TOWARDS THE SIMPLEST [^{11}C]ACETATE SYNTHESIS

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Keywords: Acetate, solid phase synthesis, SPE

[^{11}C]acetate is a well known radiopharmaceutical, obtained by reaction of $^{11}CO_2$ on methylmagnesium halide, hydrolysis and formulation. Numerous automated and improved production system have been reported ((1, 2; recently 3, 4 among others). Pitfalls in this synthesis are well known, related mainly on radiochemical impurities arising from grignard reagent in excess.

In view of recent progress in solid phase synthesis, including "loop" methods (5) and solid phase extraction purification (SPE) we aimed to combine these methods to achieve the "simplest" acetate radiosynthesis.

A first attempt based on direct carboxylation of 100 µL diluted grignard reagent (CH_3MgBr 0.5 M in THF) trapped on a dry SPE cationic cartridge confirmed (6) that if $^{11}CO_2$ trapping is complete more than 50 % of radioactivity remains on cartridge after acid elution.

We then performed carboxylation in 2 ml loops made of 1.5 mm ID PEEK, polypropylene tubing, and 1/16" SS HPLC loop. All materials have been used with the same success: 100 µL of diluted grignard reagent are introduced under nitrogen (10 ml.min^{-1}). $^{11}CO_2$ from cryogenic trap is then sent through the loop to achieve carboxylation. Trapping efficiencies are very good, in excess of 90 %, if flow is kept low. Hydrolysis in the loop is then obtained by flushing 2 ml of diluted acid. Purification is achieved either with HPLC on a C18 column (direct injection from the loop) eluted with NaCl 0.9 %, or better by a combination of SPE columns (3), cationic, Ag to remove impurities and anionic that traps [^{11}C]acetate. This cartridge is finaly washed with diluted acid, water and eluted with NaCl 0.9 % to obtain [^{11}C]acetate, in injectable form after sterilisation, with good radiochemical and chemical purities.

Corrected yields exceed 65 % in 8 minutes synthesis with a solid phase method benefiting from a minimal amount of grignard reagent and simple automation, possible on available commercial systems like the Bioscan 11-C Loop System with minor changes (CO_2 trap).

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ONE-POT SYNTHESIS OF DOCETAXEL USING RADIOLABELED CARBON DIOXIDE

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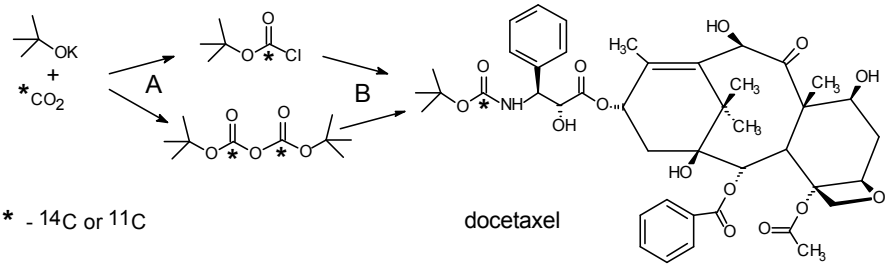
Keywords: Docetaxel, Carbon Dioxide, DiBOC, *tert*-Butyl Chloroformate, MDR

The taxane class of anticancer drugs has demonstrated remarkable activity. Docetaxel and paclitaxel are the first two drugs in this class and have already become standard treatments for several tumor types. Accumulation of docetaxel in tumor is necessary for the drug to be cytotoxic. One mode of resistance to docetaxel is through increased efflux of the drug from a tumor by multi-drug resistance transporters initially named MDR. It would be useful to have radiolabeled docetaxel to study these transporters *in vitro* and even more desirable to monitor these transporters in man and to measure the accumulation of docetaxel in tumors using [^{11}C]docetaxel as a probe. The current work is the preliminary approach to the synthesis of [^{14}C]docetaxel with the goal of translating the procedure into a method for preparation of [^{11}C]docetaxel.



A. Potassium *tert*-butoxide is mixed with carbon dioxide followed by the addition of methanesulfonyl chloride. The product of this reaction is di-*tert*-butyl dicarbonate (diBOC) as described by Kurimoto, et al, US Patent 5,151,542 or as the amount of methane sulfonyl chloride is increased the product is likely *tert*-butyl chloroformate. **B**. Docetaxel primary amine is added directly to the mixture. The sample is dried at 50°C with a stream of air. The residue is dissolved in mobile phase and can be analyzed or purified by HPLC to give docetaxel.

$Na_2[^{14}C]CO_3$ is used to synthesize [^{14}C]docetaxel and the product is confirmed by HPLC analysis using UV and radioactivity detectors. To start, sulfuric acid is added to 20 mol $Na_2[^{14}C]CO_3$. The $[^{14}C]CO_2$ generated is transferred for 10 min through a drying sulfuric acid into a stirred, room temperature solution, ~17°C, of potassium *tert*-butoxide, 25 mol, in 1 ml of methylene chloride. Methanesulfonyl chloride, 129 mol, is added and the reaction is stirred at room temperature for 10 min. A solution of docetaxel primary amine, 4 mol, in 1 ml ethyl acetate is added directly. After 10 min at room temperature the mixture is dried at 50°C with a stream of air. Analysis by HPLC gives a molar amount of [^{14}C]docetaxel that is >10% of the starting $Na_2[^{14}C]CO_3$. The procedure is performed in one pot with two 10-min reactions and does not require any blocking or de-blocking procedures.

RADIOSYNTHESIS OF A C-11 LABELED INHIBITOR OF GLYCINE TRANSPORTER TYPE-2

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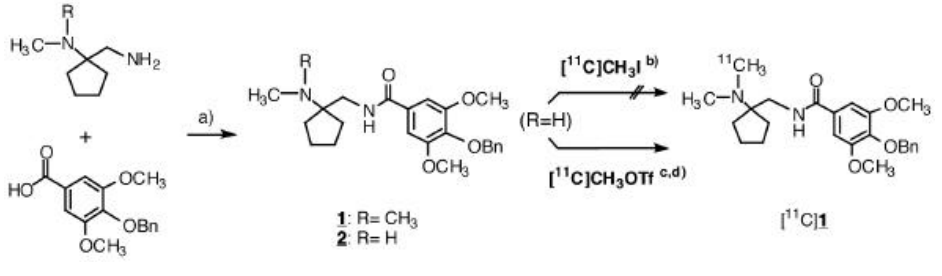
Keywords: glycine transporter type-2, inhibitor, glycine receptor, carbon-11, PET

Inhibitory neurotransmissions are mediated by two amino acids, GABA (-aminobutyric acid) and glycine, in mammalian CNS. Both of the amino acids exert the inhibitory actions through their distinct receptors coupled to ligand-gated chloride ion channels. With identification of a link between dysfunctions of GABA neurotransmission and CNS diseases such as epilepsy and Huntington's chorea, several positron-emitter labeled radioligands for GABA receptors have been developed so far to study the GABA-ergic neurons by PET. In contrast, due to a lack of selective drugs binding to glycine receptors, little is known about the involvements of glycine receptors in CNS diseases, as well as in CNS functions. Only strychnine is known to bind the glycine receptors and inhibit its neurotransmission. Therefore, the glycine receptors are so-called as strychnine-sensitive glycine receptors (ssGlyR) to distinguish from the strychnine-insensitive glycine receptors that are coupled to NMDA receptors.

Recently, first potent and selective inhibitors of glycine transporter type-2 (GlyT-2) have been developed[1)]. GlyT-2 is a Na^+/Cl^--dependent transporter and is co-localized with ssGlyR in the spinal cord and brain. Therefore, developments of positron-emitter labeled radioligands for GlyT-2 would provide a useful tool for studying the mammalian ssGlyR by PET. In this paper, we report a synthesis of C-11 labelled GlyT-2 inhibitor ([^{11}C]**1**, Scheme 1) as a potential PET radioligand.

The authentic **1** (IC_{50}= 16 nM) and a substrate (**2**) for the synthesis of [^{11}C]**1** were prepared according to the methods described in the literature[1)]. Our initial attempts to obtain [^{11}C]**1** by *N*-methylation with [^{11}C]methyl iodide were unsuccessful in the reaction conditions tested (solvent: DMF, base: NaH or TBAOH, temperature: 30–80°C). However, the *N*-methylation was successfully accomplished by use of a [^{11}C]methyl triflate ([^{11}C]CH_3OTf), an alternative and highly reactive [^{11}C]methylating agent. The reaction of **2** with [^{11}C]CH_3OTf (30°C for 3 min) in acetone and subsequent HPLC purification (C18, MeOH/H_2O/triethylamine=50/50/0.1, 6 ml/min) gave 1.5-2.1 GBq of [^{11}C]**1** at EOS, after a 5-10 min proton-bombardment at a beam current of 15 A. The total synthesis time and radiochemical purity were about 30 min from EOB and 95%, respectively. The *in vivo* evaluation of [^{11}C]**1** as a PET radioligand for GlyT-2 is currently underway.



