P318 [11C]CARBON MONOXIDE IN 11C-LABELLING OF AZABICYCLIC ARYL AMIDES, THE AGONISTS FOR α7 NICOTINIC ACETYLCHOLINE RECEPTORS

O. RAHMAN*[1] and B. LANGSTROM[2]

1. GE Healthcare, Uppsala Applied Science Laboratory, Uppsala, Sweden; 2. Uppsala University, Department of Biochemistry and Organic Chemistry, Uppsala, Sweden

Objectives: Carbonylation using [^{11}C]carbon monoxide and Synthia technique have been employed in ^{11}C-labelling of a wide range of carbonyl compounds.[1] This report describes ^{11}C-labelling of azabicyclic aryl amides which are potent and selective agonists of alfa7 nicotinic acetylcholine receptors.[2] These receptors are abundantly present in telencephalic regions such as hippocampus in the brain and can play a role in the pathophysiology of neuropsychiatric disease such as schizophrenia, Alzheimer's disease, anxiety etc.[3] The ^{11}C-labelling of the ligands for these receptors will give an opportunity to study the functions of these receptors using PET tchnique.

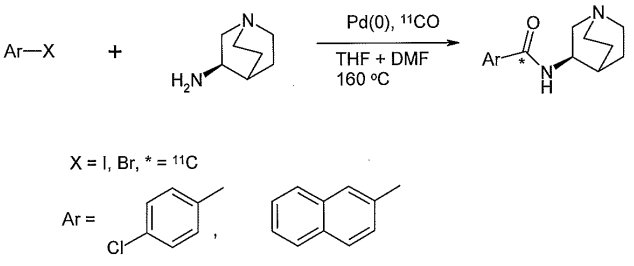
Methods: Tetrakis(triphenyl)phosphine)palladium (2.6 μmol) and aryl halides (iodides or bromides) (21.0 μmol) were taken in a vial, flashed with nitrogen and dissolved in anhydrous THF (200 μL). 1-Azabicyclo(2.2.2)oct-3-ylamine dihydrochloride (8.0 mg, 40.2 μmol) was was taken in another vial, flashed with nitrogen, dissolved in anhydrous DMF (200 μL) and treated with PMP (15 μL). The two reagent solutions were mixed together, filtered and loaded into the injection loop of the instrument from where the mixture was transfered with pressure (35 Mpa) into the micro-autoclave pre-charged with [^{11}C]carbon monoxide in helium. The micro-autoclave was heated for 5 min at 160 °C. The crude product was transfered into a vial and TFH was removed by heating at 50 °C and flashing with nitrogen at the same time. The residue was dissolved in acetonitrile (1 mL) and injected into semi-preparative HPLC system for purification.

Results: Two azabicyclic aryl amides, N-(1-azabicyclo[2.2.2]oct-3-yl)-4-chlorobenzamide and N-(1-azabicyclo[2.2.2]oct-3-yl)-1-naphthamide were labelled with ^{11}C at carbonyl position using low concentration of [^{11}C]carbon monoxide and micro-autoclave technique. Palladium mediated carbonylation using tetrakis(triphenylphosphine)palladium, aryl bromide or iodide and 1-azabicyclo[2.2.2]oct-3-ylamine was employed in the synthesis (Scheme 1). The ^{11}C-labelled products were obtained with 50-55% decay-corrected radiochemical yields and more than 98% radiochemical purity.

Conclusions: The presented approach is a novel method for the synthesis of a series of ^{11}C-labelled analogues of azabicyclic aryl amides which are potent and selective agonists of alfa7 nicotinic acetylcholine receotors.

Research Support: This work was supported by Uppsala Applied Science Laboratory, GE Healthcare.

References: [1] Langstrom B, Itsenko O, Rahman O, J Label Compd Radiopharm 2007; 50: 794-810. [2] Walker D P, Wishka D G, Jia S, et al. Bioorg Med Chem 2006; 14: 8219-8248. [3] Nordberg A. Biol Psychiatry 2001; 49: 200-210.



Scheme 1. Preparation of ^{11}C-labelled azabiclic aryl amides

P319 [CARBONYL-^{11}C]BENZYL ACETATE: SYNTHESIS FROM [^{11}C]CARBON MONOXIDE AND PET EVALUATION OF BRAIN UPTAKE IN MONKEY

S. LU*, J. HONG, T. ITOH, M. FUJITA, R. B. INNIS and V. W. PIKE
National Institutes of Health, NIMH, Molecular Imaging Branch, Bethesda, MD

Objectives: Radiolabeled acetate is a potential marker of glial metabolism in vivo, but its brain uptake is inadequate. [^{14}C] Benzyl acetate, among several ^{14}C-labeled esters of acetate, has shown much higher uptake and retention than [^{14}C]acetate in rat brain [Momosaki et al. Nucl Med Biol, 2007, 34, 939]. [carbonyl-^{11}C]Benzyl acetate ([^{11}C]BA) is therefore a possible pro-radiotracer for [^{11}C]acetate that might be used to image glial metabolism in brain with PET. Here a palladium-mediated method [Kihlberg and Langström. J Label Compd Radiopharm, 2001, 44, S990] was developed for the radiosynthesis of [^{11}C]BA from [^{11}C]carbon monoxide (Fig. 1). The brain uptake of [^{11}C]BA was assessed with PET imaging in rhesus monkey.

Methods: [^{11}C]CO was obtained by single pass of cyclotron-produced [^{11}C]CO_2 over a mixture of Mo wire and powder in a quartz tube at 845 °C. MeI (0.08 mmol), benzyl alcohol (0.08 mmol) and Pd(PPh_3)$_4$ (0.0014 mmol) were premixed in THF (150 μL) and loaded into an autoclave. The mixture was heated with [^{11}C]CO at 140 °C for 2 min. The reaction mixture was then flushed out with THF (0.7 mL) and diluted with water (1.3 mL) before being injected onto a semi-preparative size reverse phase column eluted at 4 mL/min with a linear gradient of H_2O (A)-MeCN (B), starting with 30% B for 4 min and then increasing to 60% B in 2 min. The product fraction eluting between 14.3 and 15.0 min was collected. Acetonitrile was removed at room temperature under reduced pressure. [^{11}C]BA was formulated in saline containing 10% EtOH and filtered through a 0.45 μm sterile filter. The procedure was fully automated using a modified Synthia module under control with Labview-based software. For PET imaging, [^{11}C]BA (5.99 mCi, 0.43 μg of carrier) was injected into a rhesus monkey (11.28 kg) and the radioactivity in brain monitored for 2 h on an HRRT camera.

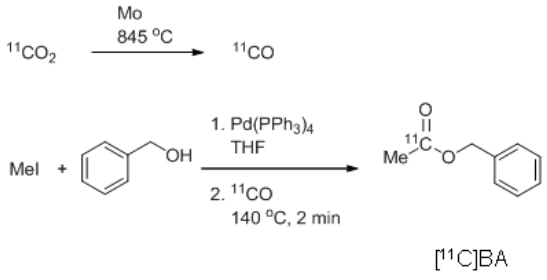


Fig. 1 Synthesis of [carbonyl-^{11}C]benzyl acetate ([^{11}C]BA) with Pd-mediated [^{11}C]CO chemistry

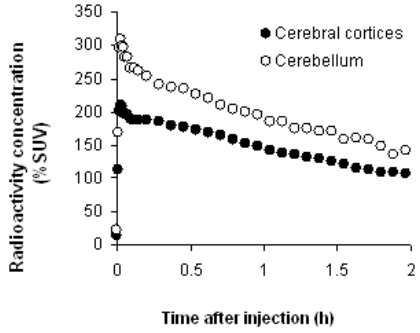


Fig. 2 Rhesus monkey brain time-activity curve after i.v. injection of [^{11}C]BA (5.99 mCi, 0.43 μg carrier)

Results: [^{11}C]BA was obtained in 8.4% (n = 17) decay-corrected radiochemical yield from [^{11}C]CO, in >96% radiochemical purity and with an average specific radioactivity of 1879 mCi/μmol (n = 12). The total radiosynthesis time was about 45 min. After administration of [^{11}C]BA to monkey, radioactivity entered monkey brain quickly and efficiently, reaching peak %SUV of 209 and 309 in cerebral cortices and cerebellum, respectively, at 1.75 min (Fig. 2). The radioactivity remained within the brain.

Conclusions: Palladium-catalyzed [^{11}C]carbonylation provides a practically convenient route to [^{11}C]benzyl acetate, avoiding air-sensitive reagents, such as Grignard reagents, formerly used to make [^{11}C]acetate derivatives. The [^{11}C]carbonylation route is expected to prove generic for the synthesis of other [^{11}C]acetate esters as potential radiotracers.

References: [1] Momosaki S, et al. Nucl. Med. Biol., 2007, 34: 939-944. [2] Kihlberg T and Langstrom B. J. Label. Compd. Radiopharm., 2001, 44: S990-992.

P320 SYNTHESIS OF [11C]ETHANOLAMINE VIA NITROALDOL REACTION

K. KATO*, M. ZHANG, K. MINEGISHI and K. SUZUKI
National Institute of Radiological Sciences, Dept of Molecular Probes, Chiba, Japan

Objectives: Phospholipids metabolism is a significant target for detecting tumor cell. Ethanolamine is one of primary components of phospholipids and the uptake is increased in accordance with the rapid proliferative rate of tumor cell. Carbon-11 labeling of ethanolamine ([^{11}C]EA) is an objective of this work.

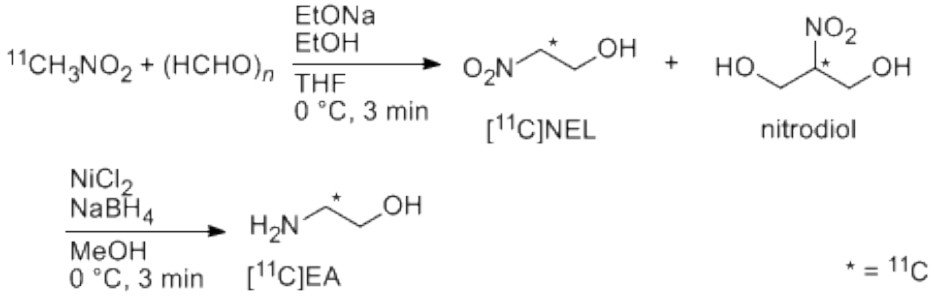
Methods: Nitroaldol reaction of nitro[^{11}C]methane ([^{11}C]NM) and formaldehyde and subsequent reduction of the nitro group is feasible and accessible for the synthesis of [^{11}C]EA. Nitroaldol reaction of [^{11}C]NM and formaldehyde should be carried out in the presence of large excess of formaldehyde. Therefore, it is significantly important to suppress the successive nitroaldol reactions that form nitrodiol and nitrotriol with keeping rapid formation 2-nitro[2-^{11}C]ethanol ([^{11}C]NEL).

Results: Results of the nitroaldol reaction were summarized in Table. All reactions were carried out in the presence of 15 μmol of EtONa and 10 μmol of $(HCHO)_n$ for 3 min. The reaction in the absence of EtOH was very slow to afford low conversion of [^{11}C]NEL (entry 1). On the other hand, low conversion of [^{11}C]NEL was improved by the addition of EtOH (entry 2−4). Increased solubility of EtONa enhanced the nicroaldol reaction. Efficient formation of [^{11}C]NEL was obtained by the addition of 5 or 10 μL of EtOH (entry 3 and 4). Increasing the amount of EtOH gave better conversion of [^{11}C]NEL, however the reaction in EtOH was slow (entry 5). Intra-molecular hydrogen-bonding might contribute selective formation of [^{11}C]NEL. Remarkable nitroalkane anomaly in protic solvent was the reason for slow reaction in EtOH. Nickel boride was chosen as an agent for the nitro group reduction of [^{11}C]NEL. Thus, a solution of $NiCl_2$ in MeOH was added to the reaction mixture and the resulting solution was transferred directly to the reaction vessel placing $NaBH_4$. After 3 min, the nitro alcohols were converted and the desired [^{11}C]EA was obtained. [Table]

Conclusions: We elucidated the method synthesizing [^{11}C]EA efficiently. The method can be applicable for the remote-controlled synthetic machine. Further progress will be presented.

