SYNTHESIS AND DIRECT FLUORINATION OF LBT-999 AND NEW CONFORMATIONALY RESTRICTED ANALOGUES

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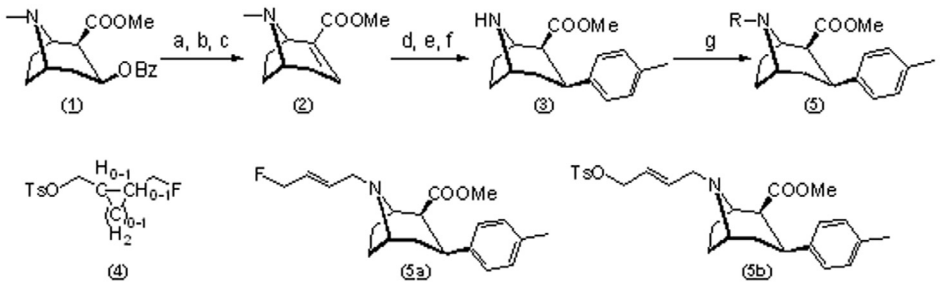
Introduction: The dopamine transporter (DAT) plays a key role in the regulation of dopaminergic signal transduction. Several neurodegenerative disorders like Parkinson's disease (PD) are characterised by an altered DAT availability. In the case of PD, a diminished DA biosynthesis effects a significant increase of available DAT binding sites. Radioligands for quantification of DAT availability are of high clinical relevance for early diagnosis of PD. 3-exo-phenyl tropane serves as lead structure for selective DAT ligands. LBT-999 **5a**, a N-4-fluorobut-2-en-1-ylated tropane derivative, exhibits excellent affinity to the DAT (K_i = 9.4 nM) together with high selectivity (SERT/DAT >100 and NET/DAT >100)[1-4]. This may be due to its conformational restricted moiety at the bridge nitrogen. We intended a systematic variation of conformational restricted LBT-999 analogues to be prepared and evaluated for DAT affinity and selectivity. In addition we decided to prepare a new labelling precursor **5b** for efficient direct nucleophilic fluorination.

Experimental: 2 was prepared from cocaine **1** as published elsewhere. Addition of toluene magnesium bromide and subsequent N-demethylation afforded compound **3**. **3** was alkylated with appropriate ω-fluoro-halides to yield **5** as references. Labelling precursors were obtained via tosylation of ω-hydroxy analogues. ^{18}F was introduced via the common ^{18}F-cryptate procedure in moderate to high yields.

Results and Discussion: 5a and **5b** have been prepared as reference and labelling precursor, respectively. Instead of the established two-step synthesis via 4-[^{18}F]fluorobut-2-en-1-yl tosylate[1,3], a labelling precursor for direct nucleophilic radio-fluorination provides [^{18}F]LBT-999 in an easy and efficient reaction. In addition, we have prepared new conformational restricted N-substituted analogues of LBT-999. All new compounds are well suited for direct radio-fluorination and fluoroalkylation.



a) HCl, reflux; b) POCl$_3$, reflux; c) MeOH, -78°C to RT; d) 4-MePheMgBr, -40°C, Et$_2$O/CH$_2$Cl$_2$; e) -78°C, TFA; f) ACE-Cl, MeOH; g) 4, dioxane, MW

Conclusion: A promising set of new tropane derivatives containing a conformational restricted C_4-chain has been prepared to be evaluated as DAT ligands. In addition, an improved labelling precursor for efficient synthesis of [^{18}F]LBT-999 is now available for comparative small animal PET studies of dopaminergic signal pathways, involving 6-L-[^{18}F]FDOPA and [^{18}F]FP.

