchers for several years. Recently, a new series of 1-(heteroarylalkynyl)-4-benzylpiperidines has been described in the literature as potent and highly selective antagonists of the NMDA receptors (1). Derivative **1**, namely 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzo-imidazol-2-one, shows a high affinity and selectivity for the $NR1_A/2B$ subtype (IC_{50} values for inhibition of NMDA responses : $NR1_A/2B$: 5.3 nM, $NR1_A/2A$: 35 µM and $NR1_A/2C$ > 100 µM). Moreover, due to its chemical structure, this derivative can be labelled with carbon-11 (half-life : 20.4 minutes) at its benzoimidazolone ring. The present work represents (a) a concise synthesis of derivative **1**, as the non radioactive reference and its precursor for labelling **2** ; (b) the labelling of **1** with carbon-11 using [^{11}C]phosgene. The synthesis of the diaminophenyl precursor **2** and the benzoimidazolone reference **1** was achieved using literature-based methods (1,2). Briefly, 4-benzylpiperidine was first reacted with propynyl tosylate. Sonogashira coupling between 4-bromo-2-nitroaniline and the 4-benzylpropynylpiperidine, using $Pd(PPh_3)_4$ as catalyst, followed by reduction of the nitro function with Raney-Ni gave the diaminophenyl derivative **2**. Cyclisation with phosgene afforded the benzoimidazolone **1** in 29% overall yield.



[^{11}C]Phosgene was synthesized from [^{11}C]methane via [^{11}C]carbon tetrachloride using a combination of published processes (3,4). The cyclisation reaction of [^{11}C]phosgene and the diaminophenyl derivative **2** in dichloromethane at room temperature was rapid and afforded pure [^{11}C]-**1** after HPLC purification (column: Merck Lichrosorb SiO_2, 250 × 10 mm; 7 µm; eluent: CH_2Cl_2/MeOH/NH_3 95/5/0.5). The total synthesis time was 28-30 minutes and the specific radioactivity was about 1 Ci/µmol (37 GBq/µmol) at the end of the synthesis.

Pharmacological profile of [^{11}C]-**1** is currently being evaluated *in vivo*. Biodistribution studies and brain radioactivity monitoring using intracerebral radiosensitive β-microprobes in rodents are underway.

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1. Wright JL, Gregory TF, Kersten SR, Boxer PA, Serpa K, Meltzer LT, Wise D, Espitia SA, Konkoy CS, Whittemore ER, Woodward RM. *J Med Chem* 2000; 32: 3408-3419.
2. Wright JL, Gregory TF, Bigge CF, Boxer PA, Serpa K, Meltzer LT, Wise LD, Cai SX, Hawkinson JE, Konkoy CS, Whittemore ER, Woodward RM, Zhou Z-L. *J Med Chem* 1999; 42: 2469-2477.
3. Landais P, Crouzel C. *Appl Rad Isot* 1981; 32: 391-392.
4. Link JM, Krohn KA. *J Label Compds Radiopharm* 1997; 40: 306-308.

SYNTHESIS OF THE DOPAMINE D3 RECEPTOR ANTAGONIST [^{11}C]GR218231

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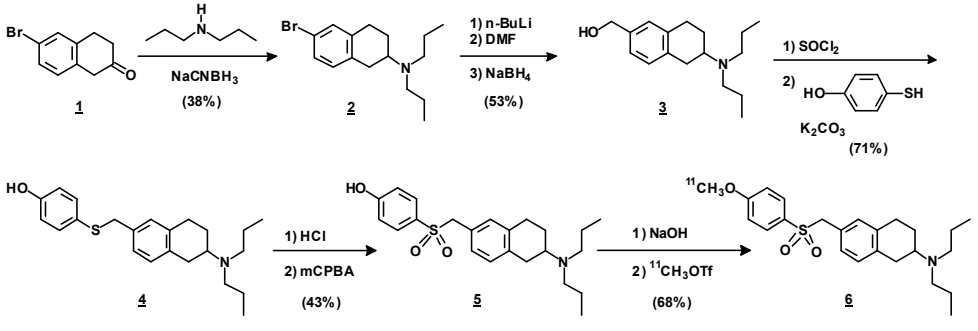
Keywords: D_3 receptor, Antagonist, Synthesis, Carbon-11, Positron emission tomography

The dopamine D_3 receptor is preferentially located in limbic brain areas and plays an important role in behavioral processes like locomotion, reinforcement and reward. Limbic brain areas are considered important targets for antipsychotic agents. The presence of the D_3 receptor in projection regions of the mesocorticolimbic dopaminergic system also suggests a potential therapeutic role in reinforcement processes and drug abuse. We aim to develop a non-invasive imaging method to study D_3 receptor distribution and binding properties in-vivo. Here we describe the synthesis of the ^{11}C-labeled GR218231 **6** as a potential PET tracer for D_3 receptor imaging. GR218231 is an antagonist with a high affinity (pKi 9.0) and selectivity for the D_3 receptor (1).



In the first step of the synthesis of [^{11}C]GR218231, 6-bromo-2-tetralone **1** was converted to amine **2** in 38% yield via a reductive amination with di-n-propylamine. Subsequent metallation, formylation and reduction afforded benzyl alcohol **3** in 53% yield. Reaction of **3** with thionyl chloride, followed by a nucleophilic substitution reaction with 4-mercaptophenol in the presence of K_2CO_3 resulted in the formation of sulfide **4** in 71% yield. After protonation of **4** to prevent oxidation of the amino group, oxidation at the sulfur atom with m-chloroperbenzoic acid gave sulfone **5** in 43% yield. Thus, tetralin derivative **5** was prepared in 8 steps in 6% overall yield.

Precursor **5** (0.5 mg, HCl salt) was dissolved in 0.3 ml of acetone and deprotonated with 2.5 l of 0.5M NaOH. [^{11}C]Methyl triflate was prepared from [^{11}C]methyl iodide as previously published (2) and bubbled through the precursor solution at ambient temperature. After the solvent was evaporated at 80 °C with the aid of an argon flow, the product was purified by reversed phase HPLC (Alphabond C18, ACN/0.1M NaH_2PO_4 (4/6), 3 ml/min). [^{11}C]GR218231 was obtained in 68 7% decay corrected yield (based on [^{11}C]methyl triflate). The overall synthesis time (EOB-EOS) was 38 2 min. The product co-eluted with a cold references sample (3), had a specific activity of >10GBq/ mol and radiochemical purity of >99%.

Thus, the labeled D_3 receptor antagonist [^{11}C]GR218231 was prepared in sufficient amounts for preclinical and human studies. At present, validation studies in rats are in progress.

References:

1. Millan M J, et al. *J Pharmacol Exp Ther.* 2000; 293: 1048-1062.
2. Någren K, et al. *Nucl Med Biol.* 1995; 22: 235-239.
3. Murray P J, et al. *Bioorg Med Chem Letters*. 1996; 6: 403-408.

IMAGING OF SIGMA RECEPTORS IN THE MONKEY BRAIN USING [^{11}C]SA5845

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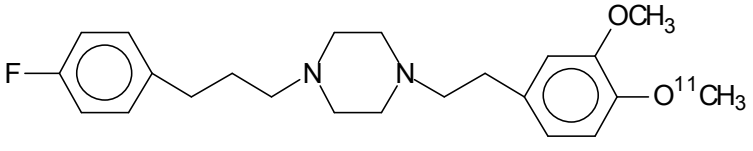
Keywords: Carbon-11, sigma receptor, PET, monkey

The sigma receptor may be involved in several diseases of the central nervous system (CNS), such as schizophrenia, depression, dementia and ischemia, as well as in peripheral nervous system diseases. Furthermore, sigma receptors have been found in the endocrine, immune and in other peripheral organ systems, and are highly expressed in a variety of human tumors such as neuroblastoma, melanoma and breast cancer. The sigma receptor is classified into two subtypes, $sigma_1$ and $sigma_2$.

PET-imaging of the sigma receptors is very helpful to understand the processes in which the sigma receptors are involved. Carbon-11 labelled SA5845 is slightly sigma-2 subtype selective and has been evaluated in the monkey for its suitability to measure sigma receptors.



[^{11}C]SA5845

The monkey (*Macaca mulatta*) was anesthetized with ketamine and was scanned with [^{11}C]SA5845 (170-270 MBq) using a Siemens HR$^+$ PET-camera. A primate chair was constructed out of styrofoam and fixed to the bed of the PET-camera. The presence of the chair caused negligible attenuation of the radiation. Brain and heart were positioned in the field of view. ROIs of the lumen of the heart were used for plasma input data. After a dynamic study of 60 min, haloperidol (1 mg/kg) was administered by i.v. injection to displace the radioligand. Regional distribution in the brain was similar to data previously obtained with [^{11}C]SA4503 [1] and its [^{18}F]fluoroethylated analogs [2]. The time activity curves of [^{11}C]SA5845 showed increasing binding for 60 min and radioactivity levels were slightly decreased at 60 min after haloperidol displacement. Venous blood samples were analyzed for metabolites. Metabolite analysis of plasma showed 76% parent compound at 20 min post injection and 52% parent at 60 min.

Preliminary kinetic analysis using a 3-compartment model revealed that determination of the true k_2/k_3 is still an issue. Because of the very rapid plasma clearance, determination of k_2 is difficult. Simulation of the free and bound components of the time-activity curves suggests that at 60 min no equilibrium exists, but that the bound fraction is still increasing, which may explain the relatively low displacement effect of haloperidol.

References

1. Kawamura et al, Neurobiological Aging (in press)
2. Elsinga et al., Synapse 43, 259-267 (2002)

SYNTHESIS AND BIODISTRIBUTION OF A ^{18}F-LABELED CORTICOSTEROID FOR MAPPING BRAIN GLUCOCORTICOID RECEPTORS (GR)

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Keywords: ^{18}F, Corticosteroids, Glucocorticoid Receptor , Biodistribution

Corticosteroids are implicated in neuropsychiatric disorders such as severe depression and anxiety. The development of corticosteroids appropriately labeled with ^{18}F would allow the non-invasive in vivo imaging and mapping of brain GRs by means of PET. Recently we have prepared a series of novel fluorophenyl pyrazolo corticosteroids which showed relative binding affinities (RBA) to the GR of up to 56% in comparison to dexamethasone (100%) [1]. One candidate was chosen for radiolabeling with ^{18}F, biodistribution studies and small animal PET imaging using male Wistar rats. The radiolabeling was accomplished by [^{18}F]fluoride ion displacement on the corresponding -keto iodide **1** to give 21-[^{18}F]fluoro compound **2** in 3-4% decay-corrected radiochemical yield after HPLC purification at an effective specific radioactivity of 25-45 Ci/mmol (Figure 1). The synthesis including HPLC separation was accomplished within 80 min after EOB, and the radiochemical purity exceeded 98%. The found low effective specific radioactivity stems from the co-eluting oxetanone **3** which was formed inevitably during the radiolabeling process.

Biodistribution in male Wistar rats (ca. 175 g) showed an initial brain uptake of 0.53 0.11 % ID/g after 5 min which slightly decreased to 0.48 0.06 % ID/g after 60 min. Brain to blood ratios at 5 min and 60 min post injection were 2.94 and 3.70, respectively. The low accumulation of radioactivity in the bone (0.55 0.11 % ID/g after 5 min and 0.68 0.10 % ID/g after 60 min, respectively) is indicative of a low in vivo defluorination of corticosteroid **2** in comparison to similar compounds reported in the literature [2,3]. The pituitary and thymus as major targets of corticosteroid hormones showed uptakes of 1.15 0.40 % ID/g and 0.55 0.11 % ID/g after 5 min, and 0.87 0.19 % ID/g and 0.68 0.10 % ID/g after 60 min, respectively. After 120 min the pituitary uptake reached 1.2 % ID/g. The uptake could be reduced to 0.74 % ID/g at 120 min in the blocking experiment (pretreatment with 2 mg of corticosterone).

In conclusion, we have synthesized a ^{18}F-labeled corticosteroid showing moderate brain uptake comparable with the best ligands reported in the literature. In association with the good in vivo stability in terms of in vivo defluorination we are encouraged to continue the work by using other ^{18}F-labeled fluorophenyl pyrazolo corticosteroids to further improve brain uptake.

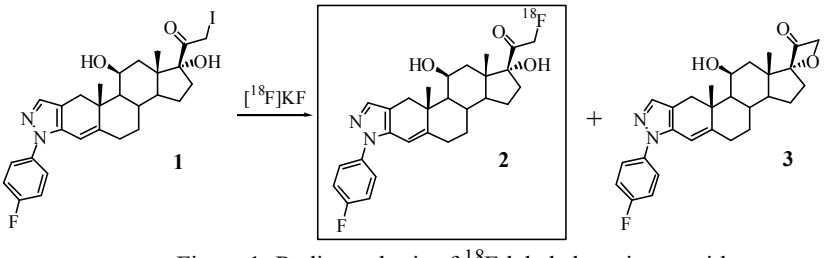

Figure 1: Radiosynthesis of ^{18}F-labeled corticosteroid

1.Wüst F, Carlson K, Katzenellenbogen JA. *Steroids.* In press.
2.Pomper MG, Kochanny MJ, Thieme AM, Carlson KE, VanBrocklin HF, Mathias CJ, Welch MJ, Katzenellenbogen JA. *Nucl Med Biol.* 1992; 19: 461-480.
3.Feliu AL, Rottenberg DA. *J Nucl Med.* 1986; 28: 998-1005.

SYNTHESIS AND IN-VIVO EVALUATION OF ^{123}I-ZIMELIDINE, A POTENTIAL SPECT-RADIOLIGAND FOR THE SERT

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Keywords : Zimelidine, Iodine-123, SSRI, Biodistribution study

Rationale : First developed SSRI-drug, zimelidine (ZIM – figure 1), has a moderate affinity for the SERT (pK_i = 7.3), and is almost devoid of action on other receptors (1, 2). However, the analogues, have a significant higher affinity (I-ZIM, pK_i = 7.5 and nor-ZIM, pK_i = 8.2). Therefore, N.C.A. ^{123}I-ZIM, and ^{123}I-nor-ZIM were screened for in-vivo evaluation in rats, as potential SPECT-ligand for the SERT.

Radiochemistry : Radioiodination of both ligands, was performed via non-isotopic exchange, with the Cu(I)-assisted nucleophilic labelling method. The precursors, ZIM and nor-ZIM, were supplied as their hydrochloride-salts. Modification to the acetate-form, with an anion-exchange column, was necessary to obtain a successful labelling yield of up to 90 % (instead of 50 %), for both radioligands. The collected RP-HPLC fraction was concentrated by C18 Sep-pak in 0.5 ml acidified ethanol, which was subsequent diluted with a saline-solution, to give a radiochemical pure product of more than 99 % .

Biodistribution : Rats were *iv* injected, with ^{123}I-ZIM or ^{123}I-nor-ZIM (approx. 3.7 MBq), and sacrificed by cervical dislocation (after 5, 30, 60 and 120 min.- n = 4x4). Several tissues were dissected and counted for radioactivity.

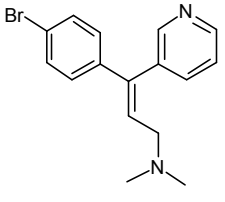

Figure 1 : ZIM

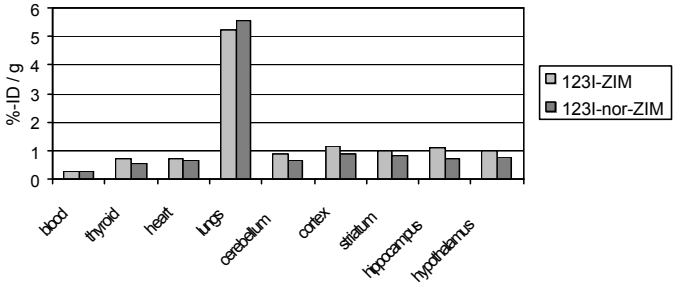

Figure 2 : Uptake-data after 60 min.

Both radioligands had a good brain penetration of 0.8-1 % ID, stable after 60 min. p.i., and a brain/blood ratio up to 3. Less specific binding for both ligands, was observed, between the different isolated brain regions. The high lung-uptake could not been associated with presence of the SERT. Blocking studies, with GBR-12909 or fluvoxamine, resp. DAT and SERT-ligands, had minor influence on the uptake of both radioligands. Modifications of the base-structure will be further investigated, in order to obtain ligands with a higher affinity and selectivity for the SERT.

1.Carlsson A, Wong D. *Life Sciences*, 1997; 61:1203.
2.Högberg T, Ross S B, Ström P, Grunewald G L, Creese M W, Bunce J D. *J Med Chem*, 1988; 31: 913-919.

SYNTHESIS AND PRELIMANARY IN-VIVO EVALUATION (PIG) OF THE C-11 LABELLED D_4 LIGAND (*S*)-1-(3-{2-[4-(4-[^{11}C]METHOXYPHENYL)PIPERAZIN-1-YL]ETHYL}-2,3-DIHYDRO-1*H*-INDOL-1-YL)ETHANONE

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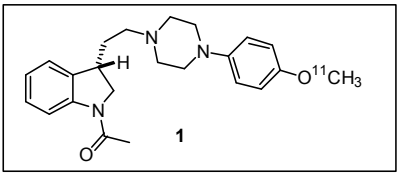
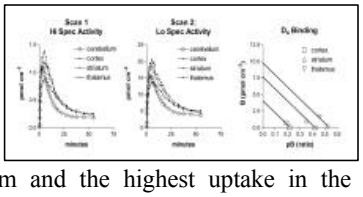
Keywords: Brain, D4-Ligands, PET



Introduction: Dopamine D_4 receptors are discussed to be involved in psychiatric disorders such as schizophrenia. The current study attempted to determine whether the selective D_4 ligand (*S*)-1-(3-{2-[4-(4-[^{11}C]methoxyphenyl) piperazin-1-yl]ethyl} -2,3-dihydro-1*H*-indol-1-yl)ethanone, compound **1**, would be a suitable PET ligand to study D_4 receptors in the living brain (pig). In vitro, compound **1** exhibited higher affinity for hD_4 receptors (Ki: 19 nM) as compared to hD_2 and hD_3 receptors (Ki: 1100nM and 7100 nM, respectively).

Chemistry: Non-radioactive compound **1** and the *O*-desmethyl precursor were prepared in a similar manner by alkylation of the appropriately para-substituted phenyl piperazines by enantiomeric pure 1-[3-(2-bromoethyl)-2,3-dihydro-1*H*-indol-1-yl]ethanone (ref: WO 98/28293, Chem Abstr 1998, 129, 95399) by use of potassium carbonate in a mixture of DMF and butanone. The final products were purified by flash chromatography on silica gel. ^{11}C-labelleled (*S*)-1-(3-{2-[4-(4-[^{11}C]methoxyphenyl)piperazin-1-yl]ethyl} -2,3-dihydro-1*H*-indol-1-yl)ethanone was readily prepared (RCY: 80% based on $^{11}CH_3I$) with high specific radioactivity (64 –85 GBq/µmol, EOS) and high purity (> 98%) by *O*-methylation in DMSO of the *O*-desmethyl compound (1 mg) with $^{11}CH_3I$ in the presence of NaOH as supporting base at 80°C within 40 min (EOB) total synthesis time.

PET study: The Danish Council of Animal Research approved all studies involving animals. Three 60-min PET scans were performed using an anaesthetised 38-kg female Yorkshire pig after an iv. injection of 418 - 484 MBq of compound **1**. In the first study the tracer was given in high specific radioactivity (HSA, 85 GBq/µmol, EOS), in the second study with low specific radioactivity (LSA, 4 GBq/µmol) and in the final study in high specific radioactivity (HSA, 64 GBq/µmol, EOS) after administration of the selective D_4 ligand L-745,870 (98 mg, 6 µmol/kg) in a displacement study. For each scan 9 arterial plasma samples were analysed by Radio-HPLC after precipitation of plasma proteins with acetonitrile for metabolite correction. **Image analysis:** Time activity curves of selected brain regions were obtained by application of a standard pig brain atlas to a MR co-registered brain.



Results: Radiolabelling can be performed in excellent yields without any problems. In pig, compound **1** was remarkably stable. After 30 min post injection (p.i.) still around 30% (22 – 34%) and at 60 min p.i. around 18% (0 – 34%) of unchanged tracer were detected. The time-activity curves from selected brain regions are shown. The brain uptake of the tracer is highly reversible, with the lowest uptake in the cerebellum and the highest uptake in the thalamus. The resulting binding potentials shown ranges from 0.5 in the thalamus to 0.2 in the frontal cortex. At a common K_d of 18 pmol/cm^3, the B_{max} ranged from 4 to 11/cm^3.

Conclusion: Despite the simple synthesis, the favourable low plasma metabolism and the fully reversible brain uptake, the ligand is not an optimal PET tracer due to the low binding potential in the range of 0.2-0.5 (optimal range 1<pB<10).

