Synthesis of [^{11}C-*methyl*]Methylisocyanate and Application with Microwave Heating to Labelling the Novel Anticancer Agent Temozolomide.

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Temozolomide (Figure 1), a novel imidazotetrazinone[1], shows promising activity against gliomas and malignant melanoma[2]. It is believed that temozolomide is a pro-drug which accesses DNA and undergoes nucleophilic induced activation within the micro-environment of the major grove of DNA[1,3]. This process is believed to consist of base promoted conversion to a methyltriazene with loss of the carbonyl group at position 4. The triazene then alkylates the nitrogen or oxygen of the central guanine of a three guanine sequence in the major grove of the DNA[3,4].

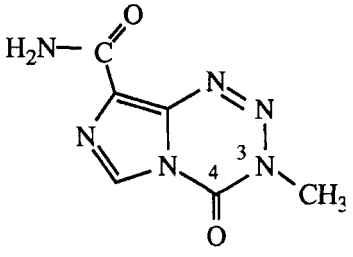

Figure 1. Temozolomide

Radiochemistry has been devised for labelling temozolomide with carbon-11 in either the 3-*N*-methyl group or the 4-carbonyl position. These labelled compounds will be used to determine the pharmacokinetics of temozolomide in man using PET in parallel with Phase II studies being carried out by the Cancer Research Campaign (CRC), U.K.. Separate PET studies with these labelled compounds may help demonstrate, *in vivo*, the postulated mechanism of action of this drug. Labelling with carbon-11 at the 3-*N*-methyl position, would result in incorporation of the radiolabel into DNA, whereas labelling in the 4-carbonyl position, would result in the loss of the label as [^{11}C]CO_2 before incorporation into DNA.

(i) [^{11}C-*methyl*]Methylisocyanate (III)

[^{11}C-*methyl*]Methylisocyanate (III) was synthesised by passing [^{11}C]iodomethane (I) in nitrogen over silver cyanate (II) (0.3 g) at 180 ^{0}C.

$$\underset{\textbf{(I)}}{^{11}CH_3I} + \underset{\textbf{(II)}}{AgO=C=N} \xrightarrow{\Delta} \underset{\textbf{(III)}}{^{11}CH_3N=C=O} + AgI$$

The conversion of iodomethane to methylisocyanate (III) has been optimised and is essentially quantitative (Figure 2).

(ii) [3-*N*-^{11}C-*methyl*]Temozolomide (Va)

Reaction of [^{11}C-*methyl*]methylisocyanate (III) with the diazo precursor (IV) gave [3-*N*-^{11}C-*methyl*]temozolomide (Va) (Figure 3). [^{11}C-*methyl*]Methylisocyanate (III) in a stream of nitrogen was bubbled into a vial containing the diazo precursor (IV) (1mg) in DMSO (0.4 mL). Cyclisation was achieved by heating the mixture for 10 min at 80 ^{0}C. The reaction mixture was purified by HPLC ("μ"-Bondapak C_{18}, 25 x 1 cm i.d., eluted with a mixture of 0.5% acetic acid : methanol [99:1] at a flowrate of 3 mL min^{-1}). The precursor eluted at 7.0

min and [3-*N*-^{11}C-*methyl*]temozolomide (Va) at 14 min. The radiochemical yield of [3-*N*-^{11}C-*methyl*]temozolomide was 5-10% from [^{11}C]iodomethane.

(iii) [4-^{11}C-*carbonyl*]Temozolomide (Vb).
Cyclisation of [*N*-^{11}C-*carbonyl*]methylisocyanate[5] with the diazo precursor (IV) gave [4-^{11}C-*carbonyl*]temozolomide (Vb) using the same reaction and purification conditions as above.

In order to improve the yield from the cyclisation of the diazo precursor with methylisocyanate, we are investigating the use of microwave heating. Reactions in DMSO were carried out in a CEM MDS-2000 microwave system using a fibreoptic temperature probe, allowing the temperature inside the reaction vessels to be monitored. Preliminary results with microwave heating show considerable enhancement of temozolomide yields in short reaction times compared to conventional heating (Figure 4). Radiochemical yields are being optimised using this approach.
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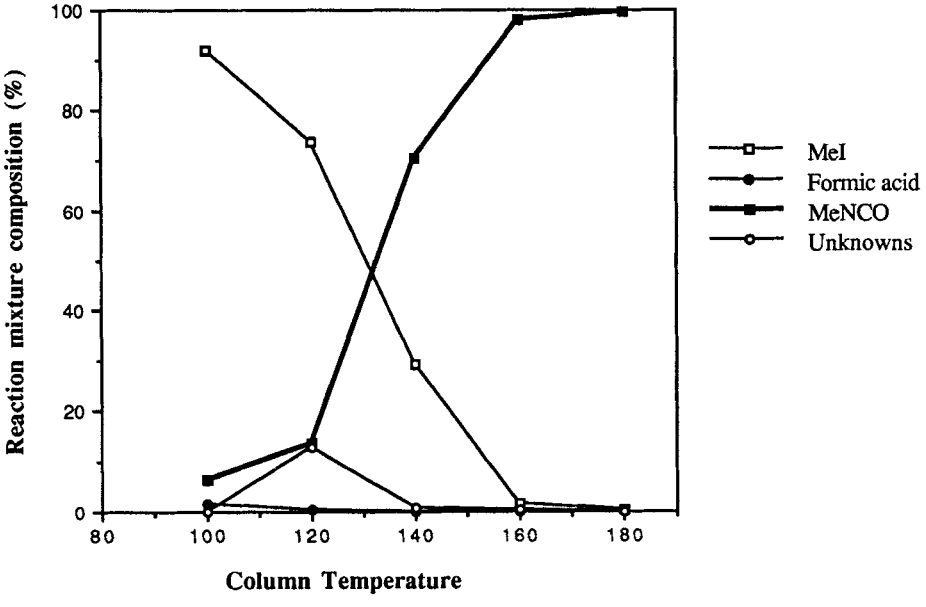

Figure 2. Effect of temperature on conversion of iodomethane to methylisocyanate over silver cyanate.

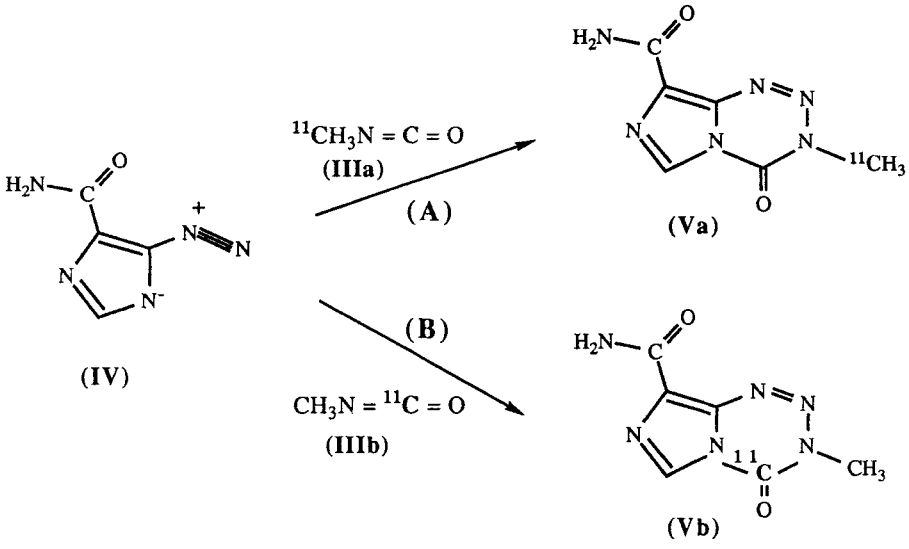

Figure 3. Synthesis of (A) [3-*N*-^{11}C-*methyl*]temozolomide (Va) and (B) [4-^{11}C-*carbonyl*]temozolomide (Vb).

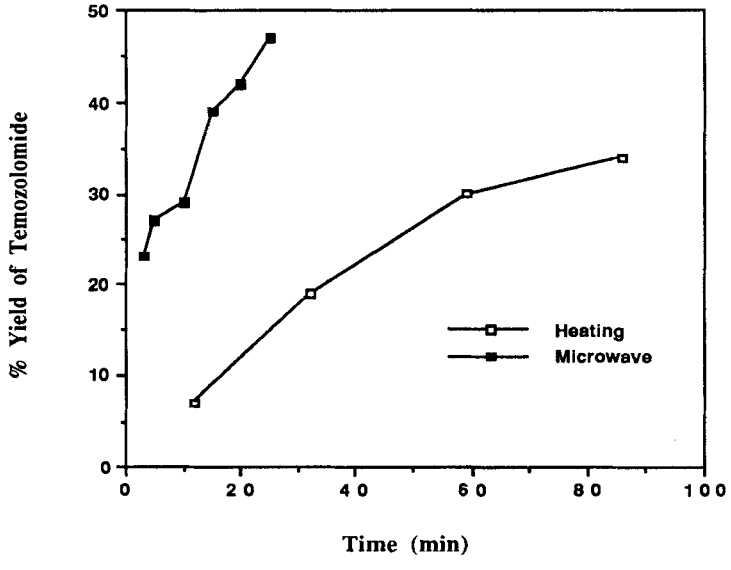

Figure 4. Comparison of conventional and microwave heating on the synthesis of temozolomide.

[^{11}C]Methylisocyanate: a new synthesis process.

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[^{11}C]methylisocyanate is a ^{11}C-precursor providing fast access to carbamates. In a paper presented at the 9th International Symposium on Radiopharmaceutical Chemistry, we described a method of synthesis by Curtius rearrangement based on acid chloride (1):

$$CH_3{}^{11}COCl \xrightarrow{NaN_3} CH_3{}^{11}CON_3 \xrightarrow{\Delta} CH_3N = {}^{11}C = O$$

Although with this synthesis (2), between 80 and 120 mCi (2,96-4,44 GBq) of $CH_3N^{11}CO$ can be obtained in 40 minutes, the process is long and leaves little time to use the ^{11}C-methylisocyanate, in view of the Carbon-11 half-life. It was thus decided to seek a simpler and above all faster method.

As we had [^{11}C]phosgene prepared, we attempted phosgenation of the methylamine, which is a conventional method of obtaining methylisocyanate:

$$CH_3NH_2 + {}^{11}COCl_2 \longrightarrow CH_3N^{11}CO + 2HCl$$

However, with this method we obtained only very low isocyanate yields, mainly because we had no accurate data as to the exact quantity of methylamine (gas) involved in the reaction, the excess methylamine forming [^{11}C]dimethylurea.