Radiochemical conversion of nitroaldol reactions

entry	solvent	EtOH (μL)	[^{11}C]NEL (% conv)	nitrodiol (% conv)
1	THF	none	<8	n.d.
2	THF	2	33.9±2.7	27.9±2.6
3	THF	5	51.3±4.0	37.2±2.5
4	THF	10	64.4±3.1	16.3±4.6
5	EtOH	—	13.5±4.3	n.d.



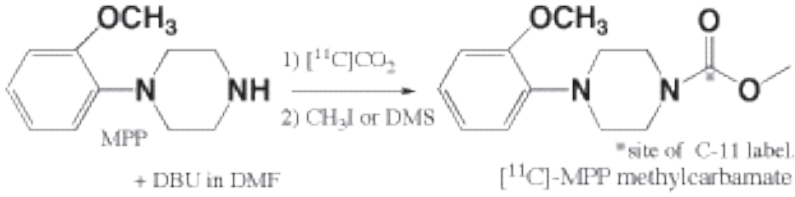
P321 DIRECT FIXATION OF [11C]-CO2 BY AMINES: FORMATION OF [11C]-CARBAMATES

A. A. WILSON*, **A. GARCIA, S. HOULE and N. VASDEV**
Centre for Addiction and Mental Health, PET Centre, Toronto, ON, Canada

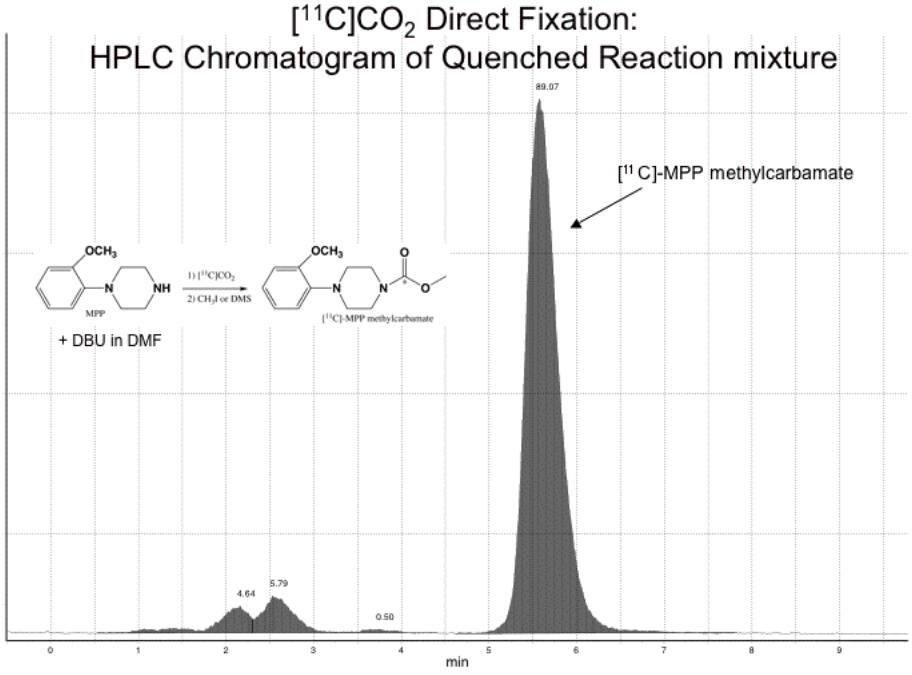
Objectives: Advances in the chemistry of trapping CO2, propagated by environmental concerns, might be exploitable in PET radiochemistry. Recent research has shown that CO2 can be efficiently trapped by amines as salts of carbamic acids, which in turn can be transformed into useful carbamates or ureas under mild conditions. We thought to apply this chemistry to the radiosynthesis of [^{11}C]-carbamates directly from cyclotron-produced [^{11}C]-CO_2. Model reactions with 2-methoxyphenylpiperazine (MPP) catalysed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), were explored. Initial conditions were best-guess based upon literature and expediency.

Methods: A solution of MPP (0.4 mg) and DBU (3 μL) in DMF (40 μL) was loaded onto a 1 mL steel HPLC injection loop and [^{11}C]-CO_2 in N_2 slowly passed through the coated loop (10 mL/min). When trapped radioactivity had peaked, the reaction proceeded for 4 min then the loop contents were eluted into a vial by a solution of either dimethyl sulphate or iodomethane (10 μL) in DMF (400 μL). Vial radioactivity was measured and aliquots of the mixture were removed for HPLC analysis at various times. [^{11}C]-CO_2 breakthrough was measured by means of an in-line NaOH trap.

Results: Trapping of [^{11}C]-CO_2 in the loop was essentially quantitative with less than 0.1% breakthrough. Elution of the loop's contents with the solution of alkylating agent was also effective; between 85-90% of trapped activity in the loop was transferred to the collection V-vial. Reaction of the formed [^{11}C]-carbamic acid salt with either alkylating agent was rapid and complete after <2 min at ambient temperature. Radiochemical conversion into the methylcarbamate of MPP was between 55 and 85% (n=9). Specific activities (EOS) of the labelled carbamate were > 60 GBq/μmol. Product identity was confirmed by co-injections with cold standard under various (column, solvent, pH) HPLC conditions.



Conclusions: Direct fixation of [^{11}C]-CO_2 by the model amine in the presence of DBU proved remarkably efficient. The described conditions are already practical for the production of PET radiotracers and presumably can be improved by optimisation of times, solvents, concentrations etc. The trapping and elution apparatus can be mounted on a 6-port, 2-position valve, readily lending the process to automation. One immediate application in PET imaging would be the synthesis of the kappa opioid radiotracer [^{11}C]-GR103545, which contains the methylcarbamate residue. Future work will explore the scope of this reaction to other amines (primary, aromatic) and alkylating agents.



P322 SYNTHESIS OF [11C]BACLOFEN VIA MICHAEL ADDITION OF NITRO[11C]METHANE

K. KATO*, M. ZHANG and K. SUZUKI
National Institute of Radiological Sciences, Dept Molecular Probes, Chiba, Japan

Objectives: γ-Amino butyric acid (GABA) is the major inhibitory brain transmitter and an important target of brain PET study. With regard to $GABA_A$ receptor, many studies have been carried out. On the other hand, very few studies are available about $GABA_B$ receptor. In this context, we focused on the ^{11}C-labeling synthesis of baclofen that is considered only agonist of $GABA_B$ receptor.

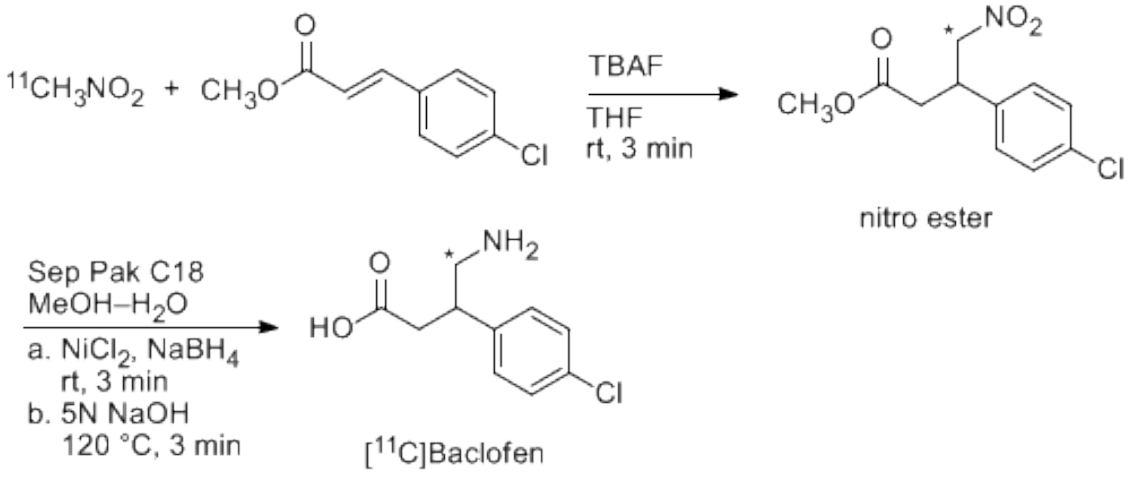
Methods: Michael addition of nitro[^{11}C]methane ([^{11}C]NM) to methyl p-chlorocinnamate and subsequent nitro group reduction and ester hydrolysis is straightforward for the synthesis of [^{11}C]baclofen. Michael addition of nitroalkane is one of the most important synthetic processes devoted to C-C bond formation, however there are no applications about the reaction of [^{11}C]NM for ^{11}C-labeling synthesis. Requirement of rapid reaction and appropriate procedure for the consecutive nitro group reduction hampered the development of synthesis.

Results: Several lines of bases were investigated for the Michael addition of [^{11}C]NM to p-chlorocinnamate. Among them, only the reaction using tetrabutylammonium fluoride (TBAF) afforded the desired Michael adduct in THF. Thus, the reaction using 5 mmol of TBAF gave the nitro ester in 67.9±1.2% conversion (entry 1). More TBAF gave better conversion of nitro ester (entry 2). On the other hand, higher reaction temperature did not cause improvement of conversion (entry 3). Good conversion of Michael adduct was obtained, however efficient procedure for the reduction and hydrolysis should be established to complete the synthesis of [^{11}C]baclofen. At first, the reduction by nickel boride in MeOH was performed directly to the reaction mixture of Michael addition. After ester hydrolysis, however, the desired [^{11}C]baclofen was not obtained. The nitro group reduction was retarded by the competing 1,4-reduction of ester precursor and decomposition of nickel boride in MeOH, resulting in incomplete amine formation. Using MeOH is crucial for the efficient reduction of nitro group. Further addition of nickel boride is not appropriate from the radiation protection view. As a result, removal of ester precursor became necessary prior to the reduction. Thus, after facile separation using C18 Sep-Pak nickel boride reduction was carried out and then successive ester hydrolysis afforded [^{11}C]baclofen.

Conclusions: We demonstrated the Michael addition of [^{11}C]NM to p-chlorocinnamate and the synthesis of [^{11}C]baclofen. Further progress will be presented.

TBAF promoted Michael Additions

entry	TBAF (μmol)	temp	nitro ester (% conv)
1	5	rt	67.9±1.2
2	20	rt	77.1±1.1
3	20	60 °C	<47



P323 LABELLING OF RTI-32 WITH C-11 IN THREE DIFFERENT POSITIONS: A STUDY OF THE INFLUENCE OF LABELLING POSITION ON BRAIN DISTRIBUTION AND METABOLITE PATTERN MEASURED IN MONKEY BY PET

S. HELIN[*1], K. NAGREN[2], A. J. AIRAKSINEN[3], C. STEIGER[4], J. P. BERGSTROM[4], A. TAKANO[4], V. W. PIKE[5] and C. HALLDIN[4]

1. Turku PET Centre, Univ. of Turku, Turku, Finland; 2. Rigshospitalet, Univ. of Copenhagen, Dept of Clinical Physiology & Nuclear Medicine, Copenhagen, Denmark; 3. University of Helsinki, Dept of Chemistry, Radiochemistry Laboratory, Helsinki, Finland; 4. Karolinska Institutet, Dept of Clinical Neurosciences, Psychiatry Section, Stockholm, Sweden; 5. NIMH, Molecular Imaging Branch, Bethesda, MD

Objectives: Over the last ten years several tropane dopamine transporter (DAT) PET and SPECT radioligands have been labelled in various positions. There is a need to compare the influence of the labelling position on PET application. Our aim was to label the tropane derivative, 2-β-Carbomethoxy-3-β-(4-methylphenyl)tropane, RTI-32 [1], in three different positions in order to study the influence on brain distribution and metabolite pattern measured in a non-human primate.

