Paper D1

INVESTIGATION OF POSSIBLE ROUTES TO [^{11}C]METHYL TRIFLATE

Douglas Jewett and Keith Mulholland.
Division of Nuclear Medicine. University of Michigan Medical School.
Ann Arbor, MI 48109-0552.

Methyl triflate is more reactive than methyl iodide, rapidly alkylating many amines at room temperature. Though less volatile (b.p. 96°) it can be distilled into a carrier gas. The availability of [^{11}C]methyl triflate would allow many [^{11}C]methylations to be accomplished more quickly and with simpler apparatus than with [^{11}C]methyl iodide. Methyl triflate alkylates 4-(4-nitrobenzyl)pyridine in acetone at room temperature to yield a chromophore measurable at the microgram level. This assay was used to examine two routes to methyl triflate under conditions applicable to carbon-11. In the first method 1 mg lithium aluminum hydride in THF was reacted with CO_2. The THF was evaporated and the residue was heated to 180° with 100 µl of triflic acid containing 20 µg triflic anhydride. The volatiles were then distilled into N_2 at 90° C. An approximate yield of 30% of methyl triflate based on CO_2 was obtained. The product was contaminated by triflic anhydride and triflic acid. Further, LAH/THF alone gave a volatile alkylating species. In the second method 200 µg CH_3Br was passed over a column (3.2 mm x 15 mm) of silver triflate at 280° to yield about 20 µg methyl triflate. While the yield increased with temperature, the column degraded above 280°. The size and geometry of the column have not yet been optimized. Similar results but lower yields were obtained for CH_3Cl. If the ionic bromination of methane (1) can be adapted for carbon-11 a simple route to [^{11}C]methyl triflate will exist.

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Paper D2

ON-LINE N-[^{11}C]METHYLATION BY [^{11}C]METHYL IODIDE

R.Iwata, C.Pascali, M.Yuasa, K.Yanai, T.Takahashi and T.Ido.
CYRIC Tohoku University, Aramaki, Aoba-ku, Sendai 980, Japan.

A large number of useful ^{11}C-radiopharmaceuticals have been prepared by [^{11}C]methylation using [^{11}C]methyl iodide. Many of them are dedicated to brain receptor studies due to their high specific activity, which can be achieved by rapid processing using HPLC purification after the [^{11}C]methylation. A few notable attempts have been made to automate the interfacing procedure between the above two steps by adapting a sample loop of an injector for the trapping and reaction of [^{11}C]methyl iodide or for the enrichment of a reaction product (1, 2). We have developed a new, general method suitable for automated synthesis by on-line [^{11}C]methylation.

Figure 1 shows a flow chart of the automated on-line [^{11}C]methylation system. A reaction substrate was coated on an inert support, mixed with the adsorber (silica gel or Porapak Q), and then charged in a short column (internal volume:55 μL), which was connected with an HPLC injector instead of a sample loop. The [^{11}C]methyl iodide carried with a He flow was first trapped in the column immersed in an acetonitrile-dryice bath (-42°C). A 200 μL portion of N,N-dimethylformamide (DMF) was loaded in the column and it was then dipped into an oil bath (80°C) for 5 min. For the on-line N-[^{11}C]methylation of spiperone, a various kind of bases was added to DMF. After the reaction the whole mixture was directly injected into a preparative HPLC column by switching the injector and the radioactive product peak was collected and analyzed.

Obvious advantages of the present system are 1) injection of a reaction mixture into a HPLC column without significant loss and 2) ready applicability to an automated system. Some typical results obtained for the present study are listed in Table 1. The optimal ratio of the adsorber to the support coated with the substrate was determined to be 35/20 (μL/μL) from the correlation curve between the trapping efficiency of [^{11}C]methyl iodide and the amount of silica gel as shown in Fig.2. Fig.3 demonstrates that a higher radiochemical yield can be obtained by increasing the concentration of substrate. Under these conditions the reaction yields listed in Table 1 are apparently comparable to or even higher than those reported in the literature (3) probably owing to another advantageous features of the present method, *i. e.* a high concentration of substrate in small portion of solvent can easily be attained and the reaction column has no vacancy inside to allow [^{11}C]methyl iodide to evaporate during the reaction.

N-[^{11}C]methylations sometimes require addition of a base to get a free base form of a substrate or mainly to convert it into a reactive form. As a typical example of base assisted [^{11}C]methylations, N-[^{11}C]methylspiperone was chosen to demonstrate wide applicability of the on-line N-[^{11}C]methylation. Among the bases investigated, sodium hydroxide dissolved in methanol gave the highest radiochemical yield. The presence of water apparently decreased the radiochemical yield, while sodium hydride gave a rather low yield showing this strong base may need a milder condition (4).

In summery, these results indicate that the on-line N-[^{11}C]methylation method improves radiochemical yields and accommodates synthetic procedures to automated syntheses.

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(2) Mulholland G.K., Jewett D.M., Toorongian S.A., *Appl. Radiat. Isot.* **39**, 373 (1988).

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Table 1 On-line [^{11}C]Methylation of Receptor Ligands Using [^{11}C]Methyl Iodide.

Product	Method	Reaction conditions						Results	
		Substrate dose (μmol)	Adsorber	Base	conc. (mM)	Reaction Time (min)	Reaction Temp. (°C)	Carrier amount (nmol)	Product yield (%)
DOX	A	1.9	SG	–		5.0	80	317	48.5
	A	3.8	SG	–		5.0	80	59	71.5
	A	3.8	PQ	–		5.0	80	46	60.2
CYH	A	3.5	SG	–		5.0	80	27	86.9
NMS	B	3.5	PQ	$TBAH^w$	25	5.0	80	47	46.0
	B	3.5	PQ	$TBAH^m$	25	5.0	80	29	60.6
	B	3.5	PQ	$NaOH^w$	20	5.0	80	42	11.6
	B	3.5	PQ	$NaOH^m$	20	5.0	80	44	65.4
	C	3.5	PQ	NaH		1.0	80	36	60.2

Product DOX: Doxepin, CYH: Cyproheptadine, NMS: N-methylspiperone
Method A: only DMF was loaded after [^{11}C]methyl iodide(MeI) trapping.
B: a mixture of DMF and a base was loaded after [^{11}C]MeI trapping.
C: NaH(1.3 mg) was sprinkled over the spiperone. DMF was loaded after [^{11}C]MeI iodide trapping
Adsorber SG: silica gel (80-100 mesh), PQ: Porapak Q (80-100 mesh).
Base TBAH: tetrabutyl ammonium hydroxide, B^m: a base in methanol, B^w:a base in water.

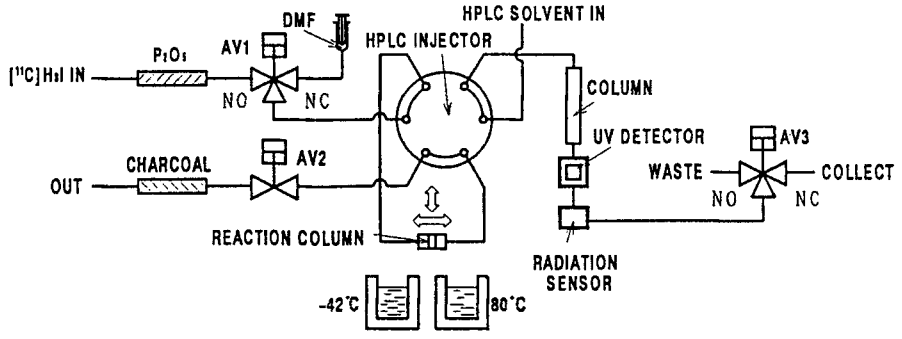

Fig. 1 A flow chart of the automated on-line [^{11}C]nethylation system

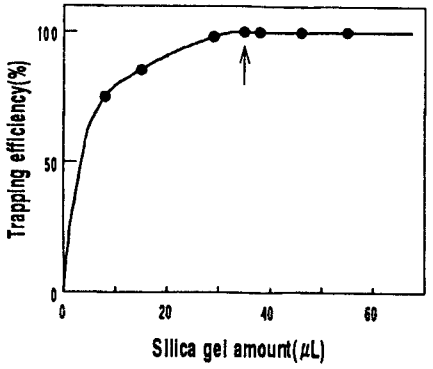

Fig. 2 Correlation between the amount of silica gel and the trapping efficiency of [^{11}C]methyl iodide

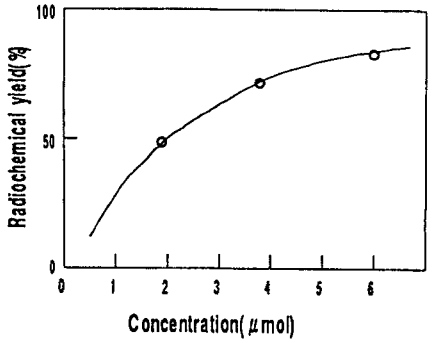

Fig. 3 Effect of the substrate concentration on the radiochemical yield in the [^{11}C]doxepin synthesis

SYNTHESIS OF [O-METHYL-^{11}C]-2-METHOXYETHANOL BY SOLID PHASE CATALYSED ADDITION OF N.C.A. [^{11}C]METHANOL TO OXIRANE

D. Bender, K. Hamacher, G. Stöcklin

Institut für Nuklearchemie, Forschungszentrum Jülich, 5170 Jülich, Germany

[O-Methyl-^{11}C]-2-methoxyethanol is a potent precursor for esterification, e.g. in the radiosynthesis of [^{11}C-methyl]nimodipine (1). We have investigated the direct synthesis of [O-methyl-^{11}C]-2-methoxyethanol by the addition of [^{11}C]methanol to oxirane in the presence of strong acidic cation exchangers or the Lewis acid γ-Al_2O_3. The addition of methanol to oxirane generally requires an excess of methanol in order to prevent the formation of oligomers and polymers. With n.c.a. [^{11}C]methanol, however, polyaddition conditions prevail. In order to suppress this a solid phase catalysed flow system was applied.

N.c.a. dry [^{11}C]methanol was prepared by standard procedures by reduction of $^{11}CO_2$ with $LiAlH_4$ and subsequent protonation of the methanolate with the high boiling alcohol diethyleneglycol-monobutylether. Radiochemical yields of about 80% and a specific activity of 40 GBq/μmol were obtained within 10 min. The reaction of oxirane with [^{11}C]methanol was performed in a heterogeneous gas-solid system using a variable He-flow to control the reaction time. The reactor was integrated in a gas chromatograph to allow preparation and purification of the [O-methyl-^{11}C]-2-methoxyethanol on line in a one step procedure.

In the presence of acidic (Dowex resin) and perfluorinated super acidic resins (Nafion^R) the heterogeneous gas phase reaction of n.c.a. [^{11}C]methanol/oxirane mixtures yielded at 140°C up to 20% of the [O-methyl-^{11}C]-2-methoxyethanol (Fig. 1). The optimum contact time on the catalyst was about 3 sec. Decreasing the He-flow rate (longer reaction time) or decreasing the resin particle size led to an increased production of [^{11}C] labelled polyaddition products, i.e. higher boiling oligomeric [^{11}C] labelled oxyethylene derivatives. The best radiochemical yields up to 38% were obtained at 120°C (Fig. 2) with γ-Al_2O_3 when oxirane and [^{11}C]methanol were injected successively with a time delay of 10 sec. Obviously the oxirane molecules chemisorbed on γ-Al_2O_3 had a limited life time, depending on the concentration of traces of oxygen in the He-carrier gas.

1. **Coenen H.H., Schüller M., Stöcklin G., Schwenner E. - J. Label. Compds. Radiopharm. 30: 245 (1991).**

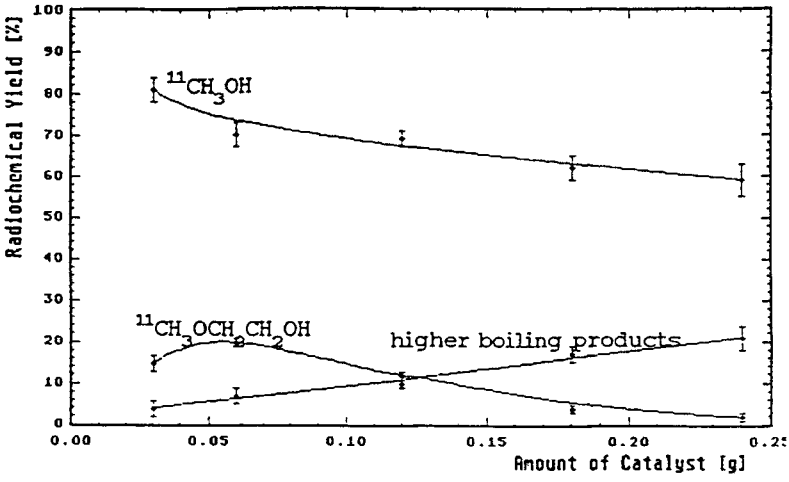

Fig. 1 Dependence of [^{11}C]-product yields on the amount of Dowex 50WX 8 (50-100 mesh) in the heterogeneous catalysis of the addition of [^{11}C]methanol to oxirane in a flow system. He flow rate: 2 ml/min, temperature: 140°C, injection of n.c.a. [^{11}C]methanol in 10 μl oxirane

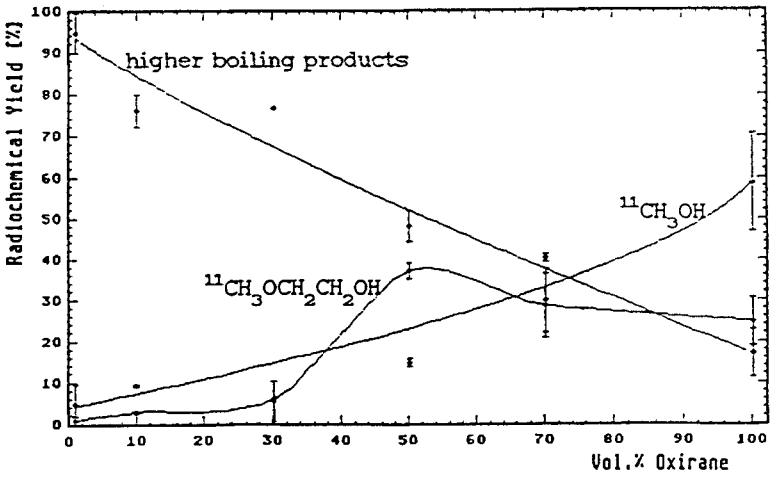

Fig. 2 Dependence of [^{11}C]-product yields on the amount of educt in the heterogeneous catalysis of the addition of [^{11}C]methanol to oxirane. Catalyst: 0.1 g γ-Al_2O_3 (80-200 mesh), He flow rate: 2 ml/min, temperature: 120°C, successive injection of oxirane in 10 μl n-heptane and after 10 sec n.c.a. [^{11}C]methanol in 10 μl n-heptane

SYNTHESIS OF [^{11}C]METHYLISOCYANATE. APPLICATION TO THE LABELLING OF A POTENT ACETYLCHOLINESTERASE INHIBITOR : [^{11}C]PHYSOSTIGMINE

S. Bonnot Lours, C. Prenant, C. Crouzel
Service Hospitalier Frédéric Joliot, CEA, DRIPP, DSV, 91406 Orsay, France.

Physostigmine, an alkaloid from the Calabar beam, is a strong inhibitor of acetylcholinesterase (K_I = 3.33 - 0.26 x 10^{-6}M) (1) and has been used clinically in the treatment of glaucoma, atropine intoxication, myasthenia gravis and more recently, in experimental trials in Alzheimer's disease.