Scheme 1. Radiosynthesis of [^{11}C]**1**. **a)** carbonyldiimidazole, THF, r.t., 3 h. **b)** **2** (1 mg), DMF (300 L), NaH or TBAOH (0-10 eq), 30-80°C, 3 min. **c)** [^{11}C]CH_3OTf was obtained by passing of [^{11}C]CH_3I through a silver triflate column (200 °C) under a stream of N_2. **d)** **2** (1 mg), acetone (300 L), 30°C, 3 min. Bn= CH_2Ph, Tf= SO_2CF_3.

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ASYMMETRIC SYNTHESIS OF ^{11}C-LABELLED a-METHYL AMINO ACIDS VIA METALLOCOMPLEX CHIRAL SYNTHONS

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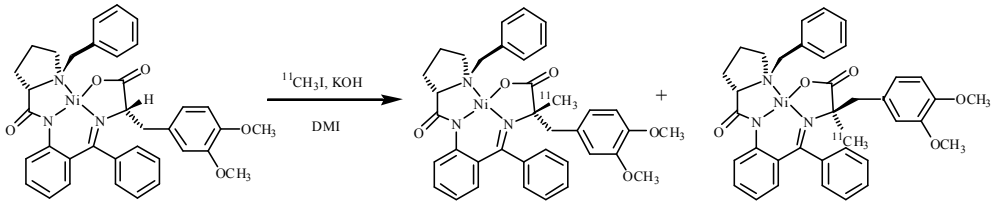
Keywords: ^{11}C, amino acids, -[^{11}C]methylDOPA, nickel

There are only few published methods for asymmetric synthesis of ^{11}C-labelled -methyl amino acids. With the exception of ^{11}C-labelled -methyltyptophan and ^{14}C-labelled -methyl-tyrosine, no other enantiomerically pure -methyl amino acids have been used for *in vivo* investigations in laboratory animals or man. The Ni(II) complex of the Schiff base of (*S*)-*N*-benzylproline (2-benzoylphenyl)amide (BPB) and -alanine has been extensively used for preparative asymmetric syntheses of non-labelled enantiomerically pure -methyl amino acids. Based on previously described application of a similar complex for asymmetric synthesis of [^{11}C]alanine, [^{11}C]methylation of the Ni(II) complex of the Schiff base of BPB and -phenyl-alanine was tested. It was reported that '… all attempts to alkylate these complexes with ^{11}C-labelled alkyl iodides were unsuccessful. The increased steric hindrance makes the alkylation reaction so slow that very little alkylation was observed even when a large excess of substrate was used.' (1) In order to re-investigate this reaction, we carried out [^{11}C]methylation of a similar complex derived from protected DOPA. Methylation of this sterically hindered substrate was acheived in the aprotic solvent 1,3-dimethylimidazolidin-2-one (DMI) within 30 min at room temperature, using a five-fold excess of methyl iodide and a large excess of dry potassium hydroxide. For [^{11}C]methylation, potassium hydroxide (2 mg of fine dry powder) was sealed in a vial, which was flushed with dry argon before addition of a solution of the complex (2 mg) and $^{11}CH_3I$ in DMI (300 l). After 2 min at 25ºC, a 9% radiochemical yield (decay corrected) of a mixture of the diastereomeric -[^{11}C]methylDOPA complexes was achieved.



Acidic hydrolysis of the diastereomerically pure complexes, after separation by HPLC, should lead to pure enantiomers of -[^{11}C]methylDOPA. The diastereomers can be separated within 10-15 min under reversed phase conditions. Optimisation of the labelling conditions and purification procedures underway.

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APPLICATION OF [^{11}C]METHYL IODIDE IN A STILLE REACTION. SYNTHESIS OF A NEW LABELLED ANALOGUE OF CITALOPRAM AS POTENTIAL TRACER FOR THE SEROTONIN TRANSPORTER

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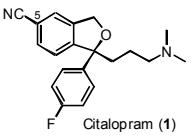
Keywords: [^{11}C]Methyl iodide, Stille reaction, specific activity, serotonin transporter, PET

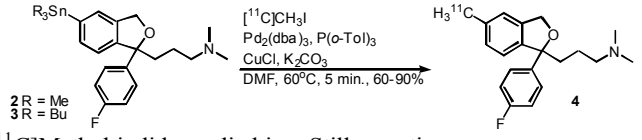
Introduction. The selective serotonin reuptake inhibitors citalopram (**1**) and its pharmacologically active enantiomer escitalopram have previously been labelled with ^{11}C in the *N*-methyl position, to validate their potential as a tracers for the serotonin transporter (SERT).[1,2] The target to background ratio observed in a human PET study with ^{11}C-labelled escitalopram was, however, low.[2] From extensive structure activity relationship studies of citalopram analogues it appears, that relatively large variations in the 5-position are allowed without subsequent loss of potency as inhibitors of the SERT and selectivity with respect to the dopamine and noradrenaline transporters.[3,4] These observations encouraged us to investigate the possibility of introducing structurally different ^{11}C-labelled substituents in the 5-position of citalopram analogues.



Citalopram (**1**)

Results and discussion. In the present study the 5-cyano group was replaced with a methyl group. The compound was synthesized and different radiolabelling strategies were investigated.

[^{11}C]Methyl iodide has previously been applied in Stille reactions for ^{11}C-C bond formations.[5] The ^{11}C-labelled methyl derivative **4** was obtained using this strategy (Scheme 1).



Scheme 1. [^{11}C]Methyl iodide applied in a Stille reaction.

The specific activity of **4** ([^{11}C]methyl iodide was obtained from [^{11}C]carbon dioxide) was dependent on the precursor. If the trimethyltin derivative **2** was applied as precursor, the specific activity of **4** was relatively low (0.1-2.5 GBq/ mol, n=6). In contrast, the specific activity of **4** was significantly higher (10-35 GBq/ mol, n=5) if the tributyltin derivative **3** was used as precursor.

In a monkey PET study **4** accumulated in agreement with the known distribution of serotonin transporters in the brain. The maximal thalamus to cerebellum ratio of 1.3 was reached 85 minutes after injection. The specific binding was only partly blocked after pre-treatment with citalopram.

Conclusion. Radiosynthesis of the ^{11}C-labelled compound **4** was successfully performed in a Stille reaction with [^{11}C]methyl iodide. The specific activity of **4** was found to be highly dependent on the choice of precursor. Compound **4** was evaluated in a monkey PET study and does not seem to exhibit appropriate properties as tracer for the serotonin transporter.

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SYNTHESIS OF NCA [*carbonyl*-^{11}C]-LABELED AMIDES USING A POLYMER-SUPPORTED CARBODIIMIDE UNDER MICROWAVE CONDITIONS

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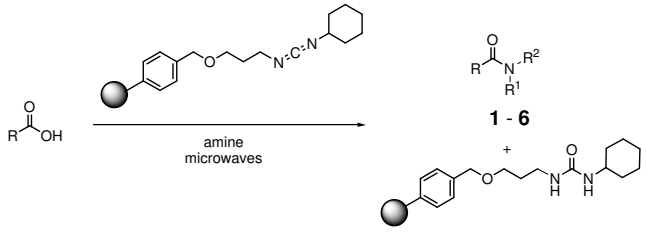
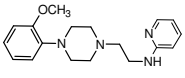
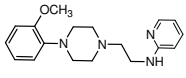
Keywords: Carbon-11, amide, microwave, PS-carbodiimide

Carbon-11 labeled amides, such as [*carbonyl*-^{11}C]WAY-100635, are an important class of PET radiopharmaceuticals. The most widely used method to prepare a no-carrier-added (NCA) [^{11}C]amide is to react the amine with the corresponding NCA [^{11}C]acid chloride. A comparable method that reacts an easily prepared NCA [^{11}C]carboxylic acid with amine to form NCA [^{11}C]amide in one pot would considerably simplify the labeling procedure.