RADIOSYNTHESIS OF [^{11}C]LBT-999, A SELECTIVE RADIOLIGAND FOR THE VISUALISATION OF THE DOPAMINE TRANSPORTER WITH PET

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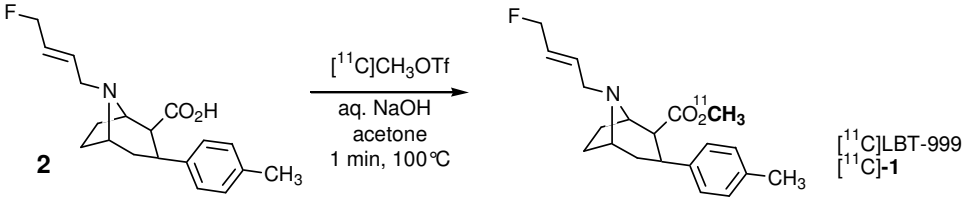
Keywords : Carbon-11, LBT-999, dopamine transporter, tropane

LBT-999 (**1**, (E)-N-(4-fluorobut-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane) is a cocaine derivative belonging to a new generation of selective dopamine transporter (DAT) inhibitors (Ki vs [^{125}I]PE2I : 11 nM). Due to its chemical structure, this compound can be labelled with carbon-11 (half-life : 20.4 min) and fluorine-18 (half-life : 109.8 min), the most widely used positron-emitting radiohalogen.

LBT-999 (**1**) was labeled in a first time with carbon-11 to evaluate the *in vivo* properties of this derivative. LBT-999 (**1**) was labelled at its methyl ester function from the corresponding carboxylic acid precursor **2** and the efficient methylation reagent [^{11}C]methyl triflate. Typically, 150 to 250 mCi of [^{11}C]LBT-999 were routinely obtained within 30 min of radiosynthesis (including HPLC purification) with specific radioactivities ranging from 0.8 to 1.2 Ci/μmol.



Cerebral biodistribution was performed in rats in order to evaluate this radiotracer *in vivo*. [^{11}C]LBT-999 presented a high uptake in the striata (5% ID/g of tissue at 30 min post injection) and very low uptake in the cerebellum and cortex (about 0.3% ID/g of tissue at 30 min). The striatum to cerebellum ratio was 18 and 27 at 30 and 60 min, respectively. Blocking studies clearly showed that striatum uptake was strongly inhibited using GBR-12909 (a reference DAT inhibitor) at the dose of 5 mg/kg whereas no effect could be detected using either paroxetine (a reference 5-HT reuptake inhibitor) nor nisoxetine (a reference norepinephrine reuptake inhibitor) at the same dose. PET studies were also performed in baboons (Papio papio). [^{11}C]LBT-999 accumulated rapidly in the striata with a maximal uptake of 0.18% ID/mL at 30 min whereas no accumulation was observed in the cerebellum. The striatum to cerebellum ratio was 33 at 30 min. Blocking studies in baboons also confirmed the excellent selectivity of [^{11}C]LBT-999 : Pre-treatment with GBR12909 (5 mg/kg) prevented the accumulation of [^{11}C]LBT-999 in striata whereas the kinetics of the radiotracer was not affected by pre-treatment with either citalopram (a reference 5-HT reuptake inhibitor, 5 mg/kg) nor maprotiline (a reference norepinephrine reuptake inhibitor, 5 mg/kg).

This *in vivo* profile suggest that [^{11}C]LBT-999 is an excellent candidate for quantification of dopamine transporter (DAT) in the brain using the high-resolution, sensitive and quantitative imaging technique positron emission tomography.

SYNTHESIS AND CHARACTERIZATION OF [^{11}C]SME-ADAM AS A POTENTIAL RADIOTRACER FOR IMAGING THE SEROTONIN TRANSPORTER WITH PET

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Keywords: [^{11}C]SMe-ADAM, serotonin transporter, PET, [^{11}C]methyl iodide

The new diphenyl sulfide derivative SMe-ADAM (N,N-dimethylamino-2-(2-amino-4-methylthio-phenylthio)benzylamine, **1**) displays a high affinity to the serotonin transporter (IC_{50} = 0.28 nM). The affinity to the norepinephrine (IC_{50} = 458 nM) and to dopamine transporter (IC_{50} = 1900 nM) is much lower. These binding data indicate that SMe-ADAM labelled with carbon-11 could be a suitable radioligand for imaging of the serotonin transporter. Compound **1** has two options for ^{11}C-labelling: the S-methyl and the N-methyl group. Here, we report on the synthesis (S-[^{11}C]methyl-thio)-SMe-ADAM and its biological characterization.

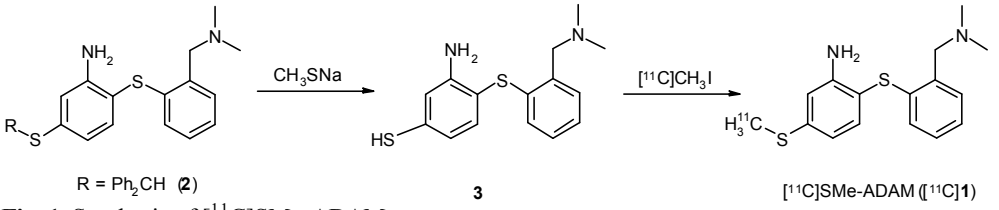

Fig. 1. Synthesis of [^{11}C]SMe-ADAM

The thiol precursor **3** was prepared starting from the diphenylmethyl derivative **2** which was obtained by reaction of 4-chloro-3-nitrodiphenylmethylthiobenzene and N,N-dimethyl-2-thiobenzamide followed by reduction of the amide moiety (borane/THF complex) and the nitro group (tin(II) chloride). The S-demethylation of **2** by treatment with sodium thiomethoxide was completed within 80 min. The crude thiol **3** can be used for the labelling reaction without purification. It can be stored at -18 °C under nitrogen for several days.

The reaction of the thiol precursor **3** with [^{11}C]methyl iodide was completed within 2 min at 40 °C. The product mixture contains more than 85 % of [^{11}C]SMe-ADAM. Side reactions with impurities of the crude precursor and at the amino function were of minor importance. The complete synthesis including [^{11}C]methyl iodide preparation, synthesis of [^{11}C]**1**, purification and formulation took 40 min. [^{11}C]SMe-ADAM was obtained in a radiochemical yield of 42 - 45% (decay-corrected, related to [^{11}C]methyl iodide). Starting from 85 GBq [^{11}C]carbon dioxide, specific radioactivities from 26 to 40 GBq/µmol (0.7 - 1.1 Ci/µmol) were reached at the end of the synthesis. The radiochemical purity of [^{11}C]**1** exceeds 98 %.

Biodistribution studies in rats demonstrated a high brain uptake (2.9%ID/g tissue) 5 min after injection, which decreased to 1.4%ID/g tissue after 60 min. The uptake in the cerebellum was 1.8%ID/g tissue (5 min after injection) and 0.4%ID/g tissue (60 min). The inspection of the microPET images showed a specific accumulation in the midbrain and thalamus regions. [^{11}C]SMe-ADAM was fast metabolised. After 10 min only 20% of the original compound remained in the plasma. Autoradiographic studies showed a high accumulation of radioactivity in the regions rich in SERT, such as amygdala, hypothalamus, superficial grey layer of the superior colliculus and substantia nigra.

These results indicate that [^{11}C]SMe-ADAM is a potential radioligand for *in vivo* imaging of the serotonin transporter with PET.

SYNTHESIS OF [^{11}C]BENAZOLINE AS A POTENTIAL PET RADIOLIGAND FOR THE IMIDAZOLINE RECEPTOR

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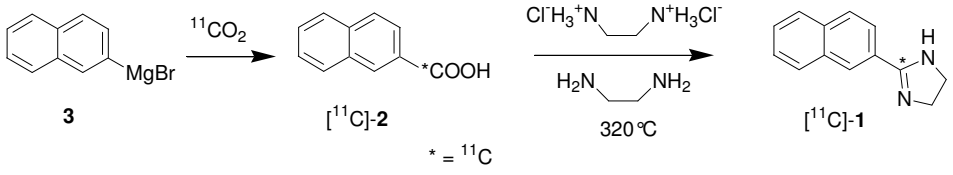
Keywords: Carbon-11, Imidazoline Receptor, Benazoline

Benazoline (2-naphthalen-2-yl-4,5-dihydro-1H-imidazole, **1**) is a high-affinity ligand for the imidazoline I_2 receptor with a high selectivity over adrenergic (α) receptors (1). We have labelled this compound with carbon-11 (half life 20.4 min) for its evaluation as a potential PET tracer for the I_2 receptor



Only carbon atom number 2 of the 4,5-dihydroimidazole ring of **1** comes reasonably into consideration for labelling. We achieved no-carrier-added radiolabelling by condensation of [^{11}C]2-naphthoic acid ([^{11}C]-**2**) with ethylenediamine at high temperature (2). [^{11}C]carboxylic acids are generally easy to obtain from the corresponding Grignard and our method possibly shows the way to a general and convenient access to [^{11}C]4,5-dihydro-1H-imidazoles. Ethyleneurea (2-imidazolidone) could possibly be an alternative to ethylenediamine (3).

The Grignard **3** was freshly prepared from 2-bromonaphthalene (8.0 mmol) and Mg (8.8 mmol) in THF (15 mL) at reflux using 1,2-dibromoethane (1.1 mmol) to initiate the reaction (4). [^{11}C]CO_2 was led into 400 µL of the Grignard solution (about 0.4M) at ambient temperature giving [^{11}C]-**2** in 60% decay-corrected yield. After 2 minutes the mixture was hydrolysed with a solution of ethylenediamine (200 µmol) and ethylenediamine.2HCl (200 µmol) in water (200 µL). The reaction vessel, having an outlet to atmosphere, was placed in a heating block of 320°C for 10 minutes during which all liquid evaporated. After cooling down, the yellow residue was taken up in water, filtered, and injected into HPLC (C18 X-Terra, water/acetonitrile/triethylamine 30/70/0.05 (v:v:v), 5 mL/min, Rt **1** = 11 min). This not yet optimised procedure gave 20% of [^{11}C]benazoline ([^{11}C]-**1**) relative to [^{11}C]CO_2 (decay-corrected).

References

1. M. Pigini *et al*, Bioorg. Med. Chem. **5**, 833-841 (1997)
2. M. Hartmann and H.O. Isler, German Patent DE 687196 (1940)
3. German Patent; I.G.Farbenind, DE 695473 (1937)
4. R. Tsuji et al, J. Org. Chem 57, 636-641 (1992)

SYNTHESIS AND PURIFICATION OF [^{18}F]FLUMAZENIL AND *IN VIVO* COMPARISON WITH [^{11}C]FLUMAZENIL IN RATS.

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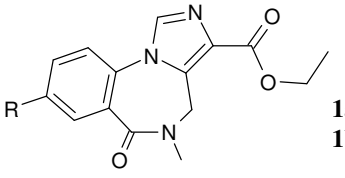
Keywords: Flumazenil, ^{18}F, ^{11}C, *In Vivo*, Rat

[^{11}C]Flumazenil is a widely used PET radioligand for the central $GABA_A$ benzodiazepine receptor and is used both in the clinic to locate epileptic foci and in preclinical research studies, e.g. for elucidation of depression processes in the brain(1-3).



1a, R = F, Flumazenil
1b, R = NO_2

Especially for small animal PET studies of receptor systems a high specific activity (SA) in the order of 10 TBq/µmol, is required (4). However, with the current production techniques of ^{11}C, labeled receptor ligands with a SA of around 100 GBq/µmol at time of injection are obtained on average. Better production methods, leading to a better SA, have been described for ^{11}C, but unfortunately these methods require extreme and unpractical production conditions. Advantages of the use of ^{18}F is that in theory one should obtain a better SA with ^{18}F and the longer half-life enables more prolonged animal PET studies Therefore, we have developed a method for the synthesis of [^{18}F]flumazenil (5). We now report on the purification and *in vivo* studies in the rat and make a comparison between ^{18}F and ^{11}C labeled flumazenil.

[^{18}F]Flumazenil was synthesized in an average decay corrected yield of 11% and purified via a double HPLC purification system. The crude reaction mixture was subjected to the first HPLC purification using an Agilent Zorbax C18 column 16x250mm 5µm (eluent : 760/240/0.5 acetonitrile/water/trifluoroacetic acid (v/v/v), flow rate 4.5 ml/min, 254 nm, Nuclear Interface radiodetection). The fraction from 18-21 minutes was collected in 50 ml of water and subsequently trapped on a SS 4.6x10 mm column loaded with activated C18 Seppak material. This column was mounted on the second HPLC system instead of the injection loop and subsequent purification using a Chromsphere C18 column 4.6x250mm 5µm (eluent : 20/80 acetonitrile/0.25 M phosphate buffer pH 3.5 (v/v), flow rate 1 ml/min, 254 nm, Nuclear Interface radiodetection) yielded (radio)chemical pure [^{18}F]flumazenil which was isolated by solid phase extraction on a C18 Seppak.

After formulation and filtration through a sterile 0.22 µm filter, 250 µl of this solution, ca 15 MBq, was injected into the tail vein of male rats (225-275 grams) and the biodistribution was obtained at 30 and 60 minutes. At these time points also a metabolite analysis was performed in the blood, liver and brain homogenates according to an analogous procedure used by Luurtsema *et al* (6). The same experiments were also performed with [^{11}C]flumazenil. A full report on the findings of these experiments and a comparison between [^{18}F]flumazenil and [^{11}C]flumazenil will also be presented.

1. Maziere *et al. Int. J. Appl. Radiat. Isot.* 1984; 35: 973-976
2. Shinotoh *et al*, *J. Nucl. Med.* 1986; 27:1593-1599
3. Savic *et al. J. Neurol. Neurosurg. Psychiatry* 1993; 56: 615-621
4. Hume *et al. Eur J Nucl Med*;1998; 25:173-176
5. Windhorst *et al. J. Labelled Compnds & Radiopharm.* 2001; 44: S930-S933
6. Luurtsema *et al.* Manuscript in preparation and abstract ISRC 2003, Sydney

RADIOACTIVE LABELING OF [^{11}C-METHYL]-NS 4194 AND COMPARISON WITH [^{11}C-METHYL]-DASB FOR PET NEURONIMAGING OF SEROTONIN REUPTAKE SITES

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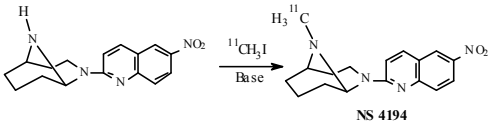
Keywords: Serotonin transporter , [^{11}C]NS 4194, [^{11}C]DASB, pig, brain

Abstract; [^{11}C]( ) 3-(6-Nitro-2-Quinolinyl)-[9-methyl-11C]-3, 9-diazabicyclo-[4.2.1]-nonane ([^{11}C]NS 4194) was evaluated as a new selective serotonin reuptake inhibitor (SSRI) and compared with [^{11}C]3-amino-4-(2-dimethylaminomethylphenylsulfanyl)benzonitrile ([^{11}C]DASB).

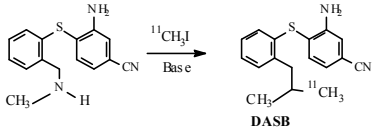
Several drugs for treating psychiatric disorders are specific blockers of the neuronal serotonin transporter (SSRIs). The aim of our present research is to evaluate a new SSRI, [^{11}C]NS 4194 and compare its suitability as a PET tracer for imaging serotonin transporters to that of [^{11}C]DASB, currently considered to be the best SSRI for imaging studies

Studies of the binding kinetics of NS 4194 *in vitro* indicate potentially useful properties for a PET tracer; NS 4194 had IC_{50} values more than 4000-fold lower for [^{3}H]serotonin than for either [^{3}H]dopamine or [^{3}H]noradrenaline *in vitro* in rat brain synaptosomes.

NS 4194 and DASB were both successfully labelled by N-methylation with a carbon-11 methyl group (see figure). Both of the synthesis and purification were completed within ca. 40 minutes and with a radiochemical purity >98 %.



NS 4194



DASB

The PET examinations were performed on three pigs. PET scans were performed sequentially with [^{11}C]NS 4194 and [^{11}C]DASB. After the baseline scans, pigs received citalopram (1 mg/kg, i.v.) in order to displace the specific binding in subsequent PET studies with the same two tracers. Arterial blood samples were taken at intervals, and the concentration of untransformed tracers in plasma measured by HPLC and gamma-counting.

The PET scans and citalopram displacement studies show that [^{11}C]NS 4194 binds to serotonin transporters in brain of living pig, with highest binding in the vicinity of the serotonin neurons. However, its specific binding was not as high as that of [^{11}C]DASB, and it furthermore had not fully reached equilibrium during the 60 minute-long PET recording. We have found [^{11}C]DASB to have high specific to nonspecific binding in brain of living pigs, and to have rapid kinetics, making a relatively brief scanning time possible. Thus, we found that [^{11}C]DASB is the best PET tracer for serotonin uptake sites yet studied at the Aarhus PET Centre. We have carried out kinetic analysis of the binding of [^{11}C]DASB in the living pig brain. This tracer should prove useful for imaging of neuropsychiatric disorders, including depression and also Parkinson's disease. In the near furture we will seek permission to use [^{11}C]DASB in human volunteers.

TC-99M LABELED BIPHENYLS AS Aß PLAQUE-SPECIFIC IMAGING AGENTS

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Key Words: Alzheimer's disease, ^{99m}Tc, Receptor, SPECT

Accumulation of Aß plaques is considered as one of the most significant factors of pathogenesis of Alzheimer's disease (AD). Therefore, developing Aß plaque-specific probes for *in vivo* imaging studies of Aß plaques may be important for diagnosis and monitoring of AD patients[1]. We have prepared and tested the binding affinity of a series of biphenyl derivatives as potential imaging agents specific for Aß plaques (Fig. 1). It is interesting to find that some of the biphenyl derivatives display an excellent binding affinity to pre-formed Aß aggregates using a ligand [^{125}I]TZDM[2].

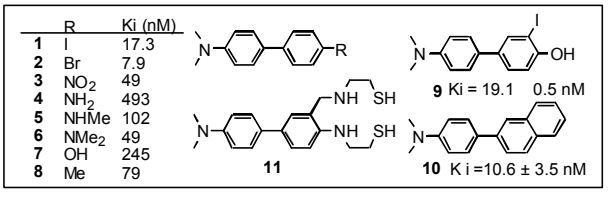

Fig 1. Chemical structures of biphenyl derivatives (**1-11**) and binding affinity. [^{125}I]TZDM was used as the ligand for competition binding to Aß40 aggregates by an *in vitro* binding assay).

The binding data for **9** and **10** suggested that there is sufficient bulk-tolerance for the U-BAT ring system (**11**) on one of the phenyl rings. The preliminary test compound, **11**, similar to U-BAT[3], did not have any gem-dimethyl function group on the N_2S_2 ligand system. Results of labeling studies were what we expected, producing two neutral compounds. It was possible to inter convert between two peaks by redox reactions. Possibly, they are $Tc^{v}ON_2S_2$ complexes, which were the oxidized (**P2**) and reduced (**P1**) forms (Fig. 2).

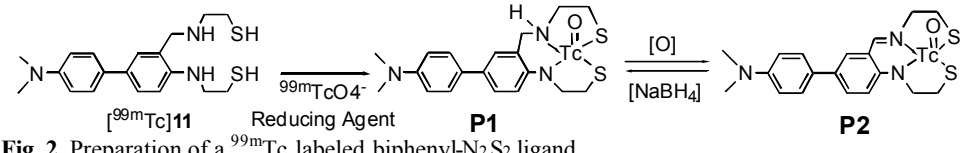

Fig. 2. Preparation of a ^{99m}Tc labeled biphenyl-N_2S_2 ligand.

However, to our surprise these complexes, peak 1 (**P1**) and peak 2 (**P2**) of [^{99m}Tc]**11,** were not stable. After the HPLC separation the compounds, **P1** and **P2**, showed increasing amount of early peak (more polar species). It is likely that the electron donating effect of the anilinic nitrogen is less than optimal leading to a weaker coordinate covalent N-Tc bonding. Previously, when the U-BAT ring system is only conjugated with one benzene ring, the stability of the final complex was excellent[3]. Unfortunately, when additional conjugation is added, like [^{99m}Tc]**11**, this electronic effect has dramatically weaken the complex. The instability observed for the U-BAT derivative (**11**) may be corrected by adding additional gem-dimethyl groups on the $Tc^{v}ON_2S_2$ ring. We are investigating the feasibility of this approach now.

References:

1. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353-356.
2. Zhuang, Z.-P.; Kung, M.-P.; Hou, C.; Skovronsky, D.; Gur, T. L.; Trojanowski, J. Q.; Lee, V. M.-Y.; Kung, H. F. *Journal of Medicinal Chemistry* **2001**, *44*, 1905-1914.
3. Oya, S.; Kung, M.-P.; Frederick, D.; Kung, H. K. *Nuclear Medicine and Biology* **1996**, *23*, 589-593.

THE RADIOSYNTHESIS OF [2-^{18}F] FLUORODEOXYGLUCOSE USING A RESIN BOUND PRECURSOR

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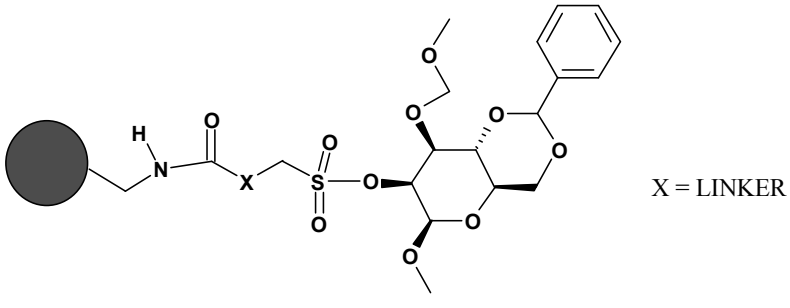
Keywords: FDG, solid phase chemistry, nucleophilic fluoridation

FDG radiosynthesis is routinely performed by the nucleophilic displacement of the tetra-acetoxy mannose triflate followed by either acid or alkaline hydrolysis, and then appropriate purification by solid phase extraction columns.