On the other hand, using di-N-trimethyl-silyl-methylamine gave satisfactory results (3).

$$CH_3\text{-}N(SiMe_3)_2 + {}^{11}COCl_2 \xrightarrow{\leq\ ^\circ OC} [CH_3\text{-}N(SiMe_3)\text{-}C(=O)\text{-}Cl] \xrightarrow{-Me_3SiCl} CH_3\text{-}N = {}^{11}C = O$$

This silylated derivative can easily be produced from methylamine and trimethylsilyl chloride. The product is in liquid form, obtained after vacuum distillation at 80° C. It is a stable product.

$$3CH_3NH_2 + 2Me_3SiCl \xrightarrow{\text{ether}} (Me_3\text{-}Si)_2N\text{-}CH_3 + 2Me\,NH_3{}^+Cl^-$$

The methylisocyanate synthesis is performed using a dilute solution in anhydrous ether of the silylated derivative at -30° C (0.6 μmole in 250 μl). The [^{11}C]Phosgene is produced using the method previously described (4):

$$^{11}CH_4 \xrightarrow[380\ °C]{\text{pumice stone } CuCl_2} {}^{11}CCl_4 \xrightarrow[310\ °C]{\text{Iron-}O_2} {}^{11}COCl_2$$

[^{11}C] CH_3 NCO is characterized by its reaction on aniline. [^{11}C]Methyl Phenylurea is formed and analyzed by HPLC: C18 column, L = 30 cm, ID = 0.9 cm, eluent = water, 20 % ETOH, pH = 2.3, flowrate = 4 ml/m, retention time = 7 min.

With this method, 150 to 200 mCi (5,55-7,4 GBq) of [^{11}C] CH_3 NCO are obtained 15 minutes after EOB, with a specific activity of 1.5 Ci/μmole (55,5 GBq/μmole).

We also attempted to use another synthesis process (5), involving the action of $^{11}CO_2$ on the di-N-trimethyl silyl methylamine:

$$CH_3\text{-}N(SiMe_3)_2 + {}^{11}CO_2 \longrightarrow CH_3\text{-}N(SiMe_3)\text{-}{}^{11}C(=O)\text{-}O\text{-}SiMe_3 \xrightarrow{100\text{-}105°} CH_3\text{-}N = {}^{11}C = O$$

This method, the advantage of which was that the $^{11}CO_2$ could be used directly, gave no result.

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Carbon-11 Labelling of 6,7-Dichloro-2,3-dihydroxyquinoxaline

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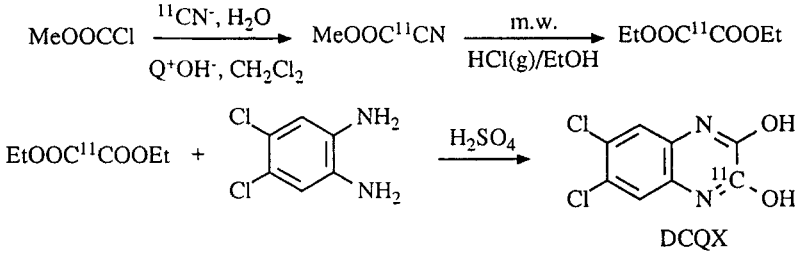
2,3-Dihydroxyquinoxaline is a basic structural unit in a series of molecules that are antagonists for the excitatory amino acid system (1). We have recently developed a general method for labelling this heterocyclic compound in a 3- or 4-step procedure starting with [^{11}C]cyanide (2), via diethyl [^{11}C]oxalate or [^{11}C]oxalic acid followed by cyclization (3) to the quinoxaline. Total synthesis times for these precursors were on the order of 6 min, using combined microwave and thermal techniques, or 15 and 20 min, respectively, by the exclusively thermal method. The subsequent cyclization was performed under thermal conditions due to problems with containing the reaction mixture in the microwave vessels used. Times required for ≥90% conversions in the cyclization using hydrochloric acid at 150 °C were 5 and 10 min using diethyl [^{11}C]oxalate and [^{11}C]oxalic acid, respectively. This study indicated that this procedure was possible to perform with good conversions in the time limits normally accepted in carbon-11 chemistry in spite of the total number of steps required for completion. Here we report the extension of this synthesis to the carbon-11 labelling of 6,7-dichloro-2,3-dihydroxyquinoxaline (6,7-dichloroquinoxaline-2,3-dione, DCQX) as shown below.



Diethyl [^{11}C]oxalate was prepared as described in (2) by the reaction of [^{11}C]cyanide with methyl chloroformate with a phase transfer catalyst at room temperature followed by microwave-assisted alcoholysis. After evaporation, the diester was cyclized with 4,5-dichloro-1,2-phenylenediamine. Different conditions were required for acceptable conversions in the cyclization reaction than had been used with the model compound 1,2-phenylenediamine: 9 M H_2SO_4 instead of 2 M HCl and 10 min instead of 5 min at 150°C.

[^{11}C]DCQX was produced in ~75% conversions from diethyl [^{11}C]oxalate according to analytical HPLC and was isolated by semi-preparative HPLC on a μ-Bondapak C-18 column (mobile phase = CH_3CN:H_3PO_4). Total isolated radiochemical yields were on the order of ~15%, decay-corrected and based on [^{11}C]cyanide, after the HPLC purification, with a total synthesis time of 35-40 min. The specific activity was >500 Ci/mmol and radiochemical purity >99%. Although this extension indicates that this method is a viable approach for labelling this type of compounds, the more stringent conditions required for the cyclization reaction indicate that even less reactive diamines may require reinvestigation of a controllable means of performing the microwave treatment in this step.

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Application of Methyl Hypofluorite Chemistry: Synthesis of Novel Steroidal Substrates and Labeling with C-11.

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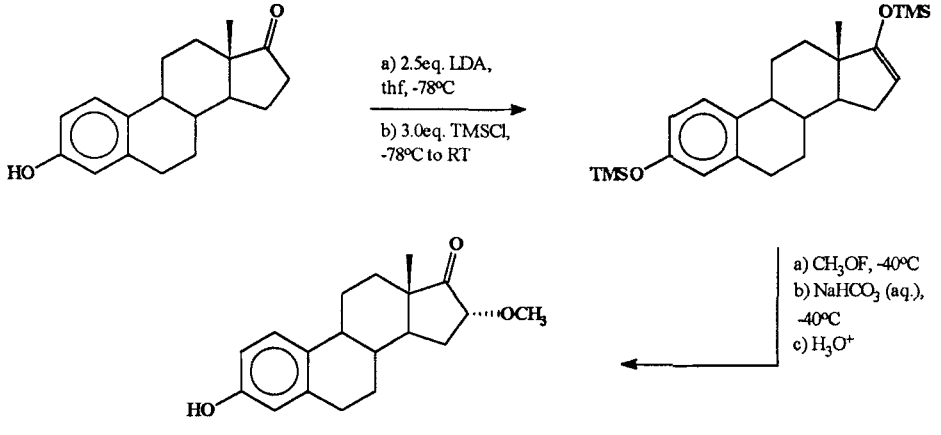
The development of novel imaging agents for tumor targeting and subsequent application to PET is always of great interest in nuclear medicine. Recent research by our group has led to the production of F-18 labeled estrogens[1] suitable for the detection of receptor positive breast tumors[2]. In addition to this a number of fluorinated progestins[3] and androgens[4] have also been described. The development of C-11 labeled steroids has been less successful, however the radiosynthesis of certain progestins[5] and estrogens[6] have been reported.

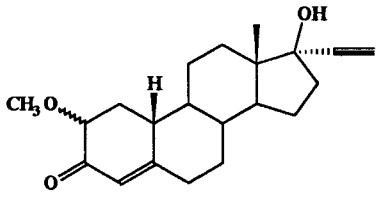
The discovery of the novel electrophilic methoxylating species, methyl hypofluorite[7] (MeOF) has accessed a new area of research for synthetic organic chemists. The reaction of enol ethers with this species will furnish, on work-up, α-methoxy ketones[8] compounds which have previously been available only through multi-step procedures. We recently reported the preparation of $^{11}CH_3OF$ and its reaction with an enol ethers to yield the corresponding [C-11]-labeled α-methoxy ketone[9] and therefore prove the feasibility of this reagent as a new method for the introduction of C-11 to organic molecules. As a result of this research we have been successful in the synthesis of both non-radioactive and [C-11] labeled 16α-methoxyestrone (Scheme) from the corresponding bistrimethylsilyl enol ether.



Scheme.

The results outlined above have given us ready access to a wide number of novel steroidal substrates which we are currently evaluating as potential ligands for tumor targeting. Of particular interest to us is the development of C-2α- and β-methoxylated androgens or progestins

(1) as these would seem favorable candidates for reduced lipophilicity and another application for radiolabeling using [C-11]methyl hypofluorite.



(1)

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Enantioselective Syntheses of n.c.a. L-(α-Methyl)-[β-^{11}C]-4-Chlorophenylalanine and L-(α-[^{11}C]Methyl)-Tryptophan

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The ideal tracer to study a metabolic process such as enzyme activity *in vivo* using external monitoring like PET should present e.g. a metabolic pathway as simple as possible in order to simplify the interpretation of the data.
The radiosynthesis of the α-methylated derivative of L-4-chlorophenylalanine (α-MPcpa) was considered for several reasons. L-4-chlorophenylalanine (L-Pcpa) is an irreversible inhibitor of tryptophan hydroxylase, the rate limiting enzyme in the biosynthesis of serotonin (1). The incorporation of halo substituted amino acids, including L-Pcpa, into protein via existing pathways of protein synthesis is well established (2). The result of this is that the incorporation of L-Pcpa is a biochemical pathway which must be taken into account. On the other hand, α-methylated substituted amino acids are known to be inhibitors of decarboxylase and hydroxylase enzymes (3 and 4).
The use of labeled L-tryptophan has not been very successful in the study of the presynaptic serotoninergic neurotransmission process due to the important incorporation into proteins and to the very rapid loss of metabolites from the brain (5). The α-methylated analog of L-tryptophan (α-MTryp) is converted *in vivo* into α-methylserotonin, a false neurotransmitter which is not substrate for MAO and thus accumulates in the brain tissue (6). Furthermore, less than 3% of α-MTryp is incorporated into brain proteins (6).

For the radiolabeling of α-MPcpa, the alkylation of imidazolidinone derivative (7), previously reported for the asymmetric synthesis of amino acids labeled with fluorine-18 (8) and carbon-11 (9, 10), was used. The radiochemical pathway is presented in Scheme 1. The highly stereoselective alkylation of the lithium enolate of (2S,5S)-*tert*-butyloxycarbonyl-2-(*tert*-butyl)-3,5-dimethyl-1,3-imidazolidine-4-one (1) with 4-chloro[α-^{11}C]benzyl bromide (2) led, after hydrolysis and HPLC purification, to L-(α-methyl)-[β-^{11}C]-4-chlorophenylalanine (4) with a radiochemical yield of 19% corrected for decay (from [^{11}C]CO_2) after a total preparation time of 45 min (Table 1). In a typical run starting from 1Ci of [^{11}C]CO_2, about 35 mCi of L-(α-methyl)-[β-^{11}C]-4-chlorophenylalanine were obtained ready for use. The enantiomeric purity was measured on the final injectable solution using two different techniques on each runs: HPLC and TLC analyses by ligand exchange chromatography. Both technique gave the same results and showed an enantiomeric excess ≥ 97% (average on twenty runs).