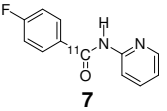
Polymer-supported carbodiimide resin (PS-CDI, *N*-cyclohexylcarbodiimide-*N*'-propyloxymethyl Polystyrene) has been used to prepare amides or amide libraries. The main advantage is less purification effort. However, these procedures usually require long reaction times (several hours or even days) and sometimes an additional activating agent, such as HOBT, is needed. Recent developments in microwave-enhanced chemistry provide a faster, cleaner, more selective and highly atom-efficient way to overcome the disadvantages of the existing procedure. Therefore the scope of this reaction can now be extended to ^{11}C-labeling.



Compound	1	2	3	4	5	6
R						
Amine						

Amides (**1**-**6**), including WAY-100635 (**3**) and *p*-MPPF (**6**), were synthesized rapidly using a microwave-enhanced one-step coupling procedure on PS-CDI. Typically, resin (33 mg, loading capacity 1.22 mmol/g), acid (0.02 mmol), amine (0.10 mmol) and solvent (300µl) are sealed in the 10 ml standard reaction tube and irradiated for 10 min at 100 ºC and 200 W power input using CEM's Discover microwave system). HPLC, MS and ^{1}H NMR analyses indicate that only the amide and the excess amine are present in the solution. Solvents, such as acetonitrile, THF, and THF with 1% 1 M HCl are tolerated. The use of THF is particularly advantageous because it is a common solvent for ^{11}C carbonylation of Grignard reagents.

The carboxyl-labeled acids in THF, from reactions of Grignard reagents with [^{11}C]CO_2, are used after a simple filtration in the subsequent microwave-enhanced coupling reactions on PS-CDI to obtain NCA [*carbonyl*-^{11}C]amides **7** and **8**. Optimization of the radiolabeling procedure is in progress.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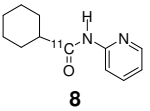
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IMPROVED SYNTHESIS OF [^{11}C]SA4503, [^{11}C]MPDX AND [^{11}C]TMSX BY THE USE OF [^{11}C]METHYL TRIFLATE

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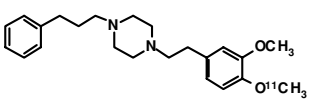
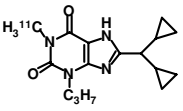
Keywords: carbon-11, methyl triflate, SA4503, MPDX, TMSX

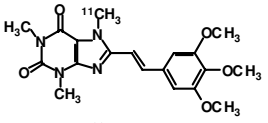
Several ^{11}C-methylation have been improved by substituting [^{11}C]CH_3I for [^{11}C]methyl triflate ([^{11}C]CH_3OTf), because [^{11}C]CH_3OTf has been shown to be a highly reactive alternative to [^{11}C]CH_3I [1]. Recently, we have used [^{11}C]SA4503 for sigma$_1$ receptor ligand [2], [^{11}C]MPDX for adenosine A_1 receptor ligand [3], and [^{11}C]TMSX for adenosine A_{2a} receptor ligand [4] in clinical studies (Fig. 1). These radioligands were synthesized by methylation of the respective demethyl-precursor with [^{11}C]CH_3I in anhydrous DMF containing NaH (1–2 mg) or Cs_2CO_3 (10 mg) as a base. In this study, we demonstrated the synthesis of [^{11}C]SA4503, [^{11}C]MPDX and [^{11}C]TMSX by the use of [^{11}C]CH_3OTf.



[^{11}C]SA4503



[^{11}C]MPDX



[^{11}C]TMSX

Fig. 1 Chemical structure of [^{11}C]SA4503, [^{11}C]MPDX and [^{11}C]TMSX

[^{11}C]CH_3OTf was prepared by passing [^{11}C]CH_3I through a silver triflate column at 200°C. [^{11}C]CH_3OTf was trapped in the solution of precursor dissolved in DMF containing 1 mol/l NaOH (0.005–0.01 ml) or Cs_2CO_3 (10 mg) at room temperature. The radiochemical yield of [^{11}C]SA4503 from [^{11}C]CH_3OTf was higher than that from [^{11}C]CH_3I, while that of [^{11}C]TMSX was improved in the presence of Cs_2CO_3 (Table 1). The radiochemical yield of [^{11}C]MPDX from [^{11}C]CH_3OTf was slightly increased, although the yield of [^{11}C]7-methyl isomer was also increased in the presence of NaOH. In conclusion, the use of [^{11}C]CH_3OTf improved radiochemical yields of [^{11}C]SA4503, [^{11}C]MPDX and [^{11}C]TMSX.

Table 1. Radiochemical synthesis of [^{11}C]SA4503, [^{11}C]MPDX and [^{11}C]TMSX

	Reagent	Base	Reaction	Radiochemical yields (%) [Ref.]
[^{11}C]SA4503	[^{11}C]CH_3I	NaH	120°C, 1 min	21– 31 [2]
	[^{11}C]CH_3OTf	NaOH	none	22– 40
		NaOH	120°C, 1 min	42– 54
[^{11}C]MPDX	[^{11}C]CH_3I	NaH	120°C, 1 min	19– 30 (0.35–1.6)[3]
([^{11}C]7-isomer)	[^{11}C]CH_3OTf	NaOH	none	25– 43 (13– 22)
		NaOH	120°C, 1 min	21– 36 (16– 21)
[^{11}C]TMSX	[^{11}C]CH_3I	Cs_2CO_3	120°C, 3 min	25– 46 [4]
	[^{11}C]CH_3OTf	NaOH	none	5.9– 12
		Cs_2CO_3	none	54– 62

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LABELING TOPOTECAN WITH METHYL IODIDE FOR METABOLISM STUDIES AND PET IMAGING OF TUMORS

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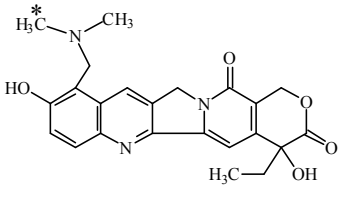
Keywords: topotecan, carbon-11, cancer therapy

Topotecan is an anticancer drug that is used for treatment of patients with ovarian and lung tumors. In cell culture, the anticancer activity of topotecan is reduced in some tumors by active efflux pumps that reduce cellular accumulation of drug. With a suitable noninvasive imaging probe, we might determine in patients which tumors accumulate topotecan, and which do not. The probe could be used to test the hypothesis that patients whose tumors have high accumulation of topotecan are more likely to benefit from this drug.

We have developed a method to efficiently prepare labeled topotecan that can be used ultimately for PET imaging. The precursor (N-demethyl topotecan) was prepared from authentic topotecan using a modification of the N-demethylation reaction of Rao, P.N. et al. (1). LC conditions were established to adequately separate precursor from product and allow for LCMS analysis. N-methylation was achieved without the use of protecting groups.



Reaction conditions were initially established with trideuterated methyl iodide. CD_3I, 0.5-5ug (3.5-30nmol), was mixed with 25-50ug (60-100nmol) precursor. In order to preserve the hydrolyzable lactone moiety in topotecan, gentle reaction conditions were used: 10% 0.02M K_2CO_3, 50C, 10min. Yields of 30% were obtained. Temperatures over 60C for 10min led to the formation of various side products. Only DMSO and DMF were found to support methylation under these conditions. During product verification by LCMS, the DMSO-based reactions were found to yield N-D_3-methyl-topotecan of 95% purity, while DMF-based reactions yielded N-D_3-methyl-topotecan with purities <50%, suggesting methyl donation from the DMF.

To yield labeled topotecan for metabolism studies in vitro, [^{14}C]-CH_3I was reacted with the precursor to yield [^{14}C]-topotecan, and confirmed the minimal production of side products.

N-[^{11}C]methyl-topotecan has successfully been produced from [^{11}C]-CH_3I in amounts sufficient to allow QC and sterility testing in preparation for patient imaging.

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SYNTHESIS OF [CARBONYL-^{11}C]KETONES USING THE SUZUKI COUPLING

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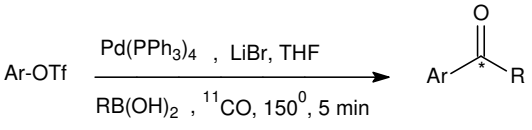
Key Words: [^{11}C]Carbon monoxide, [^{11}C]ketones, Suzuki coupling.

Carbonylation using [^{11}C]carbon monoxide has become an increasingly employed ^{11}C-labelling strategy in our laboratory and a wide range of ^{11}C-labelled carbonyl compounds including ketones,[1] amides,[2] imides,[3] carbamates[4] etc. have been synthesised using this method. The presented report describes the synthesis of ^{11}C-labelled ketones by use of the Suzuki coupling[5] of aryl triflates, aryl or alkylboronic acids and [^{11}C]carbon monoxide.