A new route to synthesis has been devised that uses a resin bound mannose precursor during the fluoride incorporation. The construct has been termed RLV (Resin-Linker-Vector) and is shown in the picture below.



Resin **L**inker **V**ector

New mannose protecting groups have been used to enable the synthesis, but can still be removed quantitatively after the labelling study. Treatment of this construct with [^{18}F]-fluoride in an acetonitrile solution containing kryptofix and potassium carbonate releases the protected FDG into solution in good yield. This solution can be simply filtered leaving many by-products behind on the solid support.

Further methodology has been developed to optimise the deprotection step in order to give good overall yields with high radiochemical purity of FDG.

SOLID PHASE [^{18}F]FLUORODEMETALLATION REACTIONS

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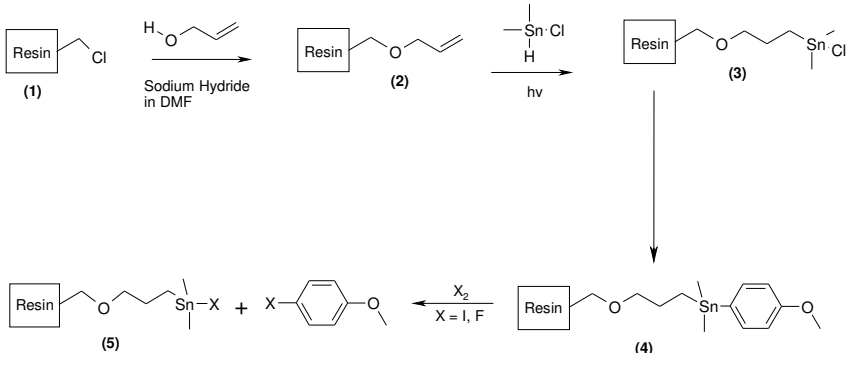
Keywords: Solid phase, [^{18}F]fluorodemetallation

6-[^{18}F]Fluoro-3,4-dihydroxy-L-phenylalanine (6-[^{18}F]fluoro-L-DOPA) is used with positron emission tomography (PET) to measure presynaptic dopaminergic metabolism to allow the study of movement disorders (eg. Parkinsons disease), schizophrenia and neurodegenerative conditions. Currently, a common radiosynthetic route to 6-[^{18}F]fluoro-L-DOPA is by [^{18}F]fluorodemetallation via the "electrophilic-type" reaction of a 6-substituted stannylated derivative with [^{18}F]fluorine. We are currently developing a novel solid phase chemistry approach to the synthesis of 6-[^{18}F]fluoro-L-DOPA using a 6-substituted stannylated derivative which is chemically attached to a modified polystyrene resin. This resin construct should present [^{18}F]fluorine with a single reactive site to allow protected 6-[^{18}F]fluoro-L-DOPA to be selectively cleaved from the resin. The resin can then be simply removed by filtration to achieve separation of unreacted stannylated precursor and stannylated reaction products.

We have prepared an allyl ether functionalised polystyrene resin **(2)** by coupling chloromethylated polystyrene **(1)** with 2-propen-1-ol. Reaction of **1** with dimethyltin chloride under photolytic conditions gave a resin bound organotin derivative **(3)**. **3** was reacted with an appropriate aryl intermediate to give a resin bound anisole tin derivative **(4)**. We have successfully cleaved the anisole substituent from 3 as either 4-iodoanisole or 4-fluoroanisole **(5)** by reaction with iodine and fluorine respectively. This synthesis has been done without the use of aryl lithium compounds and as such has been done under milder conditions. This is of particular importance for aryl compounds containing sensitive protecting groups. We are now applying this strategy to the synthesis of an aryl zinc 6-F-DOPA precursor which can be cleaved by [^{18}F]fluorodemetallation with [^{18}F]fluorine.



DESIGN, SYNTHESIS AND RADIOLABELLING OF 2,6-DIAMINOPURINE DERIVATIVES AS POTENTIAL ADENOSINE-A_{2A} RECEPTOR LIGANDS FOR PET

M. Breidenbach, M.H. Holschbach, D. Bier, W. Sihver, R.A. Olsson, H.H. Coenen

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Keywords: Adenosine-A_{2A} receptor ligand, PET, SAR-studies

The central high affinity adenosine-A_{2A} receptor (A_{2A}AR) is believed to play an important role in some neurodegenerative disorders like Parkinson's disease. This assumption makes it an attractive target for radionuclide *in vivo* imaging. Although a number of high selective and high affinity compounds have been described, due to their high lipophilicity, low water solubility and high degree of unspecific binding, none of them is promising for *in vivo* imaging[1].

The aim of the present study was to develop a new antagonistic leadstructure based on the structure-activity-relationship studies (SAR-studies) of known A_{2A}AR antagonists, namely on xanthine ligands (e.g. [^{11}C]KF17837)[2] and on derivatives of the tricyclic A_{2A}AR antagonists [^{11}C]SCH442416[3] (Fig.). This approach led to the development of 2,6-diaminopurines differentially substituted in the 2-, 8- and 9-position. A new synthetic pathway, which allows the independent and simple introduction of various substituents, was developed.

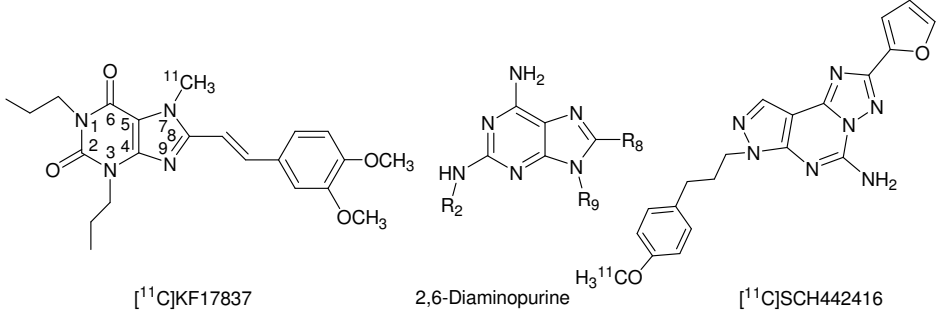

Figure: Structure of known and new AR antagonists

A series of 2,6-diaminopurine derivatives has been synthesized and evaluated *in vitro* in radioligand binding assays using A_1- and A_{2A}AR pig brain membrane preparations. 2-[2-(4-Methoxyphenyl)ethyl]amino-8-(2-furyl)-purines, differentially alkylated and fluoralkylated at the N^9-position, showed the highest A_{2A}/A_1 selectivity with A_{2A}-affinities in the nanomolar range. The rules for substitution of the purine nucleus generally followed those observed for the corresponding xanthine derivatives. Thus, short alkylchains in the 9-position and an aromatic substituent in the 8-position are necessary for high A_{2A} selectivity. Moreover an aralkyl substituent at position 2 is important for A_{2A}-affinity and –selectivity.

In comparison with other ligands, 2,6-diaminopurines exhibit increased water solubility due to their basic aminogroups; their water solubility, as determined by HPLC, being in the range of 2 to 8 µM. Tosylate and mesylate precursors for radiofluorination have been synthesized. Position 9 lends itself for methylation with carbon-11 and radioiodination has been done in the aralkyl substituent. Corresponding labelling data and *ex vivo* evaluation in rodents will be presented.

1.Holschbach M H, Olsson R A. *Curr. Pharm. Design* 2002; 8: 2345-2352.
2.Ishiwata K, Noguchi J, Toyama H, Sakiyama Y, Koike N, Ishi S, Oda K, Endo K, Suzuki F, Senda M. *Appl. Radiat. Isot.* 1996; 47: 507-511.
3.Todde S, Moresco R M, Simonelli P, Baraldi P G, Cacciari B, Spalluto G, Varani K, Monopoli A, Matarrese M, Carpinelli A, Mangi F, Kienle M G, Fazio F. *J. Med. Chem.* 2000; 39: 4359-4362.

SYNTHESIS OF ^{18}F-LABELLED 4-ETHYNYL-2-FLUOROBENZALDEHYDE, FIRST STEP OF A TpOH INHIBITOR : 4-ETHYNYL-2-[^{18}F]FLUORO-pEPA

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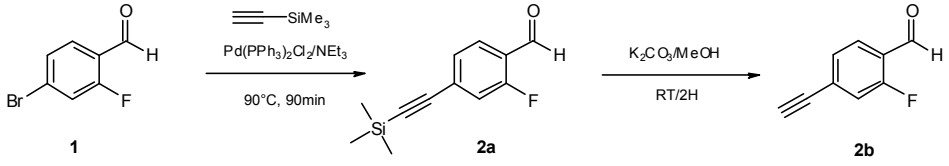
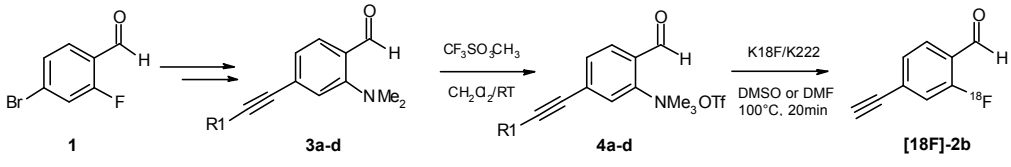
Keywords: neurotransmitter, labelling, fluorine 18, nca, cross-coupling

Stokes (1) described recently 4-ethynyl-L-phenylalanine (pEPA) as a selective and reversible TpOH inhibitor and compared it with the classically used pCPA. The aim of our investigation is the labelling of 2-[^{18}F]fluoro-4-ethynyl-phenyl-L-alanine, and quantification of the relation between 2-[^{18}F]fluoro-pEPA and 5HT by PET.

We are trying to apply the automated radiosynthesis of 6-[^{18}F]fluoro-L-DOPA realized by Phase Transfer Catalytic alkylation (2) to this novel tracer. The first cold references for the labelling were obtained by a palladium catalyzed cross-coupling reaction (3) starting from **1** using trimethylsilylacetylene, followed by a desilylation step. The compounds **2a** and **2b** were obtained with good yields (respectively 85% and 70% starting from **1**).



Compounds **3a** and **3b** were synthesized in very good yieds, 90% for the amination step and up to 95% for the cross-coupling reaction starting from **1**. Ammonium salts **4a-b** were prepared by a well known procedure (4) using anhydrous CF_3SO_3Me but gave poor yields (around 30%) of slightly coloured salts ready for the fluorination step.



a : R_1 = $SiMe_3$ c : R_1 = $Si(iPr)_3$
b : R_1 = H d : R_1 = $SiMe_2tBu$

Radiofluorination of **4b** was performed by aromatic nucleophilic substitution with the complex Kryptofix 222/K_2CO_3 activated [^{18}F]fluoride in DMSO or DMF between 100 and 120°C for 20 minutes. Purification on C18 Sep-Pak cartridge afforded [^{18}F]**2b** in 30-50% radiochemical yield. The same fluorination step starting from **4a** showed rapid decomposition, certainly due to the formation of [^{18}F]Me_3SiF. The instability of **4a** led us to prepare two other precursors with more stable alkyl silyl acetylene (**4c** : R_1 = triisopropylsilyl and **4d** : R_1 = dimethyl-tert-butylsilyl). These new precursors and cold references **2c-d** are now available and fluorination of **4c-d** are currently in progress.

1. Stokes A.H. et al. *J Neurochem* 2000, 74: 2067-2073.
2. Guillouet S. et al. *J Label Compd Radiopharm* 2001; 44: S868-S870.
3. Austin W.B. et al. *J Org Chem* 1981; 46: 2280-2286.
4. Haka M.S. et al. *J Label Compd Radiopharm* 1989; 27: 823-833.

A NEW SYNTHESIS PROCEDURE OF [^{18}F]FFNP FOR IN VIVO IMAGING OF PROGESTIN RECEPTORS

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Keywords: Progesterone receptors, Fluorine-18, [^{18}F]FFNP, Nucleophilic substitution.

The *endo* isomer of 21-[^{18}F]Fluoro-16α,17α-[(*R*)-(1′-α-furylmethylidene)dioxy]-19-norpregn-4-ene-3,20-dione, [^{18}F]FFNP, has been shown to have potential for imaging the progestin receptor (1). Therefore, an improved radiochemical yield of the [^{18}F]FFNP has been reported using a triflate precursor (2). However, the triflate precursor proved problematic due to its low stability for long times even at −78ºC and the difficulties in its synthesis from the corresponding alcohol. As a consequence methanesulfonate FNP was introduced as an alternative to the corresponding triflate as a precursor for [^{18}F]FFNP synthesis.

To carry out the synthesis a 30 - 100 mCi of [^{18}F]fluoride, was transferred to a vacutainer containing tetrabutylammonium hydroxide or Kryptofix® [2.2.2]. After the resolubilization process the residue radioactivity was dissolved in anhydrous MeCN or THF and transferred to the mesylate precursor vacutainer. The mixture reacted at 80ºC for 10 min or under microwave irradiation for 0.5 to 1.0 min. The reaction mixture then purified and concentrated *in vacuo*. The residue was then purified using radio-HPLC.

[^{18}F]FFNP was obtained in a good radiochemical yields, decay corrected to the BOS, 6 % – 21 % (n = 5) together with a high radiochemical purity, > 95 % and its specific activity > 1500 Ci/mmol.

Fluoride displacement or the nucleophilic substitution on methanesulfonate FNP did offer lower radiochemical yield than when the triflate precursor was used as starting material (45 % ± 4 %; n = 10). Nevertheless, any unreacted methanesulfonate FNP is stable and is much polar than the product [^{18}F]FFNP and therefore, it is very easily separated from the [^{18}F]FFNP product by normal phase HPLC.

This work was supported by grant number DEFG02-84ER-60218 from the United States Department of Energy.

References:

1. Buckman B.O., Bonasera T.A., Kirschbaum K.S., Welch M.J. and Katzenellenbogen J.A., *J. Med. Chem.*, 1995; 38: 328–337.
2. Al-Qahtani M.H., Hostetler E.D., McCarthy T.J. and Welch M.J., *J. Label. Compds. Radiopharm*, 2001; 44: 305–306.

DISPLACEMENT OF THE PET LIGAND ^{18}F-MPPF BY THE ELECTRICALLY-EVOKED SEROTONIN RELEASE IN THE RAT HIPPOCAMPUS

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Key words: 5-HT$_{1A}$ receptor – MPPF – serotonin release – microdialysis – -microprobe

^{18}F-MPPF is a specific radiolabelled antagonist for the study of serotoninergic 5-HT$_{1A}$ receptors with PET. Recently, we demonstrated that the specific binding of ^{18}F-MPPF was decreased after a fenfluramine-induced serotonin increase. Our aim in this present work was to study the ^{18}F-MPPF binding during an electrically-evoked serotonin release more relevant to the physiological neurotransmission process.

The raphe nucleus was electrically stimulated and the effect of the evoked serotonin release was evaluated on the binding of ^{18}F-MPPF in the hippocampus of anesthetised rats. The specific binding of ^{18}F-MPPF was measured by an implanted -Microprobe, a radiosensitive cerebral probe, and the serotonin extracellular concentration was measured by microdialysis in the same conditions.

Our results showed that the 10, 20 or 30 min electrical stimulation of the raphe nucleus (20 Hz) elicited a significant increase in extracellular serotonin, only detectable in the presence of a serotonin reuptake inhibitor in the perfusate (5µM clomipramine). Interestingly, the raphe stimulations were associated with a 27-76% reversible decrease of the ^{18}F-MPPF specific binding in the hippocampus but an unchanged extracellular ^{18}F-MPPF collected in dialysates.

Considered together, these observations suggest that ^{18}F-MPPF binding is sensitive to endogenous serotonin released at a synaptic level and compartmentally distinct from the serotonin measured at the extracellular level.

RADIOCHEMICAL SYNTHESIS OF p-[^{18}F]DESMETHYL-MPPF (p-[^{18}F]D-MPPF) FOR THE STUDY OF 5-HT$_{1A}$ RECEPTORS

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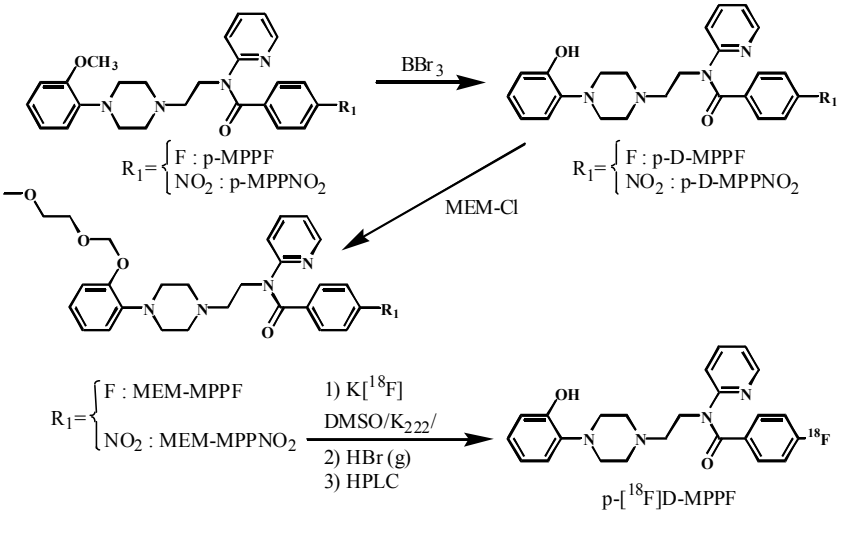
Keywords: p-MPPF, [^{18}F]fluoride, 5-HT$_{1A}$, Receptors, PET

Several studies have demonstrated the potential of p-[^{18}F]MPPF as radiopharmaceutical candidate to study the 5-HT$_{1A}$ receptors. Although the results obtained with this molecule are good, the actual amount of radioactivity getting into the brain remains low. In order to improve this point we have, at the light of the work done with WAY-100635, set out to investigate the desmethylated p-MPPF identified as p-D-MPPF.

The reference compounds p-D-MPPF and p-D-MPPNO$_2$ were obtained through direct hydrolysis of p-MPPF and of its nitro derivative with BBr_3 under classical conditions. The pure compounds were isolated with yield ranging around 75-80 % and were fully identified (^{1}H & ^{13}C NMR, LC-MS).

In the scope of an easy access to the final compound p-[^{18}F]D-MPPF, two strategies were assessed: (i) direct radiolabeling of p-D-MPPNO$_2$ and (ii) cleavage of the p-[^{18}F]MPPF methoxy moiety. Unfortunately, no positive results were obtained. According to literature, the methoxyethoxymethyl ether was selected as protecting group for the phenol, this moiety being known to be easily removed and stable under basic conditions. 2-Methoxyethoxymethyl chloride (MEM-Cl) was used to prepare MEM-MPPF and MEM-MPPNO$_2$. These two compounds were isolated with yield ranging around 35 % and were fully identified (^{1}H & ^{13}C NMR, LC-MS). MEM-[^{18}F]MPPF was obtained from the MEM-MPPNO$_2$ in the usual way (K_2CO_3 / K_{222} / DMSO) at 190°C for 4 min with a radiochemical yield of 40 % (n = 3) corrected to EOB. The instantaneous hydrolysis of the MEM protecting group was carried out with gaseous HBr. For the purpose, the MEM-[^{18}F]MPPF was extracted from the crude labeling mixture using SPE (C18 sep-pak) and directly treated with HBr on this support. After a simple nitrogen flush (disposal of HBr), the crude p-[^{18}F]D-MPPF was eluted and purified with the help of semi-prep HPLC. The final compound was obtained with an overall radiochemical yield (from [^{18}F]fluoride) of about 10 % (n = 3) corrected to EOB within 90 min. Further optimizations are underway. This new radiopharmaceutical is now ready to be evaluated in animals.



EVALUATION OF THE BOVINE NOREPINEPHRIN TRANSPORTER AS A REPORTER GENE FOR THE NON-INVASIVE MONITORING OF TRANSGENE EXPRESSION

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Keywords: norepinephrin transporter, MIBG, I-123, I-131, gene imaging, oncology

The norepinephrine transporter (NET) is a high affinity transporter for catecholamines. The expression of NET is restricted to the sympathetic nervous system and tumors derived from these cells. Radiolabeled meta-iodobenzylguanidine ([I-123/131]MIBG) is accumulated by NET expressing cells and is used as tracer in a number of oncologic and cardiologic applications. In this study we evaluated whether the NET system can be used as a reporter system for non-invasive monitoring of gene therapeutic approaches.

Human A431 squamous cell carcinoma, human HT1080 fibrosarcoma and murine CMS-5 fibrosarcoma cells were transduced with the bovine NET using a retroviral vector. Transduced (non-selected) and wild type cells were incubated in vitro for 2 hours with $1x10^{-7}$ M [I-131]MIBG. The specificity of tracer uptake was determined by adding $27x10^{-6}$ M of the NET inhibitor imipramine to the incubation medium. Rat PC12 pheochromocytoma cells served as positive controls. Retention of the tracer in the cells was studied by determining the activity accumulation in the cells 2 hours after replacing the incubation medium with tracer free medium. Furthermore, A431 wild type, A431-bNET+ and PC12 cells were xenotransplanted into nude mice, respectively and tumor uptake of [I-123]MIBG in vivo was determined 24 hours after tracer administration by planar gamma camera imaging and subsequent biodistribution studies.