In order to study the AChE activity in the brain by positron emission tomography, we have undertaken the synthesis and labelling with [^{11}C]carbon of Physostigmine.

In the first place, the starting material, Eseroline, is prepared from Physostigmine by the published procedure (2) and stored as sulfate salt.

The [^{11}C] labelled precursor is [^{11}C]methylisocyanate which is obtained by heating [^{11}C]acetylchloride with tetrabutylammonium azide in toluene.

The synthesis of [^{11}C]acetylchloride involves the carbonatation of a freshly prepared methylmagnesium bromide in tetrahydrofuran with cyclotron produced [^{11}C]carbon dioxyde under nitrogen at room temperature and the addition of phtaloyl dichloride (PDC) (3).

The [^{11}C]acetylchloride released by heating (65°C) is carried by a slow stream of nitrogen into a toluene solution of tetrabutylammonium azide which has earlier been prepared from sodium azide and tetrabutylammonium hydroxide (4).

After heating 10 minutes at 80°C, the [^{11}C]methylisocyanate is separated from the solvent by distillation and characterized by conversion to methylphenylurea with aniline or by conversion to phenylmethylcarbamate with phenol instead of the precious Eseroline.

The [^{11}C]methylisocyanate is distilled into a third vessel containing Eseroline in ether with a small piece of sodium. After 10 minutes at 25°C, the solution is purified by HPLC and the appropriate fraction collected.

Starting with 1.5 Ci of [^{11}C]carbon dioxide, few tens mCi of [^{11}C]Physostigmine are obtained 60 minutes after EOB.

Studies are now in progress in our laboratory to further characterize the pharmacology of [^{11}C]Physostigmine binding in baboon brain in view of its application to Alzheimer's disease.

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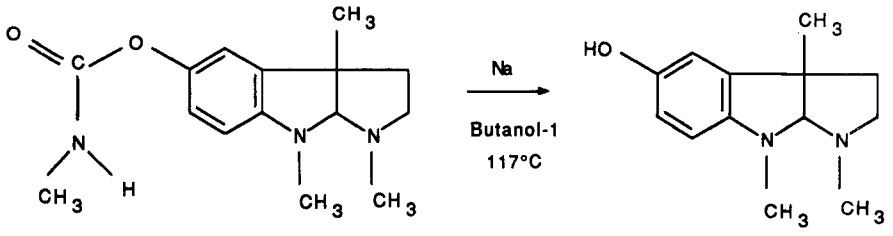
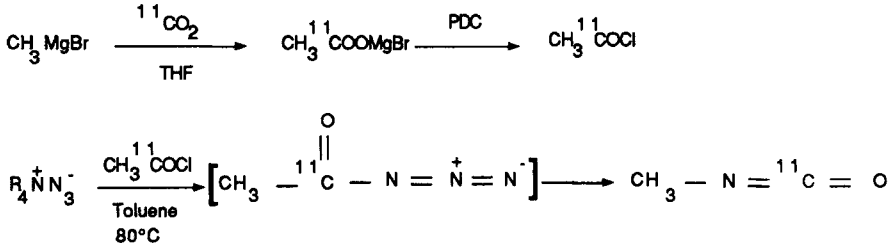
Synthesis of Eseroline

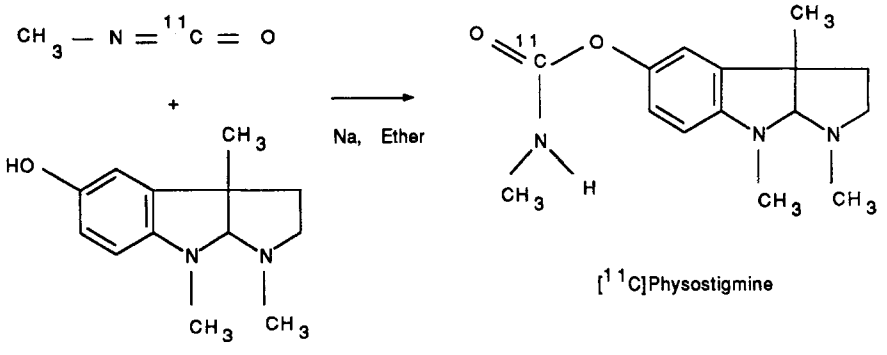


Physostigmine Eseroline

Synthesis of [^{11}C]methylisocyanate



Synthesis of [^{11}C]Physostigmine



Eseroline

[^{11}C]Physostigmine

Paper D5

MICROWAVE CAVITY: USE IN ^{11}C-ALKYLATIONS

Sharon Stone-Elander[1,2] and Nils Elander[3]

[1]Karolinska Pharmacy, Box 60024, S-10401 Stockholm, Sweden
[2]Clin Neurophys, Karolinska Hospital/Institute, Box 60500, S-10401 Stockholm, Sweden
[3]Manne Siegbahn Institute of Physics, Frescativägen 24, S-10405 Stockholm, Sweden

We recently demonstrated(1) that a coaxial resonance microwave cavity is a compact apparatus that can be used as a variable means of generating very high electromagnetic fields in small samples. It has been used in nucleophilic aromatic substitutions with [^{18}F]F$^-$ with substrates in which the leaving groups and degree of activation were varied(2). Yields comparable to or better than thermal methods were obtained with low microwave intensities for reaction times ≤0.5 min. It has also been used(3) to speed up common labelling reactions with [^{11}C]CN$^-$. Synthesis times 1/15-1/20th the literature thermal procedures result in end-of-synthesis radioactivity gains on the order of 100% .

Based on these results, a new microwave cavity has been constructed. The cavity is again separated from the electronics, keeping the space requirements in the hot cell to a minimum and allowing the use of one control unit for cavities installed in different hot cells. The method of microwave propagation has been altered from that of the previous cavity so that the yields are not as dependent on the geometry of the reaction vessel. Exchangeable sample holders permit insertion of common vessels used in PET radiochemistry directly in the microwaves. The microwave intensity is variable between 0-800 Watts and the time controllable by a stopwatch function. In multi-step syntheses, a microwave cavity is a potential apparatus simplification since no heat is retained by the cavity and different reaction conditions can be achieved immediately by simply changing the applied microwave intensity. In addition to the radiofluorinations and -cyanations cited above, the potential of this new microwave cavity for shortening reaction times for O-alkylations (ethers and esters) and N-alkylations (amines and amides) with ^{11}C-alkyl halides has now been investigated. With solvents susceptible to microwaves, good labelling yields were obtained in reaction times < 0.5 min, even with [2-^{11}C]isopropyl iodide, some of the toughest alkylations to perform thermally.

This project has been financed by grants from the Swedish National Board for Technical Development and the Karolinska Institute, which is gratefully acknowledged.

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Substrate	Reagents	$^{11}CH_3I$ Acetone[a]	$^{11}CH_3I$ DMF[b]	$(CH_3)_2{}^{11}CHI$ DMF[c]
ArCOOH	TMP, K_2CO_3	0	85	85
ArOH	NaOH	100	93	90
$ArNH_2$	----	90	100	85
$ArC(O)NH_2$	NaOH	0	92	100

[a] 300W, 60 sec [b] 300W, 20sec [c] 200-400W, ≤30sec

Yields are unoptimized with respect to both time and applied microwave intensity.

Asymmetric Synthesis of n.c.a. L-[2-^{11}C]-4-Chlorophenylalanine.

A. Plenevaux, M.J. Al-Darwich, C. Lemaire, G. Delfiore, L. Christiaens, D. Comar.
Cyclotron Research Center, Liège University, B-4000 Liège, Belgium.

L-4-Chlorophenylalanine (PCPA) is known to be an irreversible inhibitor of tryptophan hydroxylase activity, the rate-limiting enzyme in the synthesis of the neurotransmitter 5-hydroxytryptamine: serotonin (1). More recent work has shown that a single injection of PCPA totally blocked the tryptophan hydroxylase protein synthesis in the rat raphe dorsalis nucleus (the brain region displaying the greatest population of serotonin containing cells) (2,3). Another interesting property is that no such effect was observed on tyrosine hydroxylase presumably due to the very poor affinity of PCPA for this enzyme (4).

In the continuing search for a specific tool to study the neurotransmission system, enzyme inhibitors are of prime importance. Based on these considerations, PCPA appeared therefore as a potential radiopharmaceutical candidate to study the presynaptic serotoninergic neurotransmission process *in vivo* by PET.

The radiolabeling of PCPA was realized with carbon-11 at position 2, through the chemical pathway presented in Scheme 1. This radiochemical synthesis consisted of the highly stereoselective reaction between 4-chloro[α-^{11}C]benzyl bromide and the lithium enolate of (S)-(-)-1-Boc-2-*tert*-butyl-3-methyl-4-imidazolidinone (Boc-BMI). The stereoselective alkylation of imidazolidinone derivatives (5) has been previously reported for the asymmetric synthesis of amino acids labeled with fluorine-18 (6) and carbon-11 (7).

The final product L-[2-^{11}C]-4-chlorophenylalanine was obtained, ready for injection, after HPLC purification (Lichrosorb RP Select-B Merck, 250-10 mm, 0.05 M HAc pH 4 containing 5% of EtOH, flow rate : 9.5 mL/min) in ≈19% radiochemical yield corrected to EOB within 45 min with a radiochemical purity > 98%. The enantiomeric excess measured by HPLC and TLC was shown to be >98%. The final compound as well as the intermediates were identified by comparison with authentic samples characterized by ^{1}H NMR and MS.

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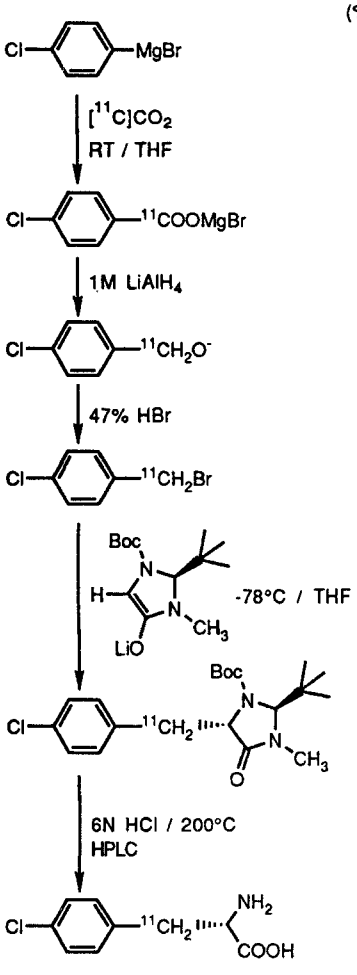
Scheme 1: Synthesis of L-[2-^{11}C]-4-chlorophenylalanine



	Yield for each step (%) EOB corrected	Time for each step (min)
^{11}COOMgBr	85	6 including [^{11}C]CO_2 recovery
$^{11}CH_2O^-$	> 95	4
$^{11}CH_2Br$	80	10
$^{11}CH_2$	50	10
$^{11}CH_2$	60	15

Paper D7

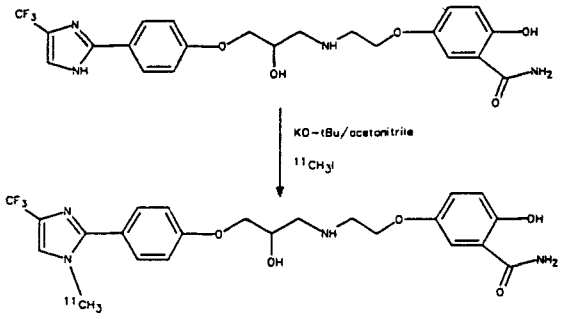
Synthesis of [^{11}C]CGP 20712A: a selective beta-1 adrenoceptor ligand for PET

P.H. Elsinga, A van Waarde *, G.M. Visser and W. Vaalburg

*PET-centre, GCCS and Cardiology Division *, University Hospital Groningen, P.O.Box 30001, 9700 RB Groningen, The Netherlands.*

Beta-adrenergic receptors play an important role in the regulation of the heart rate and the contractility. During various forms of heart failure, the beta-receptor density is known to be down regulated. Little information is available about the time course of these alterations, their spatial distribution within the heart and the influence of therapy. A suitable procedure for visualization and quantitation of myocardial beta-receptors by PET would therefore be of great clinical interest.

In this respect several positron emitting ß-ligands have been reported such as carbon-11 labelled CGP 12177, metoprolol and carazolol. However none of these are very selective for either the $ß_1$- or the $ß_2$-receptor or have a high affinity for the beta-receptor in general. Because our main interest is directed towards the $ß_1$-receptor density in the heart, we are looking for more $ß_1$-selective ligands. The most selective $ß_1$-ligand known at this moment is CGP 26505, the S-isomer of CGP 20712A. We prepared the racemic ^{11}C-analogue by methylation with ^{11}C-methyliodide of the corresponding desmethyl compound (scheme 1). The reaction was performed in acetonitrile, using KO-tBu as a base.



Scheme 1. Synthesis of [^{11}C]CGP 20712A

After evaporation of the solvent the reaction mixture was passed through an aluminium-oxyde column with methanol to remove the precursor and subsequently purified on a Zorbax-NH_2 column eluted with a dichloromethane/methanol/ammonia mixture. The total synthesis time was 50 min and the overall radiochemical yield was 15% (corrected for decay). Three radioactive byproducts (about 50% of the total radioactivity) were eluted, being the other [^{11}C]methyl isomers, e.g. the other N-imidazole isomer and products of methylation on the phenol moiety and the alkyl amino group. After evaporation of the eluent [^{11}C]CGP 20712A was dissolved in a propylene glycol-ethanol-saline mixture to prepare it for injection.

Work is in progress to synthesize the biologically active S-isomer to perform tissue distribution studies and to determine the receptor binding activity.

The authors gratefully acknowledge Dr. K. Scheibli and Dr H. Schöter, Ciba-Geigy for their kind gift of CGP 20712A and the desmethyl analogue.

Paper D8

Simplified Asymmetric Synthesis and Chiral Analysis for *S*-[*Carbonyl*-^{11}C]CGP 12177 Production

Aigbirhio F., Pike V.W., Francotte E.,[1] Jaeggi K.[1] and S.L. Waters.
MRC Cyclotron Unit, Hammersmith Hospital, Ducane Road, London W12 OHS, U.K.
[1]Ciba Geigy AG, Basel, Switzerland.