Methods: [^{11}C]RTI-32 (Figure 1) was labelled in the N-methyl (1) or O-methyl (2) positions using [^{11}C]methyl triflate and the corresponding des-methyl precursors. [^{11}C-p-methyl]RTI-32 (3) was prepared using [^{11}C]methyl iodide with a Stille coupling. The three [^{11}C]RTI-32, (1), (2) and (3), were administered during one day in separate injections to a Cynomolgus monkey by bolus injection. Each injection was monitored by acquiring PET image of the brain over 90 minutes and by taking blood samples for metabolite analysis using both acidic and basic conditions gradient HPLC. Dynamic PET scan data were analysed using the reference tissue method. Striatum and mid brain were chosen as target ROIs, cerebellum being the reference region.

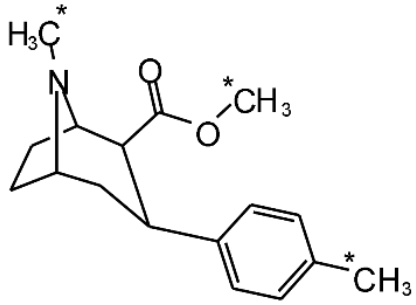


Figure 1. Structure of [^{11}C]RTI-32, with the three different labelling positions shown by asterisks.

Results: The preparation of (1) and (2) from [^{11}C]methyl triflate gave >80 % incorporation yields whereas (3) was obtained in 26-50 % yield from [^{11}C]methyl iodide. HPLC purification gave [^{11}C]RTI-32 in a radiochemical purity higher than 97%. The specific radioactivity (SA) at EOS was 160, 114 and 23 GBq/μmol for (1), (2) and (3), respectively. The metabolite study indicated no difference in the time-activity curve of the parent radioligand between any of the three labelling positions. All the observed labelled metabolites were less lipophilic compared to the parent compound. No N-demethylation was found for (2) or (3) confirmed by addition of standard. PET data of the brain uptake was very similar for (1), (2) and (3). The labelled metabolites are thus either not entering the brain or the level of brain penetration of the labelled metabolites is similar for all the three labelling positions.

Conclusions: There was no preference for any labelling position of [^{11}C]RTI-32 with regard to PET application. The only difference is a comparatively lower radiochemical yield and SA obtained using the Stille coupling for compound (3).

Research Support: VWP is supported by the Intramural Program of the National Institutes of Health (NIMH)

References: 1) Wilson AA, DaSilva JN, Houle S.: Facile radiolabeling and purification of 2b-[O-$^{11}CH_3$]-carbomethoxy-3b-aryltropanes: radiotracers for the dopamine transporter. J Labelled Compd Radiopharm 34:(1994) 759-765

P324 RADIOSYNTHESIS OF AROMATIC KETONES FROM [11C]CO2 BY PALLADIUM-CATALYZED SUZUKI-MIYAURA COUPLING

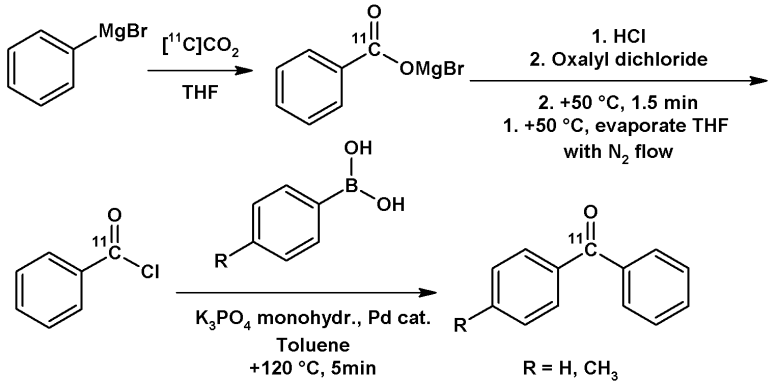
M. SARPARANTA*, J. JALOMAKI, S. MAKKONEN-CRAIG, K. HELARIUTTA and A. J. AIRAKSINEN
University of Helsinki, Laboratory of Radiochemistry, Helsinki, Finland

Objectives: Aromatic ketone substructures are important building blocks in pharmaceutical chemistry. The palladium-catalyzed Suzuki-Miyaura coupling reaction is commonly used in organic synthetic chemistry for the synthesis of aromatic ketones from arylboronic acids and acyl chlorides. In carbon-11 radiochemistry, [11C]CO has almost exclusively been used as labelling precursor for Suzuki-Miyaura coupling reactions. However, as [11C]CO2 is readily available in most facilities, it would be a more convenient labelling precursor, thereby advancing the use of this sophisticated approach to yield 11C-labelled aromatic ketones. Herein, we report studies on the optimization and further development of the Suzuki-Miyaura reaction in the synthesis of 11C-labelled aromatic ketones starting from [11C]CO2. [11C]Benzophenone, a symmetric aromatic ketone, was chosen as the model compound.

Methods: [^{11}C]Benzoyl chloride was synthesized from [^{11}C]CO_2 in a Grignard reaction with phenylmagnesium bromide followed by chlorination with oxalyl chloride. The synthesized [^{11}C]benzoyl chloride was then reacted with phenylboronic acid in a palladium(II)-catalyzed Suzuki-Miyaura reaction in basic conditions. Synthesis products were characterized with reverse-phase HPLC with H_3PO_4:acetonitrile solvent. Influence of different solvents, bases, aqueous conditions, catalysts, and temperature on the product yield was investigated. The generality of the synthetic strategy was demonstrated by the synthesis of [^{11}C]4-methylbenzophenone from p-tolylboronic acid.

Results: [^{11}C]benzophenone was obtained from [^{11}C]benzoyl chloride at decay-corrected product yields ranging from 61-98 % (mean 80±20 %, n=8) using either Pd(II)acetate catalyst with triphenylphosphine ligand or ligandless [$(C_6H_5)_3P]_2Pd(II)Cl_2$ in toluene with solid K_3PO_4 monohydrate by heating at +120 °C for 5 minutes. Other Pd(II) and Pd(0) catalysts were tested but resulted in lower product yields. Crystal water from the base was sufficient to create semiaqueous conditions favorable for the reaction, although added water seemed to improve reaction yields slightly. Replacing K_3PO_4 with potassium or cesium carbonate or TEA resulted in neglible yields. [^{11}C]Methylbenzophenone was produced with decay-corrected product yields of 84 % and 45 % (n=2). After these promising initial results, we unfortunately discovered a major problem with synthesis repeatability when product yields plummeted to 0-34 %, and an unidentified lipophilic side-product started to dominate in the reaction mixtures.

Conclusions: Our initial results demonstrate that ^{11}C-labelled aromatic ketones can be successfully prepared from [^{11}C]CO_2, but the recently encountered problems with repeatability in the synthesis have hampered the validation of the synthetic approach and its application to label compounds relevant to PET imaging. Attempts are ongoing to get to the root of this problem, identify the side-product and elevate the yields back to their original level.



P325 SYNTHESIS OF HIGH-AFFINITY SB 207710 ANALOGS AS POTENTIAL BRAIN 5-HT$_4$ RECEPTOR RADIOLIGANDS FOR PET IMAGING

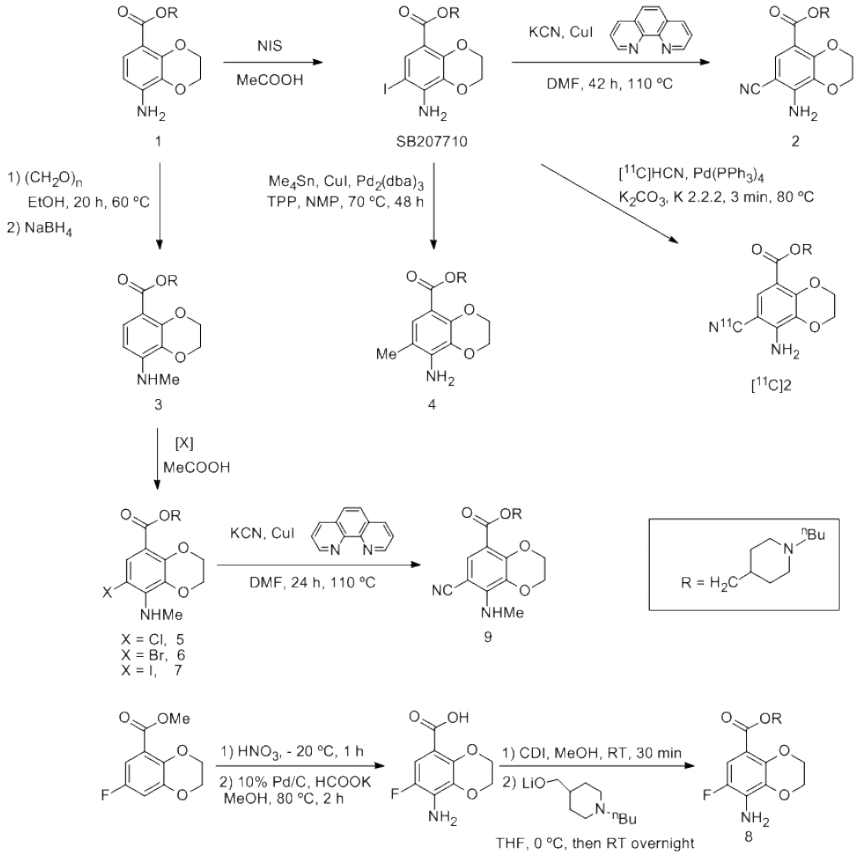
R. XU*, J. S. HONG and V. W. PIKE
National Institute of Mental Health, National Institutes of Health, Molecular Imaging Branch, Bethesda, MD

Objectives: 5-HT$_4$ receptors are implicated in neuropsychatric disorders (Eglen and Hegde, Exp. Opin. Invest. Drugs, 1996, 5, 373). SB 207710 (Scheme) is an exceptionally high affinity antagonist for 5-HT$_4$ receptors. [^{123}I]SB 207710 provided the first demonstration of brain 5-HT$_4$ receptor imaging in monkey in vivo (Pike et al., Eur. J. Nucl. Med. Mol. Imaging, 2003, 30, 1520). Some analogs, labeled with either carbon-11 or fluorine-18 in the terminal N-alkyl group, have subsequently shown promise for PET imaging (Gee et al., Curr. Radiopharm., 2008, 1, 110; Parker et al., NeuroImage, 2002, 16, part 2, S4; Kornum et al., J. Cerebr. Blood Flow Metab., 2009, 29, 186). Our objective is to develop alternative radioligands for 5-HT$_4$ receptor imaging with PET, again based on SB 207710, in which the radiolabel is located at the aryl moiety to try to avoid potential issues from radiometabolites. Our strategies include replacement of the aryl halo group and/or alkylation of the aryl amino group with alternative groups amenable to labeling with a positron-emitter.