Ar = phenyl, *p*-tolyl, *p*-methoxyphenyl; R = phenyl, methyl, * = ^{11}C

[^{11}C]Ketones were synthesized in a micro autoclave of 200 μL volume using low concentration (ca 10^{-5} M) of [^{11}C]carbon monoxide. The conversion of [^{11}C]carbon monoxide to products (trapping efficiency) were 70- 90% and the decay-corrected radiochemical yield, calculated from [^{11}C]carbon monoxide, were in the range of 15 to 64%. The radiochemical purity exceeded 98%. The specific radioactivity of the benzophenone was using an irradiation of 10 μAh, 150 GBq/μmol, 40 min after end of bombardment. A similar approach was previously used by another group, however their reaction conditions and substrates were different.[6] All of the previously reported Suzuki coupling reactions have been performed in presence of bases. In the conditions used here, the reaction worked without any additional base.
Tetrakis(triphenylphosphine)palladium(0) (5.0 mg, 4.3 μmol) was dissolved in THF (200 μL). Aryl triflate (30.8μmol) and LiBr (4.6 μmol) were added. Aryl- or alkylboronic acid (49.2 μmol) was dissolved in THF (100 μL) and mixed with the previous mixture just before use. The resulting mixture was loaded with pressure (35 Mpa) into the micro-autoclave, precharged with [^{11}C]carbon monoxide in helium. The micro-autoclave was heated to150 °C for 5 min. The product was purified by semi-preparative HPLC and characterised by LC-MS.

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AN IMPROVED SYNTHESIS PROCEDURE FOR ROUTINE PRODUCTION OF [*CARBONYL*-^{11}C] DESMETHYL-WAY-100635

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Keywords: [*carbonyl*-^{11}*C*]Desmethyl-WAY-100635, ^{11}C-DWAY, 5-HT_{1A} Receptors, Positron Emission Tomography, Cyclohexylmagnesium chloride

Desmethyl analogue [*carbonyl*-^{11}C]N-(2-(1-(4-(2-hydroxyphenyl)piperazinyl))ethyl))-N-(2-pyridinyl) cyclohexanecarboxamide (^{11}C-DWAY), which is a metabolite of [*carbonyl*-^{11}C]WAY-100635 (^{11}C-WAY), has been suggested to be superior to ^{11}C-WAY for the PET imaging of 5-HT_{1A} receptors in human brain. Its higher brain uptake and fewer labelled metabolites that cross blood-brain barrier result in improved counting statistics and modelling (1).

The reported synthesis of ^{11}C-DWAY (and ^{11}C-WAY) incorporates a 3-step reaction sequence: a) ^{11}C-carboxylation of cyclohexylmagnesium chloride with [^{11}C]CO_2, b) conversion to [*carbonyl*-^{11}C]cyclohexanecarbonyl chloride with $SOCl_2$, and c) condensation of the resulting labelled acid chloride with the precursor amine (2, 3, 4). In our hands, the first two reaction steps produced acceptable yields of [*carbonyl*-^{11}C]cyclohexanecarbonyl chloride when performed according to the methods of either McCarron et al. (2) or Hwang et al. (4), with some adjustments. Our initial attempts of the final condensation reaction, however, were less than satisfactory, yielding a mixture of radiolabelled by-products in addition to a small amount of the desired ^{11}C-DWAY. We then undertook a closer examination of this condensation reaction with the goal of increasing the yield of ^{11}C-DWAY and minimizing the formation of unwanted by-products. This report details our progress toward optimisation of this reaction, which is still ongoing.

Following the formation of [*carbonyl*-^{11}C]cyclohexanecarbonyl chloride, the excess $SOCl_2$ was completely removed and a solution of N-(2-(1-(4-(2-hydroxyphenyl)piperazinyl)ethyl))-N-(2-pyridinyl)amine (Desmethyl-WAY-100634) as a free base in anhydrous 1,2-dichloroethane (400 µL) was added. The free base was generated *in situ* from its hydrochloride (3-4 mg, 9-12 µmole) with N, N-diisopropylethylamine (10 µL, 58 µmole). After heating the mixture at 60^{o}C for 5 minutes, the solvent was removed at 90^{o}C with a stream of argon. The radioactive residue was then suspended in a solution of 20% EtOH in 1M HCl (1 mL) and loaded onto a C-18 Sep-Pak for preliminary purification. The Sep-Pak was initially washed with 20 mL of 20% EtOH in 20 mM NaH_2PO_4 (pH 6.5) to remove polar radioactive impurities and most of the unreacted precursor amine. The crude ^{11}C-DWAY was then eluted with 3-4 mL of 60% EtOH in 20 mM NaH_2PO_4 (pH 6.5). The overall radiochemical yields of this Sep-Pak purified product were 10-20% (decay corrected from trapped $^{11}CO_2$) with radiochemical purities >75 % (radio-TLC). Reverse-phase semi-preparative HPLC will be used to purify ^{11}C-DWAY before final formulation.

Following completion of this optimisation effort, it is likely that this method will result in improved yields of ^{11}C-DWAY, facilitating the application of this tracer for routine human studies.

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SIMPLIFIED SYNTHESIS OF DESMETHYL-FLB 457 IN TWO STEPS

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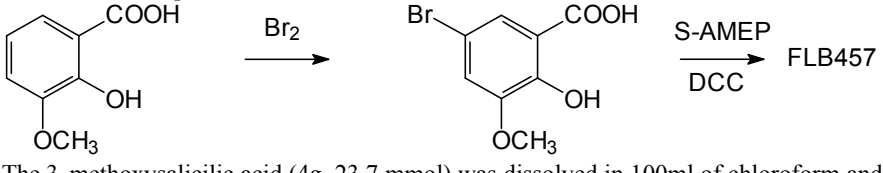
Keywords: [^{11}C]FLB 457, Desmethyl-FLB 457, PET

The dopamine D_2 receptors are a main area of interest for the study of schizophrenia and in the development of anti-psychotic drugs. In order to see these low density D_2 receptors a radioligand with high affinity must be used. [^{11}C]FLB 457 fits these criteria and is used for visualization and quantitation of extrastriatal dopamine D_2 receptors through positron emission tomography (Halldin C. et. al., J. Nucl. Med., 1995, 36: 1275-1281). According to Sandell (Sandell J, Langer O, Larsen P, et al. *J Labelled Cpd Radiopharm* 2000, 43: 331-338) the synthesis of desmethyl-FLB 457 can be done in four steps, starting from 5-bromo-2-hydroxy-3-methoxybenzaldehyde. In this study, we report a simplified method to prepare this precursor in two steps, starting from 3-methoxysalicilic acid and S-(-)-2-aminomethyl-1-ethylpyrrolidine (S-AMEP). The synthesis has been modified to eliminate the step of demethylation, thereby, resulting in no contamination of final product with cold FLB 457.



The 3-methoxysalicilic acid (4g, 23.7 mmol) was dissolved in 100ml of chloroform and 1 ml of pyridine; bromine (1.35ml, 26mmol) was subsequently added. After an hour of reaction, the reaction mixture was washed with sodium metabisulphate and water. It was then rotovaped down to clear oil and was re-crystallized from ethyl acetate and hexane to produce 1.70g of white powder of the 5-bromo compoud. NMR ($CDCL_3$): 3.85(S, 1H): 7.10(S, 1H) 7.62(S, 1H). The 5-bromo derivative was dissolved in chloroform and coupled with S-(-)-2-aminomethyl-1-ethylpyrrolidine using N, N-dicyclohexylcarbodiimide (DCC) as a condensing agent. The reaction mixture was stirred overnight and rotovaped down to yellow oil. Purification by flash chromatography (silica gel, $CHCL_3/CH_3OH$ 95/5) afforded slightly yellow oil and its chemical structure was confirmed by NMR.

In conclusion this abstract describes a simplified method to synthesize desmethyl-FLB 457 with improved specific activity for use in neural studies related to dopamine D_2 receptors.

DEVELOPMENT OF A FULLY AUTOMATED [^{11}C]-RADIOSYNTHESIS MODULE

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Keywords: PET, carbon-11, radiolabelling, automation

The automation of routinely produced [^{11}C]-radiotracers is important for any PET center. Automation should increase reliability and reproducibility while increasing productivity and minimizing exposure to the radiochemist. The use of sterile and disposable components is required in order to meet upcoming regulations for PET radiopharmaceuticals. With these aims in mind we have designed a versatile and reliable automated chemistry module for the production of [^{11}C]-radiotracers that includes HPLC purification and product formulation. The chemistry is based on the classical conversion of [^{11}C]-carbon dioxide ([^{11}C]CO_2) to [^{11}C]-methyliodide ([^{11}C]CH_3I) with lithium aluminium hydride and hydroiodic acid, followed by methylation of the relevant precursor using the "loop technique" (1).