Racemic [*carbonyl*-^{11}C]CGP 12177[1] ([^{11}C]I) has previously been described as a promising radioligand for the study of cell-surface β receptors *in vivo* with PET.[2] It is known that the *S*-isomer of CGP 12177 (*S*-I) has about 80-fold greater affinity than the *R*-isomer for β receptors.[3] Hence, use of *S*-[*carbonyl*-^{11}C]CGP 12177 (*S*-[^{11}C]I) alone would be expected to give a greater signal (ratio of β receptor-specific to non-specific binding) in PET studies. Moreover, use of the racemic radioligand might lead to difficultly interpretable data as a result of the possible differences in the pharmacodynamics of each enantiomer. Hence, recently there has been considerable interest[4-7] in developing radiosyntheses for *S*-[^{11}C]I based on the asymmetric synthesis of the *S*-di-amino precursor {*S*-[1-(2,3-diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol (VI)} for reaction with n.c.a [^{11}C]phosgene. We report a much simplified asymmetric synthesis of the *S*-diamino precursor (VI) (Scheme 1). This synthesis is a hybrid of two former methods[6,7] and embodies their best features for practical ease, efficiency (45 % yield overall), and very high enantiomeric excess (e.e. in the range 98.6 - 99.4%), namely:

1) the use of readily available 2-amino-3-nitrophenol (II),[5,7] in preference to 2,3-diaminophenol,[4] which requires extra reaction stages, or to 2,3-dinitrophenol,[6] which is difficultly available.

2) the use of readily available *S*-glycidyl-3-nitrobenzensulphonate (III)[6] as a chiral auxiliary, so permitting rapid direct attack by the phenol (II) on the carbon bearing the leaving group in III and almost complete retention of conformation, in preference to *S*-glycidyl sulphonate (slower reacting[8] and less-selectively attacked,[7,8] resulting in significant inversion[7,8]) or to *S*-3-tosyloxy-1,2-propanediol acetonide[7] (requiring extra stages in synthesis).

3) use of methyl ethyl ketone and potassium carbonate as a practically attractive reaction medium for highly selective (98.6 %) reaction between the phenol (II) and chiral auxiliary (III).

4) recrystallization of the stable intermediate *N*-*t*-butylamino compound (V) for enhancement of e e.[7]

5) 3-stage synthesis,[6] in preference to 5 stages from *S*-3-tosyloxy-1,2-propanediol acetonide.[7]

Methods that have previously been used to establish the e.e. of the di-amino precursor (VI), its precursors or of derived *S*-CGP 12177 (*S*-I) include optical rotation, circular dichroism[6] and NMR with chiral shift reagents.[7] We have developed sensitive chiral HPLC methods for the estimation of the e.e. of the compounds *S*-I and III-V so permitting the crucial reaction between the phenol (II) and chiral auxiliary (III) to be monitored for selectivity and for e.e. in product (IV), to measure the improvement in e.e. on recrystallisation of the *N*-*t*-butyl compound (V) and to measure the e.e. of derived *S*-CGP 12177 directly (*S*-I) (Table 1). These accurate methods are more convenient than NMR and, unlike the optical techniques, they do not require reference compounds of absolute optical purity and are less prone to error from optically active contaminants. The chiral HPLC analysis of *S*-I is now being explored as a routine quality control procedure for *S*-[^{11}C]I.

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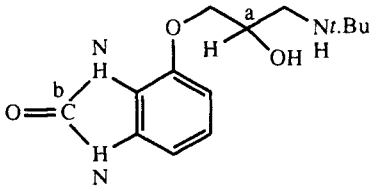
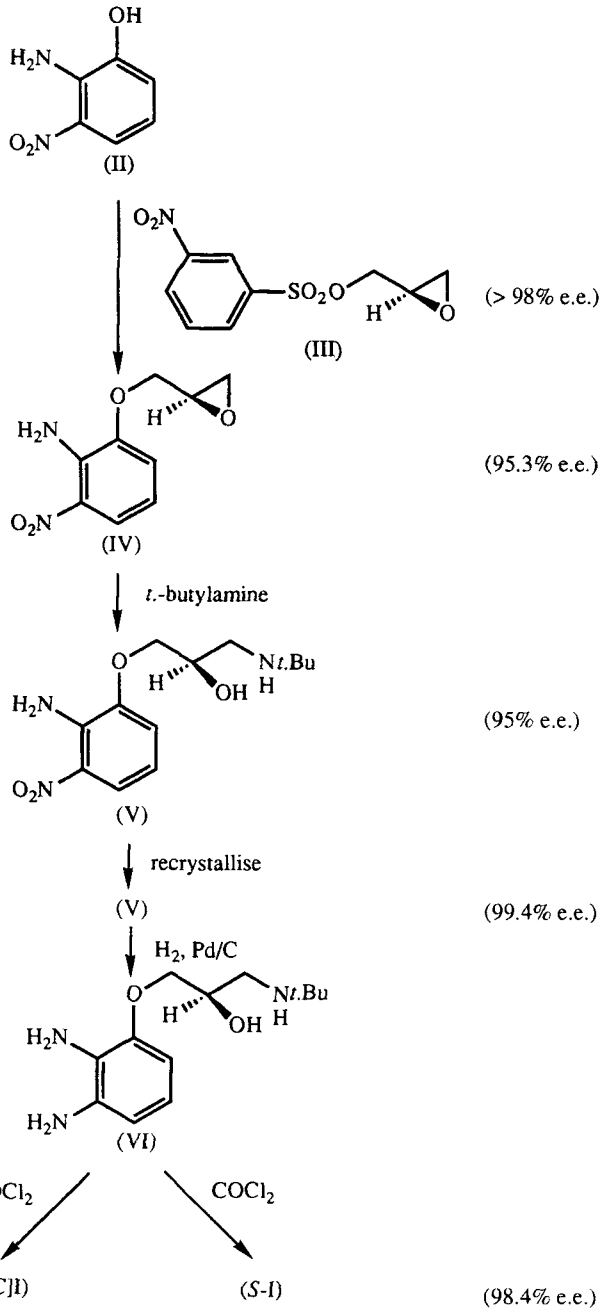


(*S*-I), a = *S*, b = $^{12/13}$C
([^{11}C]I), a = *R*,*S*, b = ^{11}C
(*S*-[^{11}C]I), a = *S*, b = ^{11}C

Compound	Column	Eluent	Flow rate (ml/min)	Retention time *R* (min)	Retention time *S* (min)	e.e. (%)
III	Chiralcel OD (250 x 4.6 mm)	Hexane/IPA (3:1 v/v)	1	19.9	19.2	>98
IV	Chiralcel OD (250 x 4.6 mm)	Hexane/IPA (4:1 v/v)	1	15.2	16.8	95.3
V (crude)	Chiralcel OD (250 x 4.6 mm)	Hexane/IPA (4:1 v/v)	1	12.1	8.1	95
V (recryst.)	Chiralcel OD (250 x 4.6 mm)	Hexane/IPA (4:1 v/v)	1	12.1	8.1	99.4
S-I	Ultron OVM (150 x 4.6 mm)	KH_2PO_4 soln. (10 mM;pH 4.6)	0.5	9.7	7.3	98.6

Table 1: Chiral HPLC analyses of compounds *S*-I, III-V. IPA is isopropanol.



Scheme 1: Simplified asymmetric synthesis of *S*-diamino precursor (VI) for the preparation of *S*- CGP 12177 (*S*-I) and *S*-[*carbonyl*-^{11}C]CGP 12177 (*S*-[^{11}C]I).

A MICROPROCESSOR-CONTROLLED SYSTEM FOR THE PRODUCTION OF MULTIPLE BATCHES OF OXYGEN-15 LABELED BUTANOL

S.M. Moerlein, G.G. Gaehle, K.R. Lechner, R.K. Bera and M.J. Welch.
Edward Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110.

Although [^{15}O]water has found widespread success as a radiopharmaceutical for the measurement by PET of regional perfusion in the brain (1) and myocardium (2), an improved radiotracer for this purpose is [^{15}O]butanol due to its optimum lipophilicity and permeability-surface area (PS) product (3,4). A major prerequisite for the institution of [^{15}O]butanol as a perfusion tracer for PET is efficient, reliable production of the radiopharmaceutical with rapid turn-around time.

The synthesis of oxygen-15 labeled butanol from organoborane substrates has been reported (5), as well as the application of this synthetic procedure to remotely-operated production systems (6,7). We describe here a microprocessor-controlled automated system capable of producing up to ten batches of [^{15}O]butanol in rapid succession.

The synthesis unit is shown in schematic form below. It was assembled from four 10-position, six 2-position and one open-close electric actuator valves interfaced with an IBM-compatible computer via two serial interfacing boxes and two RS232 ports. A Hamilton Microlab 941 dual syringe injection station was connected to a third RS232 port of the computer. The software to operate the injection station and to change the valve positions was written in GW-Basic. Ten alumina cartridges containing tri-(n-butyl)borane substrate and twenty (two per synthesis line) C18 Sep-pak cartridges for solid-phase extraction of the labeled product were used by the system. These can be easily mounted between system changes.

Oxygen-15 was produced via the ^{14}N(d,n)^{15}O reaction using the Washington University JSW BC-16/8 cyclotron. A target consisting of 0.5% O_2 in N_2 (50 psi) was bombarded with a 40 μA beam of 8 MeV deuterons for 10 minutes. The irradiated target gas containing [^{15}O]O_2 was passed at a flow rate of 1000 mL/min through an alumina cartridge loaded under argon gas with 800 μL of tri-(n-butyl)borane. The oxygen-15 labeled intermediate was hydrolyzed and eluted onto two C18 Sep-pak cartridges by the addition of 8 mL of Water for Injection, USP pressurized by helium gas. The final product was eluted from the Sep-paks with 9 mL of 5% ethanol in normal saline and sterilized on-line via passage through a Gelman 0.2 μ Acrodisc filter unit.

Quality assurance was accomplished via HPLC equipped with radioactivity and refractive index detectors. The stationary phase was an Alltech analytical C18 4.6 X 250 mm column; product fractions were eluted with a mobile phase of 20% acetonitrile/water at a flow rate of 1.0 mL/min. The radiochemical purity exceeded 99% and the specific activity of the [^{15}O]butanol was 550-700 mCi/mmol. Boric acid was the only chemical impurity; elemental analysis indicated that 93-101 ppm boron

was present per production batch.

The yield of [^{15}O]butanol ranged from 101-135 mCi EOS (n=10) for a total preparation time (including radionuclide production) of 12.2 minutes. The turn-around time (11 min) for radiopharmaceutical production was even shorter, as the cyclotron production of the successive batch of [^{15}O]oxygen commenced when the target was emptied from the preceding batch.

These results show that this automated system is a convenient means for the routine clinical production of [^{15}O]butanol.

This work was supported in part by NIH grants HL-13851 and N506833.

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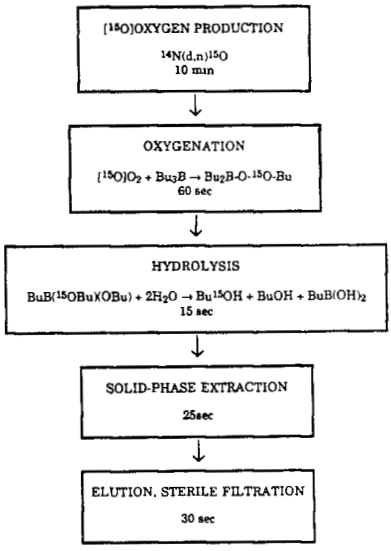
AUTOMATED PRODUCTION OF [O-15] BUTANOL



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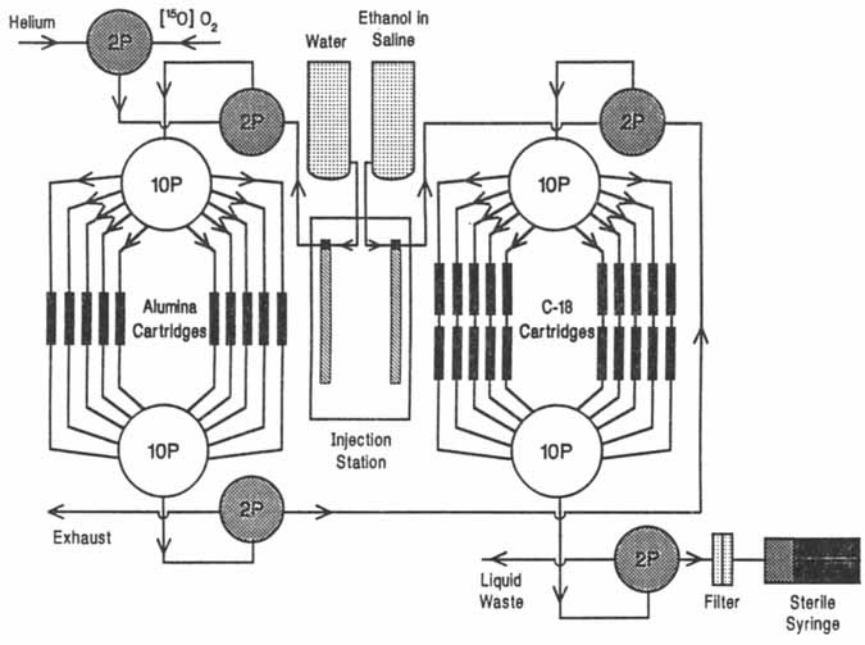
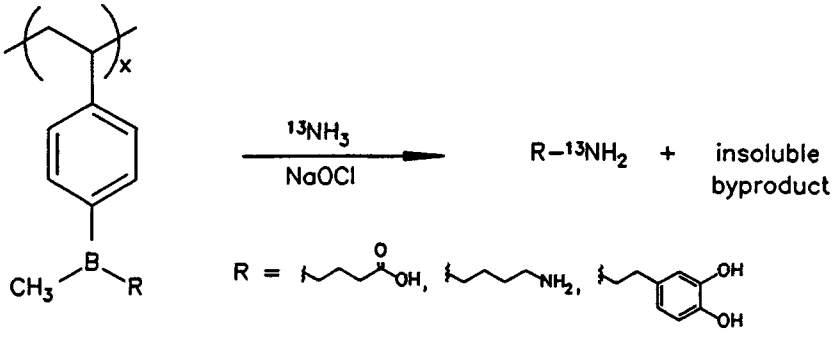

Helium
[15O] O2
2P
Water
Ethanol in Saline
2P
2P
10P
10P
Alumina Cartridges
C-18 Cartridges
10P
10P
Injection Station
2P
Exhaust
Liquid Waste
2P
Filter
Sterile Syringe

Paper D10

SYNTHESIS OF NITROGEN-13 LABELED AMINES USING ORGANOBORANE POLYMERS

G.W. Kabalka*, M.M. Goodman, J.F. Green, R. Marks and D. Longford
University of Tennessee Biomedical Imaging Center
Knoxville, TN 37920 USA Phone:(615)974-3260 FAX:(615)974-3454

Dopamine and other biologically active amines labeled with short-lived, positron-emitting isotopes have proven valuable in nuclear medicine (1,2). Nitrogen-13 labeled putrescine and gamma aminobutyric acid were recently prepared utilizing organoborane chemistry (3). However, the short 10 minute half-life of nitrogen-13 limits application of this new technology to readily isolable materials. In an effort to increase the generality of the new organoborane/nitrogen-13 incorporation technology, we have prepared a series of boronated polystyrenes which can be utilized to prepare a variety of nitrogen-13 amines. The polymers are based on the observation that methyl and phenyl groups on boron do not participate in amination reactions (4).



We have used the polymeric borane reagents to prepare no-carrier-added, nitrogen-13 labeled putrescine, dopamine and gamma-aminobutyric acid in good yields.