A different approach from the one reported by Chaly and Diksic (11) and by Suehiro (12) is proposed for the synthesis of L-(α-[^{11}C]methyl)-tryptophan. $N_α$-Methoxycarbonyl-(S)-tryptophan methyl ester cyclized with 85% phosphoric acid gives (2S,3aR,8aS)-1,2-bis(methoxycarbonyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole which on reaction with benzenesulfonyl chloride leads to (2S,3aR,8aS)-1,2-bis(methoxycarbonyl)-8-(benzenesulfonyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]-indole (13). Treatment of this protected L-(S)-tryptophan derivative (5) with lithium diisopropylamide at low temperature followed by reaction with methyl iodide results in alkylation, with retention of configuration, at C-2 of the pyrroloindole system (13). This procedure was used for the radiosynthesis of L-(α-[^{11}C]methyl)-tryptophan (7) as illustrated in Scheme 2. [^{11}C]CH_3I was trapped directly in a solution of enolate generated *in situ* by treatment of 5 with LDA. The alkylation reaction was allowed to proceed for about 5 min at -78°C. The reaction mixture was then hydrolyzed with HI followed by sodium hydroxide. After HPLC purification, L-(α-[^{11}C]methyl)-tryptophan was obtained with an overall radiochemical yield of 36% (corrected for decay) in a preparation time of 22 min from [^{11}C]CH_3I (Table

1). The HPLC analysis on the final injectable solution showed an enantiomeric excess ≥ 97% (average on ten runs).

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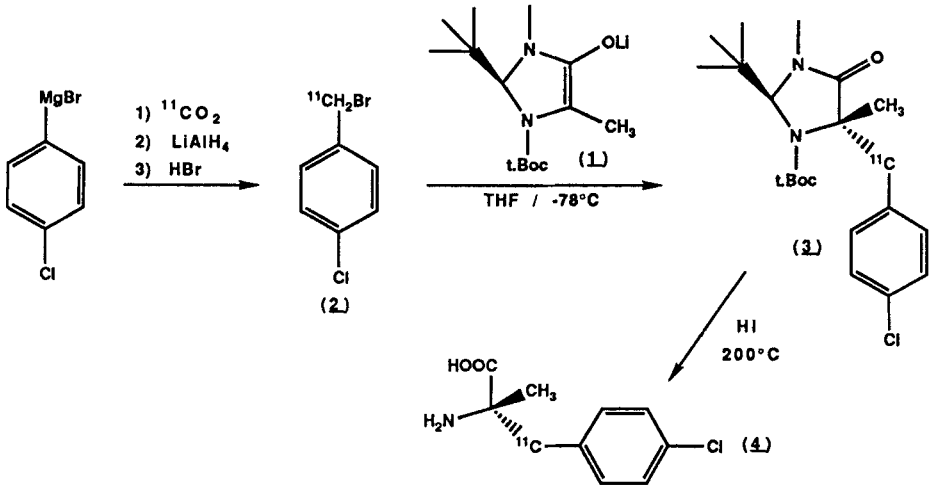
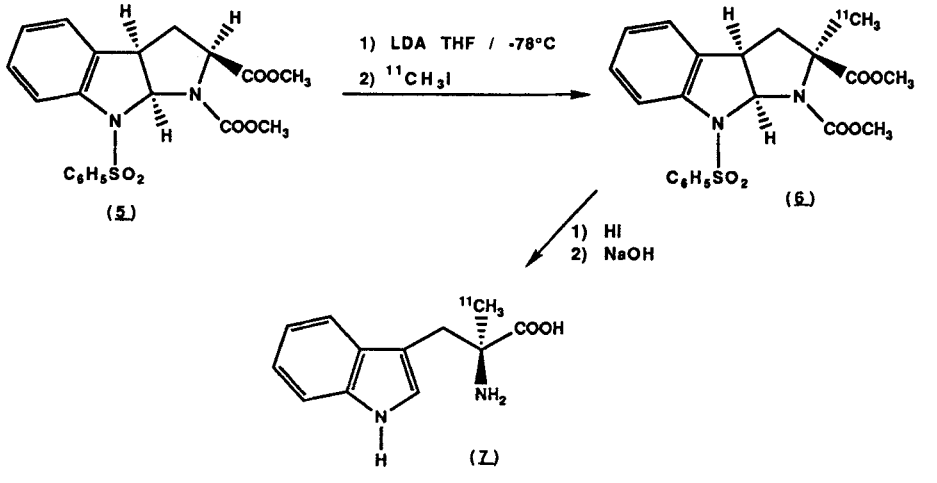
Table 1

	Yield for each step (%) EOB	Cumulative yield (%) EOB	Time for each step (min)	Cumulative synthesis time (min)
$[^{11}C]CO_2$		100		0
↓ 2	64	64	20	20
↓ 1 3	50	32	10	30
↓ HI HPLC 4	60	19	15	45
$[^{11}C]CH_3I$		100		0
↓ 5 + LDA 6	60	60	5	5
↓ HI/NaOH HPLC 7	60	36	17	22

Scheme 1: Synthesis of L-(α-methyl)-[β-^{11}C]-4-chlorophenylalanine



Scheme 2: Synthesis of L-(α-[^{11}C]methyl)-tryptophan



On-line System for Synthesis of Compounds Labelled with β^+-emitters in Supercritical Ammonia

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When designing synthetic procedures for compounds labelled with short-lived β^+-emitting radio nuclides (e.g. ^{15}O, ^{13}N, ^{11}C, and ^{18}F), special considerations must be taken compared to conventional synthetic reactions. The time factor is most important because of the short half-lives in combination with chemical yield and purity, and decides the optimum reaction times (1). In order to achieve high specific radioactivity, it is also desirable to scale down on the amounts of reactants and solvent used to avoid isotope dilution.

Supercritical fluid chromatography (SFC) (2) and supercritical fluid extraction (SFE) (3) are today well-known methods and useful because of the possibility of controlling the physical properties such as density, viscosity, dielectric constant, solubility parameter and phase behavior of the supercritical fluid by changing the temperature and pressure (4). The possibility to manipulate the supercritical fluid solvent properties can also be used to effect the selectivity and rate of chemical reactions (5-9). In the near-critical region the supercritical fluid is highly compressible and solvent-solute and solute-solute clustering give local density enhancements (10-11). This gives increased polarity around the solute and great negative activation volumes which effects the rate constant of the system (12-13). Solvent separation steps can also be avoided since selected supercritical fluids vaporizes upon depressurization to atmospheric pressure.

An objective of this study has been to investigate if supercritical fluids could be applied in synthesis of PET-tracers. A system has therefore been developed for on-line synthesis of ^{11}C-labelled molecules in supercritical fluids. Ammonia was chosen because it has high polarity, a critical pressure (P_C=112.5 atm) and temperature (T_C=132.5 °C) in a compatible range (14) and hydrogen bonding properties.

The ammonia gas is lead through a potassium hydroxide drying tower and two filters to ensure a particle free ammonia (Fig 1). A pump (308 Gilson) and pulse dampener (821 Gilson) delivers the pulse free supercritical ammonia through the system. The substrate and ^{11}C-labelled precursor are trapped in the two injection loops and as valve V_1 and V_2 are switched they are injected into the reaction cell. V_4 and V_3 are 3-port valves that can be closed during the reaction. After the set reaction time (1-5 minutes) the supercritical ammonia is depressurized through the restrictor and the products are trapped in a liquid solvent. Between each reaction the system is flushed with cleaning solvent through V_5 and then conditioned with ammonia for a few minutes.

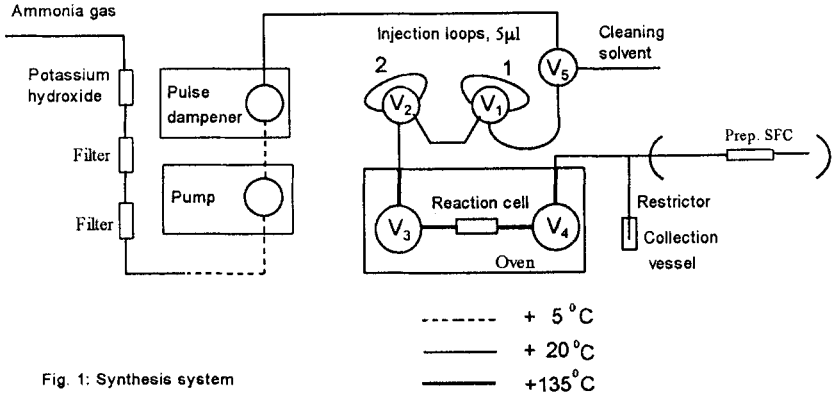

Fig. 1: Synthesis system

^{11}C-methylation of phenol to yield [methyl-^{11}C]anisole was used as a model reaction for evaluation of the system with regard to accurate control and reproducibility. [^{11}C]CH_3I was produced from $^{11}CO_2$ by adding LAH/THF and HI, and loaded into injection loop nr.1. The phenolate solution was prepared by dissolving phenol in acetonitrile and 0.8 e.q. 5 M NaOH and loaded into injection loop nr. 2. Four reaction cells, No. 1,2,3 and 4, all differing in size were compared to see the effect on reproducibility and product yield (Table 1). Five repetitive five minute reactions were performed at 145°C and 140 bar with each cell .

Table 1: Product Yield as a Function of Cell Dimension

Cell No.	Volume (µl)	cell dimension (mm) length i.d.	% [methyl-^{11}C] anisol	RSD
1	318	20 x 4.50	42 - 46	3
2	221	500 x 0.75	52 - 57	5
3	110	250 x 0.75	40 - 53	12
4	30	610 x 0.25	57 - 60	2

Several randomized 3 minute reactions were performed in cell No. 1 (V=318 µl) att different temperatures and pressures to see the effect on the product yield. The highest yields were obtained near pure ammonias critical point (Table 2).

Table 2.[a] Product Yield as a Function of Temperature and Pressure

P(bar)→ T (°C)↓	50	100	115	130	160	200
50	38	39	38	37	38	40
100	43	42	42	46	46	47
115	51	52	54	54	54	54
130	49	55[c]	55[c]	55[b]	54[b]	55
135	51	52[b]	60[c]	55[b]	55[b]	56
147	51	51	53[b]	56[b]	53[b]	54

a)Unless otherwise notified, two experiments in randomized order were performed for each set of values. b) Five and c) six experiments were performed.

The effect of substrate concentration, base used, reactant order and different trapping procedures were also tested. The following reactions: ^{11}C-methylation of aniline to [methyl-^{11}C]methylaniline (2), diethyl malonate to [methyl-^{11}C]diethyl methylmalonate (3), and homocysteine thiolactone to [methyl-^{11}C]methionine (4) were carried out to compare the reactivity of O, N, S, and C as nucleophiles in supercritical ammonia (Fig 2.). The reaction were performed at 135 °C and 115 bar for 1-3 minutes.

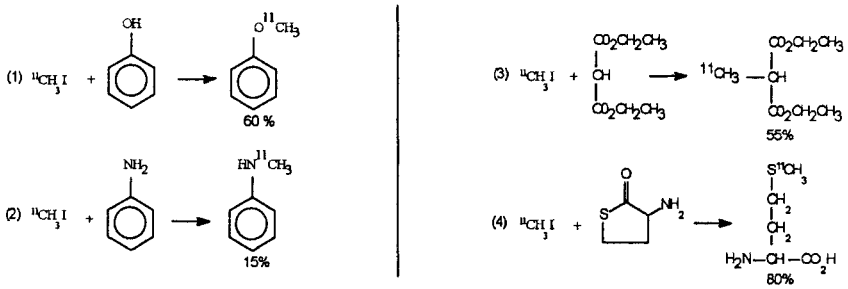

Fig 2: Reactions performed in supercritical ammonia.