The module utilizes a disposable kit assembled from commercially available components based on three 5-port manifolds (see Figure 1). The reactor vessel is a 1.5mL tapered glass vial, which is heated and cooled by air flow. After converting the [^{11}C]CO_2 to [^{11}C]CH_3I, it is distilled onto the HPLC loop containing the precursor for radiolabelling reaction. The crude labelled product is then automatically injected onto the column for purification, and the pure product is collected and reformulated in physiological solution using a C-18 SepPak cartridge. At the completion of the run the loop is washed with water and ethanol and dried with pure nitrogen ready for the next synthesis.

The synthesizer is controlled by a Siemens CPU216 PLC that is interfaced with Siemens ProTool/Pro software enabling process control and visualization. The PC interface displays the status of the synthesizer including real-time trends of all sensor information i.e. temperature, pressure radiation levels and UV absorbance. Synthesis parameters are easily changed for different compounds. All sensor information is archived, allowing comparison of trends from different syntheses.

[^{11}C]-flumazenil and [^{11}C]-SCH23390 have been successfully produced with the synthesizer. Currently 14 full syntheses of [^{11}C]-flumazenil have been performed resulting in an average yield of 84 mCi (± 29 mCi) at EOS. This is from 475 mCi (± 32 mCi) of [^{11}C]-CO_2 at EOB produced by the IBA 10/5 cyclotron. The average specific activity of [^{11}C]-flumazenil is 1000 mCi/μmol. These preliminary results show that this synthesizer fulfills all of the original aims.

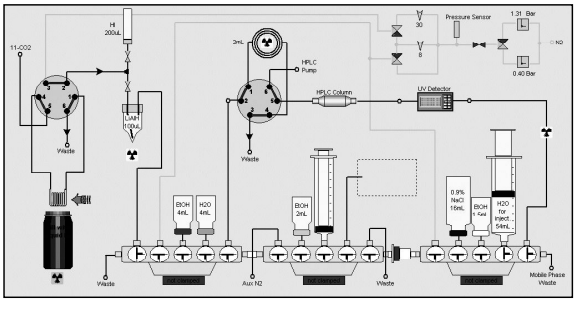

Figure 1: Schematic of the synthesiser

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SYNTHESIS OF [^{11}C]-METHYL ESTERS FROM CARBOXYLIC ACIDS USING [^{11}C]-METHANOL AND LEWIS ACID CATALYSTS

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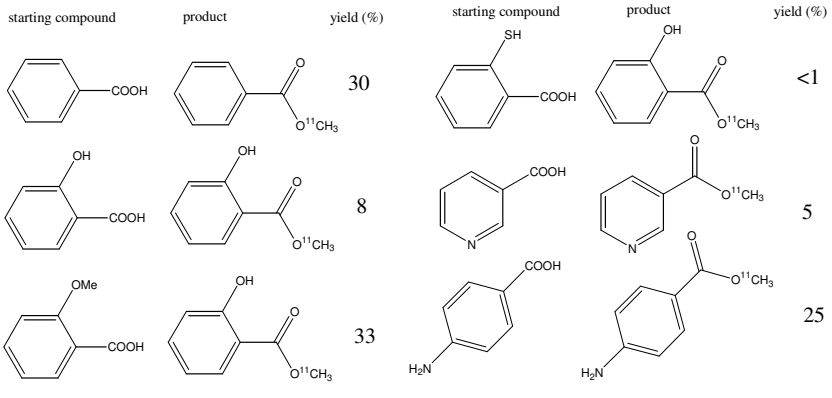
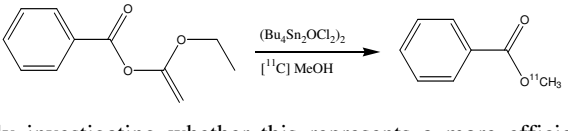
Keywords: radiolabelling; carbon-11 methanol, catalysis

The radiolabelling of carboxylic acids via a nucleophilic substitution mechanism using methyl iodide is usually complicated by the presence of other functional groups in the molecule, which compete with the carboxylate ion for the methyl iodide. This leads to labelling at multiple sites of the molecule and is therefore not a very selective strategy. An addition elimination mechanism, as found in esterification reactions, would be much more selective, since fewer functional groups are able to react in such a manner. However, due to the low reactivity of carboxylic acids, a catalyst is usually necessary to promote these types of reactions.

In classical organic synthesis, Lewis acids have long been used for this purpose. We have investigated the potential of two known Lewis acid catalysts, trimethylsilyl chloride and boron trifluoride etherate, to be used in PET radiochemistry for the synthesis of methyl esters starting from carboxylic acids and methanol. A small number of compounds has been tested and we found that only boron trifluoride etherate catalyses this reaction, whereas no product was obtained when trimethylsilylchloride was used as catalyst.



However, the high temperatures necessary to promote the catalytic effect of boron trifluoride etherate can cleave other functional groups in the molecule. For example, under the reaction conditions, *p*-anisic acid yields [^{11}C]-methyl salicylate in 33% yield but no methyl 2-methoxy benzoate could be detected. In order to circumvent this problem, we are currently also investigating a transesterification reaction starting from enol esters and [^{11}C]-methanol using a distannoxane catalyst. This transesterification is irreversible and therefore proceeds smoothly at 80°C. Also, this catalyst is not known to cleave any other functional groups.



We are currently investigating whether this represents a more efficient procedure for the production of [^{11}C]-AG957.

COMPACT SYSTEM FOR MULTIPLE [^{11}C]-METHYL IODIDE SYNTHESES

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Keywords: Automation, iodomethane, carbon-11, radiosynthesis, dispensing

The chemical scheme used in many laboratories to produce $^{11}CH_3I$ methyl iodide is the so-called "wet chemistry"[1,2]. In spite of its ubiquity in PET radiochemistry, this method has never been automated to the point where multiple successive syntheses can be carried out without substantial operator intervention. We have designed and tested such a system which also has a number of other useful properties including: 1) small hot cell space requirements, 2) ease of use with full software control and GMP documentation, 3) low cost for routine daily use, 4) short synthesis time, 5) high yield, purity, and specific activity of final product, and 6) compatibility with the ^{11}C loop methylation system developed by Wilson et al[3].

The new system uses the classical sequence: trapping of CO_2, release and reduction with $LiAlH_4$, iodination with saturated HI solution, and distillation. The size of the main module has been reduced to a package with dimensions 25 x 40 x 40 cm (W x H x D) so that it can easily fit into a mini hot cell with other synthesis modules such as the Bioscan AutoLoop system. In order to use the system for "real world" daily production of methyl iodide, it was designed so that a minimum of ten batches could be produced without requiring the operator to open the hot cell. Development focused on four important points: the CO_2 trap, the chemicals dispensing system, a reduced reactor size and custom reactor cap design, and the clean up sequence.

The use of a solid support to trap the [^{11}C]-CO_2, gives reliable, efficient trapping and avoids the requirement for liquid argon or nitrogen to cool the trap. Both chemicals – lithium aluminium hydride and hydriodic acid- are supplied in multi-doses vials which are loaded without exposure to room atmosphere and are maintained under nitrogen atmosphere. Because the $LiAlH_4$ is never exposed to ambient air, specific activity remains high and constant[4]. The vials contain enough reagents for at least ten consecutive syntheses. Precise volumes of products are delivered to the reactor with a new Bioscan patented micro-volume dispensing system which is programmable, allowing the user to increase or decrease the volume of reagents used. The reactor size has been decreased to less than 1mL which, combined with a new heater design, allows accurate and rapid control and results in a decreased total synthesis time. And finally, based on our experience of the ^{11}C AutoLoop maintenance system, the methyl iodide synthesizer is automatically cleaned at three different levels. Initially between successive syntheses; second, at the end of each day; and third, additional clean up of specific components is achieved in the maintenance mode. These three levels of cleaning significantly extend the lifetime of the valves and maintain in yield, purity, and specific activity levels.

The first tests with the system were performed at CERMEP, and showed that six consecutive runs in a day gave a decay-corrected radiochemical yield of [^{11}C]-methyl iodide of 65 to 75 %. Additional tests are being carried out at CERMEP and at Memorial Sloan Kettering to test the consistency of yield, purity, and specific activity.