Research supported by The United States Department of Energy.

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Paper D11

SYNTHESES OF HOMOCHIRAL ^{11}C-LABELLED RADIOLIGANDS FOR PERIPHERAL BENZODIAZEPINE BINDING SITES

Shah F., Pike V. W. and Turton D. R.

MRC Cyclotron Unit, Hammersmith Hospital, Ducane Road, London W12 OHS, U.K.

With the labelling[1] of PK 11195 [*N*-methyl-*N*-(1-methyl-propyl)-1(2-chloro-phenyl)isoquinoline-3-carboxamide] (I) with carbon-11 ($t_{1/2}$ = 20.3 min) it became possible to study peripheral type benzodiazepine binding sites (PBBS) in living man with Positron Emission Tomography (PET). Like PK 11195 (I), the structurally related ligands, PK 11211 (II) and PK 14105 (III), have high affinity and selectivity for PBBS.[2,3] PK 14105 (III) can be labelled with no-carrier-added (NCA) fluorine-18 ($t_{1/2}$ = 109.6 min)[4] to provide an effective radioligand for PBBS in rat kainic acid-induced striatal lesions.[5] PK 11211 (II) has not hitherto been labelled with a positron-emitting radionuclide. The presence of a methyl group at the amido nitrogen in each of these ligands (I - III) suggested that PK 11211 (II) and PK 14105 (III) would be amenable, like PK 11195 (I), to labelling with carbon-11. Though each of these ligands has a chiral centre, the affinities of the enantiomers relative to the racemates for binding to PBBS have not been reported. We set out to synthesise homochiral precursors that would allow each enantiomer of ligands (I - III) to be labelled with carbon-11 or tritium for comparison as radioligands, with the prospect of finding a better radioligand than racemic [^{11}C]PK 11195 for PBBS in man.

The enantiomers of desmethyl-PK 11195 (*S*-Ic and *R*-Ic) were each synthesised from 1(2-chloro-phenyl)isoquinoline-3-carboxylic acid (Ia) and homochiral *sec*-butylamine (Scheme 1) (by analogy with the known synthesis[6] of PK 11195). Enantiomers of desmethyl-PK 11211 (*S*-IIc and *R*-IIc) and desmethyl-PK 14105 (*S*-IIIc and *R*-IIIc) were each synthesised analogously from 1(2-fluoro-phenyl)isoquinoline-3-carboxylic acid (IIa) and homochiral *sec*-butylamine (Scheme 1). The identities and purities of all products were assessed by MS and HPLC, by ^{1}H- and ^{13}C-NMR spectroscopy and where applicable by ^{19}F-NMR spectroscopy.

PK 11195 (I) and its *S*-enantiomer (*S*-I) were each labelled by reacting the appropriate desmethyl compound (*R,S*-Ic or *S*-Ic, respectively) with NCA [^{11}C]iodomethane in DMSO with potassium hydoxide as base (Table 1). PK 11211 (II) and its *S*-enantiomer (*S*-II) were each labelled successfully by reactions on *R,S*-IIc and *S*-IIc respectively, under similar conditions (Table 1). Attempts to methylate the enantiomers of PK 14105 (*S*-III and *R*-III) with iodomethane in DMSO with potassium hydroxide as base failed due to decomposition of the precursors (*S*-IIIc and *R*-IIIc). Attempts to methylate with NCA [^{11}C]iodomethane in DMSO with potassium carbonate as base also gave only low yields. However, by using 2,6-*di*-*t*-butyl pyridine as base the desired products (*S*-IIId and *R*-IIId) were obtained in acceptable radiochemical yields (Table 1). Labelled ligands were identified by radio-HPLC and by MS on carrier in peaks having the same retention time as the authentic ligands. The optimisation of radiochemical yield, determination of enantiomeric purity, preparation of tritiated ligands and the biological evaluation of the labelled ligands is now in progress.

Acknowledgement The authors are grateful to Dr C. Guéremy (Rhône Poulenc) for the gift of PK 11195, PK 14105, PK 11211 and related compounds and to Dr S.L. Waters for mass spectrometry.

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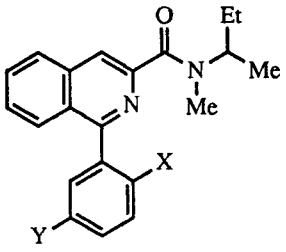
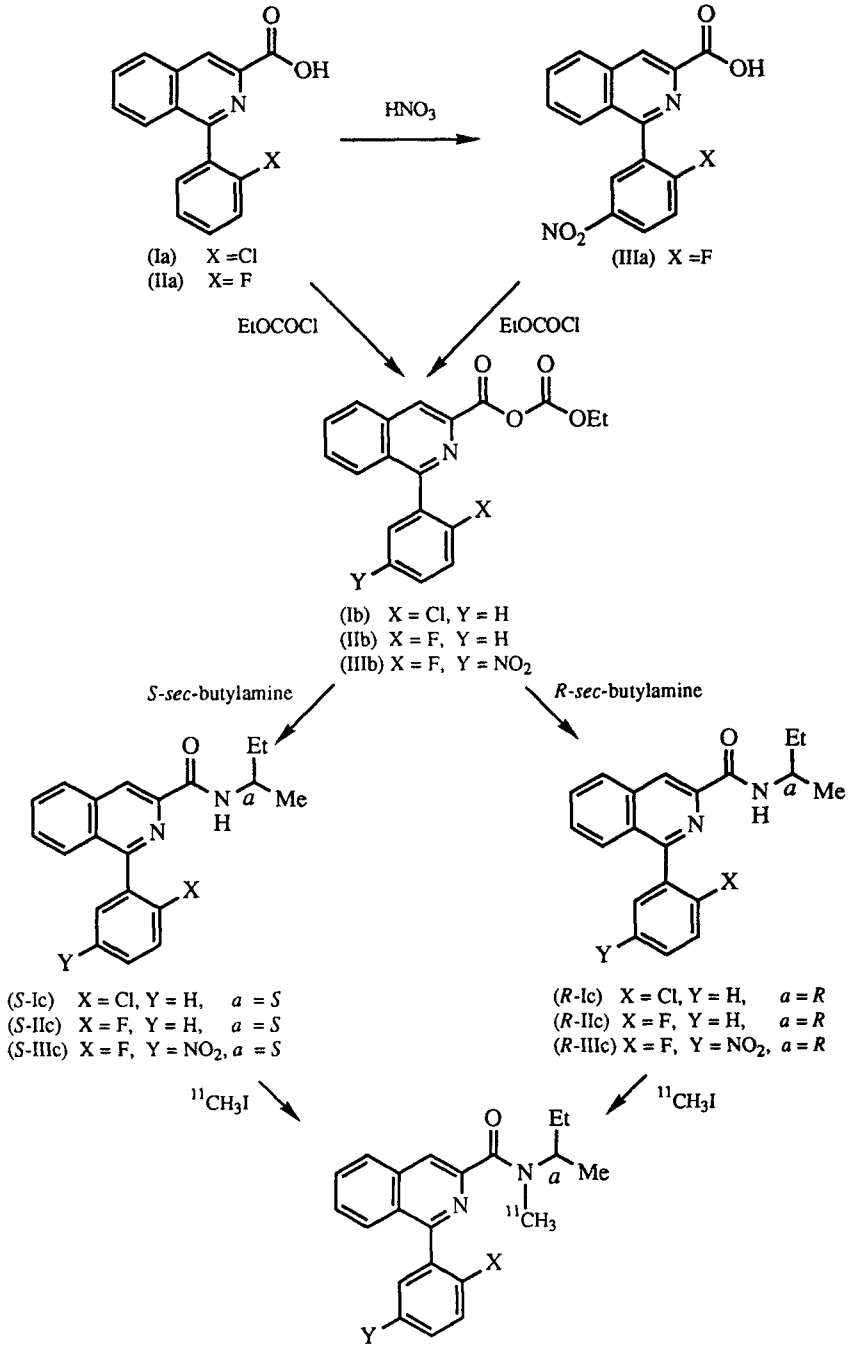


(I) (PK 11195), X = Cl, Y = H
(II) (PK 11211), X = F, Y = H
(III) (PK 14105), X = F, Y = NO_2

Precursor	^{11}C-Labelled Product	Base	Reaction Time (min)	Radiochemical Yield (%)
R,S-Ic	*R,S*-PK 11195	KOH, 10 mg	3	80
S-Ic	*S*-PK 11195	KOH, 10 mg	3	79
S-IIc	*S*-PK 11211	KOH, 10 mg	3	18
R,S-IIc	*R,S*-PK 11211	KOH, 25 mg	5	36
R,S-IIc	*R,S*-PK 11211	DTBP, 50 µL	3	2
S-IIIc	*S*-PK 14105	K_2CO_3, 10 mg	1	0.4
R-IIIc	*R*-PK 14105	K_2CO_3, 20 mg	1	3.2
S-IIIc	*S*-PK 14105	DTBP, 10 µL	1	23
S-IIIc	*S*-PK 14105	DTBP, 20 µL	3	28
R-IIIc	*R*-PK 14105	DTBP, 50 µL	3	40

Table I. Reaction conditions and yields for the labelling of PBBS ligands. All reactions were carried out with 1 mg of precursor in dry dimethyl sulphoxide (400 µL) heated by an oil bath at 90 °C. Radiochemical yields are decay-corrected from NCA [^{11}C]iodomethane and calculated from HPLC analysis of the radioactivity at the end of reaction. DTBP is 2,6-*di-t*-butylpyridine.

Scheme 1: Syntheses of homochiral ^{11}C-labelled radioligands for PBBS



(*S*-Id) (*S*-[^{11}C]PK 11195) X = Cl, Y = H, *a* = *S*
(*S*-IId) (*S*-[^{11}C]PK 11211) X = F, Y = H, *a* = *S*
(*S*-IIId) (*S*-[^{11}C]PK 14105) X = F, Y = NO_2, *a* = *S*

(*R*-Id) (*R*-[^{11}C]PK 11195) X = Cl, Y = H, *a* = *R*
(*R*-IId) (*R*-[^{11}C]PK 11211) X = F, Y = H, *a* = *R*
(*R*-IIId) (*R*-[^{11}C]PK 14105) X = F, Y = NO_2, *a* = *R*

Paper D12

The production of ^{11}C-Iomazenil

#G. Westera, #I. Eberle, * W. Hunkeler, ** S. Ametamey, ** P.A. Schubiger.
Division of Nuclear Medicine, University Hospital, 8091 Zürich, * F. Hoffmann-La Roche, Basel ** Paul Scherrer Institute, Villigen, Switzerland

Iomazenil has possibilities as a compound which maps the benzodiapine receptor (1). ^{123}I-Labeled iomazenil has been used as a tracer for SPECT.(2)

To enable a direct comparison of SPECT data with the more easily quantifiable PET results, we have prepared ^{11}C-iomazenil in a remotely controlled system.

^{11}C-Iomazenil was prepared by methylation with ^{11}C-MeI of demethyl-iomazenil (Scheme).

^{11}C-MeI was produced by the usual method via $LiAlH_4$ reduction of ^{11}C-CO_2 in THF and (after removal of the THF) reaction of ^{11}C-MeOH with HI.

The subsequent reaction with demethyl-iomazenil in 200 µl DMF, containing 1 mg/ml demethyl-iomazenil and 0.9 mg/ml NaH, proceded (2 min., 70^0) with a radiochemical yield of 20-50%.

The compound was purified by preparative reversed phase HPLC eluting with 0.1% H_3PO_4 / AcCN = 60 / 40. The eluent was removed under low pressure in a rotavapor and the product was redissolved in phosphate buffer pH 7.5.

With a total production time of 50 minutes we thus obtained an overall yield of at least 3 % radioactivity.

#_ To whom correspondence should be addressed

An experiment using a small amount of non radioactive MeI vapor (25 µl, 1 µMol) was performed and the product isolated by the same method. Subsequent NMR spectroscopy showed the N-methylated product (especially the N-methyl protons at 3.21 ppm) and no oxygen methylated analogon, which confirms the HPLC evidence for the identity of the product.

^{11}C-Flumazenil could be produced in the same manner.

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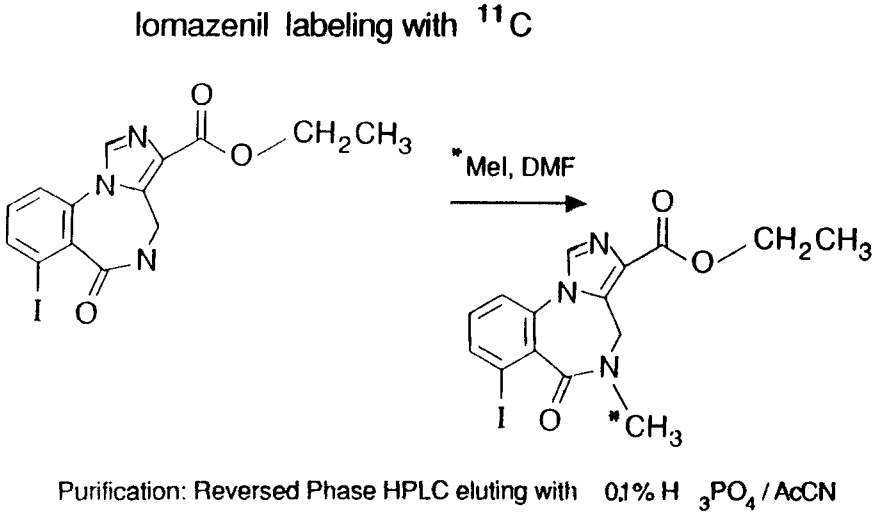
Scheme:



Paper D13

PREPARATION OF NITROGEN-13 LABELLED POLYPEPTIDES FOR POSITRON-EMISSION TOMOGRAPHY

P. Landais[1], P. Waltz[2], H. Tochon-Danguy[3], P. Goethals, K. Strijckmans[4], K. Rose[2], R. E. Offord[2].

[1] CERMEP, Cyclotron Biomédical de Lyon, 59 bld. Pinel, 69003 Lyon, France; [2] Département de Biochimie Médicale, Centre Médical Universitaire, 1 rue Michel-Servet, 1211 Genève, Switzerland; [3] Division of Nuclear Medicine, Hôpital Cantonal, 1211 Geneva 4, Switzerland (present address: Austin Hospital, Melbourne, Australia); [4] Lab. Analytical Chemistry, Inst. Nuclear Sciences, Universiteit Gent, 9000 Gent, Belgium.

It would be very useful to be able to label polypeptides for positron emission tomography, since a perturbation of the behaviour of such molecules is often the origin of a major pathological syndrome. The only positron-emitting isotopes of the elements naturally present in polypeptides have short half-lives (e.g. ^{13}N, $t_{\frac{1}{2}}$ = 10 min). This rules out labelling by de-novo synthesis of all but the very smallest such molecules.