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Keywords: DAT, LBT-999, Parkinson's Disease, Fluorine-18, Conformational Restriction

FLUORINE-18 RADIOLABELING OF S100/CALGRANULINS: A POTENTIAL APPROACH FOR CHARACTERIZATION OF RECEPTORS OF ADVANCED GLYCATION END PRODUCTS IN VIVO

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Introduction: The interaction of S100/calgranulins, a multigenic family of Ca2+-modulated proteins, with receptors for advanced glycation endproducts, e.g., RAGE and AGERs, is hypothesized to be of high relevance in the pathogenesis of various diseases including cardiovascular diseases, inflammatory processes, and cancerogenesis. However, data concerning the role of circulating S100/calgranulins in these pathologies are scarce. Furthermore, it is currently not known whether RAGE or AGERs are universal S100/calgranulin receptors in vivo. One reason for this is the shortage of suitable radiolabeling methods for direct assessment of metabolic pathways of S100/calgranulins in vivo. We report a novel radiotracer approach using radiolabeling of recombinant human S100A1 with fluorine-18 (18F) and the application of 18F-labeled S100A1 (18F-S100A1) in dynamic small animal positron emission tomography (PET) studies in rats.

Experimental: Human S100A1 was cloned in the bacterial expression vector pGEX-6P-1 and expressed in E. coli strain BL21. Radiolabeling of purified S100A1 was performed by conjugation with N-succinimidyl-4-(18F)fluorobenzoate ([^{18}F]SFB). 18F-S100A1 was used for investigations of stability in vitro and in vivo. The metabolic fate of 18F-S100A1 in rats in vivo was delineated by dynamic PET studies using a dedicated small animal PET system.

Results and Discussion: Radiolabeling of S100A1 with [^{18}F]SFB at pH 7.4 resulted in 18F-S100A1 specifically labeled at the N-terminal glycine residue with radiochemical yields of 2-6% (decay-corrected) and effective specific activities of 0.5-1 GBq/μmol, respectively. In vitro experiments, and biodistribution and metabolite studies in rats in vivo revealed high stability for the 18F-S100A1. The organ-specific in vivo distribution and kinetics of 18F-S100A1 correlated well with the anatomical localization of receptors for advanced glycation endproducts, e.g., in blood vessels and lungs. In the presence of glycated human low density lipoprotein (glycLDL), a well characterized RAGE ligand, mean plasma residence time of 18F-S100A1 increased by 40% from 29.6±1.5 min to 41.3±2.1 min and lung associated retention of 18F-S100A1 decreased by 57% first indicating circulating S100A1 to be a specific ligand for receptors for advanced glycation endproducts in rats in vivo. Data were compared to former small animal PET studies using the 18F-labeled glycLDL.

Conclusion: Radiolabeling of S100/calgranulins with 18F and the use of small animal PET provides novel probes to delineate functional expression of RAGE and AGERs under normal and pathophysiological conditions in rodent models of disease in vivo.

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Keywords: S100 Proteins, Small Animal PET, Inflammation, Cancerogenesis, Receptors for Advanced Glycation End Products

RAPID AND EFFICIENT MICROFLUIDIC PRODUCTION OF 3'-DEOXY-3'-([18]F)FLUORO-THYMIDINE (([18]F)FLT)

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Introduction: There exists a need for novel probes for biomedical applications and radiolabeled compounds for Postitron Emission Tomography (PET) as well as a need for advanced technology to deliver them in a timely manner. Conventional syntheses of PET radiotracers are hampered by complex and sensitive synthetic processes, which often produce low yields, consume costly precursors, and may take hours to complete. While [^{18}F]-2-fluorodeoxyglucose (FDG) is the workhorse of many nuclear medicine departments, there exists a barrier to introducing other potential PET biomarkers. We report the rapid and efficient preparation of 3'-deoxy-3'-[^{18}F]fluoro-thymidine (FLT) using a flow-based microfluidic chemistry system.

Experimental: A MinuteMan LF (NanoTek), liquid-flow microfluidic reactor system, was used to label, hydrolyze and purify[^{18}F]FLT from a commercially available thymidine precursor (#124 ABX - Germany). The MinuteMan LF is a modular flow-based microreactor assembly, capable of up to four reaction steps with in-line purification via HPLC based column. Syringe pumps were used to load and drive reagents through the glass microreactors at pressures up to 50 bar with microliter precision.