The supercritical fluid synthesis system can be controlled accurately to perform on-line synthesis with high reproducability. The addition of reactants effects the supercritical point of the mixture and is therefore slightly different from pure ammonia. By performing the synthesis in the near-critical region the system can easily be optimized to produce the highest yields by small changes in pressure and temperature. Further studies are in progress and the system will be combined with preparative SFC.

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Fatty Acids and Acetate Labelled in Different Positions with ^{11}C in Combination with Deuterium, using Solid Phase Synthesis.

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A significant feature of fatty acids and acetate are their fast metabolism by the β-oxidation and the citric acid cycle path ways. Due to this fact, comparative studies with PET, using these substances labelled with ^{11}C in different positions, can yield important information. Since both the β-oxidation and the citric acid cycle involves hydrogen abstraction, the combined labelling with ^{11}C and deuterium might give additional data due to an observable kinetic isotope effect. In preliminary PET experiments it has been shown that the relative metabolic rates of labelled acetate and octanoate are as follows; ($CH_3{}^{11}CO_2^-$> $CD_3{}^{11}CO_2^-$> $^{11}CH_3CO_2^-$> $^{11}CD_3CO_2^-$), ($CH_3(CH_2)_6{}^{11}CO_2^-$> $^{11}CH_3(CH_2)_6CO_2^-$> $^{11}CH_3(CD_2)_6CO_2^-$)[1]. This exemplifies the possibilities of tuning the PET-tracer.
In the case of arachidonic acid, access to the tracer, labelled in different positions, should be valuable since it could help to discriminate between metabolic breakdown and the pathways of the arachidonic acid cascade.

In all syntheses presented in this paper, except in the case of the [2-^{11}C] labelled acetates, immobilization of the Grignard reagents on a solid phase matrix has been utilized. This technique, which previously has been used in a synthesis of [1-^{11}C] palmitate[2], has a number of advantages. Since the matrix is used both for synthesis and extraction, time is saved and yields are increased due to the elimination of a transfer moment. The solid phase extraction allows one to choose the solvent to be used in the next step of purification or chemical transformation. Further on, in the case of the [1-^{11}C] acetates, the immobilization of CH_3MgBr decreases the amount of labelled acetone and t-butanol in the product. The method, which is exemplified in scheme 3. and 4., is technical convenient and offers possibilities for automatization.

Syntheses of per deuterated α,ω di bromo alkanes (scheme 1.) as well as a 1,17 di chloro tetraene (scheme 2.) has been developed. By the use of these di halides, deuterated [(ω-n)-^{11}C] labelled fatty acids (n = 0, 1, 2, 3, 8) and [19-^{11}C]arachidonic acid can be synthesized, by the methods[4] shown in scheme 4.

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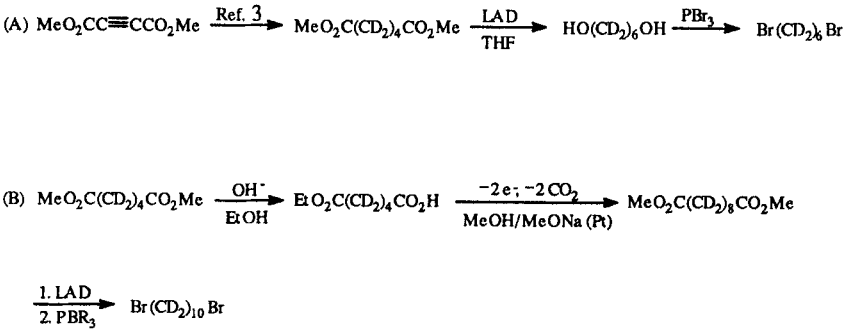
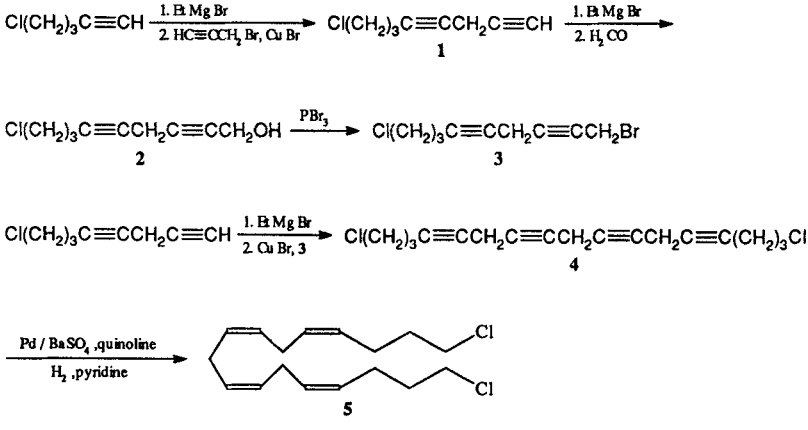
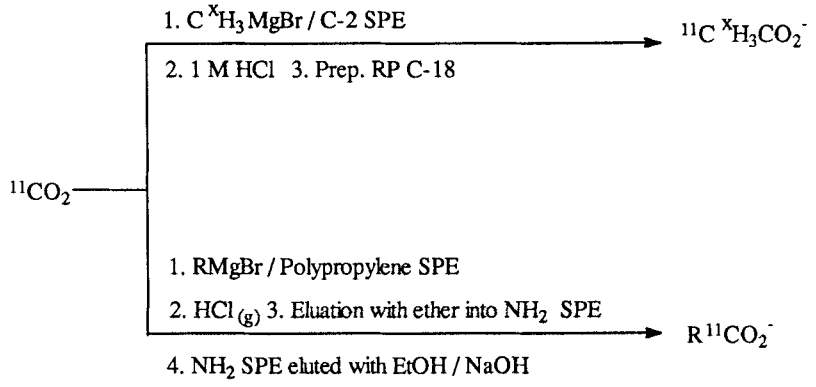


Scheme 1.

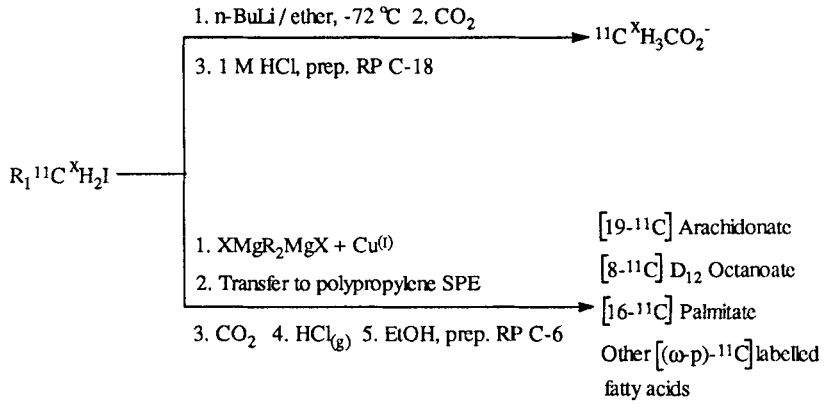


Scheme 2.



x = 1, 2 R = $CH_3(CH_2)_n$ (n = 6, 14), $CH_3(CH_2)_3(CH_2CH{=}CH)_4(CH_2)_3$

Scheme 3.



R_1 = ^{X}H, $CH_3(CH_2)_m$ m = 0, 1, 2, 3, 8 $R_2 = (C^XH_2)_n$ n = 6, 10, 14 ,

x = 1, 2 p = 0, 1, 2, 3, 8 X = Br, Cl

Scheme 4.

Biogenic Benzyl-[^{18}F]Fluorides: (1s,2s)-1-Desoxy-1-[^{18}F]Fluoro-*Meta*-Hydroxyephedrine For Mapping Cardiac Sympathetic Neurons

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Literature evidence suggests that the stereochemical configuration of the ß-hydroxyl group in sympathomimetic amines plays an important role in their neuronal storage. We have confirmed these observations in recent studies and shown that the 1S stereoisomers of *meta*-hydroxyephedrine and epinephrine display faster neuronal efflux in the dog heart than their 1R counterparts[1] Since fluorine has been suggested as an electronic mimic for the hydroxyl group[2], we were interested in developing radiotracer analogs of *meta*-hydroxyephedrine incorporating a chiral replacement of the hydroxyl with fluorine-18. Such a radiotracer would allow us to evaluate *in vivo* the biochemical equivalence of a fluorine for hydroxyl substitution.

In previous reports we demonstrated the feasibility of stereospecific replacement of the ß-hydroxyl in (1R,2S)(-)ephedrine and (1S,2S)(+) pseudoephedrine with fluorine-18 in high radiochemical yields utilizing chiral cyclic sulfamidate precursors[3]. Despite being benzylic fluorides, these tracers showed remarkable stability both *in vitro* and *in vivo*. In an extension of this approach towards synthesizing (1S,2S)-1-desoxy-1-[^{18}F]fluoro-*meta*-hydroxyephedrine, we have prepared the chiral cyclic sulfamidate **5** in five synthetic steps from (1R,2S)-(-)metaraminol in 28 % overall yield utilizing a MEM ether for protection of the phenol. Reaction with [^{18}F]fluoride provided the N-sulfonic acid intermediate **6**; subsequent removal of the MEM group to produce the final product **7** was accomplished in the same pot by *in situ* acid-catalysed hydrolysis.

Preliminary attempts at radiofluorination with K^{18}F in the presence of Kryptofix 2.2.2. in acetonitrile at 90°C for 20 minutes followed by a 30 min one-pot hydrolysis with 20 % aqueous H_2SO_4 provided [^{18}F]**7** in 20 % overall yield (decay corrected). Studies are in progress using alternative phenolic protecting groups to optimize the chemical and radiochemical yields of the unlabeled and radiolabeled fluorinated analogs, respectively.