Wild-type HT1080, A431 and CMS-5 cells showed only low uptake of [I-131]MIBG in vitro (see table). In transfected, bNET positive cells [I-131]MIBG uptake was drastically increased. Incubation with imipramine reduced [I-131]MIBG uptake of transfected cells by 97%, 99% and 99%, respectively. Between 70% and 100% of the initial radioactivity was retained in the bNET positive cells after 2 hours incubation with tracer free medium. [I-131]MIBG uptake of PC12 cells was 77 18 pmol/mg and 83% of the radioactivity was retained after 2 hours incubation with tracer free medium.

Table: [I-131]MIBG uptake in different cell lines. Values are given as pmol [I-131]MIBG / mg cellular protein.

cell line	wild type	bNET+	blocked bNET+	retention
HT1080	22 2	1601 63	48 7	1397 133
A431	21 4	1430 215	19 1	1478 135
CMS-5	16 6	2001 494	30 4	1429 119

Gamma camera images 24 hours p.i. showed clearly contrasting A431-bNET+ and PC12 tumors. In contrast, for the A431 wild type tumors no increased activity accumulation was found in the tumor compared with the background. Subsequent biodistribution studies confirmed these data and demonstrated a 33-fold higher [I-123]MIBG uptake in the A431-bNET+ than in wild type tumors (2.6 0.9 % ID/g vs. 0.08 0.01 %ID/g, PC12 tumors: 1.5 0.4 %ID/g). The tumor/muscle ratio for A431, A431-bNET+ and PC12 tumors was 0.7 0.1, 23 14 and 20 5.

In conclusion, transduction of tumor cells with bNET causes high specific uptake and significant retention of radiolabeled MIBG in vivo and in vitro. These characteristics are promising for the use of the bNET gene as a reporter gene for monitoring gene therapy.

DASF: A POTENTIAL F-18 RADIOTRACER FOR IMAGING SERT USING PET

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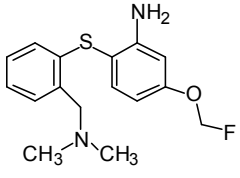
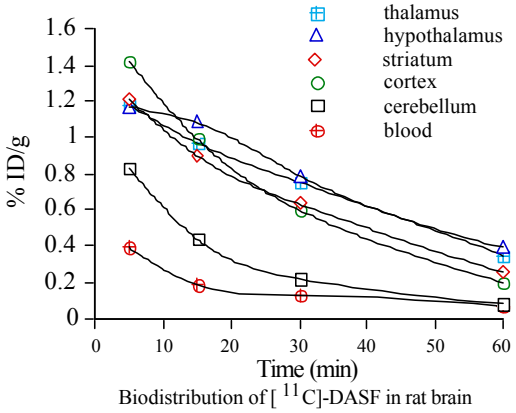
Keywords: Carbon-11, SERT, rat, biodistribution, LOOP, F-18

No good ^{18}F-labelled radiotracer for imaging the serotonin transporter (SERT) in man has been reported to date although AFM shows promise in baboons [1]. We report here the ^{11}C-radiolabelling and in vivo evaluation of DASF, a fluorine containing novel analogue of the proven SERT radiotracer DASB [2].



DASF

Methods. [^{11}C]-DASF was radiolabelled by reaction of the normethyl precursor with [^{11}C]-iodomethane or [^{11}C]-methyl triflate using our previously described loop method [3]. Rats were injected (tail-vein) with [^{11}C]-DASF and killed at various time-points after injection, brain regions dissected, counted, and weighed.



Biodistribution of [^{11}C]-DASF in rat brain

Results. Surprisingly N-normethyl DASF reacted sluggishly with [^{11}C]-iodomethane; however it was rapidly methylated using [^{11}C]-methyl triflate in fair yield to provide high specific activity [^{11}C]-DASF (57 GBq/µmole) for the animal studies. Initial brain uptake of the radiotracer was high (> 1% id/organ at 5 min) demonstrating good blood-brain barrier permeability. Wash-out of radioactivity from the SERT-free cerebellum was fast with a half-life of about 15 mins. Wash-out of radioactivity from brain regions which contained high levels of SERT was prolonged compared to the cerebellum. The regional distribution of radioactivity at later timepoints was consistent with the known distribution of SERT in rat brain. After 60 min region to cerebellum ratios of 2.6,3.3,4.5, and 5.1 were obtained for cortex, striatum, thalamus, and hypothalamus respectively.

Conclusions. It should be relatively straight-forward to radiolabel DASF with ^{18}F from its phenolic precursor using [^{18}F]-fluoromethyl bromide (a reaction already done under cold chemistry conditions). Thus [^{18}F]-DASF holds promise as a longer lived alternative to [^{11}C]-DASB for PET imaging of SERT.

References

(1) Huang, Y, et al. *J Cereb Blood Flow Metab*, 22, 1377. (2002)
(2) Ginovart, N, et al. *J Cereb Blood Flow Metab*, 21, 1342. (2001)
(3) Wilson, AA, et al. *Nucl Med Biol*, 27, 529. (2000)

SYNTHESIS AND *IN VITRO* EVALUATION OF BRIDGE MODULATED MADAM DERIVATIVES: LIGANDS FOR THE SEROTONIN TRANSPORTER EXPLORATION

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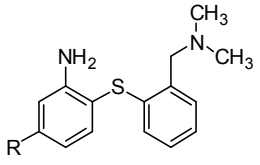
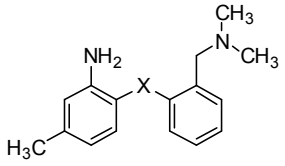
Keywords: Serotonin transporter, diphenylsulfide derivatives, MADAM

Development of radiotracers to image *in vivo* by PET or SPECT the serotonin transporter (SERT) has been a challenge for many years. Recently, compounds designed on the diphenylsulfide structure have been shown to bind with high affinity and selectivity at the SERT. For example, ADAM, labeled with iodine-123, and DABS, labeled with carbon-11 have been proposed as potential tools to explore in vivo the SERT. Our contribution in this field was the development of the methyl-analog of ADAM (MADAM) which also display, when labeled with carbon-11, promising in vivo kinetics compatible with its use in PET experiments. Moreover, we have also shown that the R, amino and N,N-dimethyl amino methyl groups of ADAM derivatives are implicated in the SERT recognition and that the atom bridging the two aromatic rings may be involved in the SERT-ligand interaction. In that aim, we have prepared a series of MADAM analogs in which the sulfur atom has been substituted by an oxygen, nitrogen, carbon or sulfur containing groups and evaluated for their in vitro binding at the three monoamine transporters.





R	
I	ADAM
CN	DASB
CH_3	MADAM

X = O, SO, SO_2, NH, NMe, NEt, NBn, NPh, CHOH, CHOMe, CO

As none of these compounds displayed affinities for the dopamine and norepinephrine transporters (*Ki's* > 100 nM), the amino and N, N-dimethyl amino methyl groups could be considered as responsible of the ADAM, DASB and MADAM's selectivities . However, only the O, SO and NH compounds exhibited affinity for the SERT as *Ki* values ranging from 0.53 to 10.28 nM have been found in competition studies involving [^{3}H]-fluoxetine. These results support that the bridging group between the two phenyl rings greatly influences the SERT affinity of such compound. Moreover, as the calculated partial charges on this linking group have been found to vary from negative (X = O, NH) to positive (X = S, SO) values, it could be assumed that the X group is not directly involved in the ligand-SERT interaction but may influence the general geometry of these compounds. After energy minimization, 3D representations of negative potential volumes, dihedral angle between the two phenyl rings and angle between the two amino groups have been calculated for each compound. Results obtained have confirmed that the X group influences not only the relative position of the two phenyl rings, but also the spatial position of the N,N-dimethyl amino methyl group related to the amino function which are believed to directly interact with the SERT. The most potent compounds at the SERT (X = O, SO and NH) have been selected for carbon-11 labeling and will be soon evaluated *in vivo*. Moreover, these results would help in the design of new derivatives by the evaluation of geometric changes induced by chemical modulations.

This work was support by the Conseil Régional de la Région Centre, FRANCE.

SYNTHESIS AND RADIOIODINATION OF SUBSTITUTED BENZAMIDES FOR THE NON-INVASIVE VISUALIZATION OF D_2-LIKE RECEPTORS

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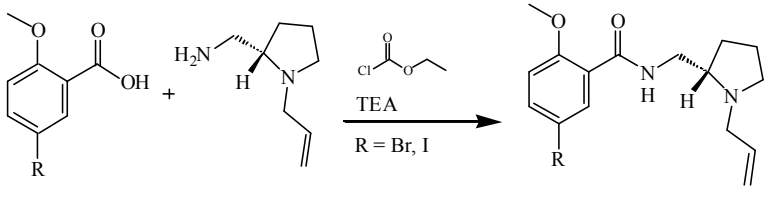
Keywords: D_2-receptors, benzamide, epidepride, SPECT

Introduction: Benzamides, especially [^{18}F]Fallypride ([^{18}F]FP) and [^{18}F]Desmethoxyfallypride ([^{18}F]DMFP) [1,2], have proven to be D_2-selective PET radioligands for the visualization of dopamine receptors. To make the favorable properties of [^{18}F]DMFP available to SPECT and have an interesting alternative to [^{123}I]IBZM, the respective iodine analogue [^{131}I]N-allyl-desmethoxyepidepride of this ligand was synthesized. Therefore the fluoropropyl moiety in the 5-position of the aromatic ring was replaced by iodine.

Synthesis: The precursor and the iodinated reference compound were synthesized by reacting the respective benzoic acid derivatives with (S)-2-aminomethyl-1-allylpyrrolidine (scheme 1).



Scheme 1: Synthesis of N-allyl-desmethoxyepidepride and its precursor

For the synthesis of (S)-N-allyl-2-aminomethyl-pyrrolidine we followed a stereoconservative route first described by Högberg et al. [3], while the benzoic acids were obtained by oxidation of the corresponding aldehydes. The benzoic acid derivatives were then reacted with chloro-ethylformiate to yield the corresponding activated anhydrides before they were reacted with (S)-N-allyl-2-aminomethylpyrrolidine to give N-allyl-desmethoxyepidepride.

Pharmacological procedures: In [H^3]spiroperidol binding assays the *in vitro* affinity was examined according to the method of a previous study [4], which showed a high affinity of the ligand to the D_2 receptor with a K_D of 4.5 nM.

The lipophilicity of the N-allyl-desmethoxyepidepride was determined using the HPLC method and Sörensen buffer as eluent, resulting in a logD of 2.7 [5].

^{131}I-Iodination: For the ^{131}I-iodination of the bromo-precursor different reaction parameters such as reaction temperatures and reaction time were examined. The highest radiochemical yields of 80% - 85% were achieved with DMSO under Cu(I)Cl catalysis at a reaction temperature of 140°C and a reaction time of about 30 min.

The ^{131}I-iodination of the corresponding stannyl-precursors is in preparation.

References:

[1] J. Mukherjee et al. *Life Sciences* 1996; No. 8, **59**: 669-678.
[2] J. Mukherjee et al. *Med. Chem. Res.* 1994: **5:** 174-192.
[3] T. Högberg et al. *Acta Chemica Scandinavica* 1989; **43**: 660-664.
[4] M. L. Rao *Psychopharmacology* 1986; **90**: 548-553
[5] OECD-guideline for testing of chemicals 1989; **117**, 1-11

SYNTHESIS AND CHARACTERIZATION OF A NOVEL FLUORINE-18 LABELED INHIBITOR OF ACETYLCHOLINESTERASE

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Keywords: Fluorine-18, Acetylcholinesterase, Emission Computed Tomography, Alzheimer's Disease

The importance of alterations of the presence and activity of acetylcholinesterase (AChE) in the central nervous system (CNS) is a well-established factor in the underlying etiology of Alzheimer's Disease (AD). The *in vivo* investigation of AChE using radiolabeled substrates (to measure enzyme activity) and inhibitors (to measure enzyme density) is therefore highly beneficial in the clinical evaluation of AD. The application of positron emission tomography (PET) or single photon emission computed tomography (SPECT) in this regard offers a highly sensitive non-invasive means to accomplish this goal.

It has been shown that significant neurochemical changes underlying AD progression result from reduction of AChE activity in cortical and hippocampal brain regions. Therefore, a suitable radiotracer for monitoring *in vivo* AChE levels non-invasively should be highly sensitive to alterations in these regions. Radiolabeled selective AChE substrates (e.g. 1-[^{11}C]methylpiperidin-4-yl propionate; [^{11}C]PMP) have been successfully developed and are being used clinically. Although radiolabeled potent AChE inhibitors (e.g. 5,7-Dihydro-7-[^{11}C]methyl-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-6*H*-pyrrolo[3,2-*f*]-1,2-benzisoxazole-6-one; [^{11}C]CP-126,998) also demonstrate potential for imaging of AChE this has only been shown in healthy control subjects.

In light of the fact that no successful fluorine-18 labeled AChE inhibitor is known we have been interested in addressing this deficiency. Fluorine-18 is the most attractive positron-emitting nuclide due to its longer half-life than carbon-11. In addition, fluorine-18 is the lowest energy positron emitter (0.635MeV, 97% abundant) and therefore affords the highest resolution images.

We have therefore focussed on development of a fluorine-18 derivative of the potent and highly selective AChE inhibitor, 5,7-dihydro-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-6*H*-pyrrolo[3,2-*f*]-1,2-benzisoxazole-6-one (CP-118,954), a structurally similar lactam benzisoxazole to CP-126,998. CP-118,954 is the most potent and highly selective inhibitor of AChE (IC_{50}=0.33nM; BuChE/AChE=23000) known to date. We have synthesized the 4-fluorophenylmethyl analog to CP-118,954, (**1**), anticipating that this will retain potent inhibitory activity for AChE as well as allow fluorine-18 labeling. Chemical synthesis of **1** was carried out by reaction of CP-144,885-51 (trifluoracetate salt of desbenzyl CP-118,954) with 4-fluorobenzyl bromide in DMF in the presence of anhydrous Na_2CO_3. Radiosynthesis of [^{18}F]**1** was achieved by heating the precursor, CP-144,885 (free base) in DMF at 105 °C for 10 min. with 4-[^{18}F]fluorobenzyl iodide. High specific activity [^{18}F]**1** was obtained (133.2 28.7 GBq/ mol; 3600 28.7 Ci/mmol; n=5) after purification on a semi-preparative Phenomenex Luna C18(2) HPLC column eluted with MeOH/0.05N NH_4OAc (60:40; 4.4 mL/min; R_t=15 min). Radiochemical and chemical purities of [^{18}F]**1** were 95% and the radiochemical yield averaged 22.1 5.3% decay corrected to solubilized fluorine-18. *In vivo* biodistribution in mice (Harlan Labs, USA) showed the highest uptake in the striatum, a brain region known to be rich in AChE. Further biological evaluation with [^{18}F]**1** is being undertaken.

The authors would like to acknowledge the assistance of Kim Black, Li Wu, Stephanie Rideout and Holly Smith in the *in vivo* studies, as well as the generous donation of CP-144,885-51 and CP-144,885 by Pfizer Inc. (Groton, Connecticut, USA).

SIMPLIFIED SYNTHESIS OF 2'-DEOXY-2'-[^{18}F]FLUORO-β-D-ARABINOFURANOSYL URACILS: FAU, FMAU, FBAU, FIAU

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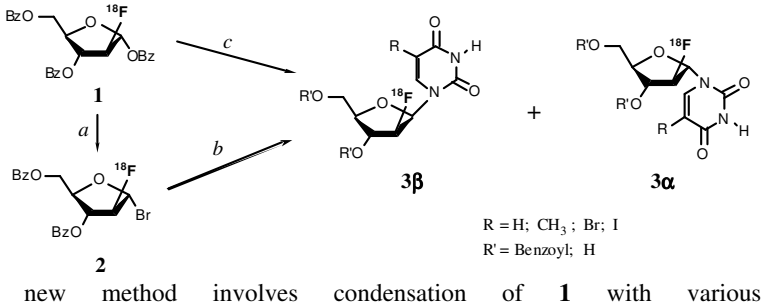
Keywords: [^{18}F]FAU, [^{18}F]FMAU, [^{18}F]FBAU, [^{18}F]FIAU, PET

We have previously reported a 4-step synthesis of a series of 1-(2'-deoxy-2'-[^{18}F]fluoro-β-D-arabinofuranosyl)uracil derivatives (**3β**, R'= H) as analogs of thymidine for imaging DNA synthesis in proliferating cells with PET (1, 2). Although the radiochemical yields obtained with this method are quite good (40 to 45% decay corrected to EOB), the synthetic sequence is time consuming (3 to 3.5 hrs) and very labor intensive. We have simplified the process by eliminating one of the synthetic steps; instead of attaching various 5-substituted pyrimidines to the tribenzoyl-protected ^{18}F-labelled sugar **1** through the bromide **2** (reactions *a, b*), as previously described, we have developed a method which produces **3** (as an isomeric mixture **3β + 3α**) directly from **1** (reaction *c*).



The new method involves condensation of **1** with various 2,4-bis-O-(trimethylsilyl)pyrimidines in the presence of trimethylsilyl triflate. The β:α ratio of the condensation products and the time and temperature necessary to drive the reaction to completion were found to be dependent on the polarity of the reaction solvent. Polar solvents (e.g. acetonitrile) facilitated the reaction (complete in 10 min @ 100°C) but favored the formation of the unwanted α isomer. The use of nonpolar solvents (e.g. chloroform, 1,2-dichloroethane) resulted in more favorable β:α ratios, but higher temperatures (150°C) and longer times (up to 60 min) were necessary. The nature of the 5-substituent on the bis-TMS pyrimidine also influences the β:α ratio; a higher ratio is obtained with the halogenated (i.e. R = Br, I) analogs versus the unsubstituted or 5-methyl compounds (R = H, Me). For full scale experimental or clinical preparations, the condensation reaction, run in a mixture of acetonitrile and chloroform (1 : 4), is complete in 30 min at 150°C. The β:α ratios (1.2 to 2.5 : 1) of the condensation products, though not as good as those obtained through the intermediate bromo compound **2** (6 to 8 : 1), are acceptable. The overall synthesis time for the final **3β** compounds (R' = H), however, after deprotection with NaOMe and semi-prep HPLC purification, is reduced from ~ 3 hrs to ~ 2 hrs. Although the overall decay-corrected radiochemical yields of 30 to 35% obtained with this method are lower than those of the 4-step sequence, the uncorrected yields (14 to 17% EOS), because of the time savings, are comparable. In addition, elimination of the bromination step (reaction *a*) and the handling difficulties associated with the very caustic and moisture sensitive HBr/AcOH, greatly simplifies the entire synthesis and reduces some of the inconsistencies observed with the original 4-step method.

1. Mangner TJ, Klecker R, Anderson L, Shields A.*J Label Compd Radiopharm* 2001;44:S912-S914
2. Mangner TJ, Klecker RW, Anderson L, Shields AF. *Nucl Med Biol* 2003; In Press.

^{18}F(2-[(2-AMINO-5-FLUOROPHENYL)THIO]-N,N-DIMETHYL-BENZENMETHANAMINE) AS A PET IMAGING AGENT FOR SEROTONIN TRANSPORTERS

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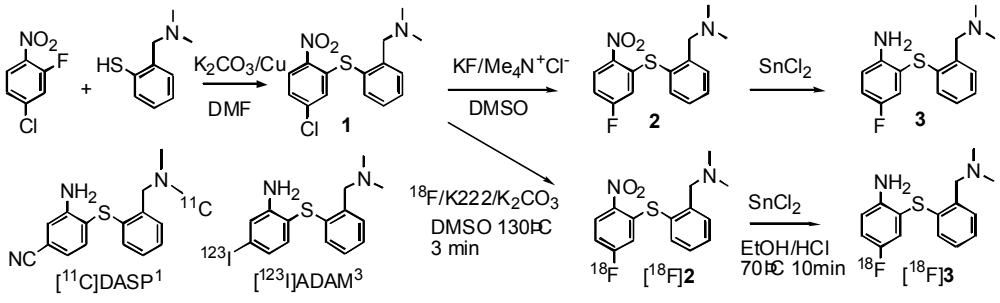
Keywords: Serotonin transporter, F-18, PET

Alterations in serotonergic neuronal function in the central nervous system (CNS) occur in patients with major depression. Imaging of serotonin transporters (SERT) in humans would provide a useful tool to understand depressive illness and other psychiatric disorders.

Recently, DASB has been reported as a useful PET imaging agent for SERT.[1] In order to improve availability, ^{18}F labeled analogs of [^{11}C]DASB with a similar SERT binding selectivity and *in vivo* kinetics will be highly desirable. We report herein an improved ^{18}F labeled ADAM derivative[2,3] as a SERT imaging agent for PET study.



The target compound **3** was synthesized as shown above. Inhibition constant of compound **3** displayed high affinity toward SERT (K_i=0.47 nM). ^{18}F labeling was performed by a substitution reaction of chloride **1** by [^{18}F]fluoride. Initial nitro product [^{18}F]**2** was reduced by tin chloride to yield desired ^{18}F labeled compound [^{18}F]**3**. Radiochemical yield was 50-60 % at the end of synthesis. The final product was purified by HPLC. Compounds **3** and ([^{18}F]**3**) showed identical HPLC profiles.

Biodistribution studies of [^{18}F]**3** in rats exhibited high initial brain uptake. Brain uptake at 2 min post iv injection was 2.86 and at 60 min 0.28 (%dose/organ). It showed high concentration in the mid brain (hypothalamus to cerebellum ratio was 4.01 to 1 at 60 min). Preliminary PET imaging of baboon's brain using [^{18}F]**3** showed an excellent localization of regions in the hypothalamus, a region with a high concentration of SERT.