Reagent solutions were prepared and loaded into the MinuteMan LF: reagent cartridge #1 was loaded with FLT precursor dissolved in anhydrous MeCN, reagent cartridge #2 was loaded with 1N HCl, and reagent cartridge #3 was loaded with no-carrier-added [^{18}F] fluoride ion in MeCN, produced from [^{18}O]-enriched target water, trapped on a micro MP-1 ion exchange column and released with a solution of kryptofix$_{222}$ and K_2CO_3 in 10% water in MeCN followed by the azeotropic removal of water at 110°C.

The microreactor temperatures for labeling and hydrolysis were set to 180°C and 60°C, respectably. Using reagent flow rates from 20 to 120 μL/min, [^{18}F]FLT was labeled and hydrolyzed within 1.6 minutes. The product flow was diverted to a C18 HPLC column for purification.

Results and Discussion: The rapid preparation and purification of [^{18}F]FLT was achieved in under 4 minutes with high radiochemical yields of 82% +/- 5% (decay corrected) and radiochemical purities >98%.

Conclusion: The average reported decay corrected yield for the production of [^{18}F]FLT is 55% using the standard macroscale labeling procedures. Compared to these methods, the microfluidic-based production of [^{18}F]FLT has been shown to be an improvement in both yield and time of synthesis.

Acknowledgement: Research support has been provided through a grant from the Department of Energy (DOE84290).

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Keywords: Microfluidics, PET Radiochemistry, Fluorine-18

SOLUBLE SUPPORTS AND THE PREPARATION OF RADIOPHARMACEUTICALS IN HIGH EFFECTIVE SPECIFIC ACTIVITY

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Introduction: With the increasing demand for targeted radiotracers and therapeutics there is a concomitant need for new methods of producing compounds in high effective specific activity. This is particularly relevant for protein targets expressed in low concentrations.

HPLC can be used to produce materials in high effective specific activity but it is not a convenient technique for routine clinical applications. As such, a number of solid-phase labelling strategies have been developed in which a substrate is bound to an insoluble polymer in such a manner that upon reaction with a radioisotope only the desired compound is released into solution. The residual support-bound ligand can be removed by simple filtration. Although convenient and easily automated, the limitation to this approach is that the loading of the precursor onto the support must be quantitative as there is no mechanism to remove impurities when working with cross-linked resins. In consequence, we have developed an analogous system using soluble supports which, unlike solid-phase approaches, is amenable to the same purification and characterization techniques employed for small molecules.

Experimental: The soluble supports were constructed by replacing the alkyl groups in trialkylstannanes with perfluorooctyl chains (i.e. fluorous supports). These were introduced into a series of aryl derivatives and the products purified by traditional means and fully characterized by IR and NMR spectroscopy, HPLC and ESI-MS.

Labeling reactions were performed using $Na^{125}I$ and iodogen and the targets isolated by fluorous solid-phase extraction (FSPE). The perfluorinated stationary phase was used to extract the starting material and other fluorous byproducts while the desired compounds were selectively eluted using alcohol-water mixtures. This approach was used to prepare a wide range of iodinated materials including high effective specific activity *meta*-[^{125}I]-iodobenzylguanidine (MIBG).

Results and Discussion: The fluorous labelling methodology (FLM) proved to be a convenient purification strategy in all cases tested. Reactions and purifications were typically completed in less than five minutes in essentially quantitative radiochemical yield. FLM is highly versatile in that it suitable for use with a wide range of isotopes. Moreover, it can be employed to prepare libraries of compounds thereby creating a new and efficient means of discovering lead agents.

Conclusion: The FLM is a convenient method for the preparation of known tracers in high effective specific activity and for the development of novel leads.

Acknowledgement: ORDCF and NSERC of Canada for funding.

Keywords: Specific Activity, Fluorous, SPE, Radioiodine, MIBG

LABELLING AND IN VIVO EVALUATION OF THE (^{123}I) LABELLED IMIDAZOPYRIDINE-3-ACETAMIDE, CLINME FOR THE STUDY OF THE PERIPHERAL BENZODIAZEPINE BINDING SITES

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Introduction: Peripheral benzodiazepine binding sites (PBBS) are implicated in a number of neurodegenerative disorders, inflammatory processes and cancers. The development of specific tracers for imaging these conditions using PET and SPECT would be highly desirable. Appropriate substitution on the imidazopyridine chemical structure has provided excellent opportunities for the development of specific PET and SPECT tracers.