Methods: Aniline 1 (King et al., PCT Int. Appl., WO93/05038, 1993) was synthesized as a key precursor from which SB 207710 was obtained by iodination. CuI-catalyzed cyanation of SB 207710 or 7 gave 2 or 9 in 19% or 7% yield. Reductive methylation of 1 gave 3 in 60% yield. Pd-catalyzed methylation of SB 207710 with Me_4Sn gave 4 in 44% yield. Treatment of 3 with N-halo succinimides (N-X; X = Cl, Br or I) gave 5–6 in moderate yields (30–46%). The fluoro analog 8 was synthesized by nitration and reduction of a fluoro benzodioxane carboxylate, followed by coupling with the requisite lithium alkoxide. The new ligands are being evaluated for affinity and selectivity for binding at 5-HT$_4$ receptors. Reaction of SB 207710 with [^{11}C]cyanide ion in the presence of Pd(0) for 5 min at 80 ºC gave [^{11}C]2 in 54% decay-corrected radiochemical yield (RCY) based on radio-HPLC analysis. [^{11}C]2 was purified with HPLC and its identity was confirmed with LC-MS.

Results: Ligands 1 and 3–7 were found to have high affinities for 5-HT$_4$ receptors with K_i values between 1 and 10 nM, among which 1 and 5 have higher affinities (K_i = 1.4 and 1.5 nM) than their analogs and SB 207710 (K_i = 2.2 nM) in the same assay. The N-methylation of 1 reduced affinity somewhat (K_i = 9.1 nM for 3). Nevertheless, ortho-halogenation of 3 recovered affinity, with chlorination increasing affinity six-fold. The chloro compound 5 also has high selectivity for binding to 5-HT$_4$ receptors versus other serotonin receptors. The radiocyanation of SB207710 gave [^{11}C]2 in high RCY.

Conclusions: Several SB 207710 analogs with high 5-HT$_4$ receptor affinity were synthesized. Single-step [^{11}C]cyanation of SB 207710 proved to be an effective radiolabeling procedure. The successful conversion of 1 into 3 suggests that reductive ^{11}C-methylation may be applicable to labeling the N-methyl compounds, 3, 5–7 and 9. Completion of the pharmacological evaluation of the new ligands and investigation of their labeling with carbon-11 is now in progress preceding their assessment as potential radioligands for 5-HT$_4$ receptors in vivo.



Scheme. Synthesis of new high-affinity 5-HT$_4$ ligands and the preparation of radioligand [^{11}C]2.

P326 RADIOSYNTHESIS OF [11C]METHYLI-LOSARTA, A POTENTIAL IMAGING AGENT FOR ANGIOTENSIN AT1 RECEPTORS

T. HADIZAD*, S. MASON, J. COLLINS, S. KIRKPATRICK, R. S. BEANLANDS and J. N. DASILVA
University of Ottawa Heart Institute, National Cardiac PET Centre, Ottawa, ON, Canada

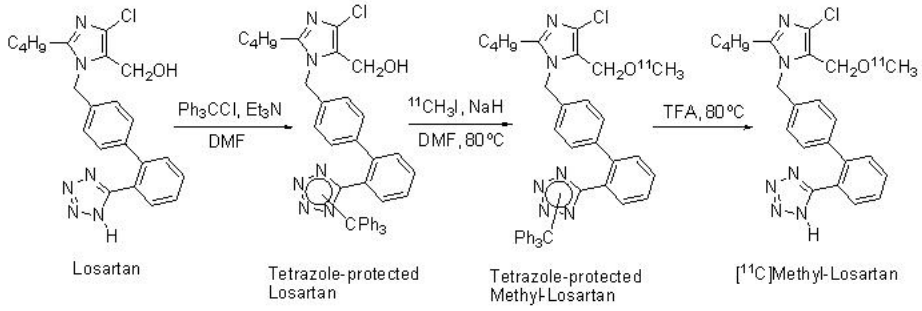
Objectives: Angiotensin II AT_1 receptor stimulation is involved in Na^+ and water balance, autonomic and cardiovascular functions, playing a key role in hypertension, ventricular remodeling, and renal diseases. Several AT_1 antagonists were previously labeled for PET imaging. We present here the synthesis of the O-[^{11}C]methylated derivative of the clinically used AT_1 receptor blocker Losartan. Methyl-Losartan was previously reported to bind with high affinity and antagonistic activity to Angiotensin II AT_1 receptors (IC_{50} Losartan 19 nM, Methyl-Losartan 32 nM).

Methods: The methyl-ester derivative was synthesized in 3 steps: 1) protection of the tetrazole group with trityl chloride; 2) O-[^{11}C]methylation with [^{11}C]methyl iodide (from [^{11}C]CO_2, LAH/THF/HI) in the presence of NaH; 3) removal of the protecting group by HCl (1N) or trifluoroacetic acid (see Scheme). [^{11}C]Methyl-Losartan was purified by semi-preparative HPLC (Luna C_{18}, 10µ, 250×10 mm, CH_3CN/0.1M AF solution: 35/65, 8 mL/min) and its identity was confirmed by reverse phase analytical HPLC compared to the standard.

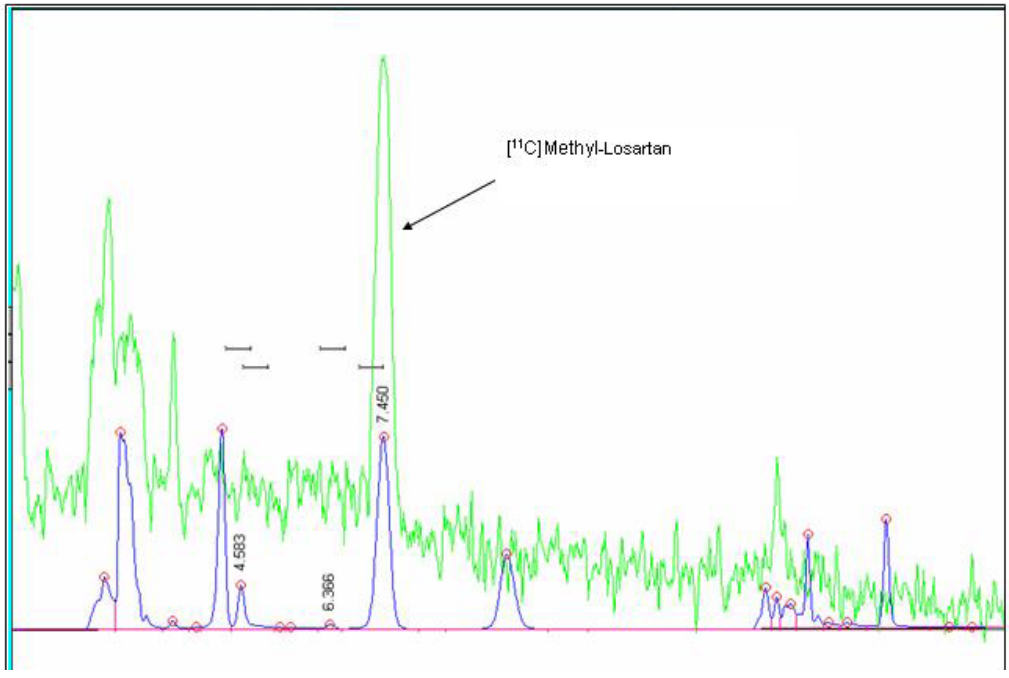
Results: A modified Williamson reaction was used for the methylation step using sodium hydride as the base. Excess in base resulted in poor yield of final product. The methylation reaction did not proceed when a weaker base such as K_2CO_3 was used while the mixture of K_2CO_3 and kryptofix was still insufficient to convert the alcohol group to the corresponding alkoxide ion. De-protection step was sensitive to the reaction time, temperature and kind of acid. Higher yields were obtained with trifluoroacetic acid to cleave the protecting group, whereas more by-products were produced with hydrochloric acid (different molarities). The structure of tetrazole-protected Losartan (precursor) and unlabeled Methyl-Losartan (final product) were confirmed by spectroscopic means (^{1}H NMR, Two-dimensional COSY, ^{1}H / ^{13}C HSQC and HRMS). [^{11}C]Methyl-Losartan was produced in 30-60% radiochemical yields, high chemical purity (>99%), high radiochemical purity (>95%) and a synthesis time of 35 min (including quality control).

Conclusions: [^{11}C]Methyl-Losartan was synthesized in high yields and purity. Higher specific activity is expected with [^{11}C]CH_3I produced from [^{11}C]methane and I_2. In vivo evaluation for imaging AT_1 receptors is currently in progress using small animal PET.

Research Support: We thank the Canadian Institutes of Health Research MOP-80203 for funding this work.



Scheme. Radiosynthesis of [^{11}C]Methyl-Losartan



P327 SYNTHESIS OF [^{11}C]RHODAMINE-123 AS A POTENTIAL TRACER FOR IMAGING P-GLYCOPROTEIN FUNCTION

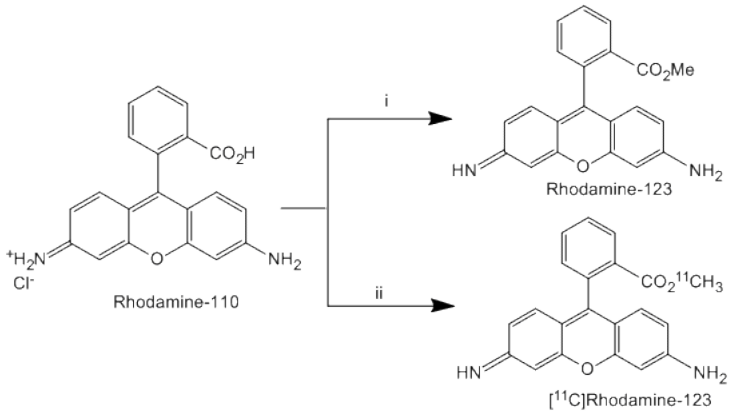
X. BAO*, C. L. MORSE, S. LU, F. G. SIMEON and V. W. PIKE
National Institutes of Health, National Institute of Mental Health, Molecular Imaging Branch, Bethesda, MD

Objectives: Rhodamine-123 is a fluorescent dye and a P-glycoprotein (P-gp) substrate that has been used extensively to study P-gp function in vitro (e.g., Chaudhary and Roninson, Cell, 1991, 66, 85; Ludescher et al., Br. J. Haematology, 1992, 82, 161). We considered that [^{11}C]rhodamine-123 could be a useful alternative tracer for imaging P-gp function at the blood-brain barrier or in tumors. Here we aimed to prepare [^{11}C]rhodamine-123 by esterification of the readily available rhodamine-110 with [^{11}C] iodomethane for evaluation in vivo.

Methods: Esterification of rhodamine-110 (Scheme): Iodomethane (0.014 mmol) was added to a solution of rhodamine-110 (mono-HCl salt; 0.014 mmol) and N,N-di-isopropylethylamine (0.028 mmol) in anhydrous DMF (500 μL) in a sealed tube. The tube was placed in a microwave cavity (CEM Discover) and irradiated (90 W, 130 °C) for 10 min. A sample of crude reaction mixture was injected onto a Luna C18 column (250 × 10 mm) eluted at 6 mL/min with a mixture of H_2O (A) and 10 mM $HCOONH_4$ in MeCN-H_2O (3: 2, v/v) (B), with B increased from 70 to 100% over 25 min (Method A). The product eluting at t_R = 10.4 min was identified as rhodamine-123 by co-injection with reference compound, and by LC-MS-MS analysis. Radiosynthesis of [^{11}C]rhodamine-123 (Scheme): [^{11}C]Iodomethane, produced with a PETtrace Microlab (GE), was delivered in helium gas (flow 17 mL/min) into a loop of stainless steel tubing (2 mL; Bioscan) pre-loaded with rhodamine-110 (0.003 mmol) and tetra-n-butylammonium hydroxide (0.006 mmol) in anhydrous DMF (80 μL). The reaction was allowed to proceed at room temperature for 5 min. Then the entire contents of the loop were analyzed by HPLC equipped with a radioactivity detector using Method A. The decay-corrected radiochemical yield (RCY) of [^{11}C]rhodamine-123 (t_R = 10.1 min) was estimated from the chromatogram. Portions of the HPLC fraction eluting between 10 and 11 min were analyzed with radio-HPLC on a Prodigy reverse phase column (250 × 4.6 mm) eluted at 1 mL/min with mobile phase A-B, with B increased from 70 to 100% over 25 min (Method B), and with LC-MS-MS of carrier. A further portion was treated with aq. KOH (~ 10% w/v) at room temperature for 40 min and then also analyzed by radio-HPLC (Method B).