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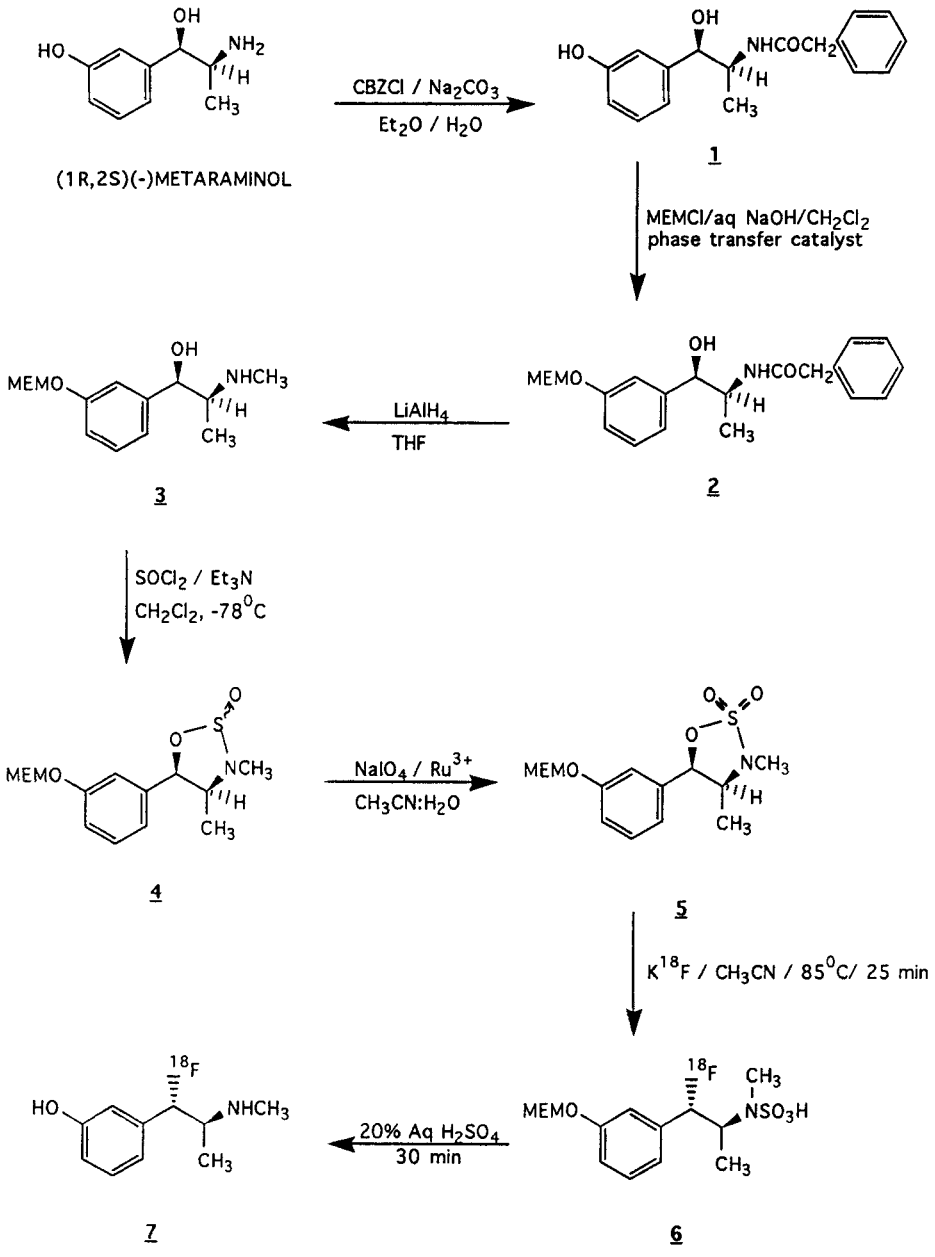
SYNTHESIS OF (1S,2S)-1-[^{18}F]FLUORO-1-DESOXY-meta-HYDROXYEPHEDRINE



Novel Use of an Isotope Separator to Determine the Position of Fluorine-18 in Labelled 1,1,1,2-Tetrafluoroethanes

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1,1,1,2-Tetrafluoroethane is a major CFC alternative and a potential drug propellant for use in multi-dose inhalers. We have reported methods[1,2] for site-selectively labelling 1,1,1,2-tetrafluoroethane with fluorine-18 ($t_{1/2}$ = 109.7 min; β^+ = 96.9%) to enable the fate of this compound after its inhalation by man to be studied using a simple whole-body counting technique.[3] For these studies it was of interest to measure the selectivity of the labelling procedures. The stability of 1,1,1,2-tetrafluoroethane and the short half-life of the label suggested that approaches involving chemical degradation would be difficult. We therefore considered a physical solution to this problem involving the use of an isotope separator.

In principle an isotope separator is similar to a mass spectrometer. In the NPL isotope separator used for this work, ions are extracted at 50 kV from an ion source similar to that described by Almén and Nielsen.[4] The ions are first focused by a cylindrical lens and then a planar lens. The latter also carries out electrostatic vertical positioning of the beams before their entry into a magnetic field of a 90 degree sector magnet (radius 1 m). Here the ions are separated according to their ratio of mass to charge (m/z). The resulting vertical elliptical beams have a separation of 10 mm at m/z = 100, typically enabling isotope enrichment factors of 2000 to be obtained. Stabilisation of beams is achieved by feeding the signal from two pins placed on either side of the beam to a logarithmic comparator, the signal from which is used to make small adjustments to the accelerating potential to correct the beam position. The beam is collected through a vertical slit (2 mm x 20 mm) (Scheme 1). The beam current is monitored continuously and is integrated to indicate the total charge collected (C). This is directly related to the mass of ions (moles) by the expression:

$$\text{Mass (moles)} = \text{Charge collected (C)}/[\text{Charge of ion } (z) \times 96{,}500]$$

The mass of a singly charged fragment (m Daltons) in the collected beam is given by the well known equation:

$$m = B^2/V \times \text{constant}$$

(where B is magnetic flux density and V is the accelerating potential). m is displayed digitally by means of a mass meter employing an NPL "Signacon" square law converter[5] applied to the output of a rotating coil magnetometer. The resulting signal, which is proportional to the square of the magnetic flux density, is compared to the applied accelerating voltage. Calibration of the mass meter is achieved by detection of the isotope beams of tungsten (m/z = 182,183,184,186) emanating from the ion source filament, and checked with the argon carrier gas (m/z = 40).

After introducing stable 1,1,1-trifluoro-2-fluoroethane into the ion source of the mass separator, stable beams were found at m/z = 69 $[CF_3]^{+\cdot}$ and m/z = 33 $[CH_2F]^{+\cdot}$. In the mass spectrometry of 1,1,1,trifluoro-2-haloethanes (halo = iodo, bromo, chloro) $[CH_2F]^{+\cdot}$ fragments are formed by rearrangement and account for > 10% of the total ionisation.[6] However, for the ionisation of 1,1,1,2-tetrafluoroethane in the mass separator it was assumed that $[CH_2F]^{+\cdot}$ fragments were formed mainly by carbon-carbon bond scission (Scheme 2). Similarly, it was assumed that $[CF_3]^{+\cdot}$ fragments were formed by the same process; consideration of the relative ion stabilities suggests that rearrangement to give $[CF_3]^{+\cdot}$ ions from carbon 2 would be highly unfavourable.[6]

For the study of each radiolabelled 1,1,1,2-tetrafluoroethane, the unlabelled fragment ion beam was collected through a slit which was positioned centrally in the collector plane of the isotope separator. On either side of the slit was placed a tantalum foil, smeared with Apiezon™ grease to ensure trapping of the arriving ions. This foil could be easily removed and replaced. Initially it was protected by a shutter. Following the establishment of a stable ion beam at an appropriate mass number (m/z = 69 or 33), the shutter was removed to expose the foil to any associated ion beams. The beam current was integrated from this time and following a suitable interval, the foil was removed and cut into pieces, each centred on the arrival position of a beam with a certain m/z value (over an m/z range of *ca* 10). The amount of fluorine-18 implanted into the foil at each position was measured at a recorded time by means of a calibrated sodium iodide well crystal. By renewing the foil, it was possible to carry out a study of more than one of the radioactive fragments using a single batch of radioactive gas.

Radioactivity associated with the ion beam of the $[CF_2{}^{18}F]^{+\cdot}$ fragment (m/z = 68) was collected, measured and divided by the integrated mass of the simultaneously collected ion beam for the $[CF_3]^{+\cdot}$ fragment (m/z = 69) to give the 'specific radioactivity' (in nCi/nmol) of the label in the 1-position. Similarly, the 'specific radioactivity' of label in the 2-position was calculated from measures of the radioactivity of the ion beam from the $[CH_2{}^{18}F]^{+\cdot}$ (m/z = 32) fragment and the integrated mass of the simultaneously collected ion beam from the $[CH_2F]^{+\cdot}$ (m/z = 33) fragment. (These measurements were corrected for contributions from dissimilar ions, ions containing natural abundance carbon-13 or ions having double charge). The selectivity of the labelling procedure for a particular position was then given by the decay-corrected ratio of specific radioactivity at that position to the sum of specific radioactivities. Labelling by nucleophilic addition of [^{18}F]fluoride to tetrafluoroethylene and by nucleophilic substitution with [^{18}F]fluoride in 2,2,2-trifluoroethyl tosylate were found to have 97.2 ± 0.4 % selectivity for the 1-position and 91.2 ± 2.2 % selectivity for the 2-position, respectively.

The very high selectivity measured for labelling in the 1-position by 'nucleophilic addition' of [^{18}F]fluoride to tetrafluoroethylene implies that rearrangement of the trifluoromethyl group to give a fluoromethyl ion, as observed in the mass spectra of 1,1,1-trifluoro-2-halo-ethanes,[6] is not an important process in the ionisation of the 1,1,1,2-tetrafluoroethane in the isotope separator. This supports the main assumption of the study.

The unexpectedly low selectivity of the nucleophilic substitution reaction for labelling in the 2-position indicates that direct exchange also occurs at the 1-position under the labelling conditions. A mechanism involving a carbanion intermediate is highly plausible, given the general ease with which other 1,1,1-trifluoroethanes carrying electron-withdrawing substituents in the 2-position (except fluoro)[7] will undergo such exchange reactions.[7,8]

The results clearly warn against any assumption of site *specificity* for labelling based on a simple formal representation of the labelling reaction. The described technique is of potentially wider value for measuring the distribution of fluorine-18 in labelled polyfluorinated molecules.

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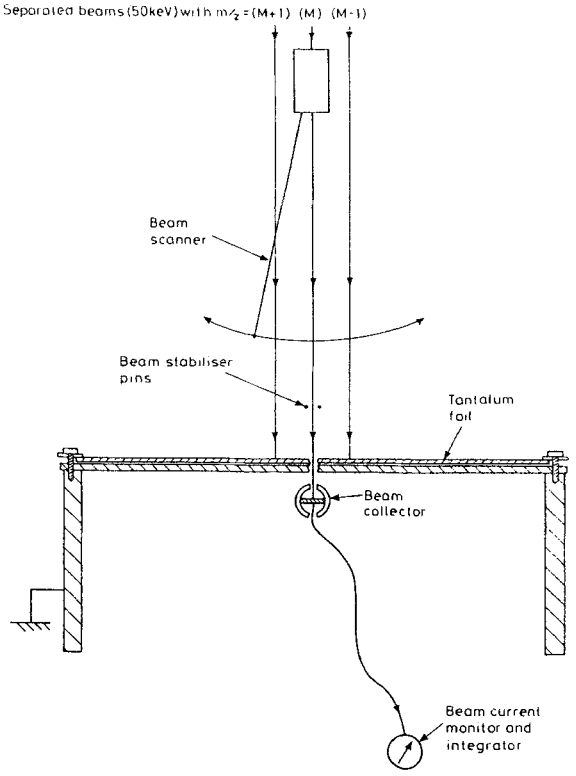

Scheme 1. Ion collector arrangement for isotope separator.

$$CF_3CH_2F \longrightarrow [CF_3CH_2F]^{+\cdot} \longrightarrow [CF_3]^{+\cdot} \; (m/z = 69) \; + \; [CH_2F]^{+\cdot} \; (m/z = 33)$$

$$^{18}FCF_2CH_2F \longrightarrow [^{18}FCF_2CH_2F]^{+\cdot} \longrightarrow [^{18}FCF_2]^{+\cdot} \; (m/z = 68) \; + \; [CH_2F]^{+\cdot} \; (m/z = 33)$$

$$CF_3CH_2{}^{18}F \longrightarrow [CF_2CH_2{}^{18}F]^{+\cdot} \longrightarrow [CF_3]^{+\cdot} \; (m/z = 69) \; + \; [CH_2{}^{18}F]^{+\cdot} \; (m/z = 32)$$

Scheme 2. Fragments generated from the carbon-carbon bond scission of unlabelled and ^{18}F-labelled 1,1,1,2-tetrafluoroethanes in the ion source of an isotope separator.