The aim of this study was to prepare and evaluate the lead [^{123}I]-imidazopyridine: *N*,*N*-methylethyl-6-chloro-(4'-iodophenyl)imidazo(1,2-a)pyridine-3-acetamide (CLINME), as a probe for the study of PBBS's in oncology and neurodegeneration using SPECT.

Experimental: [^{123}I]-CLINME has been prepared by classical iododestannylation reaction in the presence of peracetic acid. The biodistribution of [^{123}I]-CLINME was undertaken in SD rats and analysis up to 6 h p.i. in the brain and peripheral tissues was performed. Pre-treatment with PPBS specific ligands (1 mg/kg) 5 min prior to injection of [^{123}I]-CLINME was carried out. Metabolite studies using radio-t.l.c were performed in plasma, adrenals, kidney and heart up to 3 h p.i.

Results and Discussion: In vitro binding revealed that CLINME is a selective PBBS ligand (IC_{50} = 3.2 nM) with significantly lower binding to the central benzodiazepine receptors (IC_{50} = 366 nM). [^{123}I]-CLINME was synthesised in 72-80% radiochemical yield and >95% radiochemical purity. The in vivo biodistribution of [^{123}I]-CLINME indicated high uptake in tissue of known PBBS distribution with peak uptake in the adrenals of (8.3% ID/g) after 6 h. In the kidney, heart and lungs, the activity peaked at 5 min p.i. (1.8, 3.5 and 7.9% ID/g) and decreased over time to less than 0.7% ID/g at 6h. In the olfactory bulbs the activity uptake varied from 0.38 to 0.2% ID/g throughout the experiment with very low concentrations in blood (< 0.1% ID/g). Pre-treatment with PK 11195 and Ro 5-4864 reduced significantly the radioligand uptake in brain and peripheral organs whilst Flumazenil a specific CBR ligand had no effect. Metabolite analysis confirmed that in the kidney, heart, brain and adrenals more than 95% of the extractable radioactivity was shown to be unchanged [^{123}I]-CLINME. In plasma, metabolite analysis showed that only 15, 5 and 2% of the extracted activity was unchanged tracer, 15min, 1 h and 3 h p.i. respectively.

Conclusion: These results demonstrate the specific PBBS uptake of [^{123}I]-CLINME in vivo. This suggests that [^{123}I]-CLINME warrants further investigation as a potential SPECT marker for the PBBS.

Keywords: Peripheral Benzodiazepine Binding Sites, Imidazopyridines, Iodine-123, SPECT

Rh(16aneS4)^{211}At AND Ir(16aneS4)^{211}At COMPLEXES AS POSSIBLE PRECURSORS FOR ASTATINE RADIOPHARMACEUTICALS

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Introduction: Targeted radiotherapy with alpha particle emitters is a promising method for treating tiny clusters of cancer cells, because of their short tissue range and high radiobiological effectiveness. The radiohalogen ^{211}At is a potentially important alpha emitter for cancer therapy. The current study is focused on finding a stable labeled prosthetic group for use in labeling biomolecules with ^{211}At. Our idea is to attach astatide anion to soft metal cations, which are complexed by a bifunctional ligand. Rhodium and iridium were evaluated because they are moderately soft metal cations, which should form very strong bonds with the soft astatide anions. The high kinetic inertness of the low-spin d^6 Rh(III) and Ir(III) complexes is an additional advantage for the formation of a stable conjugate.