In summary, a novel ^{18}F labeled SERT ligand was developed. The ligand was easily synthesized and could be labeled with excellent yield. It showed high initial brain uptake and excellent binding properties in rats and baboon. This novel ^{18}F labeled ligand shows promise as a candidate for imaging SERT in human with PET.

References

1. Houle S, Ginovart N, Hussey D, Meyer J H, Wilson A A. *Euro J Nucl Med* 2000; **27**: 1719-1722.
2. Oya S, Choi S R, Coenan H, Kung H F. *J Med Chem* 2002; **45**: 4716-4723.
3. Oya S, Choi S R, Hou C, Mu M, Kung M P, Acton P D, Siciliano M, Kung H F. *Nucl Med Biol* 2000; **27**: 249-254.

THE SYNTHESIS OF HYDROPHILIC VAChT LIGANDS FOR ^{18}F LABELLING

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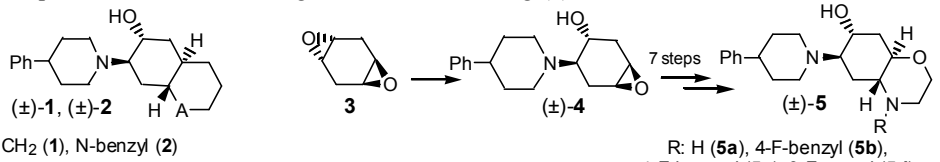
Key Words: VAChT, vesamicol, octahydro-benzo[1,4]oxazine, fluorine-18

Introduction

Deficits in central cholinergic neurotransmission have been disclosed as a consistent feature in several neurodegenerative diseases. The vesicular acetylcholine transporter (VAChT), which carries acetylcholine into synaptic vesicles, is considered as a potential candidate for *in vivo* labelling of cholinergic nerve terminals. However, many of the known radiolabelled VAChT ligands are very lipophilic and also demonstrate binding affinities to sigma (s) receptors (1).

As part of our efforts in the search for new VAChT ligands for PET with improved binding profiles we turned our interest towards more rigid structures. For instance, vesamicol derivatives such as **1** (2) and **2** (3) are known as promising candidates and **2** was subject of recently described developments of new VAChT ligands for radiolabelling (3).



The present work describes our work on the synthesis and initial biological evaluation of the fluorinated target molecules **5b-5d** starting from *trans*-1,2:3,4-diepoxycyclohexane **3**.

Results

Up to now there were no reliable routes for the synthesis of disubstituted octahydro-benzo[1,4]oxazine derivatives such as compound **5a**. *Trans*-1,2:3,4-diepoxycyclohexane **3** is known to undergo regio- and stereoselective ringopening reactions with a broad range of different nucleophiles (4). However, a partial ringopening of **3** leading to a monoepoxide **4**, which enables a convenient access to more complex reaction products has not yet been described. Starting from **4** a multistep sequence yielded **5a** as well as the fluorinated derivatives **5b – 5d**. The potential of the newly synthesized compounds to displace [^{3}H]vesamicol (VAChT), [^{3}H]haloperidol (D_2, s_1) and [^{3}H]DTG (s_1, s_2) was determined by studies on homogenates of rat brain (VAChT) and rat liver ($s_{1,2}$) and compared to the corresponding nonradioactive ligands. Thus, compound **5b** displays a moderate affinity to the VAChT. However, it shows a significant lower affinity to s binding sites.

Conclusion

We have developed a convenient access to octahydro-benzo[1,4]oxazine derivatives of type **5** starting from bis-epoxide **3**. Further enhancement of the VAChT affinity with conserving the selectivity will be sought. Appropriate candidates will be labelled for *in vivo* distribution studies.

Acknowledgement

We thank the Saxony Ministry of Science and Arts for financial support.

References

1. Efange S M N. *FASEB J* 2001; 14: 2401–2413.
2. Rogers G A, Parsons S M, Anderson D C, Nilsson L M, Bahr B A, Kornreich W D, Kaufman R, Jacobs R S, Kirtman B. *J Med Chem* 1989; 32: 1217–1230.
3. Efange S M N, Khare A B, Mach R H, Parsons S M. *J Med Chem* 1999; 42: 2862–2869.
4. Craig T W, Harvey G R, Berchtold G A. *J Org Chem* 1967; 32: 3743–3749.

EVALUATION OF ^{18}F-LABELED IMPY DERIVATIVES AS PET RADIOLIGANDS FOR b-AMYLOID IN ALZHEIMER'S DISEASE

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Keywords: ^{18}F, IMPY, -Amyloid, Alzheimer's disease.

Introduction. [^{123}I/^{125}I]IMPY (6-iodo-2-(4'-*N,N*-dimethylamino)phenyl-imidazo[1,2-*a*]pyridine) has high uptake in the normal mouse brain (7.2% dose/g) and fast clearance (peak at 2-5 min, <0.35% dose/g after 2 h). Brain radioactivity in the Tg2576 mouse model is ~ 3.3 times higher than in the age-matched control at 4 h post-injection. These desirable pharmacokinetics make [^{125}I]IMPY an attractive agent for mapping brain A plaques. Here we report our evaluation of two ^{18}F-labeled analogs of IMPY, in which one of its *N*-methyl groups is replaced with [^{18}F]3-fluoropropyl ([^{18}F]FPM-IMPY) or [^{18}F]2-fluoroethyl ([^{18}F]FEM-IMPY), as prospective PET radioligands for -amyloid.

Experimental. 6-Iodo-2-(4'-*N*-methylamino)phenyl-imidazo[1,2-*a*]pyridine (HM-IMPY) was synthesized in 3 steps. Reference FPM-IMPY and FEM-IMPY were synthesized by treating HM-IMPY with 3-fluoropropyl triflate and 2-fluoroethyl triflate, respectively. Treatment of HM-IMPY with [^{18}F]3-fluoropropyl tosylate or [^{18}F]2-fluoroethyl tosylate, generated *in situ*, in acetonitrile at 135 C gave [^{18}F]FPM-IMPY or [^{18}F]FEM-IMPY, respectively. The purified radioligands were injected i.v. into normal mice followed by sequential dynamic scanning for 2 hours using animal PET scanner (NIH ATLAS, resolution: 1.8 mm) to determine pharmacokinetics. Metabolism was determined using HPLC analysis of mice brain and plasma. The % ID/brain and % ID/g were calculated both by time-activity curve of the PET scanning and in vitro tissue counts.

Results. FPM-IMPY and FEM-IMPY were found to have high affinity for A -aggregates. The overall radiochemical yields (decay-corrected) were 22–26%. In PET experiments with normal mice, a good uptake of radioactivity was obtained in the brain after i.v. injection of each probe (6.4% ID/g for [^{18}F]FEM-IMPY, and 5.7% ID/g for [^{18}F]FPM-IMPY at 0.5-1 min). These values compare well with [^{123}I/^{125}I]IMPY (7.2% ID/g in 2 min). However, in contrast to the single exponential washout of [^{123}I/^{125}I]IMPY, the washout of radioactivity from each probe examined here was bi-phasic, being rapid over the first 20 min and thereafter very slow. Residual brain radioactivity was 4.5% ID/g for [^{18}F]FEM-IMPY, and 2.1% ID/g for for [^{18}F]FPM-IMPY at 2 h after injection. Substantial skull uptake of [^{18}F]fluoride was also clearly observed. Only a single polar radioactive metabolite was seen in plasma at < 30 min after injection of [^{18}F]FPM-IMPY.

Conclusions. The two ^{18}F-labeled IMPY derivatives enter the brain of normal mice readily and fast. However, the washout shows bi-phasic behavior. The first phase is consistent with the behavior of [^{125}I]IMPY. The brain retention of radioactivity may be due to the trap of polar metabolite(s) or non-specific binding. The probes were quickly metabolized in plasma and defluorination was clearly evident. The exact mechanism is still under study.

SYNTHESES OF (*E*)-(–)-5-AOIBV and (–)-5-FPOBV, AS POTENTIAL SPECT/PET PROBES FOR THE VESICULAR ACETYLCHOLINE TRANSPORTER

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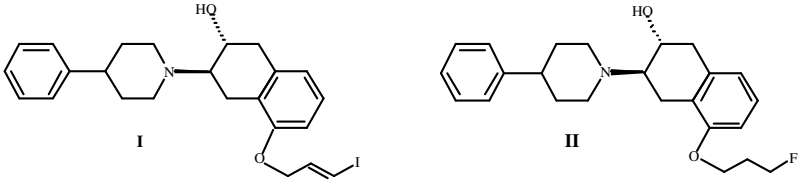
Keywords: (*E*)-(–)-5-AOIBV, (–)-5-FPOBV, SPECT, PET, VAChT

The applicability of benzovesamicol probes in studies of Alzheimer's Disease (AD) is based on the premise that over the course of the disease, VAChT levels change in parallel with other cholinergic marker proteins. Our goal was to synthesize new radioiodinated and radiofluorinated benzovesamicol derivatives, which could potentially be used as SPECT or PET radiotracers for studies involving VAChT and AD. The compounds presented here, (–)-*trans*-2-hydroxy-3-(4-phenylpiperidino)- 5-[(*E*)-3-iodoprop-2-en-1-oxy]-tetralin [(*E*)-(–)-5-AOIBV, **I**] and (–)-*trans*-2-hydroxy-3-(4-phenylpiperidino)-5-(3-fluoropropoxy)-tetralin [(–)-5-FPOBV**, II**] are two of them.



I **II**

The initial step was the synthesis of *(rac)*-5–aminobenzovesamicol [(*rac*)-5-ABV], which was accomplished through a serie of steps from 1–aminonaphthalene and 4–phenylpiperidine. The final product, a mixture of (*rac*)-5-ABV and (*rac*)-8-ABV, was separated by column chromatography. Resolution of (*rac*)-5–ABV using (*R*)-(–)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA), reductive cleavage and chromatographic purification, produced enantiomerically pure (+) and (–)-5–ABV.

The enantiomer (–)-5-ABV was subjected to diazotisation to obtain (–)-*trans*-2-hydroxy-3-(4-phenylpiperidino)-5-hydroxytetralin [(–)-5-HOBV], a common reagent for the synthesis of **I** and **II**. The next step was the preparation of the prosthetic groups: (*E*)-3-(*tri-n*-butylstannyl)prop-2-en-1-tosylate and 3-fluoropropyltosylate by methods described in the literature. Later, (–)-5-HOBVwas reacted with the butylstannyl-tosylate to generate the precursor for radioiodination, (–)-*trans*-2-hydroxy-5-[(*E*)-3-(*tri-n*-butylstannyl)prop-2-en-1-oxy]-3-(4-phenylpiperidino)tetralin [(–)-5-$Bu_3SnAOBV$], from which also authentic **I** was prepared by reaction with *N*-iodosuccinimide. Reactions of (–)-5-HOBV with 1,3-propaneditosylate and with 3-fluoropropyltosylate generated the precursor for radiofluorination, (–)-*trans*-2-hydroxy-3-(4-phenylpiperidino)-5-(3-tosyloxipropoxy)-tetralin [(–)-5-TPOBV] and authentic **II**, respectively. All the reactions were repeated using the (+)-5-ABV enantiomer.

Preliminary rat *ex-vivo* competition experiments (0.1 µmol/kg authentic compound) against [^{125}I](–)-5-IBVM, a well known VAChT radioligand, demonstrated affinity of **I** and **II** for the VAChT by blocking 38% and 74% of the IBVM binding in the cortex and 27% and 64% in the striatum respectively, at 120 min p.i. The corresponding (+) enantiomers were as expected less active: 25% and 54% cortex; 23% and 41% striatum, respectively.

NORCHLORO-FLUORO-HOMOEPIBATIDINE: ^{18}F-LABELLING AND EVALUATION OF AFFINITY AND SELECTIVITY AT NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

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Keywords: nicotinic receptor, nAChR, homoepibatidine, fluorine-18

Nicotinic acetylcholine receptors (nAChR) have important functions in the mammalian brain and play an important role in pathophysiological changes for example neurodegenerative processes like Alzheimer's disease (AD) or Parkinson's disease (PD). Thus PET-tracers for this receptor system are important tools in medical diagnostics and biomedical research. Here we describe synthesis, radiolabelling, separation of the racemate, first biological evaluation and radiosynthesis of norchlorofluorohomoepibatidine(NCFHEB) 6 -(2-fluoro-5-pyridinyl)-8-azabicyclo[3.2.1]octane.

Organic chemistry: NCFHEB and the radiolabelling precursor 8-carbethoxy-6 -(2-bromo-5-pyridinyl)-8-azabicyclo[3.2.1]octane were prepared by reductive "Heck" coupling of 8-carbethoxy-8-azabicyclo[3.2.1]oct-6-ene with 5-bromo-2-fluoropyridine or 2-bromo-5-iodopyridine. The racemate of NCFHEB was received by deprotection with iodotrimethylsilane and purified by flash chromatography. The racemate was resolved on a semipreparative Chirobiotik T column (250 mm x 10 mm) with methanol/triethylamine/acetic acid as the mobile phase. The enantiomers were received in high purity >99% ee.

Biological evaluation: (+)NCFHEB and (-)NCFHEB were assessed for binding affinity and subtype selectivity at human nAChRs expressed in HEK293 cells as well as at rat nAChRs from thalamic tissue. Binding affinities were evaluated for h $_{4\ 2}$, h $_{3\ 4}$ and r $_{4\ 2}$ by competition experiments with [^{3}H]epibatidine. The reference compound (-)epibatidine displayed similar affinities to the subtypes h $_{4\ 2}$, h $_{3\ 4}$ and r $_{4\ 2}$. The analogues (+) and (-)NCFHEB had 12 to 17-fold and 5 to 8-fold lower affinities at r $_{4\ 2}$ and h $_{4\ 2}$ than (-)epibatidine. At h $_{4\ 2}$ the analogues (+) and (-)NCFHEB displayed affinities 8 to 20–fold higher than at h $_{3\ 4}$.

Radiochemistry: A dry solution of kryptofix 222/potassium carbonate complex containing the [^{18}F]fluoride in acetonitrile was reacted with 0.4 mg of 8-Carbethoxy-6 -(2-bromo-5-pyridinyl)-8-azabicyclo[3.2.1]octane in the microwave oven (CEM-discover). Iodotrimethylsilane was added and deprotection was performed in a heating block. The product was injected on a radio-HPLC system (Chirobiotik T) column. Radiochemical yield was 2% for each enantiomer. Enantiomers and the bromo derivative resulting from deprotection of the radiolabelling precursor were well separated.

CARBON-11 LABELED 4'-SUBSTITUTED ADAM ANALOGUES FOR MAPPING THE SEROTONIN TRANSPORTER (SERT) BY PET

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Keywords: ADAM, benzylamine, carbon-11, SERT

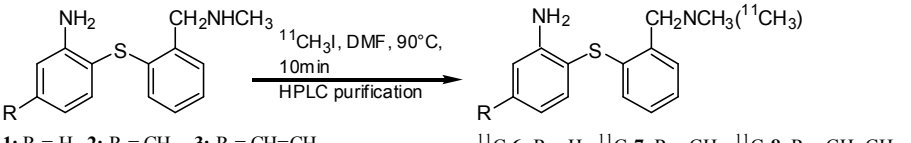
The SERT has been implicated in the pathophysiology of major depression. To elucidate the mechanisms responsible for this disorder by PET, many carbon-11 labeled *N*,*N*-dimethyl-2-(amino-4'-substituted-phenylthio)benzylamines, with different substituents at the 4'-position, for example, iodine (ADAM), cyano (DASB) and fluoromethyl (AFM) have been reported. Despite their high affinity and selectivity for SERT, some of these radioligands suffer either from a low radiochemical yield or suboptimal pharmacokinetic properties. These findings prompted us to synthesize and characterize an expanded series of benzylamines (scheme below) where the 4'-position was substituted with hydrogen (HADAM) (**6**), methyl (MADAM) (**7**), vinyl (EEADAM) (**8**), ethyl (EADAM) (**9**), hydroxymethyl (HOMADAM) (**10**), ethyl alcohol (HOEADAM), fluoroethyl (FEADAM), propyl alcohol (HOPADAM) and fluoropropyl (FPADAM) as potential PET SERT imaging agents when labeled with carbon-11.

The results of the *in vitro* binding assays of the candidate ligands in cells transfected to express human DAT, NET and SERT (Table 1) showed that the 4'-substituted analogues **6**, **7**, **8**, **9** and **10** exhibited high affinity and selectivity for SERT.

Table 1: Inhibition Constants Ki (nM) of the various SERT ligands

Ligand	DAT	NET	SERT	Ligand	DAT	NET	SERT
HADAM (**6**)	283	4.48	2.11	HOEADAM	>1000	194	50
MADAM (**7**)	557	74	0.25	FEADAM	>5000	>5000	11
EEADAM (**8**)	1000	189	1.12	HOPADAM	>1000	108	92
EADAM (**9**)	>2000	435	0.22	FPADAM	>5000	219	11
HOMADAM (**10**)	>1000	144	0.57				

Monomethylamine precursors **1**, **2**, **3**, **4** and **5** were prepared and reacted with $^{11}CH_3I$ in DMF at 90 C for 10 min to give ^{11}C-**6**, ^{11}C-**7**, ^{11}C-**9** and ^{11}C-**10** in 30-35% and ^{11}C-**8** in 5% radiochemical yield (E.O.B) following HPLC purification (scheme below). The total synthesis time was 65 min.



1: R = H, **2:** R = CH_3, **3:** R = CH=CH_2
4: R = C_2H_5, **5:** R = CH_2OH

^{11}C-**6:** R = H, ^{11}C-**7:** R = CH_3, ^{11}C-**8:** R = CH=CH_2
^{11}C- **9:** R = C_2H_5, ^{11}C-**10:** R = CH_2OH

The log $P_{S7.4}$ (n-octanol/ 0.02 M phosphate buffer partition) of radiotracers ^{11}C-**6**, ^{11}C-**7**, ^{11}C-**8**, ^{11}C-**9** and ^{11}C-**10** were 2.28, 2.46, 2.47, 2.60 and 1.60, respectively, which is optimal for initial brain uptake of CNS ligands. The regional brain uptake in monkeys of **^{11}C-HADAM**, **^{11}C-MADAM**, **^{11}C-EADAM** and **^{11}C-HOMADAM** was studied with microPET and showed that **^{11}C-HOMADAM** displayed the highest specific uptake in the SERT rich, midbrain, pons, thalamus, medulla, putamen, caudate and occipital cortex with tissue to cerebellum ratios of 3.6, 2.5, 2.4, 2.0, 1.9, 1.9 and 1.4 respectively, at 45 min post injection. These results demonstrate that **^{11}C-HOMADAM** is an excellent candidate ligand for CNS SERT imaging in humans by PET. Research supported by DOE.

NEW SEROTONIN TRANSPORTER PET IMAGING AGENT: [^{18}F]*b*FEZIENT

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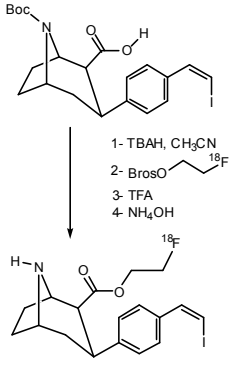
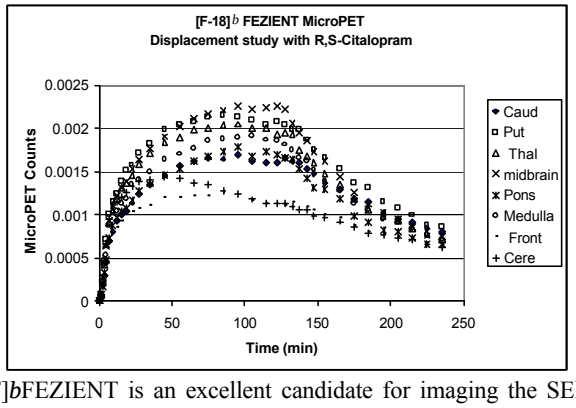
Keywords: brain imaging, serotonin transporter, radioligand, tropane, fluorine-18

Serotonin transporters (SERT) have been implicated in numerous neurological disorders and are the sites of action of many efficacious anti-depressants. To map CNS SERT density and study the pathogenesis of these disorders, we developed the synthesis of 2-[^{18}F]fluoroethyl 3*b*-(4'-((*Z*)-2-iodoethenyl)phenyl)nortropane-2*b*-carboxylate (*b*FEZIENT). The promising results previously obtained with this ligand labeled with iodine-123 (diencephalon/cerebellum=1.7) prompted us to investigate the corresponding fluorine-18 tracer as a PET imaging agent.

*b*FEZIENT was prepared from 2-fluoroethyl *N*-(tert-butoxycarbonyl)-3*b*-(4'-formylphenyl) nortropane-2*b*-carboxylate by treatment with triphenylphosphonium iodomethylene ylid followed by Boc cleavage. *In vitro* binding studies of *b*FEZIENT in cells expressing human SERT, DAT and NET gave Ki's (nM) = 0.08, 13 and 22 respectively. The required precursor of [^{18}F]*b*FEZIENT, 3*b*-(4'-((*Z*)-2-iodoethenyl)phenyl)nortropane-2*b*-carboxylic acid, was prepared from the *N*-(tert-butoxycarbonyl)-3*b*-(4'-bromophenyl)nortropane-2*b*-carboxylic acid by palladium-catalyzed cross coupling reaction with (*Z*)-bistrimethylstannylethylene then iododestannylation. Radiolabeling of [^{18}F]*b*FEZIENT was accomplished by *O*-alkylation of the corresponding acid salt with 2-[^{18}F]fluoroethyl 4-bromobenzenesulfonate followed by acid hydrolysis of the Boc group. The radiochemical yield of [^{18}F]*b*FEZIENT was around 8% (decay-corrected, non-optimized).