Fast Syntheses of Four Two-Carbon Difunctional Molecules Labelled with Carbon-11

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Precursors labelled with a short-lived isotope must be produced quickly and in high yields to be useful in subsequent radiolabelling reactions. We present here a synthetic strategy for the rapid production of four two-carbon difunctional precursors derived from [^{11}C]cyanide. Microwave treatment, which has previously been shown to be a valuable aid in reducing radiolabelling times with [^{11}C]cyanide (1), was used to speed up the time-consuming transformations requiring elevated temperatures using a prototype wave-guide cavity (2). [^{11}C]Cyanide, produced on-line from [^{11}C]carbon dioxide, was flowed through a sulfuric acid and P_2O_5 trap (3) to remove the ammonia added in the processing and trapped in ethanol. Reaction with chloromethyl pivalate generated [^{11}C]cyanomethyl pivalate (I) which was a) hydrolyzed to [1-^{11}C]glycolic acid (II), b) reduced to [2-^{11}C]2-aminoethanol (III) after evaporation of the solvent, c) esterified to ethyl [1-^{11}C]glycolate (IV) which was d) subsequently reduced to [1-^{11}C]ethylene glycol after evaporation of the solvent. The transformations are shown in the reaction scheme below.

Comparable conversions could be obtained using either thermal heating or combined thermal/microwave treatment. The conditions for the individual steps are given below:

I: In ethanol, 80°C for 2 min or 100 W for 0.25 min (~90%).
II: With 25% H_2SO_4, 120°C for 5 min or 75 W for 0.25 min (~95%).
III: After evaporation of the solvent, stirring 2 min at room temperature with 0.2 M LAH/THF (~90%).
IV: With 2 M HCl(g)/EtOH at 80°C for 10 min or 75 W for 0.5 min (~90%)
V: After evaporation of the solvent, stirring 2 min at room temperature with 0.2 M LAH/THF (95%).

The total synthesis times for I-V are given in the table below (times for evaporations not included). Although none of the reaction times given below are prohibitive, the further extension of this scheme to new radiolabelling precursors will make the combined use of microwave techniques increasingly important for reducing the total reaction times. The difunctionality of these precursors presents a possibility for utilizing only one of the functionalities in the subsequent radiolabelling reactions or for using both in, for example, cyclization reactions.

	Total reaction time (min)	
Substance	Thermal	Microwave
I	2	0.25
II	7	0.5
III	4	2.25
IV	12	0.75
V	14	2.75

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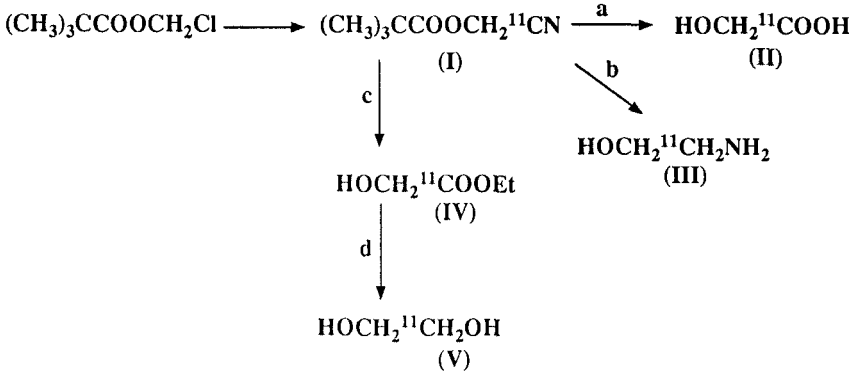
(CH3)3CCOOCH2Cl
(CH3)3CCOOCH2 11CN
(I)
a
HOCH2 11COOH
(II)
b
HOCH2 11CH2NH2
(III)
c
HOCH2 11COOEt
(IV)
d
HOCH2 11CH2OH
(V)

Selective NCA Synthesis of ^{11}C-Labelled Phenylethanolamines such as [^{11}C]Metaraminol using [^{11}C]Nitroalkanes.

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Examinations of the cardiac sympathetic neuronal system with PET has during recent years received an increasing interest for research and clinical diagnosis (1). The endogenous transmitter of highest concentration in this system is (-)-norepinephrine (NE). Metaraminol is a NE congener which unlike NE is not metabolised by MAO and COMT. Among the phenylethanolamines, metaraminol is a promising candidate to label with ^{11}C or ^{18}F (2) for quantitative imaging *in vivo*.

The first approach for synthesis of ^{11}C-labelled phenylethanolamines for PET examination of the heart was the cyanohydrin synthesis using [^{11}C]cyanide (3-5). This approach is not applicable for the ^{11}C-labelling of metaraminol. Other approaches are ^{18}F-fluorination with $^{18}F^-$ (6) or $CH_3COO^{18}F$ (7) and ^{11}C-methylation with methyl iodide (8) which have been used to label fluoro- and methyl- analogues of NE and metaraminol.

In the present project, an approach was developed with selective condensation of substituted benzaldehydes and NCA [^{11}C]nitroalkanes using tetrabutylammonium fluoride as a catalyst. This reaction show high radiochemical condensation yields (60-90 %) and a high selectivity (>80 %) for the formed [^{11}C]nitroalcohols versus the corresponding [^{11}C]nitrostyrenes. Reduction of the [^{11}C]nitroalcohols with Raney nickel gave the corresponding aminoalcohols. Benzaldehyde and 3-hydroxy-benzaldehyde were condensed directly with [^{11}C]nitroalkanes, whereas 4-hydroxy- and 3,4-dihydroxy-benzaldehyde had to be protected during condensation. The 4-methoxy- and 3,4-methylenedioxy- protecting groups were choosen for this purpose as they can be smoothly cleaved by boron tribromide.

We have applied the approach with [^{11}C]nitroalkanes for the syntheses of [1-^{11}C]labelled metaraminol, norphenylephrine, norepinephrine, octopamine and phenylethanolamine. The two- or three-step procedure is illustrated in the reaction scheme. A summary of the obtained decay-corrected radiochemical yields and total synthesis times from EOB is given in the table.

PET-examination of racemic [1-^{11}C]norepinephrine, with a specific radioactivity of 700 mCi/μmol at time of injection, in a Cynomolgus monkey demonstrated a rapid and high uptake of radioactivity in the heart. The specificity of binding was demonstrated in a pretreatment experiment with desipramine, a selective norepinephrine reuptake inhibitor. The uptake in the heart was reduced 5-fold compared to that in the baseline experiment.

These results indicate the general potential of selective condensation of substituted benzaldehydes with NCA [^{11}C]nitroalkanes for the preparation of NCA [^{11}C]phenylethanolamines for PET.

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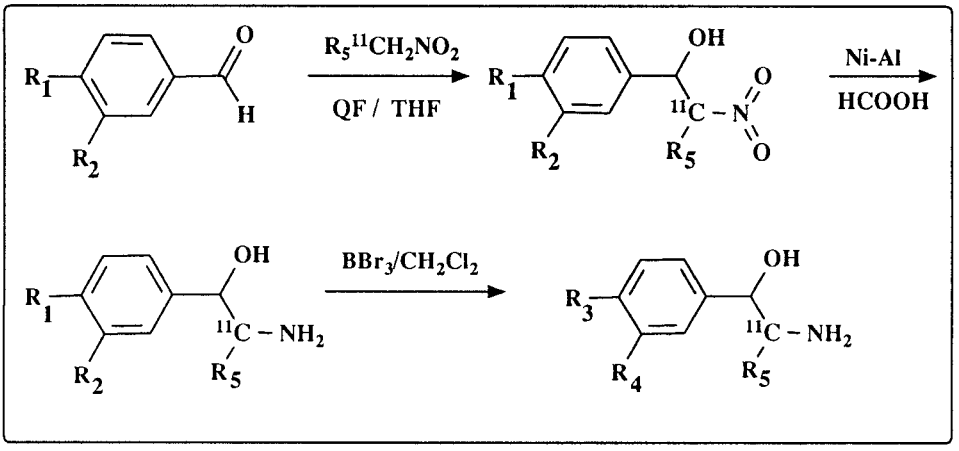

Compound	R_1	R_2	R_3	R_4	R_5	Yield[a]	Time[b]
[1-^{11}C]Metaraminol	H-	HO-	H-	HO-	CH_3-	30-45[c](13-20)[c,d]	45-55[c]
[1-^{11}C]Norphenylephrine	H-	HO-	H-	HO-	H-	35-45(21-27)[d]	40-45
[1-^{11}C]Norepinephrine	-O-CH_2-O-		HO-	HO-	H-	20-25(12-15)[d]	65-70
[1-^{11}C]Octopamine	CH_3O-	H-	HO-	H-	H-	20-25(12-15)[d]	60-65
[1-^{11}C]Phenylethanolamine	H-	H-	H-	H-	H-	37-50(22-30)[d]	40-45

[a] Decay corrected radiochemical yield (%) from ^{11}C-nitroalkane. [b] Total synthesis time (min) from EOB to final sterile filtered HPLC-purified product (rad. chem. purity >97 %). [c] Preliminary, unoptimized results. [d] Decay corrected rad. chem. yield (%) from [^{11}C]CO_2.

Synthesis of [^{11}C-Methyl]-α-Aminoisobutyric Acid (AIB)

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The Bucherer-Strecker reaction has been used to synthesize [^{11}C]-carboxyl-labeled synthetic amino acids from [^{11}C]-HCN.[1-8] [^{11}C-Carboxy]-α-Aminoisobutyric acid (AIB) has been synthesized by this route[5] and used as a tumor imaging agent in animal tumor models and in patients with soft tissue cancers[9] and melanoma.[10] AIB is an achiral, nonmetabolized amino acid which is rapidly transported into viable cells primarily by the A-type or alanine-preferring amino acid transport system. Whether due to specific alterations in cell membrane functions or to elevated transport due to cellular growth, cancer cells extract AIB to a greater extent than normal cells. AIB is not metabolized or incorporated into protein after transport. Therefore, its differential uptake and tissue distribution reflects the relative degree of A-type transport in various tissues in normal and diseased states. Since AIB is not metabolized after cellular uptake, it offers the possibility of studing amino acid transport into cells without interference by radiolabeled metabolites. In conjunction with PET, it may permit the quantitation of differences in amino acid transport between normal and malignant cells,[11] and serve as a potential agent to monitor therapeutic intervention.[12] The absence of metabolic products will yield less complex models for compartmental analysis than will metabolized natural amino acids.