Experimental: The macrocyclic sulfur ligand 1,5,9,13-tetrathiacyclohexadecane-3,11-diol (16aneS4) was chosen for these model studies because it can form a stable complex with Rh(III) and possibility be modified to create a bifunctional chelate ligand[1]. First, optimization of reaction conditions with ^{125}I/^{131}I was performed. Complexes between ^{211}At, and Rh(III) or Ir(III), and the thioether ligand were synthesized in an aqueous-ethanolic solution. The formation of complexes was studied by heating solutions for different time periods (15-120 min) and over a wide range of temperature (30-90°C). The quantity of Rh(III)/Ir(III) and 16aneS4 was evaluated in the range of 0.125-125 nmol and 2.5–250 nmol, respectively. Formation and the radiochemical yield of the complexes were estimated by paper electrophoresis, ion exchange and HPLC. Reversed phase HPLC and Sep-Pak (C18) columns were used to isolate the complexes from the reaction mixture. The *in vitro* stability of the complexes was determined in PBS at 37°C with ^{131}I isotope as an analogue of ^{211}At.

Results and Discussion: Maximum complex formation yields 75% and 80% for Rh[16aneS4]^{211}At and Ir[16aneS4]^{211}At, respectively, were obtained at pH 3-5 after heating for 1-1.5 h at 75-80°C with Rh(III) or Ir(III) concentration of 62.5 nmol and 16aneS4 - 250 nmol. Paper electrophoresis analysis indicated expected cationic character of the complexes. Both Rh(III) and Ir(III) complexes with ^{131}I showed good stability in pH 7.4 PBS after 51 h of the studies. Paired-label ^{211}At and ^{131}I biodistribution studies with Rh(III) complex in normal mice are in progress.

Conclusion: The obtained results are promising for further studies on attaching complexes to biomolecules.

Reference: [1] M. Venkatesh, et al., Nucl.Med.Biol., **23**, 33 (1996).

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Keywords: Astatine, Alpha Emitters

IAEA'S CURRENT ACTIVITIES AND FOCUS IN RADIOPHARMACEUTICAL SCIENCES OF RELEVANCE TO DEVELOPING MEMBER STATES

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Introduction: The International Atomic Energy Agency (IAEA) has been supporting interested Member States (MS) in capacity building in the production and utilisation of radiopharmaceuticals. A number of coordinated research projects (CRP) and technical cooperation (TC) projects, national and regional projects, are being implemented in this regard. Thanks to the IAEA's interaction with national laboratories and support of MS authorities, several developing MS have established and/or strengthened their expertise in the development, production and evaluation of radiopharmaceuticals. The availability of a strong cadre of radiopharmaceutical scientists in developing MS has been facilitated by the efforts of the IAEA.

Results and Discussion: The production of radiopharmaceuticals involves a number of aspects starting from the raw material medical radioisotope handling, often in large quantities, labelling or synthesis, purification, in-process control and all the processes to be done in compliance with cGMP. This is a demanding task, especially for small-scale operations and national labs and the IAEA has been helping by providing technical documents, training events and experts for consultancy. With growing interest in establishing medical cyclotron facility and production of ^{18}FDG, as well as in the products for radionuclide therapy (RNT), the demands have increased. There are challenges in ensuring a high degree of reliability of the yield and purity of ^{18}F for FDG production. Better understanding of the behaviour of targetry and associated systems is necessary in this regard. The IAEA's CRP on targetry are deovted to address this theme. There is also a need to foster development of a few other promising PET tracers for regular use and a CRP is planned based on consultants advice.

In the case of RNT, the on-going support for generator derived therapeutic radionuclides - ^{90}Y, ^{188}Re - has helped evaluate the optimal conditions and the best practices in the production, QC testing and operation of the generators. The major advantages of ^{177}Lu for world-wide deployment are driving other current efforts. A new CRP is underway to support the development of select group of important ^{177}Lu products, while a parallel CRP handled by nuclear medicine colleagues in the IAEA is focussed on the clinical evaluation of ^{177}Lu-EDTMP for bone pain palliation in prostate cancer patients.

Conclusion: The IAEA continues to play a catalytic role to help interested MS to benefit from advances in radiopharmaceutical sciences in developing, evaluating and adopting relevant new products and practices. The results of on-going CRP to update the nuclear data relevant to production, purity and decay characteristics of medical radionuclides will add synergistic strength.

Keywords: F-18 Products, Medical Cyclotron, Radionuclide Therapy, Lu-177 Products, Coordinated Research Project