Results: Rhodamine-110 was readily esterified to rhodamine-123 (39% yield). [^{11}C]Rhodamine-123 was produced in 27% RCY. Product identity was verified by radio-HPLC analysis, LC-MS-MS of associated carrier and basic hydrolysis.

Conclusions: [^{11}C]Rhodamine-123 was obtained in useful RCY, ready for preliminary evaluation as a prospective radiotracer of P-gp function in vivo.



Scheme. Syntheses of rhodamine-123 and [^{11}C]rhodamine-123. (i) MeI, DIPEA, DMF, microwave 90 W, 10 min, 130 ºC, 39%; (ii) [^{11}C]MeI, TBAH, DMF, 5 min, rt, 27% RCY.

P328 AN EFFICIENT SYNTHESIS OF [11C]FALLYPRIDE, A DOPAMINE D2/D3 TRACER

D. MURALI*[1], N. T. VANDEHEY[1], J. MUKHERJEE[2], R. J. DAVIDSON[1], R. J. NICKLES[3] and B. T. CHRISTIAN[1]

1. University of Wisconsin, Waisman Laboratory for Brain Imaging and Behavior, Madison, WI; 2. University of California, Department of Psychiatry and Human Behavior, Irvine, CA; 3. University of Wisconsin, Department of Medical Physics, Madison, WI

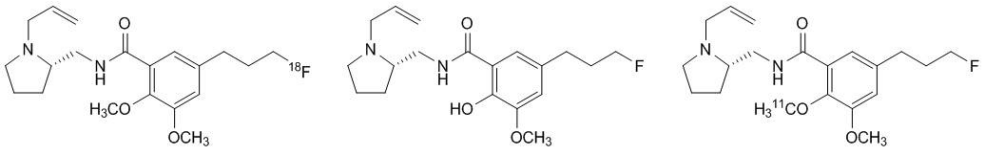
Objectives: Fallypride (FAL) is a well-studied dopamine D2/D3 receptor antagonist. In our laboratories, [^{18}F]FAL (1) has been routinely produced for human as well as non-human PET imaging studies. Recently, we become interested in same day multiple-injection imaging studies using two different D2/D3 tracers. Because of the relatively shorter half-life of ^{11}C (20min), [^{11}C]FAL (3) seemed to be well-suited for these multiple imaging sessions. However, the reported procedure[1] for the preparation of [^{11}C]FAL using [^{11}C]CH_3I produced only low yields of the tracer. We report herein a simple and efficient synthesis of [^{11}C]FAL using [^{11}C]CH_3OTf.

Methods: The ^{11}C was produced by ^{14}N (p, α) ^{11}C reaction. The [^{11}C]CH_4 was reacted with iodine vapors at ca. 750 °C to give [^{11}C]CH_3I which was then converted to [^{11}C]CH_3OTf by passing through a short silver triflate-GraphPak column at 210 °C. The [^{11}C]CH_3OTf coming out of the silver triflate column was bubbled through the reaction mixture. Semi-preparative HPLC conditions[1] are Waters Novapak C18, 7.8x300 mm and 55:45 mixture of CH_3CN-H_2O (0.1% triethylamine) at 5 mL/min with UV (254nm) and radioactivity detection.

Results: Initially, we tried alkylation of 2 using [^{11}C]CH_3I, to produce [^{11}C]FAL (3). The yields were very low and the amount of activity obtained was not optimal for animal (non-human primate) studies. Then we tried [^{11}C]CH_3OTf as the ^{11}C-methylating synthon instead of [^{11}C]CH_3I. After modifying the reaction conditions, [^{11}C]FAL was obtained in greatly improved yields. In a typical procedure, 1 mg of precursor (2) is dissolved in 300 μL of anhydrous acetonitrile in a Wheaton v-vial and to this solution 10 μL of 5N sodium hydroxide is added. The vial is capped with a Teflon septum and a vent needle with a Porapak trap is attached. Using another stainless-steel needle, the [^{11}C]methyl triflate from the silver triflate column is bubbled through the reaction mixture at room temperature. Needles were removed and the mixture stirred at 80 °C for 5 minutes with vial capped. The reaction mixture was diluted with 0.7 to 1 mL of the mobile phase and injected on to the semi-prep HPLC. The fraction containing [^{11}C]FAL was evaporated to dryness. The residue was taken up in saline (10 mL) and passed through 0.2μ modified Teflon filter (Millex-LG, 25 mm). Total synthesis time was about 20 min from [^{11}C]CH_3I trapping. The radiochemical yield was 71% (decay corrected, based on trapped [^{11}C]CH_3OTf).

Conclusions: [^{11}C]FAL was obtained in high yields using [^{11}C]CH_3OTf as the ^{11}C-methylating synthon.

References: J Mukherjee, B Shi, BT Christian, S Chattopadhyaya, TK Narayanan, Bioorg. Med. Chem., 2004, 12, 95-102.



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P329 SYNTHESIS AND BIODISTRIBUTION OF [C-11]-SN-38 FOR TREATMENT EVALUATION BY PET

S. M. APANA*[1] and M. S. BERRIDGE[2]
1. 3D Imaging LLC, Maumelle, AR; 2. University of Arkansas for Medical Sciences, Dept. of Radiology, Little Rock, AR

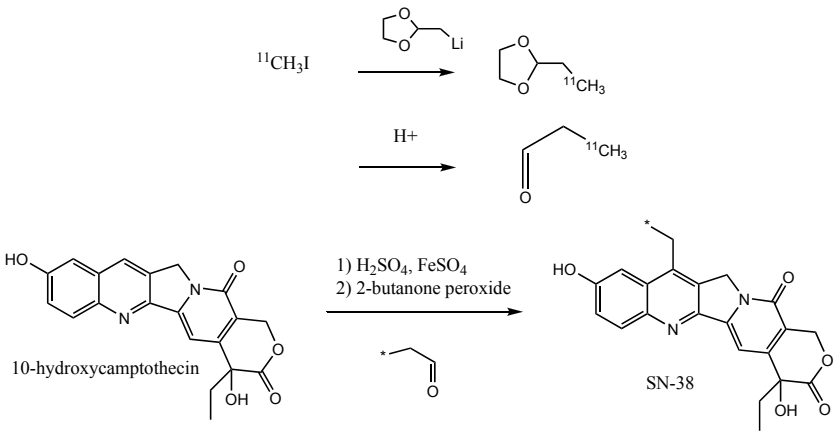
Objectives: Camptothecin is a topoisomerase I inhibitor used for chemotherapy. Camptothecin has excellent anti-tumor activity, but high toxicity. Several of its derivatives have increased solubility and lower toxicity. Irinotecan is a derivative used to treat a variety of tumors. It is inactive itself, but SN-38 is its active metabolite, 7-ethyl-10-hydroxycamptothecin. Camptothecin derivatives are believed to concentrate in tumors when they are therapeutically effective. Due to the low response rate (ca 40%), the ability to predict treatment response would be a benefit. In previous work reaction of [^{13}C]-propionaldehyde with 10-hydroxycamptothecin gave SN-38 in a Minisci-type radical reaction which decarbonylates the aldehyde, adding an ethyl radical to the electron deficient β ring. We report an optimized method to label SN-38 via 3-[^{11}C]propionaldehyde.

Methods: 3-[^{11}C]-propionaldehyde was synthesized by the iodine-catalyzed reaction of [^{11}C]-methyl iodide with 2-lithio-1,3-dioxolane, freshly prepared by metal-halogen exchange of 1-bromomethyl-1,3-dioxolane and butyl lithium. The protected aldehyde was distilled into a sulfuric acid solution containing 10-hydroxycamptothecin. Distillation prior to deprotection minimized the acetaldehyde formed by hydrolysis of unreacted lithium dioxolane. Acetaldehyde can react to form a methylated, rather than ethylated, product. After distillation and deprotection, hydrogen peroxide was added as a radical initiator for reaction of [^{11}C]-propionaldehyde with 10-hydroxycamptothecin. Labeled SN-38 was purified by reverse phase HPLC. [img] Mice (CD-1,8 male, 8 female, 25-30 g) were injected with [C-^{11}]-SN-38 (7-33 MBq, 0.2-0.9 mCi) and microPET images acquired for 2 hours. Organ regions of interest were defined and used to create residence time data that was input to OLINDA software for calculation of predicted human radiation dosimetry. Distribution was non-specific, and hepatobiliary clearance caused the upper small intestine to be the dose-limiting organ. A conservative limit for human injection based on this data is 7.5 mCi.

Results: [^{11}C]-SN-38 was obtained in 14% chemical yield from carbon dioxide at EOB +70 min, with a purity of 99+%, radiochemical yield 1.3%. Yield, biodistribution and dosimetry are amenable for human use.

Conclusions: [^{11}C]-SN-38 was synthesized in sufficient yield for human use at a 180-750 MBq (5-20 mCi) dose to measure accumulation in tumor. Murine biodistribution by microPET showed moderate uptake in most organs. Initial perfusion distribution was followed by redistribution, mainly through hepatobiliary clearance. Human dosimetry measurement by PET is planned.

Research Support: Funded by NCI Contract No. N01-CO-12400 Useful discussions with Prof. Timothy Tewson



P330 SYNTHESIS OF [C-11]-DOXORUBICIN

J. M. LINK[1], L. ANDERSON*[2] and J. COLLINS[3]
1. University of Washington, Dept of Radiology, Div of Nuclear Medicine, Seattle, WA; 2. FDA, Laboratory of Clinical Pharmacology, Silver Spring, MD; 3. NIH, Developmental Therapeutics Program NCI/NIH, Rockville, MD

Objectives: Doxorubicin is an important front line agent in a variety of malignancies. Failure of therapy with doxorubicin is thought to be due to increased activity of efflux pumps, limiting the accumulation of doxorubicin in the tumor. Our goal was to explore the utility of PET imaging of labeled doxorubicin in tumors to determine whether drug accumulation could be correlated to drug efficacy. Further studies would also monitor drug accumulation and relate those concentrations to the onset of drug resistance. Key to this proposal would be the successful synthesis of radiolabeled doxorubicin.

Methods: The 4-methoxy group offers an attractive site for labeling, and as such we acquired the 4-demethyl analog; 14-OH-carminomycin. Initial attempts to directly methylate carminomycin by conventional means using methyl iodide in various solvents including DMSO, acetonitrile, dimethylformamide and tetrahydrofuran with various bases were unsuccessful. Carminomycin contains multiple sites for methylation, and the conditions needed for deprotonation and reaction were found to be severe, resulting in formation of byproducts and degradents. A large percentage of the byproducts formed were N-methyl derivatives. To limit the N-methyl reactions, we chose to protect the 3'-amino functionality. Several blocking groups were evaluated, and the Fmoc group was used due to the non-acidic deprotecting schemes it afforded, as acidic conditions promoted pronounced aglycone formation. The addition of the Fmoc group also afforded access to additional solvent systems for use in the methylation reaction. Several solvents and bases were evaluated and alkyl ammonium and alkyl phosphonium were found to be the most effective in deprotonation. Removal of the Fmoc was effected by brief treatment with morpholine in DMF.