Synthesis of [^{11}C-carboxy]-AIB by the Bucherer-Strecker reaction has required a vigorous quality assurance program to insure that the concentration of cyanide in the final product was below certain levels.[5] Therefore, a synthetic method that did not involve cyanide was developed. Since AIB may be thought of as α-methyl-alanine, reaction of ^{11}C-CH_3I with a suitable alanine precursor was considered. The methodology for the synthesis of α-[^{11}C-methyl]-L-tryptophan had been reported[13,14] and was adapted to the synthesis of [^{11}C-methyl]-AIB (Scheme 1). DL-Alanine methyl ester hydrochloride (25.0 g, 0.18 mol) was suspended in 1 liter of CH_2Cl_2. Triethylamine (TEA) (20.0 g, 0.20 mol) was added to precipitate TEA hydrochloride. The mixture was filtered and the solvent was removed by rotary evaporation. Additional solid was collected during the evaporation process. Finally, 9.0 g (49% yield) of DL-alanine methyl ester (1) was obtained as a pale yellow liquid which solidified on standing over drierite in a refrigerator. This material (3.0 g, 29.1 mmol) was mixed with 45 mL of TEA and the mixture was cooled to -10°C. Benzaldehyde (3.1 mL, 29.1 mmol) in 56 mL TEA was added slowly with stirring over 30 min while the temperture was maintained at -10°C. Then the clear solution was stirred for four hours at -5°to -10°C. It was allowed to warm to room temperture over two hours while stirring. KOH (22.4 g) was added and the mixture was allowed to stand for 30 min without stirring. After filtering, the solvent was removed by rotary evaporation. The benzaldimine of DL-alanine methyl ester (2)

(4.0 g, 72% yield) was obtained as a pale yellow liquid. It was used without further purification. However, it's distillation has been reported (77°/0.06 mm).[15] In an unlabeled preparation of AIB, a 2M solution of lithium diisopropylamide (LDA) (15 μL, 0.03 mmol) and THF (0.5 mL) were sealed in a 5 mL reacti-vial. The THF had been distilled over sodium (benzophenone indicator) before use.

The vial was placed into a bath at -78°C for 10 min. Then the benzaldimine (2) (0.40 mL, 5.7 mg, 0.03 mmol of a solution of 43.6 mg of (2) in 3 mL of THF) was added. This converted the benzaldimine (2) to the anion (3). The color of the solution changed from orange to yellow. After 20 min, CH_3I (22 μL, 2.1 mg, 0.015 mmol of a solution of 0.38 mL, 0.87 g, of CH_3I in 9 mL THF) was added. After 1 min, the solution was placed into a bath at 35° for 5 min. The benzaldimine of AIB methyl ester (4) was hydrolyzed with 0.45 mL of 1N HCl at 110°C for 1.5 min. Argon was passed through the solution during the hydrolysis. AIB methyl ester (5) was then hydrolyzed with 0.29 mL of 2N NaOH at 35°C for 3 min. The solution was adjusted to pH 6-7 with 300 μL of 1N HCl. The solution containing AIB (6) was diluted to 2 mL with acetonitrile. All of the solution was injected through a 2 mL loop onto a Whatman Partisil 10 SAX 10 x 500 mm HPLC column (UV detection: 210 nm, 1.0 AUFS). AIB was eluted in about 17 min with 800 mL CH_3CN/200 mL buffer (1000 mL H_2O, 0.41 g KH_2PO_4, 2 drops H_3PO_4, pH 3.5) at 9 mL/min. The AIB fraction was collected in 35 mL of eluant. After it's concentration on a rotary evaporator to 3 mL, it was shown to be AIB by CI(NH_3) MS.

The above procedure was used to synthesize (^{11}C-methyl)-AIB from the benzaldimine (2) and ^{11}C-CH_3I (unlabeled CH_3I was not added to the reaction mixture). In one experiment, the final preparation was spiked with a commercial sample of AIB. The retention time of the AIB spike in the UV trace was identical to that of the retention time of [^{11}C-methyl]-AIB in the radioactive trace. In experiments carried out without spiking, the [^{11}C-methyl]-AIB fraction was collected and reinjected. Radiochemical detection indicated that the purity of the fraction was greater than 99%. The radiochemical yield was about 55%. The synthesis time was about 70 min, which included rotary evaporation of the fraction under high vacumn, formulation of the product in 10 mL of physiological saline, and sterilization through a 0.22 μm filter. To date, only low levels of ^{11}C-CH_3I activity have been employed. Preparations are in progress with higher levels of ^{11}C-CH_3I activity for overall yields and specific activity determinations.

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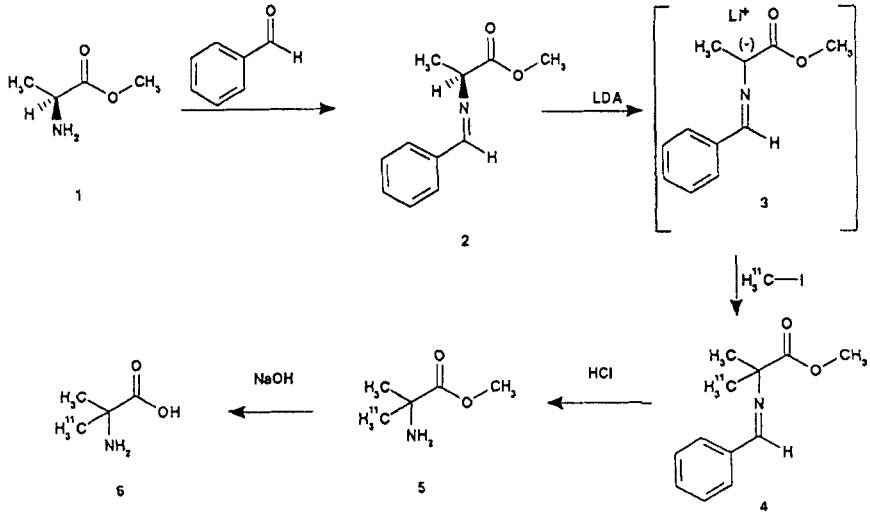

Scheme 1: Synthesis of α-(^{11}C-methyl)-aminoisobutyric acid (AIB)

Full Automated and Compact Chemistry Module for the [2-^{11}C]-Thymidine Synthesis from [^{11}C]-HCN.

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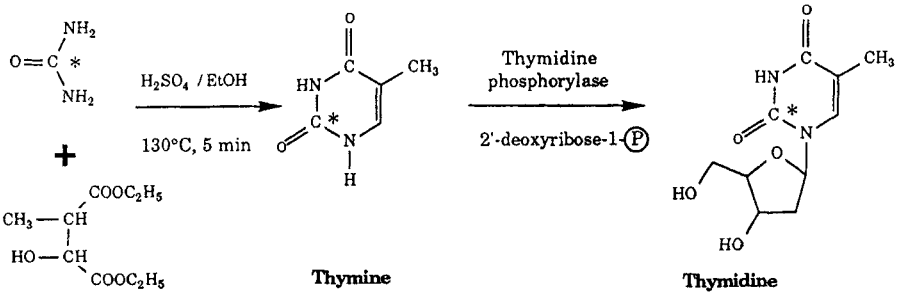
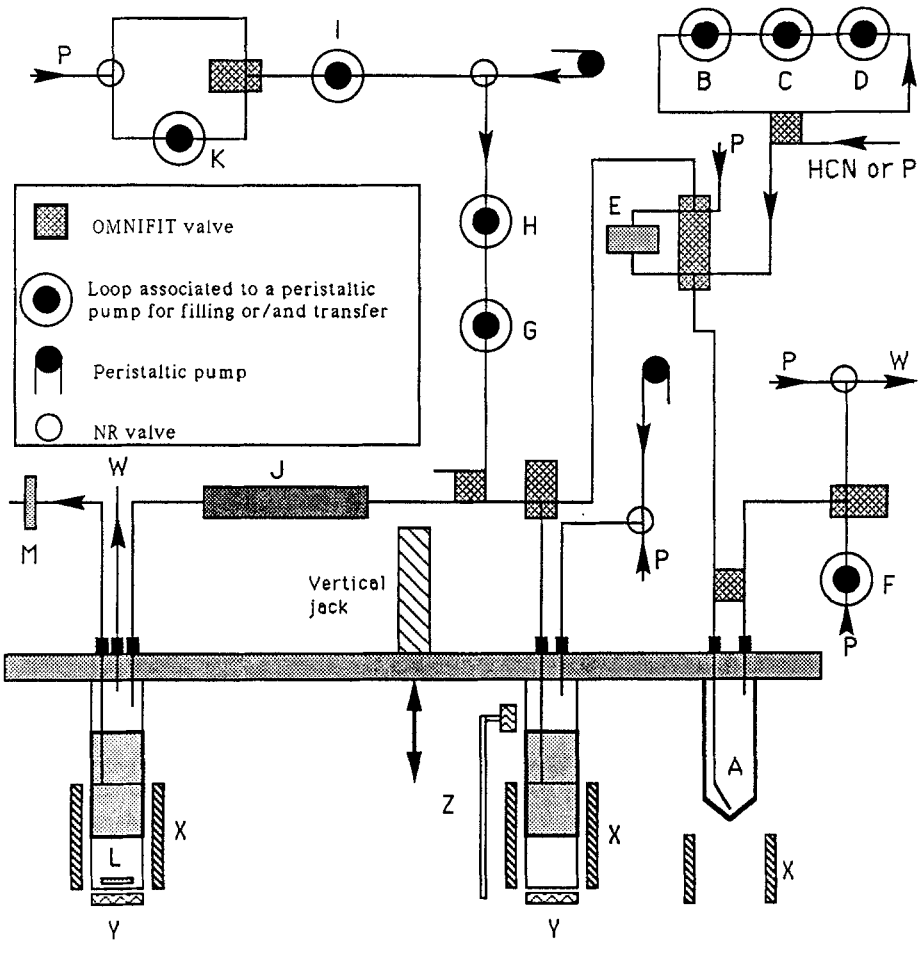
Since we have presented the [2-^{11}C]-Thymidine synthesis and have proposed this tracer for measuring cellular proliferation by PET, it was obvious that an automated synthesis process was required to prepare adequate supply of this tracer (0.3 to 1.0 GBq). The full automated synthesis process we present here, follows closely the chemical procedure we have previously described (1).



Technical data: Our procedure uses a SIEMENS 928 processor driven by keyboard and display, and using a CP527 graphic interface. The dimensions of the chemistry module are: 65cm wide, 57cm high and 35cm deep. Liquids are transferred through 1/16" PTFE tubing, pneumatically operated "OMNIFIT" or "NR" PTFE valves using argon low pressure or peristaltic pumps. Vials tightness is ensured by using PEEK caps including "Blue Septa"(Alltech) and PEEK fingertight fittings. A fast warming of the vials is performed with a controlled hot-air blast and the cooling by CO_2 expansion.