We have got round this problem by developing a rapid, enzymic method of ^{13}N labelling of polypeptides, which is in principle applicable to any polypeptide that contains glutamine. The enzyme in question is transglutaminase, which although normally destined to promote protein crosslinks through the side chains of glutamine residues,

$$-CH_2CH_2CONH_2 + H_2N\text{-}R = -CH_2CH_2CONH\text{-}R + NH_3$$

should, as a consequence of its mechanism, catalyze the exchange of the side-chain amide group of glutamine residues with $^{13}NH_3$ in solution.

$$-CH_2CH_2CONH_2 + {}^{13}NH_3 = -CH_2CH_2CO{}^{13}NH_2 + NH_3$$

There seems to be no prior report of such an approach to labelling of a polypeptide with ^{13}N, although some have been modified by reaction with isotopically substituted amines such as putrescine, and even with the stable isotope ^{15}N, used in the form of $^{15}NH_3$ [1].

The $^{13}NH_3$ was produced using the $^{16}O(p,\alpha)^{13}N$ reaction on a water target. The various ^{13}N species produced were reduced to ammonia by treatment with Devarda alloy and NaOH, and the gaseous $^{13}NH_3$ distilled into a water trap [2]. We report preliminary, unoptimized experiments in which ^{13}N was then successfully incorporated into vasoactive intestinal peptide (VIP), neuropeptide Y, secretin, glucagon, and insulin. In a typical preparation [^{13}N]VIP was recovered after reverse-phase h.p.l.c. at a radiochemical yield of about 3% ($t_{\frac{1}{2}}$ at 40 min after the end of bombardment. Only insulin no longer had authentic chromatographic or electrophoretic behaviour after labelling, which we attribute to attack on its disulphide bridges.

We conclude that this method merits further evaluation and development. It would be desirable to remove the metallic impurities in the $^{13}NH_3$ solution (which come from the Devarda alloy), and which occasionally poison the enzyme. We will also need to turn to preparations at least at the 200 MBq (5 mCi) scale, ten times greater than that used so far.

We thank Mme. J. Gerlach and Mr. E. Gillis for skilled technical assistance, and the Fonds National Suisse and the Région Rhône-Alpes (France) for financial assistance.

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SYNTHESES OF [^{11}C]EPINEPHRINE AND OTHER BIOGENIC AMINES BY DIRECT METHYLATION OF NORMETHYL PRECURSORS.

P. K. Chakraborty, D.L. Gildersleeve, S.A. Toorongian, M.R. Kilbourn, M. Schwaiger, D.M. Wieland. Division of Nuclear Medicine, University of Michigan, Ann Arbor, MI. 48109-0552

N-[^{11}C]methylation with [^{11}C]methyl iodide is an increasingly popular route to radiopharmaceuticals for *in vivo* imaging by PET. N-[^{11}C]methylations are usually rapid, high-yield reactions adaptable to remote control. A large number of useful tracers can potentially be synthesized in one-step reactions from their normethyl precursors. We have reported the synthesis of N-[^{11}C]methyl-*meta*-hydroxyephedrine (MHED) from metraminol free base by methylation with [^{11}C]methyl iodide (1). This agent is now routinely made in our center for clinical studies (2,3). In a continuing effort to develop radiotracers to probe the biochemical workings of the adrenergic neuron, we have synthesized three additional N-[^{11}C]methyl biogenic amines: N-[^{11}C]methyl-*threo*-metaraminol ([^{11}C]-*threo*-MHED), [^{11}C]epinine and [^{11}C]epinephrine.

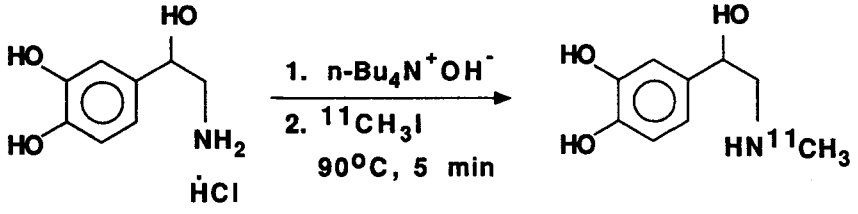
The synthesis of [^{11}C]-*threo*-MHED from *threo*-metaraminol was identical to that of MHED which used the isolated free base as the normethyl precursor (1). For the syntheses of the two catecholamines, [^{11}C]epinine and [^{11}C]epinephrine, however, this method was modified because of the susceptibility of their free base precursors dopamine and norepinephrine to air oxidation. This modification simply incorporated the *in situ* generation of free base precursor from its hydrochloride salt. The procedure has been successfully applied to the syntheses of [^{11}C]epinine and [^{11}C]epinephrine (see Table). A two-step, enzymatic pathway to [^{11}C]epinephrine has been reported (4) but the method described here is more amenable to routine clinical production. It is noteworthy that direct methylation of a primary amine with [^{12}C]methyl iodide is not a viable preparative route to the respective N-methylamine but is a useful route using tracer amounts of [^{11}C]methyl iodide.

In a typical procedure, 1.0 mg of R-(-)-norepinephrine hydrochloride was weighed into a reaction vial and 0.8-0.9 eq. of (n-Bu)$_4$N$^+$OH$^-$ in methanol was added via syringe. Following evaporation of the methanol with nitrogen purge, 0.25 mL of DMF/DMSO (3/1) was added. The vial was placed in a heating/cooling block and [^{11}C]CH_3I was bubbled through the reaction mixture at -30^{o}C. When maximum radioactivity had accumulated, the vial was sealed and heated at 90^{o}C for 5 min. The reaction mixture was cooled to ambient temperature and purified by HPLC. Pure R-(-)-[^{11}C]epinephrine was eluted at *ca.* 11 min (SCX column, 0.01 M NaH_2PO_4, 2.8 ml/min). The unoptimized yield was 5-10 % (EOB) in 40 min synthesis time.

The procedure described here appears to be a general method applicable to other N-[^{11}C]methyl phenolamines and catecholamines.

Table

Radiotracer	**Radiochemical yield % (EOB)**	**Radiochemical purity %**
Threo-MHED	20-35	>98
Epinine	5-10	>98
R-(-)-Epinephrine	5-10	>98



R-(-)-Norepinephrine **R-(-)-[^{11}C]Epinephrine**

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REMOTE SYSTEM FOR THE ROUTINE PRODUCTION OF [^{11}C]-METHYL ALBUMIN. APPLICATION TO PULMONARY STUDIES.

Carmen S. Dence, Keith R. Lechner, and Michael J. Welch
Mallinckrodt Institute of Radiology, Washington University Medical School, St. Louis, MO 63110

We have expanded the use of our remote controlled synthetic system (RCSS) unit, originally developed for the routine production of [^{11}C]-palmitic acid (1), [^{11}C]-butanol (2,3), [^{11}C]-acetate (4) and other low MW fatty acids and alcohols, to include the routine production of $^{11}CH_3I$. This last agent is then used to label human serum albumin for pulmonary clinical studies. The entire synthetic apparatus (vessels, valves, heating/cooling system) occupies a volume of less than $0.04M^3$ ($< 1.5\ ft^3$). It can be moved easily in and out of the hood, it is built with easy to obtain parts and it can deliver consistent clinical doses for animal or patient studies with minimum radiation dose to the operator (Fig. 1).

The synthesis of $^{11}CH_3I$ was an adaptation of a published procedure (5). The preparation of $^{11}CH_3$-HSA first described by Turton (6), was modified to ensure reproducibility in our RCCS unit. The purification step was simplified by the use of four Sephadex G-25 columns placed inside a centrifuge. These columns were loaded directly from the RCCS unit. The purified eluate containing the $^{11}CH_3$-HSA was adjusted to pH 7.8 with 0.1M $NaHCO_3$, and filtered through a 0.25μ filter to render a sterile and pyrogen free solution.

More than 20 preparations have been carried out with yields of about 30% based on starting $^{11}CH_3I$. The radiochemical purity of the product is between 98-99% as determined by HPLC on a Shodex protein column eluted with water at 1.0 mL/min.

The $^{11}CH_3$-HSA is being used for pulmonary studies and compared to results obtained with ^{68}Ga-transferrin. Comparative studies to evaluate PTCER, (pulmonary transcapillary escape rate) an index of pulmonary vascular permeability to large proteins (7), are under investigation

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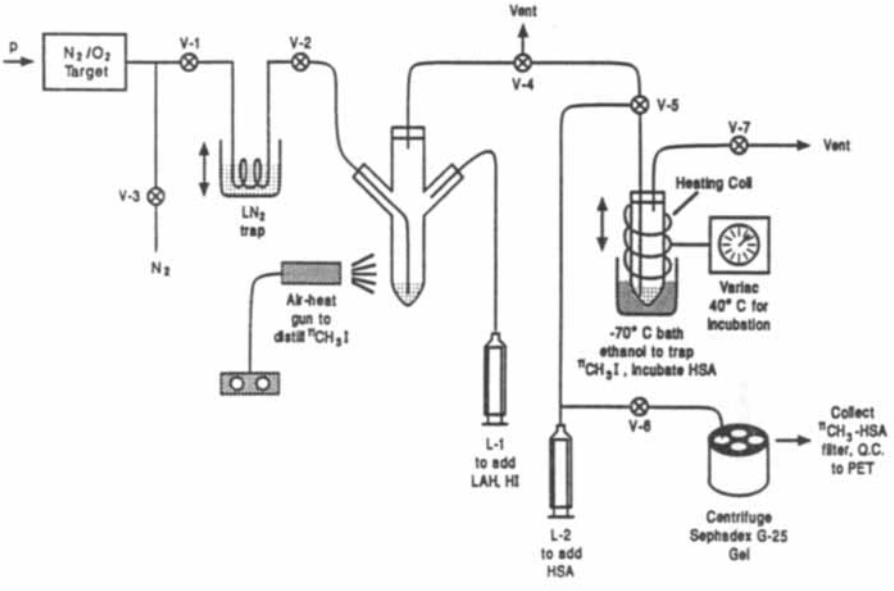
Figure 1

Remote System for the Synthesis of $^{11}CH_3$-HSA



Paper D16

Specific Activity of $^{11}C-CO_2$ Determined By Reaction With Tritylllithium Yielding ^{11}C-Triphenylacetic Acid

B. Schmall, N.R. Simpson, and W.C. Eckelman
PET Department, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD 20892

We have investigated a rapid and selective chemical method for the determination of the specific activity of $^{11}C-CO_2$. With minor modifications, this abstract describes our efforts to utilize the method described by Tewson et al (1) for the synthesis of tritylllithium and its reaction with $^{11}C-CO_2$. Triphenylmethane (244 mg, 1 mmol), which had been recrystallized from hot alcohol, was dissolved in 10 mL of THF (freshly distilled from sodium under argon with benzophenone as an indicator), and 0.625 mL (1 mmol) of a 1.6 M solution of n-butyllithium in hexane was added. The solution was contained in a 20 mL vial that was sealed and stirred at ambient conditions for 45 minutes under argon. A stock solution was freshly prepared before use. Typically, 1 mL aliquots of this solution were withdrawn and added to a 5 mL reacti-vial containing 1 mL of THF. One aliquot was taken which served as a blank for the reaction solution. It was quenched with 15 μL of sterile water, and 1 mL of THF was added. Then another aliquot was taken for reaction with $^{11}C-CO_2$. $^{11}C-CO_2$ that had been trapped on a Porapak Q column was passed through the aliquot in a stream of ultra-pure helium at 20 cc/min. After the $^{11}C-CO_2$ was trapped, the reaction solution was still red. Products of the reaction of the tritylllithium are pale yellow, so there was excess tritylllithium as desired. The reaction solution was then quenched with 15 μL of sterile H_2O. Helium was passed through the solution for 1 minute, and 1 mL of THF was added.

An authentic sample of triphenylacetic acid using an Axxiom 5 μ C-18 analytical column (4.6 mm x 25 cm) with a solvent mixture of CH_3CN/H_2O (0.1% H_3PO_4), 52/48, and a flow rate of 3 mL/min had a retention time of 14-14.5 minutes (UV: 240 nm, 0.01 AUFS). Using an Altex 5 μ C-18 semi-preparative column (10 mm x 25 cm) with a 60/40 solvent mixture and a flow rate of 5 mL/min the retention time of the acid was 16-17 minutes. Reaction solutions analyzed with either HPLC system showed absorbance of the acid at the corresponding retention times, but the peaks usually exhibited a shoulder, which interfered with the integration of the peaks. In the case of the blank solution, there was sometimes absorbance in the region of the acid. The origin of this absorbance is not clear. An attempt was made to eliminate any extraneous absorbance in the acid region by passing the reaction solution through Sep Pak columns before HPLC analysis. Solutions were placed onto a Silica Sep Pak column which was in series with two Alumina (basic) Sep Pak columns. The columns were rapidly eluted with 200 mL of ether followed by elution with 100 mL of 1% HOAc in ether. Triphenylmethane and by-products were eluted with ether. 1% HOAc in ether was required to elute ^{11}C-triphenylacetic acid. In two experiments, 59 and 75% of the acid was eluted from the columns. The respective solvents were evaporated, and the resultant residues were taken up into 3 mL of HPLC solvent, and aliquots were analyzed by HPLC. The residue obtained after evaporation of the ether fraction of the blank solution showed absorption in the acid region. There was no absorption in this region for the residue obtained for the blank solution after evaporation of the 1% HOAc in ether fraction. Therefore, potential interference in the region of the acid can be eliminated by the Sep Pak method. In the case of the reaction solution, the UV or radioactivity peaks for triphenylacetic acid did not show an interfering shoulder after workup by the Sep Pak procedure. After collection of the acid by HPLC, the radiochemical yield of the acid was 48 and 58% (n = 2). The total time from EOB was about 45 minutes.

The specific activity of the triphenylacetic acid was determined by collecting the acid from the HPLC and assaying its activity. The mass of the peak was determined from a calibration plot of mass vs integration units. The target irradiations were of short duration and low beam currents (10 μA/10 min). This yielded ^{11}C-triphenylacetic acid with a specific activity of 230 and 270 Ci/mmol (EOB) from approximately 90 mCi of $^{11}C-CO_2$ for the two experiments. In conclusion, we have demonstrated the feasibility of using a direct chemical method to determine the specific activity of $^{11}C-CO_2$.

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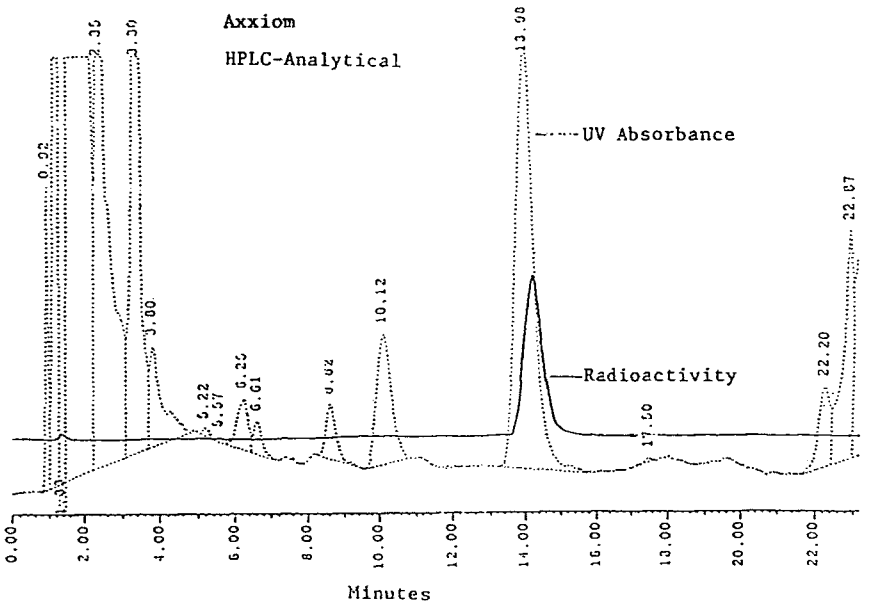

HPLC of Reaction Solution

The UV or radioactivity peaks for triphenylacetic acid (TPAA) in the region of 14-14.5 minutes did not show an interfering shoulder after workup by the Sep Pak procedure. The peak at 13.98 min represents 3.53×10^{-3} μmol of TPAA.