Results: There was no measurable radioactive reaction yield with methyl iodide. The use of methyl triflate instead of methyl iodide allowed for lower reaction temperatures, reducing the alkaline degradation of product and precursor, to further improve yields. Multiple methylated species were still produced, but authentic doxorubicin could be isolated. We found that use of aromatic solvent systems gave better yields than classical solvents. Studies using 75ug (100nmol) of the 14-OH carminomycin in chlorobenzene and base and 2ug (15nmol) of D3-methyltriflate would typically yield 300-400ng (3-5% of methyl-triflate) doxorubicin. Authenticity of the doxorubicin was confirmed by LC-MS/MS analysis. Following a similar synthetic scheme yielded ^{11}C-doxorubicin. Conventional Graphpac substrate for silver triflate to produce the ^{11}C-methyl triflate was incompatible with the critical pH requirements for the reaction and solid silver triflate was used. Thus far, 38 mCi of methyl triflate has yielded 1.8 mCi of doxorubicin Fmoc in 2 min. Work continues to improve the carbon-11 synthesis.

Conclusions: ^{11}C-Doxorubicin can be produced in sufficient quantities and quality to be used as a PET-imaging probe.

Research Support: Support provided by FDA, NCI/SAIC BOA 24XS036 and NIH CA42045.

P331 EFFICIENT METHOD TO SYNTHESIZE 13N-NITROSAMINES

V. GOMEZ-VALLEJO[*1], K. KATO[2], M. HANYU[2], K. MINEGISHI[2], J. I. BORRELL[3] and J. LLOP[1]

1. CIC biomaGUNE, Radiochemistry Department, Molecular Imaging Unit, San Sebastian, Spain; 2. National Institute of Radiological Sciences, Department of Molecular Probes, Molecular Imaging Center, Chiba, Japan; 3. Institut Quimic de Sarria, Universitat Ramon Llull, Grup d'Enginyeria Molecular, Barcelona, Spain

Objectives: To develop a fast and reproducible method for the preparation of ^{13}N-labelled nitrosamines.

Methods: Among all PET radionuclides, carbon-11 and fluorine-18 have been the most widely used due to their versatile chemistry and relatively long half-life. In some particular cases, however, the use of other radioisotopes (e.g. nitrogen-13) might be very useful for the development of synthetic strategies to label molecules in different positions, thus giving further understanding of specific biological and/or physiological processes. One example is found in nitrosamines. In this case, a better understanding of the in vivo mechanism by which they exert their carcinogenic effect is highly desirable, and ^{13}N labeling would represent a powerful tool to perform in vivo pharmacokinetic studies in animals. In this work, we present a simple method to synthesize ^{13}N-Nitrosamines by N-nitrosation of secondary amines using a heterogeneous combined approach: reaction of resin-supported $^{13}NO_2^-$ with Ph_3P/Br_2/amine solution in organic media. Nitrogen-13 was produced in a cyclotron via $^{16}O(p,\alpha)^{13}N$ nuclear reaction. The resulting solution, containing $^{13}NO_2^-$, $^{13}NO_3^-$ and $^{13}NH_4^+$ was passed through a glass column filled with cadmium/sand mixture to quantitatively reduce $^{13}NO_3^-$ into $^{13}NO_2^-$. The solution was directly introduced to an anion exchange solid phase extraction cartridge to trap $^{13}NO_2^-$. A freshly prepared solution containing Ph_3P/Br_2/amine (25/25/20 μmol) in dry dichloromethane was circulated through the cartridge at a flow rate of 0.4 mL/min. The eluted solution was recovered in a vial, dried under continuous nitrogen flow and reconstituted with water. The resulting solution was eluted through a C-18 SPE cartridge. After rinsing with water, the desired radiotracers were eluted with ethanol/water mixture and reconstituted with physiologic saline solution.

Results: Average radiochemical yields (decay corrected) for the preparation of ^{13}N-nitrosopiperidine, ^{13}N-nitrosopyrrolidine, ^{13}N-nitrosodiisopropylamine and ^{13}N-nitrosodiethylamine were 37.8±3.1%, 40.7±8.0%, 34.0±7.3% and 36.4±5.0%, respectively. Radiochemical conversion ($^{13}NO_2^-$ into ^{13}N-Nitrosamine) was over 45% in all cases. Total synthesis time (including purification) was less than 10 minutes and radiochemical purity was always above 99%.

Conclusions: The reaction of $^{13}NO_2^-$ (obtained after reduction of cyclotron produced $^{13}NO_3^-$) with secondary amines yields the formation of ^{13}N-labelled nitrosamines with good radiochemical yields.

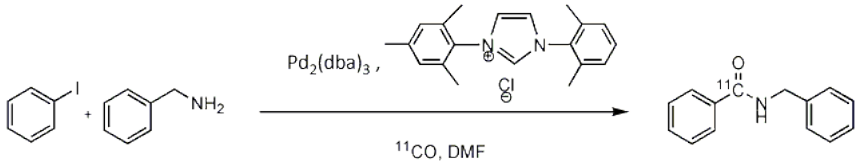
P332 PALLADIUM-NHC MEDIATED CARBONYLATION FOR THE PRODUCTION OF [11C]-AMIDES IN PET RADIOLABELLING

L. JENNINGS*[1], N. LONG[1], A. GEE[2], P. MILLER[1] and S. KEALEY[2]
1. Imperial College London, Department of Chemistry, London, United Kingdom; 2. GSK, Clinical Imaging Centre, Hammersmith Hospital, London, United Kingdom

Objectives: To investigate and optimise the incorporation of ^{11}CO in carbonylation reactions, specifically in the synthesis of ^{11}C-amides, for positron emission tomography (PET) by altering the ligand set on the palladium mediating reagent. Phosphine, diphosphine and N-heterocyclic carbene (NHC) systems were extensively investigated under cold conditions in batch reactions and using microfluidic devices prior to choosing the best performing systems for application with ^{11}CO.

Methods: A model cross-coupling carbonylation reaction forming N-benzylbenzamide was used to test a series of palladium catalysts. The use of NHCs is novel in aminocarbonylation from primary amines and aryl halides. Five imidazolium and imidazolinium salts (IAd.HCl, IPr.HCl, IMes.HCl, SIMes.HCl, SIPr.HCl) were combined with either $Pd(OAc)_2$, $Pd_2(dba)_3$ or $PdCl_2$ in different stoichiometries to create the active catalyst in situ. $Pd(IMes)_2Cl_2$ was also synthesised and tested for a comparison with pre-formed NHC catalysts. Phosphine containing complexes tested include; $Pd(PPh_3)_4$, $Pd(PPh_3)_2Cl_2$, $Pd(dppp)Cl_2$, Pd(dppf)Cl_2, Pd(BINAP)Cl_2. Analysis of amide production was assessed after 10 and 120 minutes and at 100 °C and 150 °C. Furthermore, microfluidic chip devices were used to carry out carbonylations at 100 °C, 120 °C and 150 °C with similar Pd-NHC systems. $Pd_2(dba)_3$, $PdCl_2$, IMes.HCl, SIAd.HCl and SIPr.HCl were all taken forward to synthesise ^{11}C labelled N-benzylbenzamide. In these cases a Cu(I)tris(3,5-dimethyl)(pyrazolyl)borate complex was used to trap ^{11}CO and released with PPh_3.

Results: The Pd-NHC catalysts are comparable to other more commonly employed Pd-phosphine catalysts for the synthesis of amides. Yields of N-benzylbenzamide achieved when carrying out the reactions on a microfluidic chip device were also comparable to the highest performing catalysts ($Pd(dppp)Cl_2$ and $Pd(dppf)Cl_2$) when using the chip devices. An average of 78% yield was achieved after 10 minutes at 150 °C. Although parity was achieved with other catalysts, the variables assessed in the study of NHCs as ligands did not yield trends to be used for optimisation; however, the best performing catalysts were used to make ^{11}C labelled N-benzylbenzamide.



Initial findings have given radiochemical yields of up to 62% with the $Pd_2(dba)_3$ and IMes.HCl with a radiochemical purity of up to 89%. The radiochemical purity of amide was diminished by competing production of benzoic acid and N, N'-dibenzylurea. It was noted that this process was especially prevalent when using a palladium (II) source. A competing catalytic pathway yielding the urea has been postulated.

Conclusions: Palladium NHC systems provide a convenient and effective method for amide production under cold conditions. It has also been illustrated that these results can be replicated in the radiochemical laboratory. Furthermore, encouraging results have been achieved when using these systems on microfluidic devices. This work opens up the potential advantageous use of NHCs in other areas of radiolabeling chemistry for PET.

P333 CARBON-11 LABELING OF SSR180711, A NOVEL α7-SELECTIVE LIGAND FOR PET IMAGING OF NICOTINIC ACETYLCHOLINE RECEPTORS

Y. BRAMOULLE[1], P. GEORGE[2], H. VALETTE[1], M. BOTTLAENDER[1] and F. DOLLE*[1]
1. CEA, I2BM Service Hospitalier Frederic Joliot, Orsay, France; 2. Sanofi Aventis, SAR&D, Bagneux, France

Objectives: The α7 nicotinic acetylcholine receptor subtype has recently been the focus of growing interest due to increasing evidence suggesting its involvement in psychiatric and neurological conditions such as schizophrenia and Alzheimer's disease. Recently, a novel series of highly potent α7-selective ligands was developed by Sanofi-Aventis. Within this series, SSR180711 [1,2] (1,4-diazabicyclo[3.2.2]nonane-4-carboxylic acid 4-bromophenyl ester) was selected on the basis of its pharmacological and biological characteristics (Ki of 22 nM and 14 nM for rat and human α7 n-AChRs, respectively) as a potent candidate for PET imaging and labeled with carbon-11 using [^{11}C]phosgene.

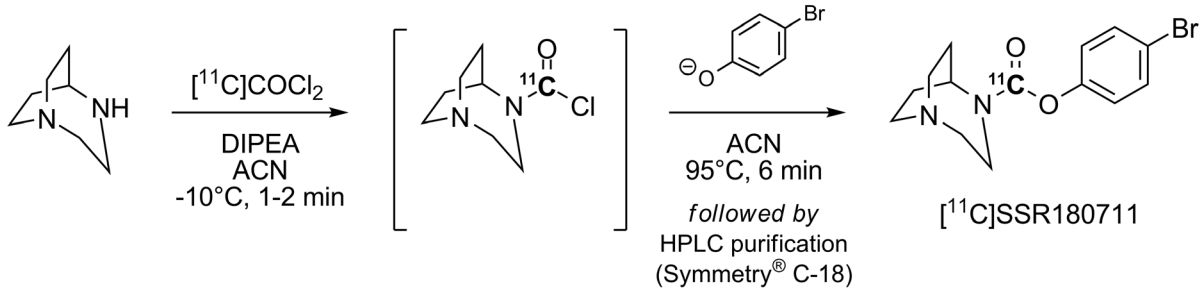
Methods: Carbon-11-labeling of SSR180711 comprises (1) trapping at -10°C of [^{11}C]phosgene (radiosynthesized from cyclotron-produced [^{11}C]methane via [^{11}C]carbon tetrachloride using minor modifications of published processes [3,4]) in acetonitrile (0.5 mL) containing 1,4-diazabicyclo[3.2.2]nonane dihydrochloride (0.24 mg, 1.2 μmoles) and DIPEA (2.5 μL) ; (2) addition of an excess of 4-bromophenolate (about 1.75 mg, 10 μmoles) in acetonitrile (0.5 mL) and heating at 95°C for 6 min; (3) purification using semi-preparative reversed-phase HPLC (Waters Symmetry® C-18 - eluent : ACN / H_2O / TFA : 24 / 76 / 0.3 (v/v/v) - flow rate : 8 mL/min - detection at 219 nm) and (4) SepPak®Plus C-18-based formulation for i.v. injection.