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RADIOLYTIC DECOMPOSITION OF THE STRUCTURALLY SIMILAR ^{11}C LABELLED PET RADIOPHARMACEUTICALS FOR BENZODIAZEPINE RECEPTORS

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Keywords: Radiolysis, [^{11}C]Ro 15-4513, [^{11}C]iomazenil, Hydrated Electrons

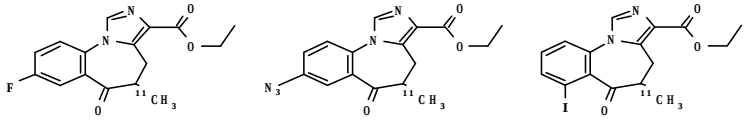
^{11}C-labelled imidazobenzodiazepine derivatives of [^{11}C]flumazenil, [^{11}C]Ro 15-4513 and [^{11}C]iomazenil have been used as PET radiopharmaceuticals for benzodiazepine receptors (Figure 1). We often observed the decrease in the radiochemical purity of [^{11}C]Ro15-4513 and [^{11}C]iomazenil with time, specially in high radioactivity concentration and high specific activity, whereas [^{11}C]flumazenil was stable for a long time. In the present study, we examined the decomposition of [^{11}C]Ro 15-4513 and [^{11}C]iomazenil to prepare these PET radiopharamaceuticals in high radiochemical purity.

[^{11}C]Ro 15-4513 and [^{11}C]iomazenil were prepared by N-methylation of desmethyl precursors with [^{11}C]CH_3I in the presence of NaH. HPLC purification of the reaction mixture was carried out using CH_3CN/H_2O mixture as an eluent. The radioactive fraction, which corresponded to the ^{11}C-labeled imidazobenzodiazepines was evaporated to dryness and then the residue was dissolved in pure water. The obtained aqueous ^{11}C labeled PET radiopharmaceutical solutions were allowed to stand and the radiochemical purity was determined periodically. The effects of additives were examined in the presence of selective OH radical scavenger (EtOH, HCOONa) or selective hydrated electron scavenger ($NaNO_3$). Determination of molecular weight of the degradation products was performed by LC/MS.

The radiochemical purity of [^{11}C]Ro 15-4513 and [^{11}C]iomazenil in an aqueous solutions was decreased with time. Specially, in the high specific activity and high radioactivity concentrations tended to facilitated the decomposition. The decomposition of [^{11}C]Ro 15-4513 and [^{11}C]iomazenil was significantly suppressed by addition of selective hydrated electron scavengers. In contrast, the decomposition of [^{11}C]Ro 15-4513 and [^{11}C]iomazenil was accelerated in the presence of selective OH radical scavengers compared with control (no additive). These results showed that the decrease in radiochemical purity of [^{11}C]Ro 15-4513 and [^{11}C]iomazenil was attributed to the radiolysis of H_2O by its own radiation and hydrated electrons played an important role in the radiolysis of both compounds.

LC/MS analysis of degradation products of [^{11}C]Ro 15-4513 and [^{11}C]iomazenil suggested that degradation products of [^{11}C]Ro 15-4513 and [^{11}C]iomazenil were the reduced product (NH_2 instead of N_3) and the deiodinated compound, respectively. Estimated structures of the degradation products were consisted with those of the possible products generated by the typical reactions of hydrated electrons with solute (reduction and dehalogenation reaction).

The difference in the stability against the radiation among these three imidazobenzodiazepine derivatives (Figure 1) would be explained by the stability and reactivity of the functional group on aromatic ring with hydrated electrons. (Azido group is subject to reduction and the reactivity of halogenated benzene with hydrated electron is $FC_6H_5 < IC_6H_5$)



[^{11}C]Flumazenil [^{11}C]Ro 15-4513 [^{11}C]Iomazenil

Figure 1. Structurelly Similar PET Benzodiazepine Receptor Ligand

A SIMULTANEOUS ION-CHROMATOGRAPHIC ANALYSIS OF [^{11}C]CHOLINE AND IT'S PRECURSOR, DEANOL, WITH APPLICABILITY TO A CLINICAL SETTING

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Keywords: [C-11]Choline, Ion Chromatography, Ion Suppression

Although the synthesis of [^{11}C]choline from the simple precursor, (N,N-Dimethylaminoethanol; Deanol; DMAE) is readily achieved and [^{11}C]choline has been described as a useful agent for imaging in many oncologic situations where FDG is unsuitable, e.g. prostatic cancer (*1*), its application has been limited by a sensitive assay for choline and deanol.

In our hands and others (*2*) UV and refractive index detection of the non-chromogenic choline and deanol are far too insensitive for the levels expected for a clinical radiopharmaceutical. The use LC-MS has been successfully applied, but this type of equipment is expensive and not normally found in a clinical PET operation (*3*). Deanol is sufficiently volatile for GC analysis (*2*). However, we preferred to have a method in which all components could be assayed in one simple, simultaneous, analysis. Attempts using Evaporative Light Scattering detection (ELSD) failed since deanol is too volatile to be separated from the water required for elution.

Since both choline and deanol are primary alcohols we investigated the use of integrated amperometry that could potentially utilise the same system which we routinely use for FDG and ClDG analysis. This method would also negate the potential confounding effects of isotonic saline on alternate ion chromatographic methods. The system was exquisitely sensitive to deanol, but choline gave no signal, which we ascribe to some internal neutralization of the resulting anion by the quaternary ammonium cation. We thus turned our attention to conductivity detection.

An ion-chromatographic analysis was developed using a reverse phase copolymeric column, Surfactant/R (Alltech), methanesulfonic acid$_{(aq.)}$ as eluant, and conductivity detection with auto-suppression in eluant recycle mode (Dionex). In the absence of isotonic saline a baseline separation of choline and deanol was readily achieved. In the presence of isotonic saline the sodium ion peak was so great as to interfere with the resolution. We found that a 1:5 dilution of saline (0.018%) gave an acceptable elution from the cation exchange cartridge used during [^{11}C]choline preparation and at the same time reduced the sodium cation level to the extent that a baseline separation of choline and deanol was easily achieved. 1.4 mM Methanesulfonic acid$_{(aq.)}$ at 0.7 mL/min. provided the best compromise between separation of the components and analysis time. This method allowed for the quantitative separation of choline (7.5 min.) and deanol (6.0 min.) with a resolution of 15.2 and detection limits of <12.5ng for each compound, well within the mass range (sub to low ppm) of typical amounts found in the [^{11}C]choline preparations. The total analysis time was <10 min and permitted accurate determination of chemical and radiochemical purity, and specific activity of the labelled [^{11}C]choline. Our results compare most favourably to those presented recently (*4*).

A rapid, simple, specific, and highly sensitive assay for [^{11}C]choline and deanol has been realised. The method should be adaptable to metabolite studies, e.g. [^{11}C]betaine. The interference of NaCl in the rapid analyses and sensitivity required for PET radiopharmaceuticals highlight the need for methods of selective ion suppression of Na cation in isotonic pharmaceutical preparations.

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APPLICATION OF ULTRA HIGH SPECIFIC ACTIVITY [^{11}C]COMPOUNDS TO IN VITRO ARG

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Keywords: ultra high specific activity, [^{11}C]Ro15-4513, [^{11}C]PE2I, Single pass I_2 method, In vitro ARG

Various kinds of radioisotopes and their labeled compounds have been used for investigation of the brain receptors with autoradiography (ARG) and positron emission tomography (PET). Recently, several neuroreceptors with quite a low concentration have been reported, e.g., dopamine D_2 receptors in the cerebral cortex (1). For visualization and quantitative analysis of the receptors with such low densities in the brain, a quite small amount of tracer must be injected into the subject to perform these studies, which requires extremely high specific activity. However, it has been difficult to achieve such high specific activity level. Recently, we have constructed an automated synthesis device using single pass I_2 method coupled with the in situ [^{11}C]CH_4 production method, and optimized the experimental conditions for the production of ultra high specific activity [^{11}C]CH_3I (2). Using this [^{11}C]CH_3I, various kind of [^{11}C]compounds with ultra high specific activity could be synthesized (> 50Ci/ mol). In this study, we tried to show the usefulness of the ultra high specific activity [^{11}C]compounds by in vitro ARG using imaging plates (IP). As model compounds, Ro15-4513 and PE2I were used, because [^{11}C]Ro15-4513 have super high affinity binding site for benzodiazepine receptor in the hippocampus and [^{11}C]PE2I is known to be a dopamine transporter which exists in cortex at extremely low density (3,4).