Paper D17

Synthesis of 2-[^{11}C]thymine from [^{11}C]phosgene; a precursor for 2-[^{11}C]thymidine.

C.J.Steel, G.D.Brown, K.Dowsett, D.R.Turton, S.K.Luthra, H.Tochon-Danguy, S.L.Waters, P. Price and F.Brady.
MRC Cyclotron Unit, Hammersmith Hospital, Ducane Road, London W12 OHS, UK.

2-[^{11}C]thymidine has recently been proposed as an agent for measuring cellular proliferation *in vivo* by PET (1,2). Loss of the carbon-11 label from the 2-position as [^{11}C]carbon dioxide early in the catabolic pathway reduces the number of metabolites and should simplify the modelling of this tracer.

Synthesis of 2-[^{11}C]thymidine has been achieved by enzymatic addition of the ribose moiety to 2-[^{11}C]thymine (2). 2-[^{11}C]thymine itself is synthesised by the cyclisation of diethyl-2-hydroxy-2'-methylsuccinate (diethyl-β-methylmalate) with [^{11}C] urea (2). The latter synthesis requires anhydrous [^{11}C]urea , however ,the reported methods of syntheses from [^{11}C]cyanide (3) or [^{11}C]phosgene (4) produce [^{11}C]urea as an aqueous solution, thus necessitating removal of water by heating. Gentle heating of urea is known to produce the dimer biuret and rapid heating the cyclic trimer, cyanuric acid. To avoid possible complications from these compounds we have devised an alternative procedure for synthesising [^{11}C]urea. This utilises reaction of [^{11}C]phosgene with liquid ammonia followed by removal of excess ammonia thus providing anhydrous urea directly without heating. The [^{11}C]urea can then be condensed with diethyl-β-methylmalate to give [^{11}C]thymine and subsequently [^{11}C]thymidine as previously reported (2).

[^{11}C]methane,produced using the ^{14}N(p,α)^{11}C reaction on nitrogen containing 5% hydrogen, is first converted to [^{11}C]CCl_4,by reaction with chlorine over $CuCl_2$/pumice at 400^{0}C, and then to [^{11}C]$COCl_2$ by oxidation of the latter over an iron catalyst at 290^{0}C (5). Production time is 20 minutes and the typical decay corrected radiochemical yield of phosgene is 50%. Liquid ammonia *ca* (300μl) is condensed in a reaction vessel at -70^{0}C and [^{11}C]phosgene is passed through the ammonia, starting the passage during the last 10 minutes of phosgene production . The cooling bath is then removed and the liquid ammonia evaporated at room temperature under a flow of inert gas. [^{11}C]urea is typically produced in 45% radiochemical yield in 22 minutes from EOB. A mixture of diethyl-β-methylmalate (10μl), fuming sulphuric acid (100μl) and ethanol (10μl) is added from a glass syringe and the mixture heated at 130^{0}C for 7 minutes. The solution is neutralised by addition of sodium hydroxide and tris-buffer and desalted by passing through an ion retardation resin AG 11A8. Conversion of [^{11}C]urea to [^{11}C]thymine is achieved in 60% radiochemical yield in 10 minutes. The synthesis is carried out using a microprocessor controlled remote handling system (Fig 1) which is being extended for conversion of [^{11}C]thymine to [^{11}C]thymidine by the published procedure (2). Precursors, intermediates and products were characterised by mass spectrometry and ^{13} C NMR where appropriate.

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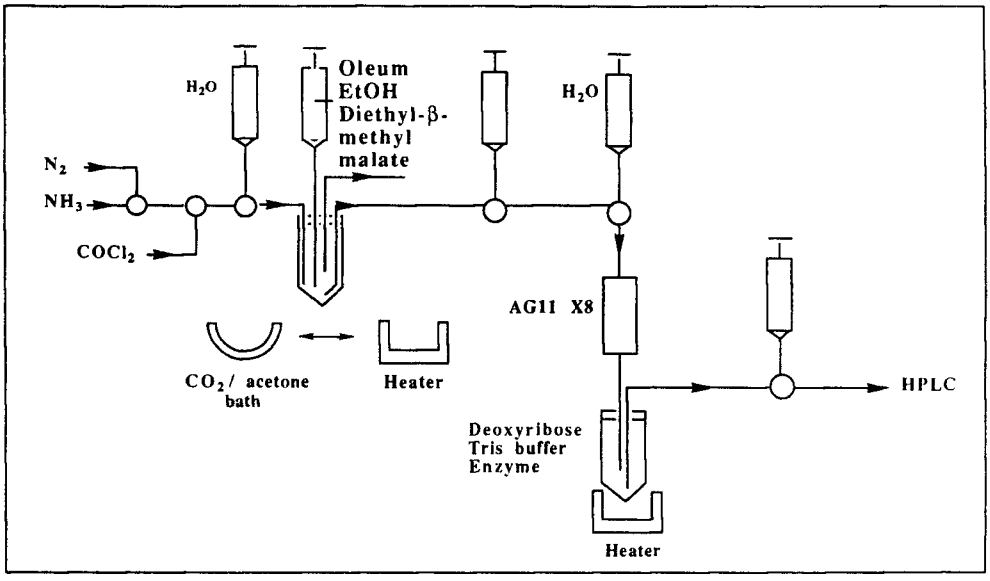


Figure 1. Schematic for the preparation of [^{11}C]thymine from [^{11}C]phosgene and its conversion to [^{11}C]thymidine.

NO CARRIER ADDED [^{11}C] ALLYLATION OF AMINES

M.C. Lasne, B. Moreau, L. Barré+, Ph. Cairon, and C. Ducandas

Unité de Recherche Associée au CNRS, Institut des Sciences de la Matière et du Rayonnement, 14050 Caen ; + CEA, DSV/DPTE Cyceron, Bd H. Becquerel, 14021 Caen ; FRANCE.

As a part of our research program on the development of unsaturated [^{11}C] precursors and on their uses for the labelling of radiopharmaceuticals for positron emission tomography studies (1) we have investigated different methods (scheme 1) for the preparation of N-[^{11}C] 2-propenyl amines (N-[^{11}C] allyl amines). We report here our results using tetrahydroisoquinoline 1 as a model amine.

The most commonly used method (route A) was based on an alkylation reaction . The [^{11}C] iodide 2 was prepared from [^{11}C] carbon dioxide according to the method we have previously described (1, 2), and was allowed to react with a large excess of amine. When the reaction was carried out in DMF at 95°C, a time of 15 min was necessary to ensure an incorporation of more than 50 % of the total radioactivity. Analysis of the crude mixture by TLC or HPLC showed the presence, with the expected [^{11}C] adduct 3 , of a more polar product which was identified as the [^{11}C] ammonium salt 4 (up to 15% yield). Under these conditions, the amine 3 was isolated pure in 5% yield (decay corrected, from [^{11}C] CO_2, 60 min overall synthesis time). This low yield and the possibility of side-reactions due to the contamination of iodide 2 with [^{11}C] methyl and propyliodides (1) led us to search a more selective way of [^{11}C] allylation.

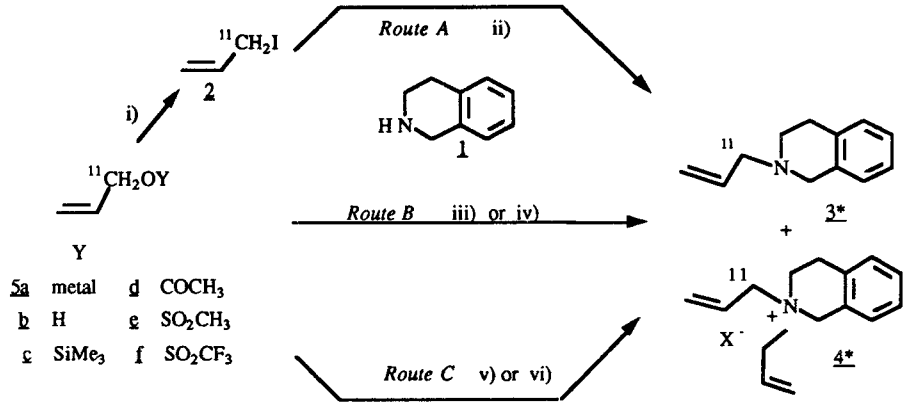
In route B, we made use of ability of allylic trifluoromethanesulfonates to undergo readily an SN2' reaction (3). With this aim, we treated the crude [^{11}C] alcohol (as the metallic salt 5a) obtained after [^{11}C] carbonation of vinylmagnesium bromide with methanesulfonyl chloride. After 15 min at room temperature [TLC of the crude mixture showed the presence of 43% of the expected mesylate 5e (4)] 15 µL of the amine 1 were added and the mixture heated for 5 min at 80°C. Under these conditions, the N-[^{11}C] allylamine 3 was isolated in 10 % yield (after HPLC). No improvement, either the yield or purity were observed when using the trifluoromethane sulfonate 5f (5) instead of the mesylate 5e . This may be a result of the high reactivity of the triflate in alkylation reactions (6).

Finally, the third approach (route C) was based on the now well documented reaction of nucleophiles on allylic compounds in the presence of palladium complexes (7, 8). When the alcohol 5b, obtained by hydrolysis of 5a (300 µL of HCl, 1M) was treated by tetrakistriphenylphosphinepalladium for 25 min at 90°C in DMF, the amine 3 was isolated in 13% yield.

All the reactions were carried out in non radioactive chemistry and the reaction times were not optimized. Work is in progress to improve the yield and reduce synthesis time with the aim of labelling naloxone, a selective antagonist of opioid receptors.

TLC analysis were performed on silica plates using as eluent a mixture of dichloromethane and methanol (v/v : 85/15, Rf 3 : 0.78 ; 4 : 0.55 ; N-methyl and N-propyltetrahydroisoquinoline : respectively 0.61 and 0.72). HPLC were carried out either on a reverse phase [C_{18} μ Bondapack, eluent : CH_3OH/NH_4OH 0.06 M (v/v : 85/15)] or a normal phase [μ Porasil, eluent : CH_2Cl_2/CH_3OH (v/v : 96/4), retention time of 3 : 11 min].

Scheme 1 : synthetic routes to N [^{11}C] allyltetrahydroisoquinoline



* the position (either in 1' or 3' in the allylic chain) of the labelled atom could not be precised ; i) distillation of 5c over solid P_2I_4, 80-90°C, 3 min, 10^{-2} torr ; ii) 1 (200 μL) , DMF (500 μL) 15 min, 95°C ; iii) 5a , CH_3SO_2Cl (60 μL) pyridine (65 μL) 25°C, 15 min then 1 (15 μL) 15 min, 95°C ; iv) 5a , CF_3SO_2Cl (50 μL) NEt_3 (40 μL) 25°C, 15 min then 1 (25 μL) , DMF (200 μL) 15 min, 25°C ; v) 5b , $(Ph_3P)_4Pd$ (2.5 mg) , DMF, 1 (5 μL) 25 min, 90°C ; vi) 5a, $(CH_3CO)_2O$ (19 μL) $(Ph_3P)_4Pd$ (2.5 mg) , DMF, 1 (5 μL), 25 min, 90°C .

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Paper D19

A MODIFIED PREPARATION METHOD FOR ^{11}C ACETATE, PREVENTING LIQUID PHASE EXTRACTION STEPS.

G-J. Meyer, K. Günther, K.-H. Matzke, Th. Harms, H. Hundeshagen. Abteilung Nuklearmedizin und spezielle Biophysik, Medizinische Hochschule Hannover, D-3000 Hannover 61, Germany

^{11}C Acetate has become in demand for routine application since its validation as a tracer for the measurement of myocardial oxygen consumption (1).

The preparation of ^{11}C Acetate, although simple and described early (2,3), often suffers from inappropriate set-ups which complicate the handling, inconsistent yields and insufficient purity. A semi-automated system has been designed, tested, and used in routine production which overcomes these problems very effectively.

A fast production without phase-separation purification require a set-up as shown in Fig. 1

After addition of 0.1 mMol of methylmagnesium bromide in 2 ml ether to the trapped ^{11}C Carbondioxide in a special reaction flask and 2.5 min reaction time at room temperature, the reaction mixture is hydrolysed with 100 µl of water. The mixture is then heated to 100°C and the solvents are evaporated to complete dryness. The residue is then taken up by 2 ml of 1% phosphoric acid. To remove any ^{11}C Carbondioxide which may not have reacted properly with the girgnard reagent, the acidified aqueous reaction mixture is degassed with a controlled stream of Heluim (50 ml/min) at room temperature for 3 min. Tests have shown that this procedure under aqueous acidic conditions removes over 90% of ^{11}C Carbondioxide, while it retains over 90% of the ^{11}C- Acetic acid.

The reaction mixture is then withdrawn into a motorized syringe. From there it is driven through combination of a Dionex Silver-Oxide column, which retains all bromide and a cation exchange cartridge, to remove magnesium ions. Subsequently the mixture passes a 0.22µm filter for sterilization before it is collected in a sterile flask containing 6ml 0.9% saline and 1.8ml 1% NaOH in order to buffer the phosphoric acid. (Since some loss occurs during the transfer of the phosphoric acid to the motor driven syringe, the amount of NaOH is a little smaller than stochiometric.) In order to increase the recovery, the coulmn- and filter-system is finally washed with 2 ml of sterile water. The collected solution is ready for injection

Quality control has been performed with HPLC using an ion-exchange column (REZEX organic acid from Phenomex), and a weak aqueous mineral acid mobile phase, (0.005n H_2SO_4). Using an RI detector system salt concentrations in the order of a few µmol could be detected.

Sterility and apyrogenicity of the product are checked frequently in routine production runs (every month) by using samples and testing these by the standard procedures.