Results: Starting from a 26 GBq cyclotron-produced [^{11}C]methane batch, 0.55 to 0.74 GBq of [^{11}C]SSR180711, > 99% radiochemically pure and ready-to-inject, were obtained in a one-pot two-step process within 35 min (including HPLC-purification, R_t : 7-8 min, and formulation). Specific radioactivities ranged from 74 to 111 GBq/μmol.

Conclusions: SSR180711 was labeled with carbon-11 at its carbamate function using [^{11}C]phosgene. The decay-corrected overall yields for the preparation of [^{11}C]SSR180711 were 7.0%-9.4% (n=5). Dynamic PET studies in baboons (including pre-saturation experiments with nicotine and non-labeled SSR180711) are currently underway to evaluate the potential of [^{11}C] SSR180711 to image central α7 nicotinic acetylcholine receptors in vivo.

Research Support: Supported by the EC - FP6-project DiMI (LSHB-CT-2005-512146).

References: [1] Biton et al. Neuropsychopharmacol. (2007), 32, 1-16. [2] Pichat et al. Neuropsychopharmacol. (2007), 32, 17-34. [3] Link et al. J. Label. Compds Radiopharm. (1997), 40, 306-308. [4] Dolle et al. Bioorg. Med. Chem. Lett. (2003), 13, 1771-1775.



P334 SYNTHESIS OF [11C]TRIS VIA FLUORIDE ASSISTED RAPID NITROALDOL REACTION

K. KATO*, M. ZHANG, R. NAKAO, M. TAKEI, K. MINEGISHI and K. SUZUKI
National Institute of Radiological Sciences, Dept Molecular Probes, Chiba, Japan

Objectives: Inflammation is general target for the diagnosis by PET imaging. FDG-PET has been employed for in vivo analysis of inflammation so far because high uptake of FDG is observed for the inflammatory tissue. However, FDG is taken up to high glucose metabolism cells other than inflammation. Differentiation of inflammation from other physiological changes is important for the treatment. Therefore, new probe particular for inflammation is required.

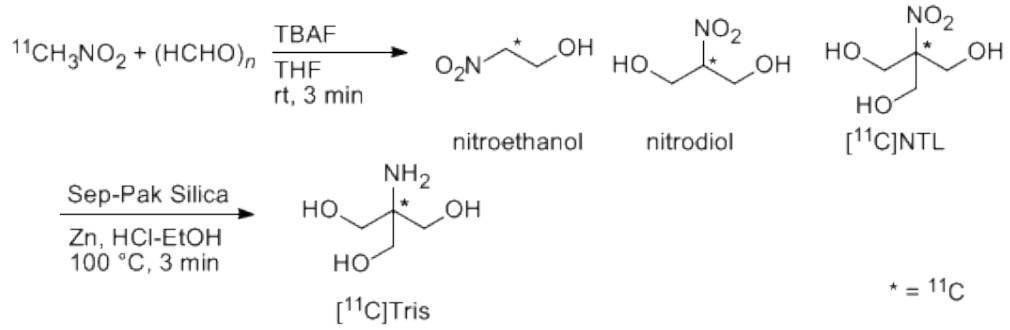
Methods: We focused on the acidosis accompanied with inflammation. Acidosis is phenomena that physiological equilibrium moves to acidic and neutralizing by alkaline agents is employed for the treatment. Thus, labeling alkaline agent with positron emitter became a considerable approach for the acidosis PET. Carbon-11 labeling of Tris is our target in this work. Tris has been used as a buffering medium in many biological applications with buffering pH range is 7 to 9. From the point of ^{11}C-labeling synthesis, the nitroaldol reaction between nitro[^{11}C]methane ([^{11}C]NM) and formaldehyde and subsequent reduction of nitro group of 2-(hydroxymethyl)-2-nitro[2-^{11}C]propane-1,3-diol ([^{11}C]NTL) are feasible for the synthesis of [^{11}C]Tris.

Results: The nitroaldol reaction between[^{11}C]NM and formaldehyde using EtONa as a base was enhanced by the addition of EtOH to afford nitroethanol in THF at 0 °C (entry 1 and 2). When the reaction temperature was wormed up to 40 °C, the reaction became faster and nitrodiol was obtained efficiently (entry 3). Additional higher temperature, however, did not cause further addition of formaldehyde to nitrodiol forming [^{11}C]NTL (entry 4). The stable intra-molecular hydrogen-bonding of nitrodiol might retard the further addition to form [^{11}C]NTL. Thus, efficient disruption of hydrogen-bond of nitrodiol is crucial for the reaction forming [^{11}C]NTL. Fluorine is the most electronegative element and the strong H-F bond energy furnishes the potential ability of fluoride acting as a base. Moreover, there are many reports that alkylation on the hetero atom with acidic proton is promoted via hydrogen bonding with fluoride. Thus, the fluoride assisted nitroaldol reaction using [^{11}C]NM was carried out in the presence TBAF. As a result, the desired [^{11}C]NTL was obtained very efficiently at room temperature (entry 5). After Sep-Pak separation, the reduction of nitro group by Zn in acidic solution was carried out at 100 °C for 3 min to give [^{11}C]Tris. The formation of [^{11}C]Tris was confirmed by LCMS using HILIC as a stationary phase. [Table]

Conclusions: [^{11}C]Tris was synthesized via nitroaldol reaction using [^{11}C]NM and subsequent reduction. Fluoride assisted nitroaldol reaction between [^{11}C]NM and formaldehyde was rapid and afforded [^{11}C]NTL efficiently.

Radiochemical conversion of nitroaldol reactions

entry	base (μmol)	EtOH (μL)	temp (°C)	nitro-ethanol (% conv)	nitrodiol (% conv)	[^{11}C]NTL (%conv)
1	EtONa	none	0	<8	n.d.	n.d.
2	EtONa	10	0	64.4±3.1	16.3±4.6	n.d.
3	EtONa	10	40	29.2±5.2	67.0±5.9	n.d.
4	EtONa	10	100	2.9±1.4	85.3±2.1	n.d.
5	TBAF	none	rt	n.d.	n.d.	97>



P335 HIGHLY EFFICIENT SYNTHESIS OF [11C]CELECOXIB BY PALLADIUM(0)-MEDIATED RAPID C-[11C] METHYLATION USING AN ORGANOBORON PRECURSOR AND PET IMAGING OF COX-2 EXPRESSION IN RAT BRAINS

M. TAKASHIMA-HIRANO*[1], Y. CUI[2], T. TAKASHIMA[2], E. HAYASHINAKA[2], Y. WADA[2], Y. WATANABE[2], H. DOI[3] and M. SUZUKI[1]

1. RIKEN Center for Molecular Imaging Science (CMIS), Molecular Probe & Drug Design Laboratory, Kobe, Japan; 2. RIKEN Center for Molecular Imaging Science (CMIS), Molecular Probe and Dynamics Laboratory, Kobe, Japan; 3. RIKEN Center for Molecular Imaging Science (CMIS), Drug Synthesis & Molecular Probing Unit, Kobe, Japan

Objectives: Cyclooxygenase (COX)-2, a rate-limiting enzyme of the arachidonic acid cascade, is up-regulated in response to inflammatory stimuli, cytokines, and mitogens, and regulates a variety of patho-physiological processes, such as cancer, arthritis, ischemic heart disease, stroke, organ rejection, Alzheimer's disease and Parkinson's disease. For in vivo imaging of COX-2 using non-invasive positron emission tomography (PET) technique, we designed and synthesized Carbon-11 labeled Celecoxib, a selective COX-2 inhibitor, as a PET tracer. Small animal PET imaging was performed in rat model of neurogenic inflammation to confirm the evaluation of COX-2 expression.

Methods: [^{11}C]Celecoxib was synthesized by cross-coupling between [^{11}C]methyl iodide and a corresponding pinacol borate precursor under the standard radiolabeling conditions of $Pd_2(dba)_3$/P(o-tolyl)$_3$/K_2CO_3 (1:4:4) in DMF at 65 °C for 4 min, using a Sumitomo CYPRIS HM-12S cyclotron and an automated synthesis system in RIKEN CMIS. The specificity and Blood-Brain Barrier permeability of the tracer was confirmed in the rats generated unilateral cortical spreading depression (SD) characterized by the propagation of neuronal/glial membrane depolarization followed by intense expression of COX-2 in the cerebral hemisphere. For the displacement study, NS-398, a highly selective COX-2 inhibitor, was used.

Results: The chemical synthesis was realized by application of the Pd(0)-mediated rapid C-[^{11}C]methylation using an organoboron precursor, with the aim of establishing the one-step ^{11}C-labeling without deprotection reaction. Chemical results of [^{11}C]Celecoxib synthesis are shown as follows: decay-corrected radiochemical yield after HPLC preparative isolation based on [^{11}C]CH_3I, up to 97%; decay-corrected radiochemical yield after prescription procedure based on [^{11}C]CH_3I, 59%; obtained radioactivity for administration, 5.9-6.2 GBq; specific radioactivity, 87±2 GBq/μmol (n = 3); radiochemical purity, >99%; and total synthesis time, 35 min. The [^{11}C]Celecoxib-PET studies revealed that radioactivity of [^{11}C]Celecoxib was dominantly increased in the cerebral hemisphere in which the intense expression of COX-2 was induced by unilateral cortical SD, and clearly was displaced by excess unlabeled NS-398.

Conclusions: Thus, we have succeeded in establishing the efficient synthesis of [^{11}C]Celecoxib and visualizing the spatial-temporal distribution of COX-2 in the rat brain by PET imaging technique. In conclusion, [^{11}C]Celecoxib will be a potentially useful PET probe for evaluating COX-2 expression and drug development.

P336 AN EFFICIENT METHOD FOR THE INCORPORATION OF A POSITRON-EMITTING 11C RADIONUCLIDE INTO VARIOUS HETEROAROMATIC FRAMEWORKS BY Pd(0)-MEDIATED RAPID COUPLING OF METHYL IODIDE WITH HETERO-ARYLSTANNANES

M. SUZUKI[1], K. SUMI[2], H. KOYAMA*[2], SIQIN[2], T. HOSOYA[2], M. TAKASHIMA-HIRANO[1] and H. DOI[1]
1. RIKEN, Center of Molecular Imaging Science, Kobe, Japan; 2. Gifu University, Graduate School of Medicine, Gifu, Japan

Objectives: In order to synthesize PET tracers, we have developed the rapid cross-coupling of methyl iodide with excess amounts of aryltributylstannanes. However, as indicated by us and others,the reaction conditions for such a rapid C-methylation using the stannane seem to be not optimized yet, particularly for heteroaromatic frameworks in the core structure. Here, we investigated the Pd(0)-mediated rapid trapping of methyl iodide with an excess amount of a hetero-aromatic ring-substituted aryltributylstannane with the aim to incorporate a short-lived ^{11}C-labeled methyl group into a heteroaromatic carbon frameworks efficiently to synthesize a PET tracer in high yield.