For in vitro ARG study, sagital brain sections (30 m) were incubated with 50mM Tris-HCl buffer (pH 7.4) containing various concentrations of [^{11}C]Ro15-4513 or [^{11}C]PE2I for 60 min at 25 °C. After incubation, the brain sections were rinsed twice in cold fresh buffer, once in distilled water, and then exposed to an IP for 60 min. After exposure, the radioactivity in IP was quantified by using Bio-Imaging Analyzer System (BAS1800).

By the use of ultra high specific activity [^{11}C]Ro15-4513 (63.4 Ci/ mol), brain imaging could be performed in wide range of concentrations (0.49pM~8.5nM). Accumulation in the hippocampus was successfully visualized even at extremely low concentration of 0.047pM (1 Ci/200ml) Ro15-4513. This concentration level corresponds to 10^5 lower compared with that used in the [^{3}H]Ro15-4513 (5). With the ultra-high specific activity [^{11}C]PE2I (50 Ci/ mol), accumulation in cortex was slightly higher than that in cerebellum as a reference. But in both cases, the significant difference of ratios, hippocampus/pons for [^{11}C]Ro15-4513 and cortex/cerebellum for [^{11}C]PE2I, were not observed in spite of the big difference of specific activity.

In this study, we succeeded in performing the imaging studies at extremely low concentration of the tracer with ultra high specific activity. However, the significant difference of accumulation by the specific activity was not observed in the present study with [^{11}C]Ro15-4513 and [^{11}C]PE2I.

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A NEW PRODUCTION METHOD OF [^{11}C]CH_3I USING A LOOP TECHNIQUE

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Keywords: [^{11}C]CH_3I, Loop Method, High Specific Activity, Repeated Production, PET

[^{11}C]methyl iodide is the most important precursor since the majority of the C-11 labeled compounds have been synthesized with [^{11}C]CH_3I and [^{11}C]methyl triflate prepared from [^{11}C]CH_3I . For a long time, [^{11}C]CH_3I has been synthesized by the reaction of [^{11}C]CO_2 with $LiAlH_4$ in THF and the successive reaction with HI. This method gives a relatively good yield, but it requires the tedious preparation before starting the synthesis and it is difficult to repeat productions in a short time. Furthermore, the method is not suitable to achieve very high specific activity since non-radioactive CO_2 from the atmosphere is easily absorbed in the $LiAlH_4$ solution and decreases the specific activity of [^{11}C]CH_3I (1). Another production method of [^{11}C]CH_3I using the reaction of [^{11}C]CH_4 with I_2 vapour was developed(2). This method has many advantages of high specific activity, easy preparation prior to the synthesis and repeatable production at short time intervals although the radiochemical yield is not good enough compared to the $LiAlH_4$/THF method (3). On the other hand, the loop method of preparing a thin layer of a Grignard reagent on the inner surface of a thin tube is known in the preparation of [carbonyl-^{11}C]WAY100635 (4). This loop method might be useful to decrease the amount of regents and to achieve high specific activity. In this study, we intended to develop an improved method of the current $LiAlH_4$/THF method, which enables us to produce [^{11}C]CH_3I repeatedly at short time intervals with high specific activity, high radiochemical yield and a little preparation work.

We have developed an automated device to synthesize [^{11}C]CH_3I with $LiAlH_4$/THF, using a loop technique. The device is composed of a main part, two injection parts for a solution of $LiAlH_4$/THF and HI, and a washing part with two bottles for 0.1 N HCl/acetone and acetone. The main part of the device is composed of a 6-way valve and a thin loop tube (o.d. 1/16", i.d. 0.75 mm, Teflon), which can withstand corrosion, pressure and temperature. Before synthesis, $LiAlH_4$/THF was introduced into the loop, and then purged off with a He gas flow. [^{11}C]CO_2 was then introduced into the loop and allowed to react with remaining $LiAlH_4$. A small amount of HI solution was injected into the loop and the loop was closed by switching the 6-way valve, heated to ~180 ? for 5 minutes. The generated [^{11}C]CH_3I was recovered in a vial containing a DMF solution of desmethyl Ro15-1788 and NaH. By the irradiation of 300 mCi for ^{11}C, 87 +/- 17 mCi of [^{11}C]Ro15-1788 could be obtained with the specific activity of 16 +/- 7 Ci/ mol (EOS) (n=3). The production of [^{11}C]CH_3I could be repeated without disassembling the device between the productions.

The new method developed in this study was quite useful to produce [^{11}C]CH_3I with high specific activity in high radiochemical yield. This method had further advantage of being able to repeat the productions without changing reaction vessels and chemicals. This automated device might be used for the production of [^{11}C]methyl triflate and other ^{11}C-labeled compounds with the Grignard reaction with minor modification.

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CYCLOCONDENSATION OF RADIOLABELLED CARBOXYLIC ACID WITH 1,2-DIAMINOBENZENE USING MONOMODAL MICROWAVE HEATING

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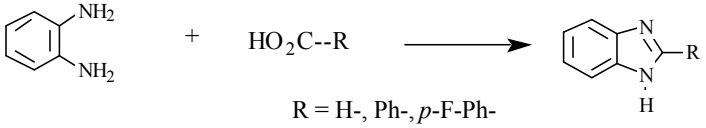
Keywords: benzimidazole, cyclocondensation, positron, microwaves

Imidazoles and benzimidazoles are basic structures in endogenous compounds such as histidine, purines and pyrimidines and are important building blocks in pharmaceuticals[1]. We have been using UV monitoring to follow the progress of the microwave-assisted syntheses of 6/5 membered fused heterocycles. The microwave-assisted reaction[2] between 1,2-diaminobenzene and formic acid (R=H) (Fig) produced benzimidazole in a few minutes with rate enhancements over the conventionally-heated reactions over a range of concentrations and compositions. The study has recently been extended to include un- and substituted benzoic acids, which have, however, predictably been more difficult to perform.



R = H-, Ph-, *p*-F-Ph-

A wide range of carboxylic acids labelled with ^{11}C and ^{18}F have been synthesized[3]. Microwave heating has been shown to be useful in accelerating radiolabellings with positron-emitters[4]. We have thus begun an investigation of the applicability of this cyclocondensation as a general method for labelling benzimidazoles with positron-emitters. [4-^{18}F]Fluorobenzoic acid, prepared by hydrolysis of the corresponding [4-^{18}F]fluorobenzonitrile, was separated by SPE and reacted with 1,2-diaminobenzene in solvents of varying microwave susceptibility and with acidic and basic catalysis. Though optimal conditions have not yet been identified, the trends observed follow the same patterns as those found under non-labelling conditions. Reaction is thus observed within 5-10 min when the most polar solvents (ethanol, DMSO, DMF, propylene carbonate) with acids (e.g. HNO_3, p-TsOH) were rapidly brought to high temperatures with the microwave treatment.

The formation of phenylbenzimidazoles typically requires prolonged heating at high temperatures and often high pressures. The ability to effect reaction after such short times encourages further optimization for PET labelling conditions. Furthermore, it has recently been reported[5] that ^{11}C-labelled formic acid can be easily synthesized. Synthesis of the ring-^{11}C-labelled benzimidazole should be easier to achieve with this technique.