The overall synthesis time, including the recovery of $^{11}CO_2$ from the target, is about 20 min. Yields of up to 40 GBq have been produced for test purposes. In routine production runs a 10 mC irradiation (ca 20 µA for 8.5 min) result in ca 10 GBq of ^{11}C Acetate ready for injection

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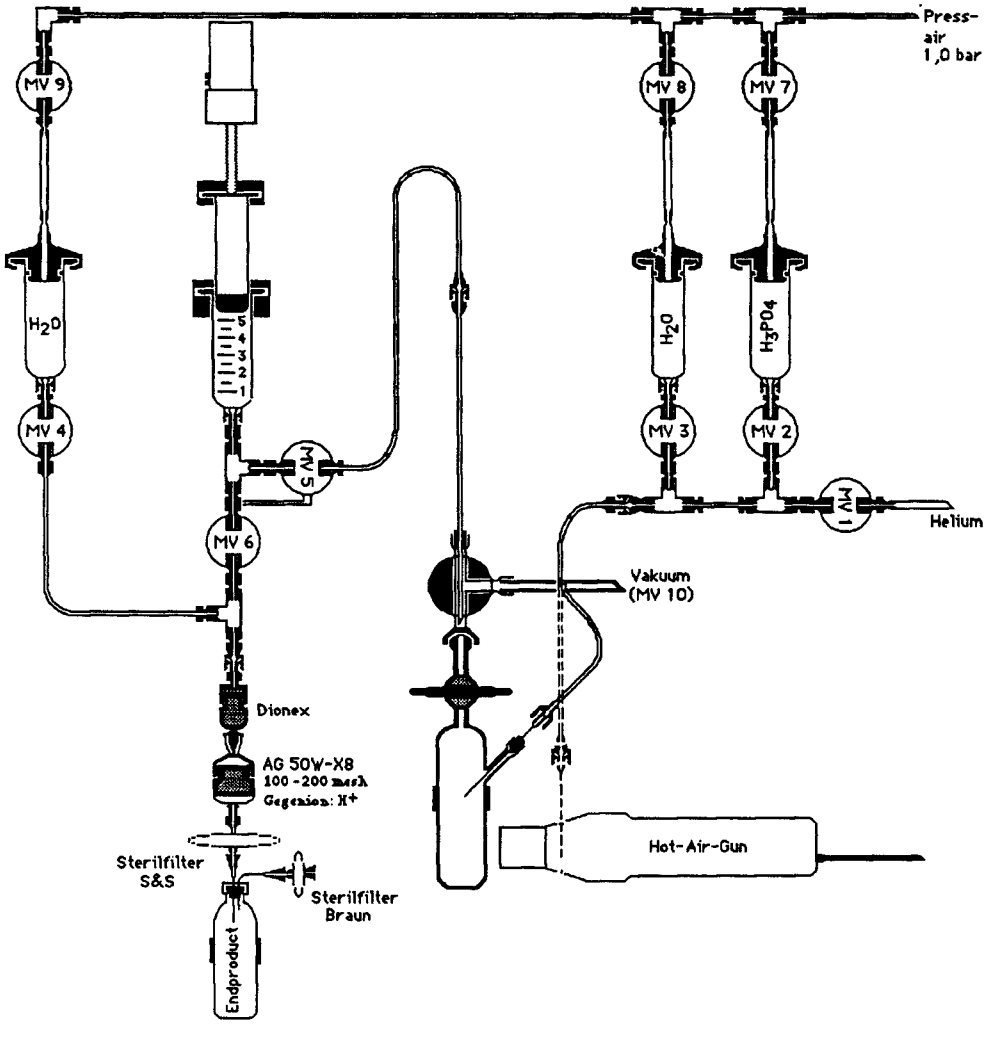


Paper D20

A ROBOT-BASED SYSTEM FOR THE DEVELOPMENT OF PRODUCTION METHODS FOR RADIOTRACERS FOR PET

Göran Appelqvist[1], Christian Bohm[1], Hans Eriksson[1] and Sharon Stone-Elander[2,3]

[1]Dept. of Physics, University of Stockholm, Vanadisvägen 9, S-10405 Stockholm, Sweden
[2]Karolinska Pharmacy, Box 60024, S-10401 Stockholm, Sweden
[3]Clin Neurophys, Karolinska Hospital/Institute, Box 60500, S-10401 Stockholm, Sweden

Production of PET radiotracers requires the construction of systems for handling the radioactivity used during development as well as during routine production. These systems range from operator-interactive manual- or remote-controlled systems to automated dedicated devices. Robotics have been used to perform either sequential or simultaneous radiotracer syntheses. To minimize the time to routine production, the programming procedure should be easy, the vessels should be standardized and stations should be available for performing common synthesis steps. These demands served as guidelines when designing the Karolinska robotic system.

The system uses a robot arm (RTX from U.M.I.) together with specially designed supporting equipment in a Van Gahlen hot cell. A PC-AT serves as a system controller and an easy-to-use process editor creates synthesis specific command sequences by familiar editing commands from the keyboard or by use of the mouse.

The system has now been tested in synthetic procedures common to PET radiochemistry: production of and alkylation with [^{11}C]alkyl halides, ^{11}C-cyanations and nucleophilic displacements with [^{18}F]fluoride. These procedures include set-up of glassware, trapping of radioactivity in air-sensitive reagents, removal of solvents, addition of reagents, distillation of radioactivity from one vessel to another, heating, cooling, opening and closing valves, stopwatch functions and monitoring levels of radioactivity. The system allows three levels of operation: the "edit" mode in which only robot movements are performed or two "run" modes in which all commands are performed either automatically from the beginning to the end or step-wise with the possibility of operator intervention to adjust arm movements, times, temperatures, flows etc. In the run mode, if conditions specified in the control sequence are not met, the process is aborted. Step-wise runs are particularly useful in the development of new sequences for which all procedures are not completely optimized. At the end of the reactions the arm removes contaminated glassware and wash routines can be run before set-up of the next synthesis, allowing quick assess to the hot cell. Executions of robot arm movements and of the various control sequences have been found to be reproducible with results comparable to remote-controlled systems.

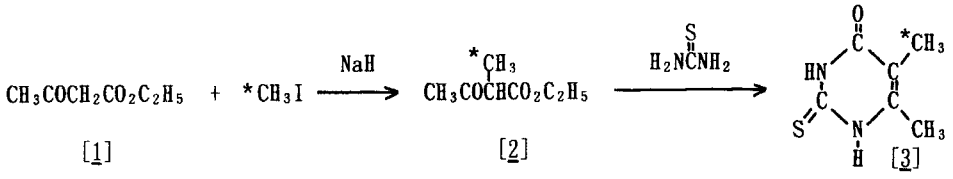
Paper D21

RADIOMETHYLATION OF ETHYL ACETOACETATE AND ITS USE AS AN INTERMEDIATE FOR SOME ^{11}C-RADIOPHARMACEUTICALS

K. Ogawa and T. Nozaki
School of Hygienic Sciences, Kitasato University, Sagamihara, 228 Japan

Formerly we reported the synthesis of α-methyl[$^{11}CH_3$]-fatty acids by the reaction of $^{11}CH_3I$ on diethyl alkyl-malonates and the behaviour of them in mice (1). Similar ^{11}C-methylation is practicable for other substrates with reactive methylene groups, such as ethyl acetoacetate, acetylacetone and ethyl cyanoacetate, and the products can be used as intermediates for some potential radiopharmaceuticals (2,3).

We have studied in detail the radiomethylation of ethyl acetoacetate [1] to form ethyl α-aceto-β[^{11}C]-propionate [2] and its condensation with thiourea to give 5-methyl[$^{11}CH_3$]-6-methyl-2-thiouracil [3], using ^{14}C more often than ^{11}C. Table 1 shows



[1] [2] [3]

the radiomethylation yield of 2 under various conditions. As the solvent tetrahydrofuran was found to be suitable, but its presence deteriorated the efficiency of HPLC purification. For the synthesis of 2 in a good yield, $^{11}CH_3I$ is trapped in ethanolic solution of 1 (0.1 mol/l, 50 μl), which is heated to 70 °C for 10 min, and the product is purified by HPLC (adsorbent: Free Fatty Acid Analysis, 3.9 X 150 mm; eluent: water, 2ml/min). From 100 MBq of $^{11}CH_3I$, about 13 MBq of 2 is obtained in 40 min. The hydrolysis of ethyl α-acyl-propionates, to which 2 belongs, gives α-acyl-propionic acids, which are intermediates of the β-oxidation of α-methyl-fatty acids in vivo. We measured the tissue distribution of 2 and its free acid, α-aceto-β[^{11}C]-propionic acid in the mouse. As shown in Fig. 1, Compound 2 demonstrated a very high accumulation in the lung immediately after the intravenous injection; the free acid, on the other hand, behaved similarly with α-methyl[^{14}C]-butyric acid, as is expected.

Compound 3 was synthesized as follows: ethanolic solution of thiourea (0.5 mol/l, 0.5 ml) was added to the as-obtained solution of 2 (0.1 mol/l, 1 ml), and the mixture was heated to 120 °C for 10 min; and the solvent was removed by evaporation under N_2 gas bubbling, the residue dissolved in 7 % aqueous $NaHCO_3$ solution, and 3 thus formed was separated in pure state by HPLC (adsorbent: RP-5 LiChrosorb 4 X 250 mm; eluent, 1 % aqueous $NaHCO_3$ solution). About 8 MBq of 3 was thus obtained from 100 MBq of $^{11}CH_3I$ in 60 min. By similar condensation of ethyl methyl[$^{11}CH_3$]-alkyl-malonates with urea or thiourea, 5-methyl[$^{11}CH_3$]-alkyl-barbitrates or -thiobarbitrates were obtained. Similar synthesis is under way for more variety of compounds with potential radiopharmaceutical usefulness.

1) K. Ogawa, K. Niisawa, M. Sasaki, and T. Nozaki, J. Label. Compds. Radiopharm., XXX, 417 (1991).
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3) A. Gee, Acta Universitatis Upsaliensis (Fac. Sci.), No. 299, 1991, Upsala.

Table 1. Methylation of Ethyl Acetoacetate with $^{14}CH_3I$

Temperature	Solvent	Time	Yield
room temp.	Ethanol	5 min	11.0 %
		10	16.6
		20	23.4
		40	40.0
50℃	Ethanol	5	33.9
		10	68.1
		20	79.0
		60	100
70℃	Ethanol	5	80.2
		10	99.0
		20	100
50℃	Methanol	5	28.3
		10	41.6
		20	58.9
		40	77.1
50℃	THF	5	89.3
		10	100

The reactions were carried out in reaction mini vial containing 1 ml of 0.1 mol/l ethyl acetoacetate solution.

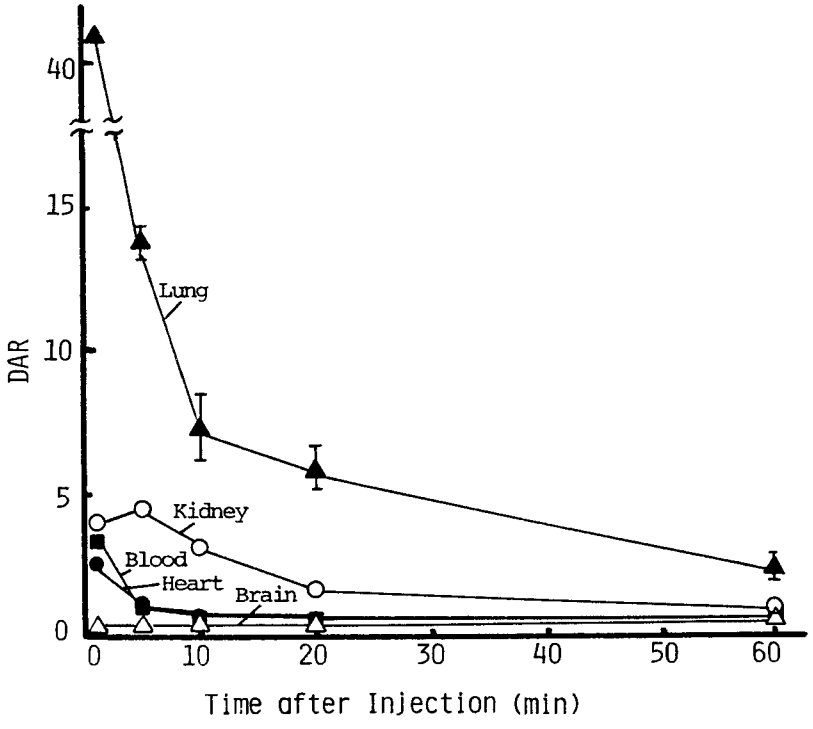

Figure 2. Distribution of Ethyl α-Aceto-β[^{14}C]-Propionate in Mice

Paper D22

[^{11}C]-N-METHYL AND ETHYL NOR-FENFLURAMINE AS SEROTONIN LIGANDS FOR PET.

D. R. ELMALEH*, X. MENG*, R. J. WURTMAN ** T. M. SHOUP*, A. J. JONES***, A. H. KIZUKA*, H. W. STRAUSS* AND A. J. FISCHMAN *.

*MASSACHUSSETTS GENERAL HOSPITAL, BOSTON MA 02114
**MASSACHUSSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE MA 02139
***HARVARD MEDICAL SCHOOL BOSTON MA

Fenfluramine (N-ethyl-β-methyl-3-trifluoromethyl-phenethylamine) is a substituted phenehtylamine which blocks serotonin. D-Fenfluramine is taken up into 5-HT (5-hydroxy-tryptamine) neurve terminals in order to produce these effects[1-4]; long-lasting decreases of 5-HT[1,2] and decreased trypyophan hydroxylase activity[4]. The labeling of its D active enantiomer with a positron radionuclide should provide a useful tracer for imaging serotonin binding sites with PET.[5]

In this work, we will report on the synthesis of nor-fenfluoramine (2-methyl-3-trifluoromethyl-phenethylamine) **1** and the labeling of its D,L- [^{11}C]-N-methyl and ethyl derivatives **2** **3**.

Nor-fenfluoramine **1** was prepared from 3-(trifluoromethyl)-phenylacetone via an amination-reduction [6]. High specific activity labeling of [^{11}C]-N-methyl-nor-fenfluoamine compound **2** was achieved by reacting nor-fenfluoramine (**1**) with $^{11}CH_3I$ [7] at 100^{0}C in acetonitrile for 5 minutes (scheme 1). In the same manner [^{11}C]-N-ethyl-nor-fenfluoramine was prepared by heating compound **1** and $CH_3{}^{11}CH_2I$. The C-11 labeled ethyl iodide was obtained by the automated reaction sequence outlined in scheme 2. The chemical purity of compound **1** and **2** was determined by TLC and identified by comparison with the respective cold standards. The preparation of the D and L specific precursors and their labeling are being devloped.

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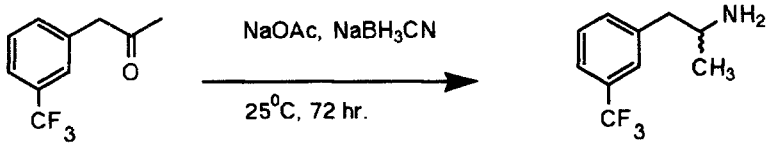
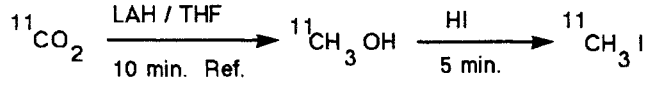
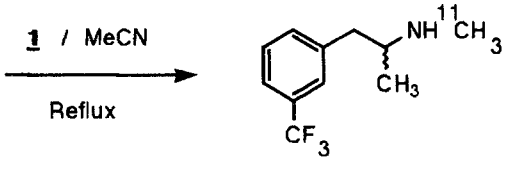
7. Kizuka, H. and Elmaleh, D. R., *Absctract of Sixth International Symposium Radiopharmaceutical Chemistry*, p.17 (1986).