The *in vivo* regional brain uptake of [^{18}F]*b*FEZIENT was determined in an anesthetized cynomolgus monkey using a Concorde microPET P4. The images acquired from 0 to 4.2 hours demonstrated high radioactivity uptake in the midbrain (MB), pons (P), medulla (M), putamen (Put) and thalamus (T) with tissue to cerebellum ratios at 4.0 h after injection of 4.35, 3.25, 3.25, 3.09 and 3.08 respectively, and lower but significant uptake in the caudate (C) and the frontal cortex (FCX) (ratios versus cerebellum of 2.28 and 1.75 respectively at 4 h post-injection). A displacement study carried out by administration of the SERT ligand citalopram (1.5 mg/kg) 2 h after injection of [^{18}F]*b*FEZIENT showed significant washout of the radioactivity from the SERT rich regions, 67%, 63%, 62%, 62%, 59%, 50% and 41% from MB, T, P, M, Put, C and FCX respectively at 4 h post-injection, proving SERT specificity of this compound.





These findings suggest that [^{18}F]*b*FEZIENT is an excellent candidate for imaging the SERT in humans by PET. Research supported by NIH.

SEROTONIN TRANSPORTER LIGANDS: SYNTHESIS OF (±)-[^{11}C]2'-METHOXYMETHYL-6-NITROQUIPAZINE

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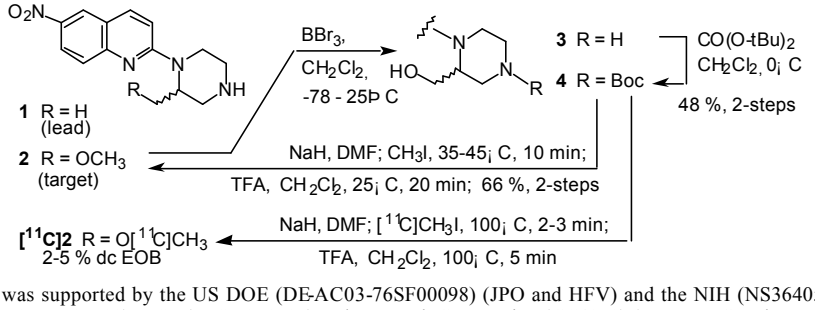
Keywords: SERT, serotonin transporter, carbon-11, 6-nitroquipazine, PET

The serotonin transporter (SERT) found on pre-synaptic terminals of serotonin (5-HT) neurons is responsible for clearance of the endogenous neurotransmitter 5-HT from the synaptic cleft. Abnormalities in SERT function and/or changes in SERT receptor densities, have been implicated in a variety of mental health disorders, including depression. Investigations employing ^{11}C and ^{18}F radiolabeled established SERT-selective antidepressants have shown only limited success, suggesting custom SERT PET agents may be required. Recently, innovative ligands such as [^{11}C]DAPA (2) and [^{11}C]5-methyl-6-nitroquipazine (3) have emerged as promising SERT PET agents. The potent lead agent, (±)-2'-methyl-6-nitro-quipazine **1** has been investigated (1) along with the analogous 2'-methoxymethyl-6-nitroquipazine **2.** We describe the production of [^{11}C]2'-methoxymethyl-6-nitroquipazine ([**[^{11}C]2**).

The cold, racemic target ligand **2** was produced by a route analogous to that used earlier for the synthesis of lead agent **1**. (1) 2-chloroquinoline was coupled with 2-methoxymethyl-4-triphenylmethylpiperazine, followed by N-trityl deprotection and nitration (not shown). Ligand **2** was characterized by *in vitro* competitive binding pharmacological measures (partially purified rSERT, [^{3}H]paroxetine) and was found to be of similar potency to lead agent **1**. For the radiochemical synthesis, the requisite precursor **4** was produced by the demethylation of the target methoxymethyl ligand **2**, followed by protection of the terminal piperazine nitrogen as a *t*-Boc derivative. Utilizing sequential reactions related to radiolabeling methods, intermediate **4** was subjected to methylation and then *t*-Boc group deprotection conditions followed by HPLC purification to reproduce the target ligand **2** (two steps, 66%).

[^{11}C]2 was prepared as shown below. N-tBoc protected (±)-2'-hydroxymethyl-6-nitro-quipazine **4** was deprotonated and added to a DMF solution containing [^{11}C]methyl iodide (0.25 mL), sealed and heated for 2-3 minutes @ 100°C. The crude protected intermediate was isolated on a C-18 Sep-Pak and eluted with dichloromethane. TFA was added in a 1:9 ratio and the mixture concentrated in vacuo @ 100°C providing crude **[^{11}C]2**. Purification on reversed phase semipreparative HPLC provided title compound **[^{11}C]2** in 2-5% decay corrected EOB yield.



This work was supported by the US DOE (DE-AC03-76SF00098) (JPO and HFV) and the NIH (NS36405, NS39814 and NCRR P20 RR15583), NSF (EPS-0091995) and Research Corporation (CC4299) (DBB, JMG and BRK).

References:

1) Gerdes JM, et al., (2000). Bioorg Med Chem Lett 10:2643-2646.
2) Huang Y, et al., (2002) Nuc Med Biol 29:741-751.
3) Sandell J, et al., (2002) Nuc Med Biol 29:651-656.

SYNTHESES AND APPLICATIONS OF THREE ^{18}F-FLUOROALKYLATING AGENTS IN A COMMERCIAL AUTOMATED RADIOSYNTHESIS APPARATUS

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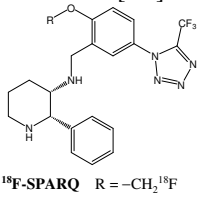
Keywords: Fluorine-18, Automation, ^{18}F-FCH$_2$Br, ^{18}F-F(CH$_2$)$_2$OTs, ^{18}F-F(CH$_2$)$_3$OTs

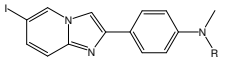
Introduction: The GE TRACERlab FX$_{F-N}$, an automated '[^{18}F]nucleophilic substitution module' offered by GE Medical Systems, is primarily designed for general single-step ^{18}F-labeling of PET radiopharmaceuticals by nucleophilic substitution with cyclotron-produced no-carrier added (NCA) [^{18}F]fluoride. However, many radiofluorinated compounds are made in two or more steps with ^{18}F-labeled agents, which are themselves prepared from NCA [^{18}F]fluoride. We aimed to develop simplified radiosynthetic procedures that would enable this particular automated apparatus to be used to prepare known and potential radiopharmaceuticals from three useful radiolabeling agents, namely [^{18}F]fluoromethyl bromide ([^{18}F]FMB), [^{18}F]2-fluoroethyl tosylate ([^{18}F]FET) and [^{18}F]3-fluoropropyl tosylate ([^{18}F]FPT) derived from cyclotron-produced [^{18}F]fluoride.

Experimental & Results: [^{18}F]FET and [^{18}F]FPT were each made non-automatically and reproducibly in 70-99% radiochemical yield (decay-corrected) from [^{18}F]fluoride. The automated radiosyntheses of these labeling agents also gave radiochemical yields in this range. It was established that these two labeling agents could be used *in situ* for successful 'one-pot' radiosyntheses of the prospective β-amyloid radioligands, [^{18}F]FEM-IMPY and [^{18}F]FPM-IMPY (Table 1). [^{18}F]FMB was more difficult to prepare and use in the automated apparatus. Highest radiochemical yields of isolated [^{18}F]FMB were achieved from reactions of 'naked' [^{18}F]fluoride with neat dibromomethane, rather than dibromomethane in acetonitrile solution (Table 1). Separation of [^{18}F]FMB by gas-phase transfer was necessary for the preparation of the known NK$_1$ receptor radioligand, [^{18}F]SPARQ (Table 1), in a secondary vessel containing *des-fluoromethyl-N-Boc*-SPARQ at 0 °C in DMF-Cs$_2$CO$_3$. Deprotection was performed in the same vessel with trifluoroacetic acid.

Table 1. Radiochemical Yields from [^{18}F]Fluoride (Decay-corrected to Start- and End-of Synthesis)



^{18}F-SPARQ R = –CH$_2$^{18}F



^{18}FEM-IMPY R= –CH$_2$CH$_2$^{18}F
^{18}FPM-IMPY R= –CH$_2$CH$_2$CH$_2$^{18}F

Compound	Radiochemical yield (%)	
	Start-of-synthesis	End-of-synthesis
^{18}F-CH$_2$-Br*	19.9	15.7
^{18}F-CH$_2$-Br**	37.2	28.5
^{18}F-SPARQ	4.9	2.1
^{18}FEM-IMPY	21.3	8.2
^{18}FPM-IMPY	22.3	8.9

* Prepared in acetonitrile solution; Δ~100 °C for 10 min. ** Prepared without solvent; Δ~100°C for 10 min.
Gas-phase transfer was made at 50 °C with N$_2$ flow (~ 5 mL/min) until trapping was complete by radiodetection.

Conclusion: The GE TRACERlab FX$_{F-N}$ was adapted successfully for the preparation of three ^{18}F-fluoroalkylating agents for reactions *in situ* ([^{18}F]FET, [^{18}F]FPT) or in a second vessel ([^{18}F]FMB).

DEVELOPMENT OF ^{123}I-LABELLED AGENTS FOR AMYLOID IMAGING

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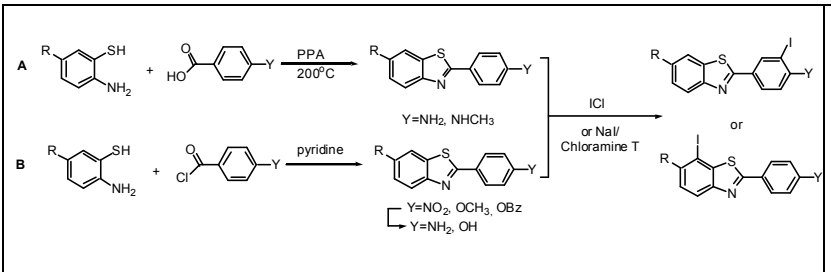
Keywords: Alzheimer's disease, amyloid, 2-aryl benzothiazoles, PET, SPECT

Background: Amyloid-ß (Aß) production and aggregation are targets for therapeutic intervention in Alzheimer's disease (AD). Anti-amyloid therapies currently under development would benefit from efficacy evaluation through in vivo detection and quantitation of amyloid deposits in the brain. **Objectives:** the goal of this study is to develop amyloid-imaging agents for single photon emission computed tomography (SPECT). We report design and synthesis a series of iodinated 2-aryl benzothiazole derivatives capable of I-123 incorporation for amyloid imaging with SPECT. **Methods:** The syntheses of iodinated 2-aryl benzothiazole derivatives were achieved through conventional chemical approaches. In vitro binding affinity was determined using synthetic Aß(1-40) fibrils and AD brain homogenates. After radiolabelling, the in vivo studies were conducted in normal control mice to determine brain entry and clearance. Film autoradiography was conducted using AD brain tissue sections to determine in vitro binding specificity. **Results:** A series of novel 2-aryl benzothiazole derivates substituted with iodine were synthesized as amyloid-binding ligands. The affinity of these compounds for synthetic amyloid-ß(1-40) fibrils ranged from 0.67-15.1 nM. Compared to non-iodinated analogues, introduction of an iodo group in the position ortho to an amino group increased the binding affinity, while introduction of an iodo group ortho to a hydroxyl group decreased the binding affinity. Selected compounds with high binding affinity and acceptable lipophilicity (logP values 1.65 to 3.90) were radiolabelled and evaluated in normal control mice for brain entry and clearance. At early time points, radiolabelled compounds entered the brain well with radioactivity concentration ranging from 4.4-9.1%ID/g at 2 min post i.v. injection. At later time points, radiolabelled compounds cleared from the brain with 2 min-to-30 min ratios ranging from 1.6-15.7. Structure-activity relationship (SAR) studies showed a strong correlation between the lipophilicity of the iodinated compounds and the binding affinity as well as the non-specific brain clearance. As the lipophilicity increased, the affinity for Aß(1-40) fibrils was enhanced, while non-specific binding clearance rate decreased in the mouse brain. **Conclusion:** These results provide important SAR information to guide the development of novel amyloid-binding agents and provide further insights into the molecular interaction of 2-aryl benzothiazole ligands with Aß fibrils. Supports from the Institute for the Study of Aging, Alzheimer's Association, and National Institute on Aging are gratefully acknowledged.



R = H, OCH_3, Br, NO_2, $COOCH_3$, OCH_2OCH_3, and OH.
Y = NH_2, $NHCH_3$, OH, OCH_3

R = NH_2, OH
Y = OCH_3, OH.

DEVELOPMENT OF RADIOTRACERS FOR MEASURING CEREBRAL REDOX STATE USING REDOX CONVERSION OF DIHYDROPYRIDINE-PYRIDINIUM SALT AND METABOLIC TRAPPING PRINCIPLE

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Keywords: Cerebral redox state, Dihydropyridine-pyridinium salt

The homeostases of redox state in the organism are maintained by a number of prooxidant/antioxidant systems. Recent studies have shown that the breakdown of the homeostases caused by imbalances of these systems induced oxidative stress and finally a variety of diseases. Accordingly, development of radiopharmaceuticals that measure the redox states in the living brain would be important and useful for estimating disease states, therapeutic efficacies and diagnosis of the cranial nerves disorders. We focused our efforts to develop radiopharmaceuticals for measuring cerebral redox states using the redox conversion of dihydropyridine-pyridinium salt (DHP-Py^+) (1) and the metabolic trapping principle. In this study, five N-[^{14}C]methyl-3 or 3,5-subustituted-1,4-dihydropyridines (^{14}C-MeDHPs) with different oxidation rates were designed and synthesized, and evaluated their abilities as the irreversible type radiotracers of the metabolic trapping principle.

All pyridinium salt and dihydropyridine derivatives were prepared as described (2). N-[^{14}C]methyl-3 or 3, 5-subustituted pyridinium iodides (^{14}C-$MePy^+$s) were radiolabelled by N-methylation using [^{14}C]methyl iodide. The radiochemical yields and purities of ^{14}C-$MePy^+$s were 50-92% and 95-99%, respectively. Five ^{14}C-$MePy^+$s were reduced in the presence of $NaHCO_3$ and $Na_2S_2O_4$ to generate the corresponding ^{14}C-MeDHPs in 55-90% radiochemical yields and 90-96% radiochemical purities. The partition coefficients for ^{14}C-$MePy^+$s and ^{14}C-MeDHPs in octanol/phosphate buffer (PB) showed that ^{14}C-MeDHPs possess lipophilicity sufficient to penetrate the blood-brain barrier (log P = 0.22-1.55). Indeed, when injected to mice (ddY, male, 6 weeks, 30-36 g, n=3-5), 2.84 to 4.22% dose/g of ^{14}C-MeDHPs were incorporated in the cerebrum after 1 min. On the other hand, the log P values for ^{14}C-$MePy^+$s were ranging from -2.64 to -0.22, which was reflected in low cerebrum uptake as compared to those of ^{14}C-MeDHPs. The oxidation potentials of MeDHPs electrochemically determined with cyclic voltammetry were correlated well with the oxidation rates of ^{14}C-MeDHPs in PB and in mouse brain homogenate when determined by TLC/BAS methods (3). For estimating the oxidation rates in the brain in vivo, the retention fraction (RF), 30 min-to-1 min uptake ratio after i.v. administration of each ^{14}C-MeDHPs, was calculated to approximate the fractions of ^{14}C-MeDHPs trapped in the brain. The RF values were also well correlated with the oxidation potentials and the oxidation rates of the compounds. Furthermore, an increase in the RF of N-[^{14}C]methyl-3-acetyl-1,4-dihydropyridine was observed in the brain of mice pretreated with diethylmaleate (DEM). Since DEM treatment depletes glutathione of the brain, these results showed that it was oxidized faster in DEM-treated mice than in control mice, due to the oxidative stress induced by GSH deficiency.

These results suggested that ^{14}C-MeDHPs entered from the blood to the brain and were retained depending on their oxidation rates. The findings in this study also suggested that radiopharmaceuticals based on DHP-Py^+ and the metabolic trapping principle would be applicable to measuring the redox states in the brain.

1. Bodor N, Farag H H. *J Med Chem* 1983; 26: 313-318.
2. Brewster M E, Simay A, Czao K, Winwood D, Farag H, Bodor N. *J Org Chem* 1989; 54: 3721-3726.
3. Irie T, Fukushi K, Namba H, Iyo M, Tamagami H, Nagatsuka S, Ikota N. *J Nucl Med* 1996; 37: 649-655.

^{18}F-FLUORINATED AGONISTS AND ANTAGONISTS FOR IMAGING OF μ-OPIOID RECPTORS: APPROACHES TO ^{18}F-CARFENTANIL, ^{18}F-SUFENTANIL AND ^{18}F-CYPRODIME

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Keywords: μ-opioid receptor ligands, fluorination, pain

Background: Recent work has emphasized the potential of μ-opioid receptor (μ-OR) selective PET-ligands for the determination of pain-induced release of endogenous opioids. So far, only ^{11}C-labeled Carfentanil is available. With respect to studies requiring a pharmacological intervention or stimulation and thus a prolonged scanning period, synthetic approaches to ^{18}F-labeled μ-OR ligands with high affinity and lower agonistic potency than Carfentanil or even antagonistic activity were investigated. The aim of this study was the synthesis and evaluation of F-18-labeled analogues of the μ-OR agonists carfentanil **(1)** and sufentanil **(2)** and the μ-selective antagonist cyprodime **(3)**.

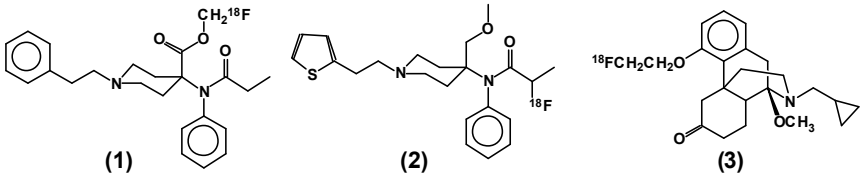

Fig.1: ^{18}F-Labeled μ-opioid receptor ligands investigated: ^{18}F-carfenta **(1)** , ^{18}F-sufenta **(2)** and ^{18}F-cyprodime **(3)**.

Results: 2-[^{18}F]Fluoropropionyl-anilido-sufentanil (^{18}F-Sufenta, **(2)**) was obtained in 50% radiochemical yield (RCY) by direct ^{18}F-fluorination of N-(2-bromopropionyl)anilido-sufentanil. Although a variety of column/mobile phase combinations were tested, the corresponding hydroxy- and elimination products were coeluted with **(2)**. Thus, this compound could only be obtained in low chemical purity so far. ^{18}F-Fluoromethylation of the carboxylic acid precursor of carfentanil yielded the [^{18}F]fluoromethyl ester **(1)** (^{18}F-Carfenta) in high RCY and chemical purity (SA >37GBq/μİmol) in ~80 min. High and selective uptake of ^{18}F-Carfenta in brain regions with high μ-OR expression (frontal cortex, amygdala, thalamus) 10 min p.i. was demonstrated by ex vivo autoradiography of rat brain slices. Specificity of uptake was confirmed by co-administration of naloxone (1mg/kg). The tracer uptake kinetics in mice revealed a fast decrease of brain activity (3.69±1.69 %ID/g, 1.27±0.46 %ID/g and 0.33±0.08 %ID/g at 5, 20 and 60 min, respectively). Since no metabolites were detected in brain homogenates, fast ester hydrolysis followed by a washout of [^{18}F]MeOH from cerebral compartments seems to be the dominant metabolic fate. Plasma metabolite analysis showed the presence of three more hydrophilic metabolites. ^{18}F-cyprodime **(3),** a ^{18}F-labelled analogue of the μ-OR antagonist cyprodime, was prepared by ^{18}F-fluoroethylation of 4-O-desmethyl cyprodime (90 min, SA>74 GBq/μmol). Tracer uptake in mice brain reached 3.31±0.41, 2.00± 0.46 and 1.84±0.35 %ID/g at 5, 20 and 60 min. Plasma metabolite analysis revealed 80% and 40% intact tracer at 5 min and 20 min p.i., respectively. Metabolites were also detected in brain homogenates. Autoradiography of rat brain slices revealed weak to moderate selectivity of ^{18}F-Cyprodime for μ-OR binding sites.

Conclusion: Procedures for radiosynthesis of ^{18}F-Carfenta, ^{18}F-Sufenta and the nonpeptide antagonist ^{18}F-Cyprodime have been established. Preliminary biological evaluation of ^{18}F-Carfenta and ^{18}F-Cyprodime indicate a moderate to high uptake of the tracers in brain with however a less than optimal stability towards metabolic degradation.