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SYNTHESIS OF 11C-LABELED SODIUM 2-[*o*-[(2,6-DICHLOROPHENYL)-AMINO]PHENYL]ACETATE ([11C]DICLOFENAC SODIUM) FOR PET STUDIES

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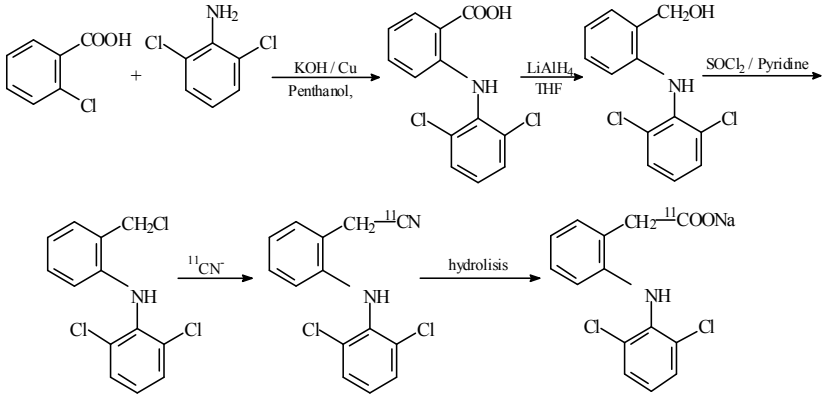
Keywords: sodium [^{11}C]cyanide, carbon-labelling, [^{11}C]diclofenac sodium, NSAID, PET

Sodium 2-[*o*-[(2,6-dichlorophenyl)amino]phenyl]acetate, better known as Diclofenac Sodium, is a potent nonsteroidal anti-inflammatory drug (NSAID) used in the treatment of inflammatory, rheumatic and non rheumatic diseases. Previous studies on pharmacokinetic and biodistribution of this molecule in humans were performed using Diclofenac labelled with stable or long half-life isotopes such as deuterium or carbon-14. The aim of our work was to improve the knowledge on the biological behaviour of this drug, using nuclear imaging. In particular, the goal of this study was the evaluation of the transdermal penetration into the tissue of a commercial preparation of a particular formulation of Diclofenac gel (Dolaut) using Positron Emission Tomography (PET). To maintain the same chemical structure and biological activity of Diclofenac, the use of carbon-11 was compulsory. The industrial procedure of Diclofenac preparation does not allow the introduction of carbon-11 in the molecule. A new method of synthesis, that involves the preparation of a chlorinated intermediated that can be labelled by cyanation with sodium [11C]cyanide, has been set up and optimised. Carbon-11 cyanide has been prepared starting from [11C]carbon dioxide produced from cyclotron. $^{11}CO_2$ is before reduced to $^{11}CH_4$ by reduction with hydrogen on Ni catalyst at 485°C. [11C]-CH_4 is than converted to H^{11}CN by reaction with gaseous NH_3 on Pt wool at 1050°C. Cyanation was performed on 30 mg of 2-[*o*-[(2,6-dichlorophenyl)amino]benzyl]chloride dissolved in 1.2 ml of dimethylsulfoxide. Purification, was performed by high performance liquid chromatography (HPLC) on reverse phase (C_{18} X-Terra, Waters) using CH3CN/phosphate buffer 0.01 M (pH 3.5) - 60/40 as mobile phase. A scheme of the overall synthesis procedure is reported below:



The transdermal penetration of the drug was assessed adding the purified 11C- to Dolaut in its final container (sprayer) and used for PET studies as formulation on healthy volunteers. Studies are still ongoing. Preliminary tests showed in depth penetration of the product up to 22 mm (FHWM = 5.6 mm) at 40 min. from administration. PET may be used to study dynamic penetration of topic drugs in humans.

A FULLY AUTOMATED PRODUCTION OF [CARBONYL-^{11}C]WAY-100635 FOR CLINICAL PET STUDIES

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Key Words: automation, carbon-11, on-line carboxylation, [carbonyl-^{11}C]WAY-100635

Fast implementation of PET into clinical studies and research has resulted in high demands in the automated modules for the preparation of PET radiopharmaceuticals in a safe and reproducible manner. [Carbonyl-^{11}C]WAY-100635 (**I**) is a well-established radioligand for PET evaluation of brain 5-HT_{1A} receptors (1). No commercial modules are available for its routine production. A problem is that the introduction of the label into carbonyl position require the reaction of [^{11}C]CO_2 with cyclohexylmagnesium chloride followed by treatment of the resultant complex with thionyl chloiorde to give [^{11}C]cyclohexanecarbonyl chloride (**II**). The acylation of a secondary amine, WAY-100634, with (**II**) produces the radioligand which is purified by HPLC (2). The use of an immobilised reagent technique β) for a synthesis of [**II**] is advantageous since the reactions proceed fast with low amounts of reagents facilitating HPLC purification. This approach has recently been implemented into custom-modified PET tracer synthesizer (Nuclear Interface) (4).

Our goal was to design a fully automated synthetic module to operate synthesis of (**I**) as well as cleaning procedure with a minimized radiation burden to a personal. The module is built with all the commercially components placed inside a stainless steel box with a front door (Laboratech, Sweden). Electronic parts, cables and I/Os, were placed inside the box, while all the valves, tubing, heating block, automated Kloehn syringe and HPLC column were positioned on a front door. The transfer of reagents and solvents for a cleaning was achieved via a Kloehn syringe-driven module, equipped with multi-positioned valve and a web of 3-way valves (Burkert Conpromatic). For radioactivity monitoring replaceable commercially available scintillation type detectors (Carroll-Ramsey Associates, USA) with time-radioactivity recording via the LabView program were used.

The critical step of the synthesis is the reaction of [^{11}C]carbon dioxide with an immobilized cyclohexylmagnesium chloride. We found that high entrapping efficiency (>90%) was achieved in a loop made from standard Teflon tubing (1/16", 80 cm length) at the flow rate of 15 ml/min and 0.5M concentration of the Grignard reagent in THF/diethyl ether. The success of this step greatly depends on proper cleaning and drying of tubing provided by auto cleaning procedure using several solvents. The next reaction of the [^{11}C]adduct with thionyl chloride proceeds instantly during the pass of $SOCl_2$ through the loop. Acylating agent (**II**) was collected directly into the reaction vial containing 3 mg of WAY-100634 in 150 l of TEA. The reaction mixture was kept at 70°C for 6 min with continuous mixing of the reagents by nitrogen flow. The residual was dissolved in 1 ml of MeOH/0.1M ammonia formate mixture (80/20) and injected into the C18 HPLC column. In the synthesis of (**I**) HPLC separation was often associated with difficulties [2]. However with our new technique only two radioactive peaks where exhibited on the chromatogram with the RT of the product of 7-8 min (flow 8 ml/min). Specific radioactivity of (**I**) was in range 74-185 GBq/ mol (EOS, synthesis time 28-30 min). About 1500 MBq of the product was obtained in a typical run using 30 min bombardment at 55 A beam current. Auto cleaning procedure can be started immediately after EOS without opening the hot cell end next production can start within 60 min.

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[^{11}C] LABELLING OF L- AND D-LACTIC ACID

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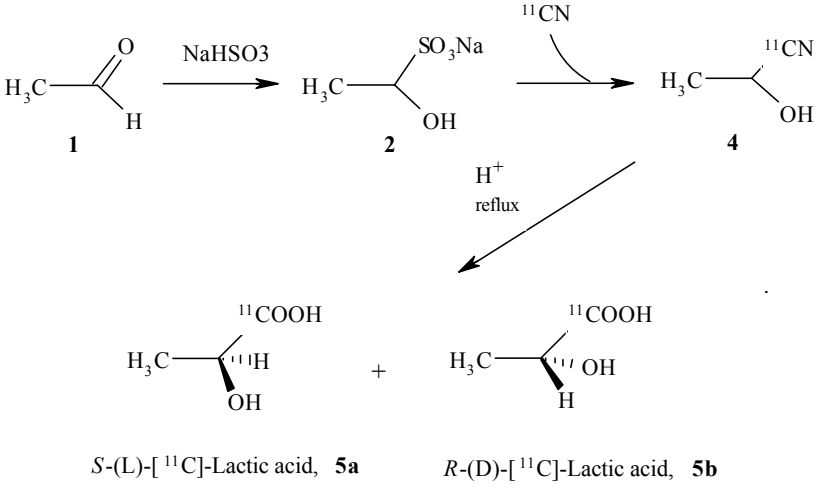
Keywords: lactate, carbon-11

The trophic relations between astrocytes and neurons in the brain are still not enough understood. The [^{11}C]-labelled D-enantiomer of lactic acid was recognised as a possible tool for the investigation of these relations by positron emission tomography (PET). For this purpose the radiosynthesis of [^{11}C]-labelled racemic lactic acid (**5a** + **5b**) was achieved starting from acetaldehyde (**1**), which was transformed to its bisulfite adduct (**2**). Compound **2** was the substrate for the further radiolabeling substitution reaction with [^{11}C]-CN$^{?}$ to yield the [^{11}C]-(±)-2-hydroxypropionitrile (**4**) which was hydrolysed to the racemate of the [^{11}C]-labelled lactic acid (**5a** + **5b**) in the presence of conc. HCl. Both enantiomers of [^{11}C]-lactic acid (**5a**, R_t 12 min) and (**5b**, R_t 16.2 min) were isolated by preparative chiral HPLC by the Davankov's method using a CLC-D column *Astec* and 0.005 M $CuSO_4$ as the mobile phase. The physiological experiments with the [^{11}C]-D-lactic acid (**5b**) are in progress.



1 **2** **4**

S-(L)-[^{11}C]-Lactic acid, **5a** *R*-(D)-[^{11}C]-Lactic acid, **5b**