Methods: We chose various basic and non-basic heteroaromatic type aryltributylstananes, where the reaction using the 1:40 ratio of methyl iodide and a tin substrate was set up by keeping the actual PET tracer synthesis in mind. Firstly, we reinvestigated the conditions so far accumulated in our group for the rapid methylations using aryl, alkenyl, and alkynylstannanes. Consequently, we discovered the novel conditions applicable to all compounds taken up in this study, and the utility of the rapid methylation was well demonstrated by the synthesis of an actual PET tracer. Sumitomo CYPRIS HM-12S cyclotron and an automated synthesis system in RIKEN CMIS were used in the PET tracer synthesis.

Results: The study was done firstly using our previously developed CH_3I/stannane/$Pd_2(dba)_3$/P(o-tolyl)$_3$/CuCl/K_2CO_3 (1:40:0.5:2:2:2) combination system in DMF at 60 °C for 5 min (condition A), but the reaction gave low yields for various kinds of heteroaromatic structures. The increase of the amount of an added bulky phosphine (condition B) improved the reaction yield to a considerable extent, but the conditions were still insufficient in terms of a range of adaptable heteroaromatic structures. Another CH_3I/stannane/$Pd_2(dba)_3$/P(o-tolyl)$_3$/CuBr/CsF combination system (condition C) also provided a similar result as the condition B even under an increased amount of the phosphine. Consequently, we found that the problem was overcome by replacing DMF with N-methyl-2-pyrolidinone (NMP) as a solvent. Thus, the reaction in NMP at 60–100 °C for 5 min using a CH_3I/stannane/$Pd_2(dba)_3$/P(o-tolyl)$_3$/CuBr/CsF (1:40:0.5:16:2:5) combination system (condition D) gave the methylated products in >80% yields (based on the reaction of CH_3I) for all of heteroaromatic compounds listed in this study. The method were applied to the synthesis of an actual PET tracers, 2-[^{11}C]methylpyridine and 3-[^{11}C]methylpyridine, using stannane/$Pd_2(dba)_3$/P(o-tolyl)$_3$/CuBr/CsF (3:1:16:2:5) at 60 °C for 5 min, giving the desired product in 88 and 91% HPLC analytical yields, respectively.

Conclusions: We elaborated an efficient protocol for the rapid C-methylation by the reaction of methyl iodide with an excess amount of a heteroaryltributylstannane useful for the synthesis of a short-lived ^{11}C-incorporated PET tracer.

P337 A REVERSIBLE EVAPORATOR/CONDENSER AND ITS USE IN PRODUCTION OF [11C]METHYL IODIDE

B. ALVORD*, T. GRAVES, N. C. MURPHY and P. ROY
Siemens Healthcare, Molecular Imaging, Knoxville, TN

Objectives: [^{11}C]Methyl Iodide is produced by introduction of iodine vapor into a stream of helium containing [^{11}C]Methane at 700 °C[1].[2]. Typically the iodine is generated in a small evaporator, and unused iodine is condensed out downstream of the labeling step with a different device. Previous studies[2] had indicated a higher labeling yield over 0.5 mg/cc densities, so we were interested in building and testing a device that could generate stable iodine densities in the 1-4 mg/ml range. We also wanted to eliminate accumulation of crystalline iodine deposits downstream of the condenser that are a serviceability issue in other modules. Finally, we wanted to significantly reduce intervention in the methyl iodide system to improve overall specific activity. Our chosen path was to design a high efficiency evaporator/condenser that could be used reversibly at both ends of the process. [1] Larsen, P. et al., Appl. Radiat. Isot. 48, 153-157 (1997) [2] Link, J. et al., Nuc. Med. Biol. 24, 93-97 (1997)

Methods: Initially calculations were performed to simulate deposition rates on a 1-D plate at various temperatures, densities and flows. This led us to develop an ideal temperature profile for a channel to evenly deposit iodine in condensation mode. Multiple prototypes were made to better approximate this temperature profile until a suitable design was reached. This design was incorporated into a synthesis module which was used to produce methylated compounds.

Results: The device performs to specification both as an evaporator and a condenser. As an evaporator it is capable of reliably delivering 0.2 to 3.3 mg/ml in 20 sccm helium flows. At 0.2 mg/ml and 20 sccm the evaporator has the capacity for 40 hours of continuous operation before exhausting iodine. Operating as a condenser, the device traps iodine with 98% efficiency at the maximum flow density of 3.3 mg/ml and 120 sccm. Condensing efficiency increases to 99.5% at a more nominal density of 0.05 to 0.13 mg/ml and 120 sccm flows. This design enables running easily up to 8 hours in one flow direction, after which flow is reversed, temperatures are adjusted slightly, and it is possible to operate for another 8 hours. 43 runs have been performed in one direction, and 87 runs have been performed without any user servicing. Methyl Iodide yields (decay corrected, from CO_2, approx. 10 minute synthesis) are routinely 18% at low iodine densities, but yields as high as 44% have been recorded at high iodine densities.

Conclusions: The design enables Methyl Iodide production at reasonable yields with absolutely minimal intervention. Further work is indicated on yield optimization. The utility of the evaporator/condenser for removing contaminants from iodine and other specific activity enhancing techniques have yet to be fully investigated but are extremely promising.

P338 DETERMINATION OF LABELED METABOLITES OF [11C]METHYL-CANDESARTAN IN RAT PLASMA AND KIDNEY USING A COLUMN-SWITCH METHOD

S. KIRKPATRICK, T. HADIZAD*, M. LORTIE, R. A. DEKEMP, R. S. BEANLANDS and J. N. DASILVA
University of Ottawa Heart Institute, National Cardiac PET Centre, Ottawa, ON, Canada

Objectives: Angiotensin II AT_1 receptors (AT_1R)s play a role in the pathogenesis of cardiovascular and renal diseases. Studies have reported that the O-methyl-ester derivative of candesartan binds with high affinity and antagonistic activity to AT_1Rs (IC_{50} Me-candesartan 66 nM, candesartan 99 nM). Hydrolysis of O-alkyl esters was reported to occur in vivo. Our recent work with [^{11}C]Me-candesartan, a novel AT_1R radioligand, has shown high specific binding in the kidneys at 15 min and rapid clearance. We present here the relative proportions of labeled metabolites of [^{11}C]Me-candesartan using a reverse-phase capture and analytical HPLC column-switch method.

Methods: Male Sprague-Dawley rats (210-325 g, n=4) were injected iv with 8-13 mCi of [^{11}C]Me-candesartan (>300 mCi/μmol specific activity) and sacrificed 15 min post-injection. Trunk blood was collected and centrifuged at 4,000 rpm for 5 min. Kidneys were quickly dissected out, homogenized in 5-7 ml of 80/20 ethanol/water (v/v), and centrifuged at 22,000 rpm for 15 min. The supernatant was evaporated and reconstituted in 1/99 acetonitrile/water (v/v). Plasma protein binding was disrupted by adding 0.4-1.0 g/ml urea to plasma and tissue supernatant samples. Samples were analysed by column-switch HPLC utilizing a capture column packed with Oasis HLB sorbent and a reverse-phase analytical column with UV absorbance (280 nm) and radioactivity (coincidence detection, Bioscan) detection. Control plasma (n=4) and kidney (n=4) samples were collected and processed as above in the presence of authentic [^{11}C] Me-candesartan. Data were expressed as mean percent of total radioactivity for each sample.

Results: Control samples of plasma and kidney displayed >97.8% unchanged [^{11}C]Me-candesartan confirming our methodology. Fifteen min post-injection, unchanged [^{11}C]Me-candesartan (10.4 min post-switch) accounted for 91.1 ± 6.8 % and 70.7 ± 10.2 % of the total radioactivity in rat plasma and kidney respectively. Rat plasma and kidney samples showed three hydrophilic radiolabeled metabolite peaks. Peak 1 (0-2 min capture column elution) represents 4.8 ± 3.2 % of the total signal in rat plasma and 0.8 ± 0.9 % in rat kidney. Peak 2 (3.6 min post-switch) accounts for 2.2 ± 2.6 % of the total signal in rat plasma and 6.2 ± 3.9 % in rat kidney. Peak 3 (5.4 min post-switch) comprises 1.9 ± 2.0 % and 22.3 ± 7.9 % of the total radioactivity in rat plasma and kidney samples respectively.

Conclusions: At fifteen min post-injection, greater than 70% activity corresponds to unchanged [^{11}C]Me-candesartan in kidney, whereas this proportion is greater than 90% in plasma. High levels of unchanged [^{11}C]Me-candesartan in plasma is consistent with the expected dealkylation mechanism of the C-11 methyl at the carboxylic acid. The presence of the C-11 labeled hydrophilic metabolite in the kidney suggests its production in situ. Attempts to identify this derivative and its interaction with AT_1 receptor are currently underway.

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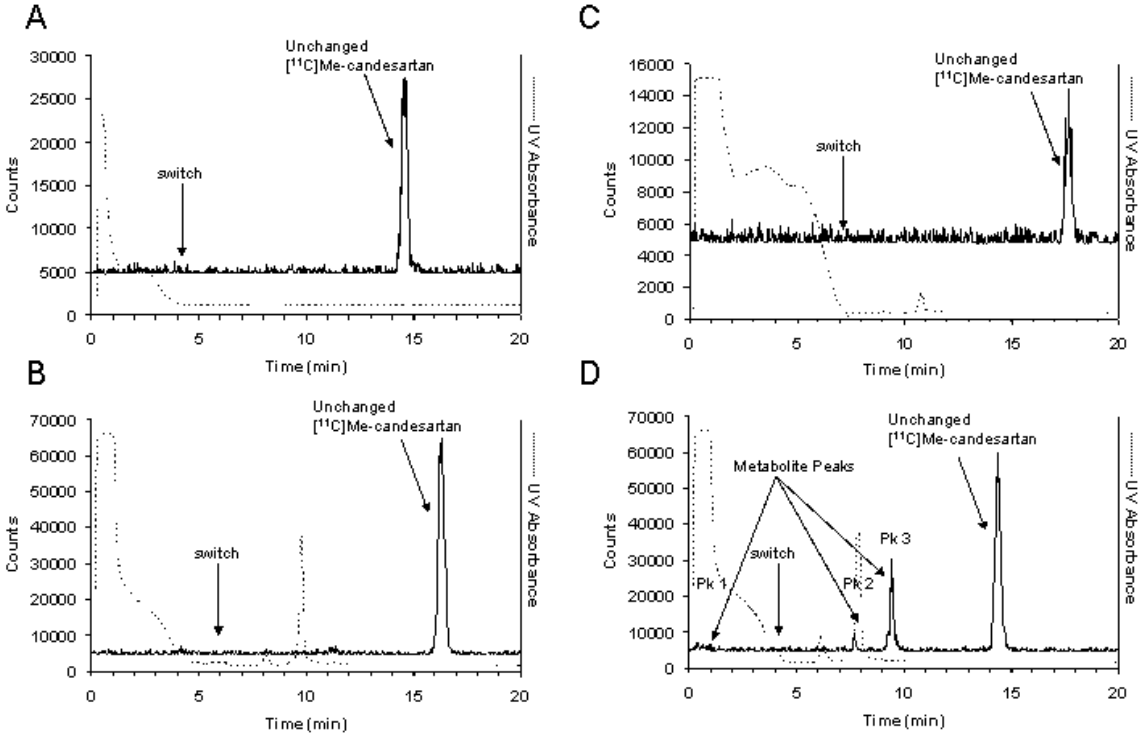

Figure. Sample chromatograms depicting the radioactive peaks in: (A) control plasma or (B) control kidney homogenate in the presence of authentic [^{11}C]Me-candesartan, and (C) *in vivo* rat plasma or (D) kidney homogenate 15 min following [^{11}C]Me-candesartan administration. Columns and solvents were switched at times indicated.