PREPARATION AND PET EVALUATION OF [^{11}C]FALLYPRIDE – FOR EXTRASTRIATAL DOPAMINE D2/D3 RECEPTORS

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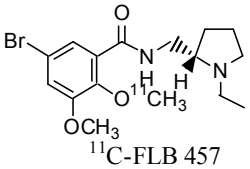
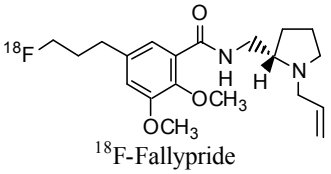
Keywords: Carbon-11, dopamine D2/D3, PET, extrastriatal, brain

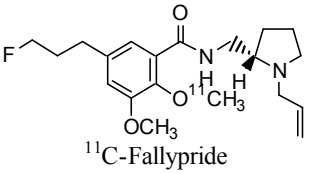
Fallypride is a dopamine D2/D3 receptor antagonist which has been previously labeled with fluorine-18 for PET studies in monkeys and humans (1-2). Fallypride is a substituted benzamide which contains a dimethoxy substituent group in the benzamide ring. The 2-methoxy group is a common feature in this class of compounds which can be replaced with a carbon-11 label. This has been done with [^{11}C]FLB 457 successfully (3-4). Carbon-11 labeled fallypride may also serve as a good imaging agent for use in PET studies. It may in particular be useful for a comparison between [^{11}C]fallypride and [^{11}C]FLB 457 examining the competition of endogenous dopamine in the extrastriatal brain regions.



^{11}C-FLB 457



^{18}F-Fallypride



^{11}C-Fallypride

The synthesis of the precursor, (*S*)-*N*-[(1-allyl-2-pyrolidinyl)methyl]-2-hydroxy-3-methoxy-5-(3'-fluoropropyl) benzamide was accomplished starting with the commercially available 3-methoxysalicylic acid and (*S*)-prolinamide in 12 steps. [^{11}C]Fallypride was prepared by O-methylation of the desmethyl precursor with [^{11}C]methyl triflate in a 30% incorporation yield. The specific radioactivity was 74 GBq/ mol with a radiochemical purity >99%. [^{11}C]Fallypride was injected i.v. into a Cynomolgus monkey and examined during baseline and pretreatment (raclopride, 1 mg/kg) conditions with PET. Cerebellum was used as a reference region for free radioligand and non-specific binding. Radioactive metabolites in plasma were measured by HPLC. After injection of [^{11}C]fallypride, radioactivity in brain peaked at 4 min (3.4% injected dose). PET images were consistent with high uptake of radioactivity into supposed dopamine D2 brain regions. Radioactivity levels in striatum, thalamus, mesencephalon were 20-, 5- and 5-fold higher than in cerebellum at 60-70 min. In the pretreatment experiment, these ratios were reduced markedly to the level of cerebellum. Labeled metabolites in plasma were more polar than the parent radioligand (50% parent at 45 min). These results indicate that [^{11}C]fallypride is an excellent PET radioligand for imaging extrastriatal D2 receptors *in vivo*.

1. Mukherjee J, Yang ZY, Das MK and Brown T. *Nucl Med Biol* 1995; 22: 283-296.
2. Mukherjee J, Christian BT, Dunigan K, et al., *Synapse* 2002; 46:170-188.
3. Halldin C, Farde L, Högberg T, et al., *J Nucl Med* 1995; 36: 1275-1281.
4. Farde L, Suhara T, Nyberg S, et al., Psychopharmacol 1997; 133: 396-404.

SYNTHESIS AND IN VITRO CHARACTERIZATION OF 2-[^{131}I]IODOSTRYCHNINE

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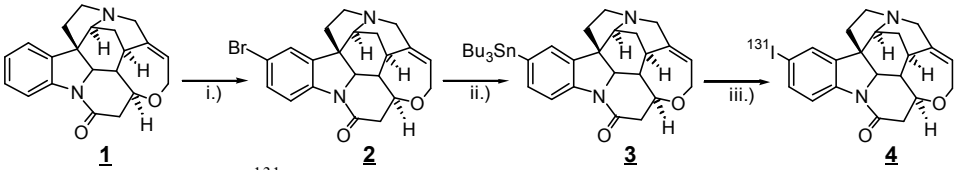
Keywords: glycine receptor, strychnine, SPECT

The inhibitory glycine receptor (GlyR) represents a family of ligand-gated chloride channels and exists in developmentally regulated isoforms composed of distinct subunit varients (1-4) (1, 2). In the CNS glycine receptors predominate in the spinal cord and brain stem. Disturbances of GlyR function are caused by alkohol and anaesthetics (3). Interestingly, the functional role of the isoform GlyR$_N$, which is abundantly expressed in rat cerebral cortex during a short postnatal period, remains unclear.

Here, we report the radiosynthesis and in vitro characterization of 2-[^{131}I]iodostrychnine as a potential GlyR antagonist.

Radioiodination of strychnine (1) was performed following the strategy as depicted in scheme 1. Briefly, strychnine was selectively brominated at the 2-position using standard reaction conditions to afford 2. Treatment of the bromo derivative 2 with hexamethyldistannane and tetrakis(triphenylphosphine)palladium in refluxing toluene gave compound 3 (55% yield). Iododestannylation of 3 using I$_2$ in THF afforded 2-iodostrychnine as a standard for analytical purposes. Radioiodination of 3 was realized using no-carrier-added [^{131}I]iodide in ethanol/ 1N HCl (1:1) and hydrogen peroxide (3%) as oxidant. The product, [^{131}I]iodostrychnine (4), was isolated by HPLC purification (RP-8, CH_3CN/50mM KH_2PO_4 (10:88 v/v), 2%Et_3N, pH 3). Binding studies were performed using [^{3}H]strychnine and membrane preperations of HEK 293 cells expressing the GlyR 1 subunit. Membrane incubation was performed in sodium-free buffer (25mM KH_2PO_4, 200mM KCl, pH 7.4) for50 min. K_d values were determined by the use of Origin (Microcal).



1 2 3 4

Scheme 1: Synthesis of 2-[^{131}I]iodostrychnine (i.) AcOH, Br_2, 0°C, ii.) (Bu_3Sn-)$_2$, $[(Ph)_3P]_4Pd$, toluene (reflux), iii.) n.c.a. [^{131}I]iodide, ethanol/1N HCl, 3% H_2O_2)

Strychnine was selectively radiolabelled at the 2-position by iododestannylation with a radiochemical yield of 85% (t=1min). The receptor affinity of 2-iodostrychnine was determined to be K_d=3.9 nM. In comparison to the parent compound strychnine (K_d=5.2 nM), no loss of binding affinity was observed.

Work is in progress in order to characterize the binding properties of 2-[^{131}I]iodostrychnine by autoradiography using tissue slices of rodents. Moreover, radioiodinated strychnine may serve as a tool for in vivo glycine receptor imaging by SPECT when labelled with iodine-123.

References

1. Becker CM. *Handbook of Experimental Pharmacology*, vol102. Springer-Verlag: Berlin, 1992.
2. Breitinger HG, Becker CM. *Chem Bio Chem* 2002; 4: 1042-1052.
3. Harris RA. Alc *Clin Exp Res* 1999; 23: 1563-1570.

SYNTHESIS, CHARACTERIZATION OF [^{11}C]ZBrENT AS MICROPET IMAGING OF THE SERotonin TRANSPORTER (SERT)

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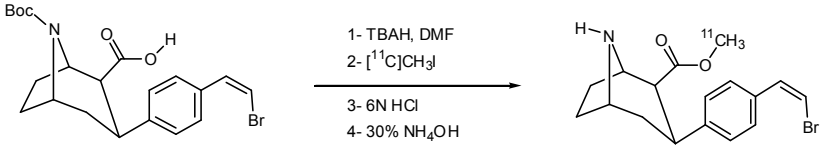
Keywords: brain imaging, serotonin transporter, radioligand, tropane, carbon-11

Alterations in SERT density have been associated with the psychiatric disorders such as suicide and depression. Monitoring disease progression and following the effectiveness of psychotropic drugs may be achieved using highly selective SPECT or PET radioligand. Recently, we reported [^{123}I]ZIENT, 2*b*-carbomethoxy-3*b*-(4'-((*Z*)-2-iodoethenyl)phenyl)nortropane, as an attractive SPECT imaging agent for studying the CNS SERT. In order to further investigate the structure activity relationship of 4'-(*Z*)-vinyl halide substitution on SERT affinity and selectivity, we prepared its bromo analog ZBrENT. ZBrENT, which can be labeled either with carbon-11 or bromine-76, was first studied by incorporation of readily available carbon-11.

2*b*-carbomethoxy-3*b*-(4'-((*Z*)-2-bromoethenyl)phenyl)nortropane (ZBrENT) was prepared from the 2*b*-carbomethoxy-3*b*-(4'-bromophenyl)nortropane by palladium-catalyzed cross coupling reaction with (*Z*)-bis(trimethylstannyl)ethylene followed by halodestannylation using *N*-bromosuccinimide. The *in vitro* competition assays of ZBrENT in cells transfected to express human SERT, DAT and NET gave Ki's (nM) of 0.037, 3.95 and 2.5 respectively. The acid **1**, precursor of [^{11}C]ZBrENT, was prepared from *N*-(tert-butoxycarbonyl)-3*b*-(4'-bromophenyl)nortropane-2*b*-carboxylic acid following an analogous series of reaction used for ZBrENT. [^{11}C]ZBrENT was labeled in a four-step reaction sequence: deprotonation of acid **1** using aqueous tetrabutylammonium hydroxide was followed by *O*-alkylation with [^{11}C]methyl iodide in DMF at 85°C, acidic hydrolysis of the Boc group with 6N HCl at 85°C and basification with ammonium hydroxide. The radiochemical decay corrected yield of [^{11}C]ZBrENT was 50% with a total synthesis time of 1 hour.



1 **[^{11}C]ZBrENT**

Brain imaging performed in monkeys using a Concorde microPET P4 showed high radioactivity uptake in the putamen, caudate, midbrain, thalamus, medulla and pons with tissue to cerebellum ratios of 2.24, 2.07, 1.80, 1.67, 1.63 and 1.60 respectively, at 115 min post injection. In order to determine the selectivity of this radioligand, block and/or displacement studies were performed by injection of different monoamine transporter ligands. The results of these studies demonstrated that administration of SERT ligand citalopram (1.5 mg/kg) at 30 min after injection of [^{11}C]ZBrENT clears the radioactivity from SERT rich regions except the putamen and the caudate. Nevertheless, studies carried out by pre-treatment with citalopram followed by a chase with the DAT ligand RTI-113 or *vice versa* showed that [^{11}C]ZBrENT uptake in striatum can be displaced.

In conclusion, the radiosynthesis of [^{11}C]ZBrENT has been successfully achieved. The *in vivo* properties of this new radioligand were not found to be an improvement over ZIENT. Research supported by DOE.

CONFORMATIONALLY-FLEXIBLE BENZAMIDE ANALOGUES AS DOPAMINE D_3 RECEPTOR IMAGING AGENTS FOR PET AND SPECT

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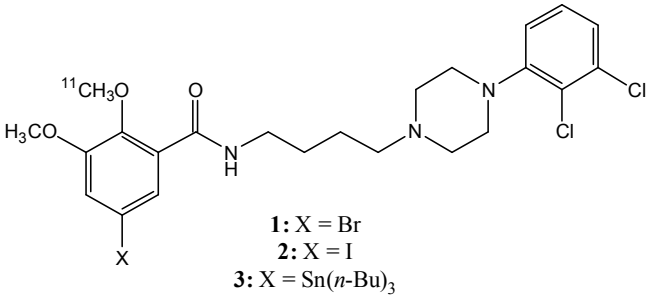
Keywords: PET, SPECT, dopamine D_3 receptors

Over the past fifteen years there has been a tremendous amount of research conducted in the development of PET and SPECT radiotracers for studying dopamine D_2 receptor function in vivo. This research has been largely driven by the availability of a number of antipsychotics possessing a high affinity for dopamine D_2 receptors, and the alteration of D_2 receptor density identified in postmortem brain samples from a variety of CNS disorders such as schizophrenia, Parkinson's Disease and substance abuse. However, radiotracers that have been developed to date are not capable of discriminating between the different subtypes of the D_2-class of receptors, particularly dopamine D_2 and D_3 receptors. Therefore, measurement of "D_2 receptor binding potential" obtained with PET radiotracers such as [^{11}C]raclopride consist of a composite of D_2 and D_3 receptors.

A great deal of experimental evidence suggests that the D_3 receptor plays a critical role in a number of CNS disorders. Furthermore, there is evidence suggesting that D_2 and D_3 receptors are differentially regulated in a variety of disease conditions. Therefore, radiotracers having a higher affinity for D_3 versus D_2 receptors, and vice versa, would be of tremendous interest to the PET and SPECT research community.

Over the past six years, our group has synthesized a number of heterocyclic and benzamide analogues with the goal of identifying compounds having a high affinity for D_3 versus D_2 receptors that could serve as lead compounds for PET and SPECT radiotracer development. The results of this effort led to the discovery of the conformational-flexible benzamide analogues, **1** and **2**, which may be useful radiotracers for PET and SPECT studies of the dopamine D_3 receptor.

Initial radiolabeling studies have focused on preparing [^{11}C]**1**. This was readily achieved via O-alkylation of the des-methyl precursor with [^{11}C]methyl iodide. The labeling yield was ~10% and [^{11}C]**1** was obtained in a specific activity of >1,000 mCi/ mol. The corresponding tributyltin precursor, **3**, has also been prepared for radiolabeling studies with $^{125/124}$I and ^{76}Br. These radiotracers may be useful probes for imaging dopamine D_3 receptors with PET and SPECT.



1: X = Br
2: X = I
3: X = Sn(*n*-Bu)$_3$

1	**2**
D_2 = 60 nM	D_2 = 62 nM
D_3 = 2 nM	D_3 = 9 nM
D_4 = 800 nM	D_4 = ND

Acknowledgement. This research was supported by NIH grants DA 12647 and DA16181.

SYNTHESIS, EVALUATION AND [^{11}C]LABELLING OF A NEW EPIBATIDINE ANALOGUE AS A NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) PET LIGAND

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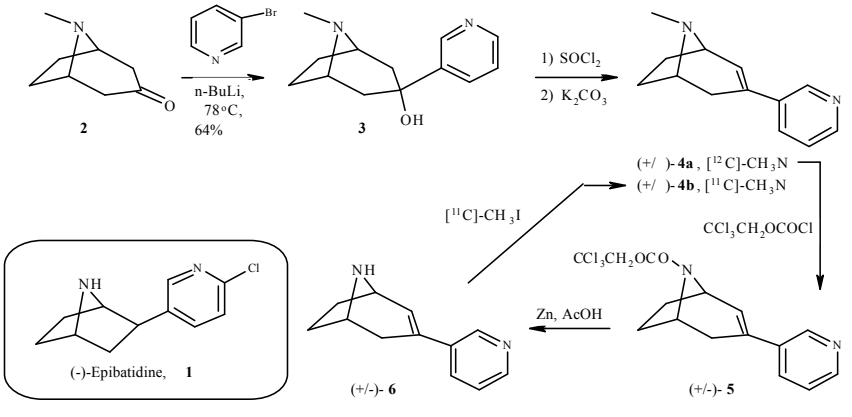
Keywords: epibatidine analogue , nAChR, carbon-11, receptor-affinity

The potential benefit in a variety of CNS neurodegenerative diseases has energized efforts for development of new neuronal nicotinic acetylcholine receptor (nAChR) subtype selective ligands. Additionally, radiolabelled analogues of such compounds could be used as radiotracers for PET imaging of the density and distribution of the brain nAChRs which is of great importance for the early diagnosis of a number of CNS disorders including Alzheimer's and Parkinson's diseases. The alkaloid epibatidine (**1**) exhibits the highest known affinity (subnanomolar range) toward the central brain 4 2 and 7 nAChRs. However, the possible therapeutic and/or radiotracer use of epibatidine (**1**) is prevented by its enormous toxicity. Recently we synthesized a series of new epibatidine analogues. The *in vitro* binding affinity studies of these compounds showed that compound (±)-**4a** (synthesis shown in the Scheme) possesses high affinity (2 nM) towards 4 2 nAChRs, and subtype selectivity for the 4 2 (4 2/ 7 affinity ratio >100) and > 50 times lower toxicity in mice (LD_{50} > 0.5mg/kg body weight) than that of epibatidine (**1**). These results characterize compound (±)-**4a** as a very selective nAChRs ligand and its [^{11}C]-labeled analogue (±)-**4b** (its radiosynthesis is shown in the Scheme) as a useful nAChRs PET tracer.



Scheme

RADIOLABELLING OF THE KAPPA-OPIOID RECEPTOR LIGAND [^{123}I]MCL-118 AND IN VIVO EVALUATION IN RATS AND NONHUMAN PRIMATES

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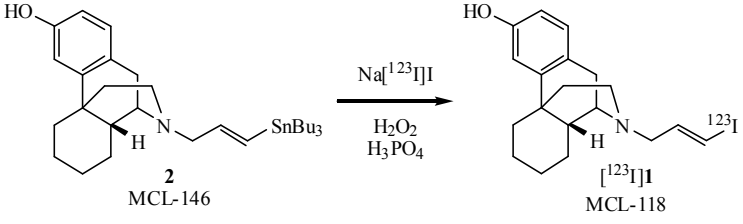
Email ronald.baldwin@yale.edu.

Keywords: Iodine-123; kappa opioid receptor; in vivo;



2
MCL-146

[^{123}I]**1**
MCL-118

Interactions of agonists at the -opioid receptor subtype are associated with analgesic, neuroprotective, and anticonvulsant properties and in the behavioural effects of cocaine. The *N*-substituted morphinan derivative MCL-118 (**1**) showed high affinity and selectivity at the and subtypes (K_i 0.0048 nM at , 0.037 nM at) (*1*). warranting examination as a potential SPECT agent. [^{123}I]**1** was prepared by iododestannylation of the stannyl precursor **2** with H_2O_2. HPLC purification(C_{18}, $CH_3OH/H_2O/Et_3N$, 75/25/0.1, 1.0 mL/min, t_R 13.5 min) yielded 37.8% [^{123}I]MCL-118 in 94.2% purity.

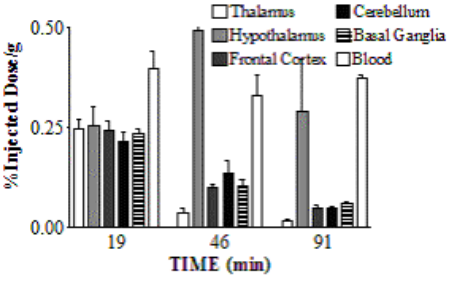


Fig 1. [^{123}I]MCL-118 Uptake in Rat Brain

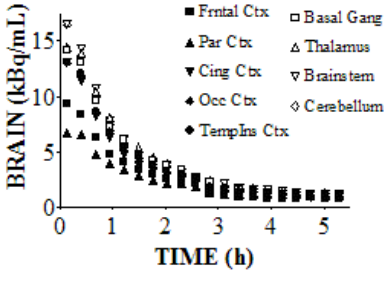


Fig 2. [^{123}I]MCL-118 Uptake in Baboon Brain

Administration of [^{123}I]MCL-118 to rats gave an indication of high uptake in hypothalamus, an area of relatively high opioid receptor density (Fig 1). However, SPECT imaging of baboons given [^{123}I]MCL-118 showed homogeneous brain uptake without selective accumulation in opioid-rich tissues (Fig 2), and pre-administration with naloxone or GR89696 did not alter the uptake. We conclude that in contrast to the in vitro binding results, the in vivo behaviour of [^{123}I]MCL-118 is dominated by non-specific uptake.

This work was supported in part by NIDA (DA 14251) and NINDS (NS4587).

REFERENCES

1. Neumeyer JL, Gu X-H, van Vliet LA, DeNunzio NJ, et al. *Bioorg. Med. Chem. Lett.* (2001) **11**: 2735-2740.

[11C]NAFADOTRIDE AS A POTENTIAL PET RADIOLIGAND FOR THE DOMAPINE D3 RECEPTORS: PREPARATION AND IN VIVO EVALUATION

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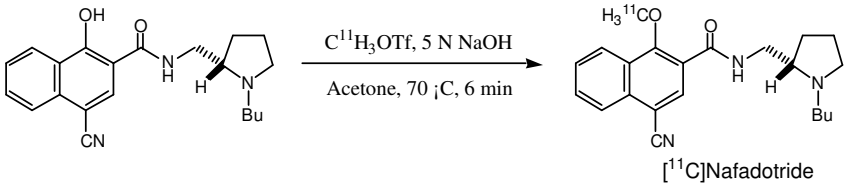
Key words: Dopamine, D3 receptor, Radioligand, PET

The dopamine D3 receptor has been implicated in schizophrenia and drug abuse, and several selective D3 antagonists are under development as antipsychotic drugs. However, no PET radiotracers are currently available to image this important target in vivo and to investigate the dose-occupancy relationship of these new drugs. Here we report the sythesis and in vivo evaluation of [11C]nafadotride, a benzamide with a modest in vitro binding selectivity for D3 over D2 receptors (Ki 0.3 nM for D3, and 3 nM for D2 receptors), as a potential PET imaging agent for the dopamine D3 receptors.

[11C]Nafadotride was prepared by *O*-methylation of the naphthol precursor with [11C]methyl triflate (see scheme). Total synthesis time was 32 – 35 min. Specific activity at end of synthesis was 1216 ± 523 Ci/mmol (n = 8). Radiochemical yield was 15 ± 4 (n = 8). Radiochemical and chemical purity of the final product was > 98%.