Scheme 1



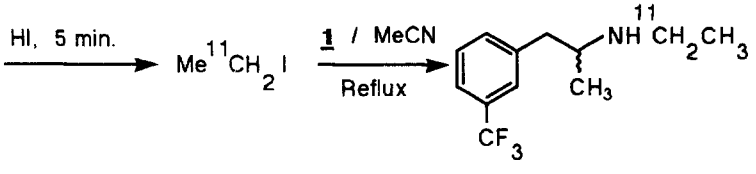
1





2

Scheme 2



3

Paper D23

[O-METHYL-^{11}C]NIMODIPINE: A HIGH YIELD, ONE POT SYNTHESIS

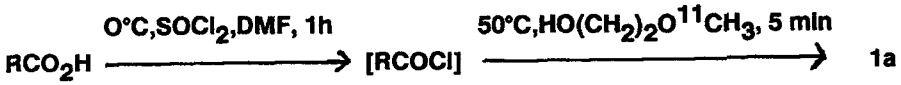
M. Holschbach, H.H. Coenen, M. Schüller, S. Goldmann*, G. Stöcklin
Institut für Nuklearchemie, Forschungszentrum Jülich, 5170 Jülich, Germany
***Bayer AG, Wuppertal, Germany**

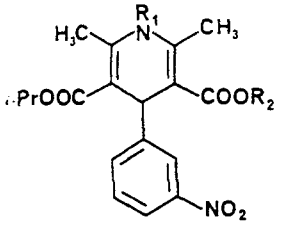
In view of the potential usefulness of carbon-11 labelled nimodipine 1a for the study of brain and/or heart functions and pharmacokinetics of this calcium channel blocker with PET, new approaches to its n.c.a. synthesis were undertaken. Considering its metabolism (1) labelling of the methoxy-function seems to be the method of choice. Nimodipine has already been labelled in the isopropylester moiety (2) and at the methoxy-function (3). In the latter case reaction and purification procedures were complicated and lengthy, thus resulting in low yields.

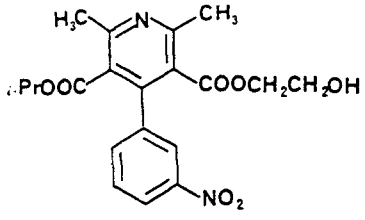
Because of the ready availability of the hydroxy derivative 2a and the monocarboxylic acid 11a (4), these compounds were chosen as starting materials for a new synthetic apporach to [O-methyl-^{11}C]nimodipine 1a. However, direct methylation of the hydroxy derivative 2a with [^{11}C]methyliodide in a Williamson-type ether synthesis resulted in the exclusive formation of the N-methylated compound 3a. Methylation of the N-protected compounds 4a-7a under the conditions described in Table 2, only gave rise to the aromatised compound 2b. Quaternization of the pyridine-nitrogen was in no case observed. Reference substance 2b was synthesized by ceric ammonium nitrate oxidation of the corresponding dihydro derivative 2a and its structure confirmed by ^{1}H-NMR, IR and MS.

In order to enhance the reactivity of the OH-functions of the N-protected derivatives, compound 4a was chosen as a model substance and submitted to various functional group manipulations. Thus, groups with good leaving abilities such as Tf, Ts and $PO(OC_6H_5)_2$ were introduced, giving the activated compounds 8a-10a. Subsequent methylation with [^{11}C]methanol resulted in N-deprotection and aromatisation to yield 2b.

A successful approach consisted in the reaction of the monocarboxylic acid 11a with $SOCl_2$ and subsequent alkylation of the in situ formed acid chloride with [^{11}C]methoxyethanol (3). Under carefully controlled reaction conditions (as shown below) this reaction sequence gave, after a quick HPLC purification, [O-methyl-^{11}C]nimodipine in a radiochemical yield of 40 ± 4 % and a purity > 99% in an overall synthesis time of 35 min (EOB). The specific activity of the product was 48 TBq/mmol.







2b

	R_1	R_2
1a	H	$(CH_2)_2O^{11}CH_3$
2a	H	$(CH_2)_2OH$
3a	CH_3	$(CH_2)_2OH$
4a	MOM	$(CH_2)_2OH$
5a	MEM	$(CH_2)_2OH$
6a	t-BOC	$(CH_2)_2OH$
7a	VOC	$(CH_2)_2OH$
8a	MOM	$(CH_2)_2OTf$
9a	MOM	$(CH_2)_2OTs$
10a	MOM	$(CH_2)_2OP(OC_6H_5)_2$
11a	H	H

Abbreviations: Tf: triflate, Ts: tosylate, MOM: methoxymethyl, MEM: methoxyethoxymethyl, t-BOC: tert.butyloxycarbonyl, VOC: vinyloxycarbonyl

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Paper D24

The Routine Synthesis of Carbon-11 Phosgene, an Intermediate to Carbon-11 2-Thymine.

F.R. Dobbs, M. Franceschini, and T.J. Tewson. University of Texas Health Science Center at Houston, Cyclotron Facility, 6431 Fannin, Houston, Texas 77030, USA.

We have designed and constructed an apparatus for the routine synthesis of carbon-11 phosgene, an intermediate to carbon-11 2-thymine (Scheme 1). Based on the chemistry of Crouzel (1), the routine synthesis of carbon-11 phosgene involved the reduction of carbon-11 CO_2 to carbon-11 methane over a nickle catalyst at 430°C followed by the chlorination of carbon-11 methane over a copper chloride catalyst at 400°C. Chlorine gas was mixed with the methane by loading both of them into a syringe. Emptying the syringe then drove the gas mixture over the chlorination catalyst. After the removal of excess chlorine over antimony, the oxidation of the intermediary carbon tetrachloride to carbon-11 phosgene was accomplished over an iron metal filings catalyst at 300°C using 1% O_2 balance nitrogen.

Using a 5 uamp bombardment for 5 minutes (Table 1), carbon-11 carbon tetrachloride was the major product detected in the chlorination step using 20 wt.% $CuCl_2$ on pumice catalyst at 400°C. utilizing GC analysis on Porapak Q column with both a radiation detector and TCD. Decomposition to CO_2 was about 15% yield loss at essentially 100% conversion of methane. A lower catalyst loading of 10wt.% $CuCl_2$ gave dichloromethane and chloroform in addition to carbon tetrachloride without any methyl chloride detected.

The overall yield of carbon-11 phosgene from carbon-11 CO_2 was about 20% with the major yield loss to carbon dioxide and carbon monoxide due to thermal decomposition of phosgene. Phosgene was analyzed in the routine synthesis as the diphenylurea derivative at 244nm using HPLC analysis on a Whatman Partisil 10 ODS-2 column with radiation and UV detectors. The specific activity of carbon-11 phosgene appears high based on these scouting studies. An optimization of the routine synthesis is underway to minimize thermal decomposition by reduction of contact time.

Work is in progress to isolate the thymine precursor 2-methyl-2-hydroxy-3-aminopropionamide, so that the reaction with carbon-11 phosgene can be studied. Carbon-11 2-thymine could elucidate the DNA synthetic pathway better in cells since degradative products would be eliminated as labelled carbon dioxide (2,3). The epoxidation of methacrylic acid with alkaline hydrogen peroxide in the presence of a sodium tungstate catalyst followed by amination of the acid chloride with concentrated ammonium hydroxide is being evaluated for synthesis of the thymine precursor.

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Scheme 1. The Synthesis of Carbon-11 2-Thymine from Carbon-11 Phosgene.

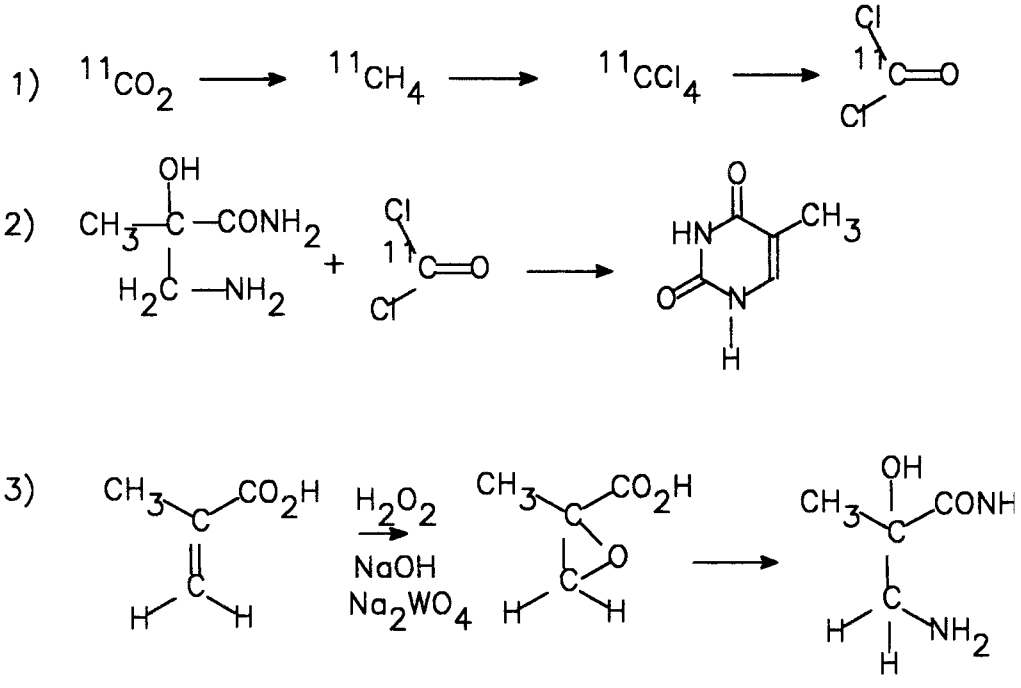


Table 1. Routine Synthesis of Carbon-11 Phosgene.

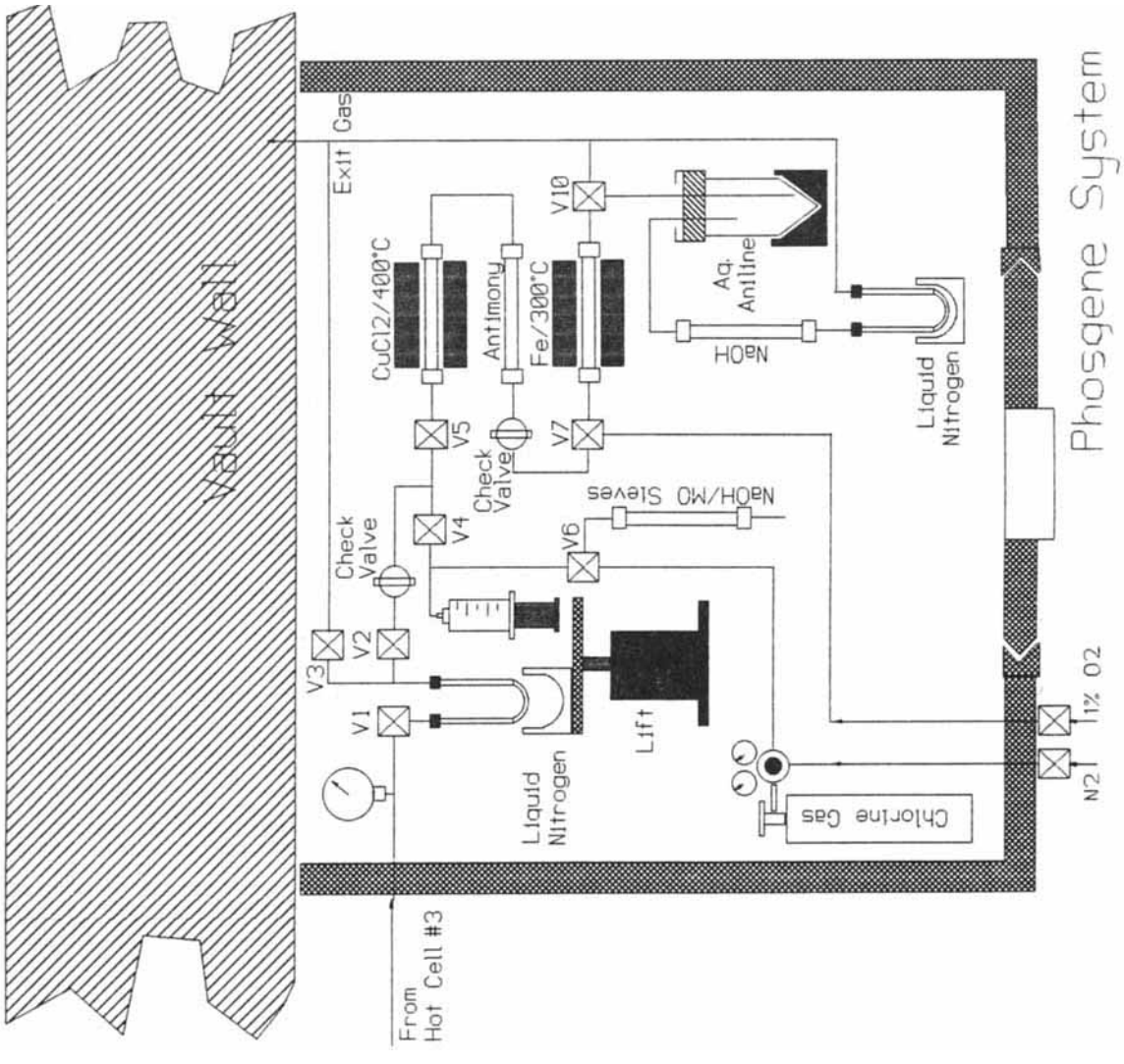
A. Evaluation of the Chlorination Step.

Catalyst Loading (wt.%)	Oven Temp (°C)	Flow Rate (cc/min)	Product Composition (%) CCl4	CO2	CH4
20	400	5	64.2	35.2	0.1
20	400	5	78.8a	15.3	5.9
20	400	5	90.1a	8.5	0.4
10	400	25	80.9b	17.9	1.0

B. Evaluation of Both Chlorination and Oxidation Steps.

Oven Temp (°C)	Flow Rate (cc/min)	Product Composition (%) Phosgene	CO2	CO	CCl4
300	25	20.7	61.1	14.5	3.5
200	25	5.1	24.2	8.2	62.5
250	25	26.3	32.2	9.2	32.2
300	25	11.5	69.6	17.3	1.5
300	5	17.9	67.9	14.1	0.0
275	5	0.1	48.4	50.8	0.0
300	5	0.1	95.3c	3.0	0.1
300	5	0.1	88.2	10.4	0.1
250	5	1.7	69.4	28.8	0.0

a. GC analysis shows 100% CCl4.
b. GC analysis shows 48% CCL4,31% CHCl3, and 20% CH2Cl2.
c. 1% Oxygen was not used in this run.


Vault Wall
Exit Gas
CuCl2/400°C
Antimony
Fe/300°C
V10
Aq. Aniline
NaOH
Liquid Nitrogen
V5
Check Valve
V7
NaOH/MO Sieves
Check Valve
V4
V6
V2
V3
V1
Lift
Liquid Nitrogen
Chlorine Gas
N2
1% O2
From Hot Cell #3
Phosgene System