[11C]Nafadotride

PET imaging experiments were conducted in two baboons, under control conditions (n = 4), and following pre-treatment with the selective D3 antagonist BP 897 or agonist 7-OH DPAT, or the D2/D3 antagonist haloperidol. Emission data were collected for 90 min with the ECAT EXACT HR+ scanner. Arterial input function was measured and corrected for metabolites. Distribution volumes (V_T, mL/g) were derived with graphical analysis. The medulla was used as the reference region devoid of D3 receptors and specific binding was assessed by V_3", calculated as [(V_T in striatum / V_T in medulla) - 1].

In the blood, [11C]nafadotride was metabolized fairly fast, with parent fraction of 35 ± 11% (n = 8) at 30 min after radiotracer injection. Free fraction in the plasma was 6.9 ± 1.9% (n = 8). In the brain, peak uptake in the striatum was achieved 5 to 15 min after radiotracer administration, indicating a fast kinetics. Peak striatum to medulla activity ratio was ~1.4 during the period of 10 to 40 min post-injection. Medulla V_T was 3.9 ± 2.0 mL/g (n = 8) and unaffected by pre-treatment with BP 897, 7-OH DPAT, or haloperidol. Under control conditions, striatal V_3" was 0.29 ± 0.12 (n = 4). When the baboon was pre-treated with BP 897 (1 mg/kg, i.v., n = 1) or low dose of 7-OH DPAT (0.2 mg/kg, i.v., n = 2), [11C]nafadotride V_3" in the striatum was reduced by 97 and 64%, respectively, while pre-treatment with haloperidol (0.5 mg/kg, i.v., n = 1) reduced striatal V_3" by 52%. These results suggest that , in the striatum, [11C]nafadotride specific binding to the D3 receptors is detectable, and also displaceable by selective D3 receptor ligands. In conclusion, [11C]nafadotride appears to be a potential PET radioligand for imaging the dopamine D3 receptors in vivo.

TWO NEW PET RADIOTRACERS FOR THE SEROTONIN TRANSPORTER: SYNTHESIS AND PHARMACOLOGICAL CHARACTERIZATION OF [^{11}C]AFA AND [^{11}C]AFE

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Key words: Serotonin transporter, Radioligand, PET

We have previously reported the synthesis and characterization of [^{11}C]AFM as a highly specific PET ligand for the serotonin transporter that provides high signal to noise contrast in imaging studies in baboons[1]. In our continued effort to develop appropriate PET radiotracer for the serotonin transporters (SERT) that can be labeled either with C-11 or F-18, we have synthesized [^{11}C]AFA {(2-[2-[(dimethyl- amino)methyl]thiophenyl]-5-fluorophenylamine)} and [^{11}C]AFE {(2-[2-[(dimethylamino)methyl]thio- phenyl]-5-fluoroethylphenylamine)}. Here we reported the preparation and evaluation [^{11}C]AFA and [^{11}C]AFE as SERT radioligands in PET imaging studies in baboons.

[^{11}C]AFA and [C-11]AFE were prepared by C-11 methylation of their respective monomethyl-amino precursor with [^{11}C]methyl iodide. Total ynthesis time for both radioligands was ~30 min. [^{11}C]AFA and [^{11}C]AFE were produced in high specific activity and >98% chemical and radiochemical purity. In vitro, AFA and AFE binds with SERT with Ki's of 1.46 nM and 1.88 nM, respectively, compared with a Ki of 0.42 for AFM. Imaging experiments in baboons indicated that the highest activity uptake of [^{11}C]AFA and [^{11}C]AFE was in the thalamus and midbrain, followed by striatum and hippocampus, in a pattern consistent with the distribution of SERT in the baboon brain. For [^{11}C]AFA, peak uptake in the thalamus was achieved 10 to 25 min after radiotracer administration. For [^{11}C]AFE, peak uptake was 15 to 30 min post-injection. These compare with a peak uptake time of 40 to 60 min for [^{11}C]AFM. Peak thalamus to cerebellum activity ratio was ~2.5 at 90 min after radioactivity injection for both [^{11}C]AFA and [^{11}C]AFE, compared with ~4.0 for [^{11}C]AFM. In vivo binding of [^{11}C]AFA and [^{11}C]AFE appears to be specific to the serotonin transporter, as pretreatment of the baboons with citalopram (4 mg/kg, i.v.) reduced radioactivity in SERT-rich regions such as thalamus, midbrain and striatum to the level of cerebellum. Kinetic analysis was performed using a two-compartment model and metabolite-corrected blood input function. Non-specific distribution volume (VT in the cerebellum) of [^{11}C]AFA and [^{11}C]AFE (16.3 and 15.3 mL/g) were lower than that of [^{11}C]AFM (31.2 mL/g). The specific to non-specific equilibrium partition coefficients {V3", defined as [(VT ROI / VT Ref) – 1]} were 1.09, 0.54, 0.43, 0.19, 0.20 for [^{11}C]AFA and 1.03, 0.48, 0.37, 0.17 and 0.19 for [^{11}C]AFE in the thalamus, striatum, hippocampus, temporal cortex and cingulate cortex of the baboon brain. These compare with 2.46, 1.06, 0.61, 0.35 and 0.30 for [^{11}C]AFM in the same regions.

In conclusion, [^{11}C]AFA and [^{11}C]AFE are two new PET tracers for SERT that display faster brain kinetics and lower non-specific binding than [^{11}C]AFM in the baboon brain. Both [^{11}C]AFA and [^{11}C]AFE appear to be specific PET tracers suitable for the imaging of SERT in vivo.

Reference:
1. Huang Y, Hwang DR, Narendran R et al. *J Cereb Blood Flow Metab* 2002; 22:1377-1398

THE SYNTHESIS AND CHARACTERIZATION OF MGLUR5 RECEPTOR PET LIGANDS

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Keywords: mGluR5, Autoradiography, PET

Glutamate is a major excitatory central nervous system (CNS) transmitter and is involved in many physiological processes acting through ionotropic glutamate receptors. Glutamate also binds to G-protein-coupled metabotropic glutamate receptors (mGluR) which activates modulatory pathways. The mGluR5 subtype shows widespread CNS distribution and has been suggested to be a potential target for a number of CNS disorders including Parkinson's disease, pain and anxiety. We are interested in developing PET tracers to further study the mGluR5 receptor and have synthesized analogs of 3-methoxy-5-(pyridin-2-ylethynyl)pyridine (Methoxy-PEPy[1]), and 5-[(2-methyl-1,3-thizaol-4-yl)-ethynyl]pyridine (MTEP[1]) (Figure 1) with either carbon-11 or fluorine-18.

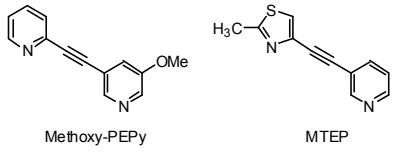

Figure 1. Chemical Structures of mGluR5 Receptor Antagonists.

These mGluR5 PET tracers were synthesized and autoradiographic studies and PET imaging studies have been carried out. Figure 2 shows the time-activity curves in dog and monkey that results from an analog of MTEP. The results from the autoradiographic studies and the PET studies will be presented.

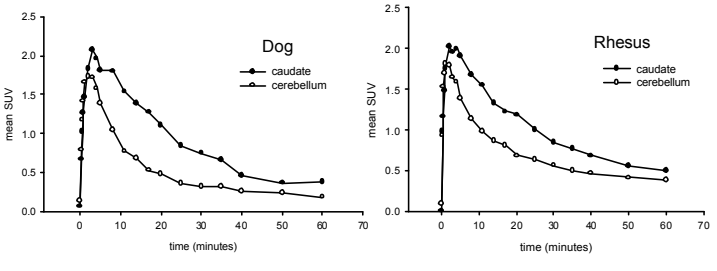

Figure 2. Time Activity Curves in Dog and Rhesus Monkey.

1. Cosford NDP, Roppe J, Tehrani L, Schweiger EJ, Seiders TJ, Chaudary A, Rao S, Varney MA. *Bioorg Med Chem Lett* 2003; 13: 351-354.

SYNTHESIS AND BIOLOGICAL EVALUATION OF ^{131}I- AND ^{18}F-LABELLED PYRAZOLO[1,5-*a*]PYRIDINES AS SUBTYPE SELECTIVE D4 RECEPTOR LIGANDS FOR SPECT AND PET

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Keywords: D4 receptor, pyrazolo[1,5-*a*]pyridine, F-18, autoradiography

The D4 subtype of the dopamine receptors has been recently cloned (1); its precise function and exact distribution in the central nervous system are of great interest, as disturbances of this receptor subtype have been implicated in the genesis of a broad range of psychotic disorders such as schizophrenia. As yet, there is no suitable radioligand for the non-invasive assessment of the dopamine D4 receptors in vivo. We aim at filling this gap by developing selective D4-radioligands in order to determine the receptor density and affinity in vivo by PET and SPECT.

The radiosynthesis of [4-(4-[^{18}F]fluoroethoxyphenyl)-piperazin-1-ylmethyl]-pyrazolo[1,5-*a*]pyridine (4-[^{18}F]FPMP) and the biological evaluation of [4-(4-[^{131}I]iodophenyl)-piperazin-1-ylmethyl]-pyrazolo[1,5-*a*]pyridine (4-[^{131}I]IPMP) and [4-(4-chlorophenyl)-piperazin-1-ylmethyl]-7'-[^{131}I]iodopyrazolo[1,5-*a*]pyridine (7'-[^{131}I]IPMP) in comparison to 4-[^{18}F]FPMP are presented. The design of these candidates as SPECT and PET radioligands was essentially supported by SAR-studies (3). Receptor binding assays were performed using human D2R, D3R and D4R expressed in CHO-cells and striatal bovine membranes (D1R) for evaluating receptor affinities and selectivities. [^{3}H]Spiperone and [^{3}H]SCH23390 were used for competition binding analysis. For biodistributional studies Sprague-Dawley rats were sacrificed by decapitation at designated times (5, 10, 20 min). Autoradiography of rat brain slices was analysed by using the MicroImager (Biospace).

4-[^{131}I]IPMP and 7'-[^{131}I]IPMP were obtained as described before (2). Radiosynthesis of 4-[^{18}F]FPMP by the use of [^{18}F]fluoroethyltosylate in DMF gave an overall yield of 50% within 30min. Moreover, the aromatic ^{18}F-fluorination of the corresponding 7'-bromo precursor was possible yielding 7'-[^{18}F]FPMP ([^{18}F]F^{-}, DMSO, 140°C, 10%). The molar activity of ^{18}F-labelled ligands was 2.2 Ci/µmol, radiochemical purity for all radioligands was >95% (HPLC). Binding assays demonstrated that 4-[^{131}I]IPMP and 4-[^{18}F]FPMP are highly selective for the D4 receptor and have high receptor affinities (K_i(D4R)= 3.1 - 5.8nM). 7'-[^{131}I]IPMP (K_i(D4R)=2.6nM) showed a further improved D4/D2-selectivity (pK_i(D2/D4)>3.4) as compared to the other putative D4-ligands. Biodistributional studies revealed rapid clearance of 7'-[^{131}I]IPMP in kidney and heart. Brain uptake of 7'-[^{131}I]IPMP was highest in frontal cortex when compared to cerebellum. Maximum cortex-to-cerebellum ratio was 1.6 after 10min as supported by autoradiography. Pretreatment experiments using L-750,667 had no significant effect on cortex-to-cerebellum ratio, but a more homogeneous distribution in other brain regions was observed.

Due to our in vitro and preliminary in vivo results 4-[^{131}I]IPMP and 7'-[^{131}I]IPMP are potential SPECT tracers when labelled with iodine-123. In addition, a ^{18}F-fluorinated pyrazolo[1,5-*a*]pyridine (4-[^{18}F]FPMP) of higher specific activity as a putative dopamine D4 receptor ligand suitable for PET is now available for further in vivo studies.

Acknowledgement
This work is supported by the Deutsche Forschungsgemeinschaft (DFG) grant PR677/2.

References

1. Van Tol HHM et al. *Nature* 1991; 350: 610-614.
2. Löber S, Hübner H, Gmeiner P. *Bioorg Med Chem Lett* 1999; 9: 97-102.
3. Prante O, Löber S et al. *J Labelled Cpd Radiopharm* 2001*;* 44: 849-858.

SYNTHESIS OF F-18 LABELED *N,N*-DIMETHYL-2-(ARYLTHIO)BENZYLAMINES AS SEROTONIN TRANSPORTER IMAGING AGENTS

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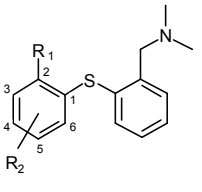
Keywords: F-18, Serotonin Transporter Imaging, PET

There has been considerable interest in the development of PET radioligands that are useful for imaging serotonin transporters (SERT) in the living human brain. For the last decade, [^{11}C](+)McN 5652 is the most promising PET agent for studying SERT in humans. However, this agent has some limitations. We recently reported the synthesis of a new F-18 labeled SERT PET radioligand, *N,N*-dimethyl-2-(2-amino-4-[^{18}F]fluorophenylthio)benzylamine (4-[^{18}F]-ADAM, **2**) (1) which may have advantages compared to C-11 labeled radioligands. The purpose of this study was to synthesize 5-[^{18}F]-ADAM (**4**) and compare it with compound **2** as a SERT imaging agent.

Compound **4** was synthesized by nucleophilic substitution of the multi-step synthesized *N,N*-dimethyl-2-(2-nitro-5-bromophenylthio)benzylamine (**3**) with K[^{18}F]/Kryptofix $_{2.2.2}$ in DMSO at 130^{0}C for 25 min followed by reduction with $NaBH_4$-$Cu(OAc)_2$ in EtOH at 78^{0}C for 20 min and purification with HPLC (10 x 250 mm, Phenomenex Luna 2; CH_3CN:0.1 M HCO_2NH_4 (30:70) containing 0.3 v% of acetic acid; 5 ml/min) in ~ 5% yield with a synthesis time of 150 min from EOB (Figure 1).

Biodistribution studies of **4** were performed in Sprague-Dawley rats (n=3) at 2, 30, 60 and 120 min. Organs and different brain regions were dissected, weighed and the radioactivity in each tissue was measured in a NaI detector and expressed as % injected dose/g tissue. For blocking experiments, rats were pre-treated with (+)McN 5652 (2 mg/kg, iv) 5 min prior to the tracer administration (iv) and sacrificed 1 h post-injection. The results showed a high initial uptake of **4** in all organs studied, followed by rapid wash-out. The initial uptake in the brain was also high (1.77%/organ at 2 min post-injection) and then declined rapidly (0.09 and 0.02%/organ, respectively, at 60 and 120 min post-injection).The ratios of the radioactivity in the target tissue/cerebellum were 3.37 and 1.63 for hippocampus, 2.78 and 2.65 for hypothalamus, and 2.60 and 1.87 for thalamus, respectively, at 1 and 2 h post-injection. The corresponding ratios for **2** were 4.26 and 5.97 for hippocampus, and 5.57 and 12.49 for hypothalamus. Pre-treatment of rats with (+)McN 5652 inhibited the uptake of **4** in the SERT-rich areas. The ratios of target tissue/cerebellum were reduced from 3.37 to 1.80 for hippocampus, from 2.78 to 1.40 for hypothalamus, and from 2.60 to 1.20 for thalamus, respectively.



1. $R_1 = NO_2$, R_2 = 4-NO_2;
2. $R_1 = NH_2$, R_2 = 4-^{18}F;
3. $R_1 = NO_2$, R_2 = 5-Br;
4. $R_1 = NH_2$, R_2 = 5-^{18}F.

Figure 1

In conclusion, compound **4** binds with high uptake to SERT-rich areas, but washes out from brain rapidly. Compared to compound **2**, compound **4** may not be as useful a SERT imaging agent as **2**.

1. Shiue GG, Fang P, Shiue C-Y. *Appl Radiat Isot* 2003; 58: 183-1

STRUCTURE ACTIVITY RELATIONSHIPS OF NEW VESAMICOL DERIVATIVES WITH RESPECT TO THEIR BINDING CAPACITY TO THE VESICULAR ACETYLCHOLINE TRANSPORTER IN BRAIN TISSUE

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Keywords: vAChT, vesamicol derivatives, binding affinity, PET

Aim: The brain vesicular acetylcholine transporter protein (vAChT) is considered to be a powerful target structure for binding of vesamicol (phenylpiperidinylcyclohexanol) derived PET radioligands. Suitable ^{18}F labeled vesamicol derivatives in combination with PET represent a valuable tool in diagnosis and follow up of patients with cholinergic deficits.The aim of the study was to evaluate binding affinity and specificity of potential PET ligands derived by systematic chemical modifications of the ring structures of the vesamicol molecule.

Methods: We studied a) six 4-O-fluorobenzylether of vesamicol: I, II, III, IV, V (piperazine instead of piperidine), VI (cyclohexyl opened) b) VII: 4-O-fluoropropylether of vesamicol c) VIII: fluorobenzylmorpholine derivative of vesamicol in concentrations from 10^{-12} M to 10^{-5} M for their potency to compete with [^{3}H]vesamicol binding to rat brain membranes. To evaluate any non-specific binding to sigma receptors (described to be present in many parts of the brain), the sigma receptor-specific ligand 1,3-Di-O-tolylguanidine (DTG) was used as displacer. The affinities of the compounds cited to sigma receptors were measured by their competition with [^{3}H]DTG binding in liver tissue membranes.

Results: a) I, III, IV competed with high affinity to [^{3}H]vesamicol binding sites (K_i: 10 – 50 nM). Methylation of the piperidine ring (II), opening of the cyclohexyl ring (VI) and exchange of piperidine by piperazine (V) slightly reduced the affinity. b) Exchange of benzyl moiety of the ether compounds by a propyl function (VII) restored high affinity to vAChT. c) Modification of vesamicol to a morpholine derivative (VIII) impaired the affinity to vAChT.
Competition curves were best fitted by a one site competition nonlinear regression. Displacement experiments with [^{3}H]DTG in liver tissue revealed differential affinities to sigma binding sites of the compounds tested, while in rat brain tissue (excluding cerebellum) sigma receptors could be detected only at low level. Blocking of sigma binding sites in brain with 200 nM of DTG was without effect on curve fitting and K_i-values when tested with [^{3}H]vesamicol for V and VIII.

Conclusion: Four of the eight compounds tested showed a promising high affinity binding to the vAChT, but exhibited also binding capacities to sigma receptors. It remains to be elucidated whether this lack of specificity may affect their usefulness in quantitative vAChT imaging by PET.

SYNTHESIS AND EVALUATION OF [^{11}C]M-FPEP AS A PET LIGAND FOR IMAGING THE METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 5 (MGLUR5)

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Key words: [^{11}C]M-FPEP, mGluR5, biodistribution, PET

Research in the area of metabotropic glutamate receptors (mGluRs) suggests that modulators of the subtype mGluR5, which belongs to class I of the mGluRs, could be potential candidates for a variety of brain disorders such as pain, epilepsy, focal and global ischemia and neurodegenerative diseases. To date no useful PET ligand exists for the *in vivo* imaging of these glutamate receptors. In the pursuit of suitable PET imaging agents for the mGluR5, we prepared carbon-11 labelled 2-methyl-6-(3'-fluoro-phenylethynyl)-pyridine ([^{11}C]M-FPEP) and evaluated its potential as a PET imaging agent.

The radiosynthesis of [^{11}C]M-FPEP was accomplished in a two-step reaction sequence as depicted in Fig. 1. The appropriate bromo precursor was reacted in THF at –85°C with butyllithium for 5 min. $^{11}CH_3I$ was added at –85°C to the lithium salt and the mixture was warmed up to 45°C. After a reaction time of 8 min, the reaction mixture was quenched with water and the product was purified by a reversed-phase HPLC using a C-18 μ-Bondapak column and a mobile phase consisting of 0.01M H_3PO_4/MeCN (7:3) at a flow of 5 ml/min. The total synthesis time was 50 min and radiochemical purity was greater than 98%. Radiochemical yield was on average 10 % and specific radioactivity ranged from 2700-3400 Ci/mmol. Using the shake-flask method with octanol and phosphate buffer (pH 7.4), a log P value of 2.7 was obtained for [^{11}C]M-FPEP.

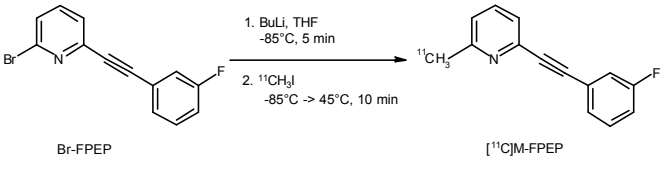

Fig. 1: Radiosynthesis of [^{11}C]M-FPEP.

Scatchard analysis of [^{11}C]M-FPEP binding to rat brain homogenates (whole brain without cerebellum) revealed a single high affinity binding site with a K_D of 1.2 ± 0.1 nM and a B_{max} of 84.5±18.5 fmol/mg protein. [^{11}C]M-FPEP was injected i.v. into rats and the tissue distribution was measured at 15, 45 min postinjection (p.i). Brain radioactivity levels at 15, 45 min were 0.14 and 0.06% ID/g, respectively. Radioactivity uptake was similar for all the brain regions (hippocampus, striatum, cortex, midbrain and cerebellum) examined. A PET study using the ultra-high resolution QUAD HIDAC scanner in a rat brain showed a rapid and high initial uptake. Radioactivity peaked at 3 min p.i. and was followed by a fast wash-out phase. [^{11}C]M-FPEP binding in rat brain reached a plateau after 50 min and remained at this level for the rest of the study. Blockade studies are currently underway to determine receptor-specific uptake.