Synthesis procedure: [^{11}C]-HCN is trapped in the KOH-$KMnO_4$ solution (A) and oxidized into cyanate by heating at 80°C for 3.0 min. After a short cooling, H_2O_2 (B), $(NH_4)_2SO_4$ (C) and ethanol (D) contained in prefilled loops are added. The vial is hermetically sealed and heated at 185°C for 3.0 min. After cooling till R.T., [^{11}C]-urea is cleared of MnO_2 by filtration through a hydrophilic 0.45µm filter preconditioned with ethanol (E). The filter is rinsed with 0.8 ml ethanol (F) and the filtrate is evaporated to dryness by heating at 130°C for 4.0 min under an argon flow. To the anhydrous residue, kept under argon during cooling, are added the solution of diester in ethanol (G) and the fuming sulfuric acid (H). The reaction mixture is magnetically stirred at R.T. for 1.0 min and then heated at 130°C for 5.0 min. After cooling, 1.0 ml water (I) is added and a peristaltic pump loads the acidic solution onto the ion retardation resin (J). The resin is then eluted by 4.0 ml water (K) and the [2-^{11}C]-thymine is incorporated in the enzymatic reaction mixture (L) which is magnetically stirred at 40°C for 4.0 min.This mixture containing the [2-^{11}C]-thymidine is then filtered through a hydrophilic 0.45µm filter and transferred to the HPLC system we have previously described.

Following this procedure, we are now able to prepare injectable [2-^{11}C]-thymidine in 62 min with a 2.5 ± 0.5 % non-decay corrected radiochemical yield, calculated from the initial estimated [^{11}C]-HCN activity.



A: 200µl of 32mM $KMnO_4$ and 50µl 2.0N KOH; **B**: 6% H_2O_2; **C**: 100µl 1.5M $(NH_4)_2SO_4$; **D**: 100µl ethanol; **E**: hydrophilic 0.45µm membrane, d: 13mm; **F**: 0.8ml ethanol; **G**: solution of 50µmol of diethyl-β-methylmalate in 10µl absolute ethanol; **H**: 100µl of fuming sulfuric acid (±10% SO_3) kept under argon; **I**: 1.0ml water; **J**: 4.0ml dry retardation resin (AG 11A8, Bio-Rad); **K**: 4.0ml water; **L**: 50µl of a 0.15M aqueous solution of 2'-deoxyribose-1-phosphate and 25 U.I. of thymidine phosphorylase; **M**: hydrophilic 0.45µm membrane, d: 25mm; **P**: argon low pressure; **W**: waste; **X**: heating and cooling cavity; **Y**: magnetic stirrer; **Z**: rotating jack with stirring bar.

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Robotic synthesis of L-[1-^{11}C]-tyrosine

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The rate of incorporation of L-amino acids into proteins is correlated with the protein synthesis rate (PSR) in tissue. In principle, local PSR in tissue can be quantified *in vivo* by the use of PET with ^{11}C-labeled amino acids[1].

In our laboratory L-[1-^{11}C]-tyrosine is synthesized via ^{11}C-carboxylation of an α-lithiated isocyanide[2]. Because for routine purposes large amounts of initial radioactivity are required and to avoid excessive cumulative radiation doses, a remote controlled synthesis was a prerequisite. Since the synthesis of the α-lithiated isocyanide is prone towards moisture, the synthesis by a remote controlled apparatus was problematic. In addition the application of HI for the hydrolysis of the methoxy group prompted us to design an easy to clean system, which can maintain a high vacuum. With the installation of an Anatech robot, delivered by Scanditronix, in our PET-center we were able to handle the before mentioned problems. A robotic system imitates the manipulations carried out by the laboratory worker. For this reason no compromise has to be made to the synthetic procedure.

The production system consists of four units. a) The basic component in the Anatech Robot Controller (ARC)-system is the robot arm RB-86. The robotic arm is mounted on a rail to facilitate a flexible positioning of several parts of the synthetic system in different hotcells. These parts are also used for syntheses of other radiopharmaceuticals. The robot is controlled by a sequencer, which in turn is controlled by a Personal Computer with ARC-software. The ARC-program, written for the synthesis of L-[1-^{11}C]-tyrosine consists of a main program divided in 30 subprograms. If necessary troubleshooting can easily be performed during the run of the program. b) Reaction unit, in which the chemistry is performed. The reaction vial is connected to a two-way high vacuum valve, which is operated by the fingerhand of the robotic arm. In this way switches are possible between the argon and the vacuum line. c) The neutralization unit for pH adjustment. Using an autoburet and a pH-electrode the crude product is neutralized to a pH of 5-7 before injection on the first C-18 HPLC-column. d) HPLC unit. After purification by RP-HPLC, D,L-[1-^{11}C]-tyrosine is switched onto a chiral HPLC-column to separate the two enantiomers. Finally a solvent change from buffer to saline is performed by switching the L-isomer to a C-18 column with saline as eluent. After passing through a sterile filter and QC, L-[1-^{11}C]-tyrosine is suitable for injection. The injection valve and switching valves are controlled by the sequencer of the robotic system.

The total synthesis time is 60 minutes (EOB), including HPLC-purification. The overall radiochemical yield of L-[1-^{11}C]-tyrosine is 10% (corrected for decay to the end of bombardment) starting from [^{11}C]CO_2. The specific activity is >1000 Ci/mmol.

Following this strategy a reliable synthesis method of L-[1-^{11}C]-tyrosine has been developed for routine patient studies.

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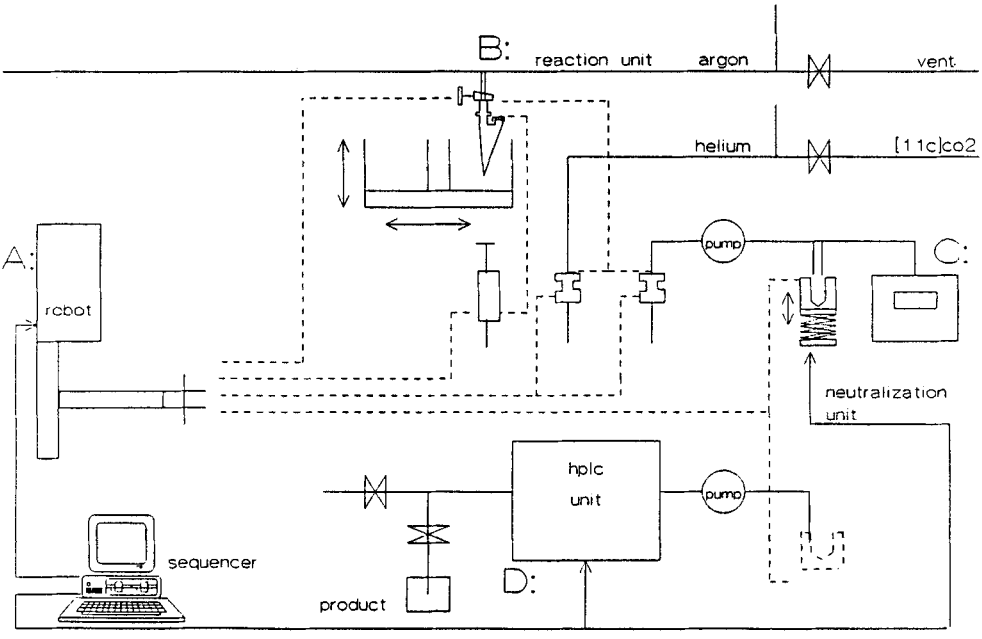
Performance of the robotic L-[1-^{11}C]-tyrosine production system

	Average	SD	EOB
Integrated beam (μAh)	36	6	
Activity [^{11}C]CO_2 (mCi)	2000	300	6
Product before HPLC (mCi)	109	28	40
Endproduct (mCi)	25	6	60
% Yield (cfd)	10	2	

Average is from 9 preparations, SD = standard deviation
EOB is minutes

Schematic representation or the production system.



Solid lines are gas or liquid lines.
Dashed lines represent operations of the robotic arm.

On-line Preparation of ^{11}C-labelled Acetate and Palmitate by Column Extraction.

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Evaluation of myocardial metabolism is one of the important subjects in routine clinical PET studies. They utilize mainly ^{11}C-labelled acetate and palmitate, which are prepared by the Grignard reaction from [^{11}C]carbon dioxide with a remote-controlled or automated system (1-6). The [^{11}C]carboxylation is generally carried out by bubbling [^{11}C]carbon dioxide through the Grignard reagent solution and quenching the reaction mixture followed by purification using extraction or distillation from acid solutions. We developed the new on-line method for [^{11}C]methylation by the on-column reaction of [^{11}C]methyl iodide and demonstrated its excellent advantages over many conventional batchwise methods (7-9). This approach has been successfully applied to the preparation of ^{11}C-labelled fatty acids using column extraction techniques.

The on-line preparation of [^{11}C]acetate was carried out using the semi-automated system shown in Fig.1. [^{11}C]Carbon dioxide, concentrated in a cold trap cooled with liquid argon, was introduced with a He flow of 30 *ml*/min into a short reaction column packed with well dried Extrelut (Merck), loaded with 0.1-0.2 *ml* of commercially available 1M methylmagnesium bromide in THF. The Grignard reaction mixture was quenched by injecting 0.1 *ml* of 3N HCl into the reaction column and the [^{11}C]acetic acid formed was extracted with 5 *ml* of diethyl ether and transferred from the reaction column to a glass vessel. The ether was rinsed with He bubbling for 1 min to remove the unreacted [^{11}C]carbon dioxide and then passed through a subsequent Extrelut column absorbing 1 *ml* of a 7 w/v% sodium bicarbonate solution, where the [^{11}C]acetic acid was extracted back. It was finally eluted from the column with 10 *ml* of saline. For the preparation of [^{11}C]palmitate freshly prepared 1M n-pentadecylmagnesium bromide in THF and hexane were used instead of methylmagnesium bromide and diethyl ether. The [^{11}C]palmitic acid extracted in the same way was then adsorbed with a short silica gel column (Sep-Pak Silica, Waters) and eluted with 10 *ml* of ethanol.

Extrelut is a commercially available column packed with granular kieselguhr of giant pores, capable of taking up and holding water. It is largely used for column extraction of lipophilic compounds from biological samples such as serum and urine. To our knowledge the present study is the first application of column extraction with Extrelut to the preparation of positron-emitting radiopharmaceuticals. The extraction of [^{11}C]fatty acid from the acidic reaction mixture into the ether or hexane layer and the back extraction of [^{11}C]acetic acid from the ether layer into the sodium bicarbonate solution using Extrelut were almost quantitative as expected. It should be noted that [^{11}C]carbon dioxide was efficiently trapped into the Grignard reagent solution absorbed by Extrelut from the flowing He. Thus [^{11}C]acetate was obtained in overall radiochemical yields of over 80% by the present on-line preparation method within 10 min including the recovery and concentration steps of [^{11}C]carbon dioxide, while [^{11}C]palmitate was prepared in 30-40 % radiochemical yields. HPLC analysis showed over 95% radiochemical purities for these [^{11}C]fatty acids.

In summary, the present work has demonstrated that the on-line method by column extraction offers the convenient and efficient preparation of [^{11}C]fatty acids. It can be concluded that the use of Extrelut will greatly simplify synthetic procedures for [^{11}C]fatty acid preparations by the Grignard reaction and, as a result, provide a facile way to their automated preparations. In addition, such an automated system can be used for the routine preparation of both [11C]acetate and [11C]palmitate practically in the same procedures. It can be expected that Extrelut will find versatile applications in preparations of positron-emitting radiopharmaceuticals.

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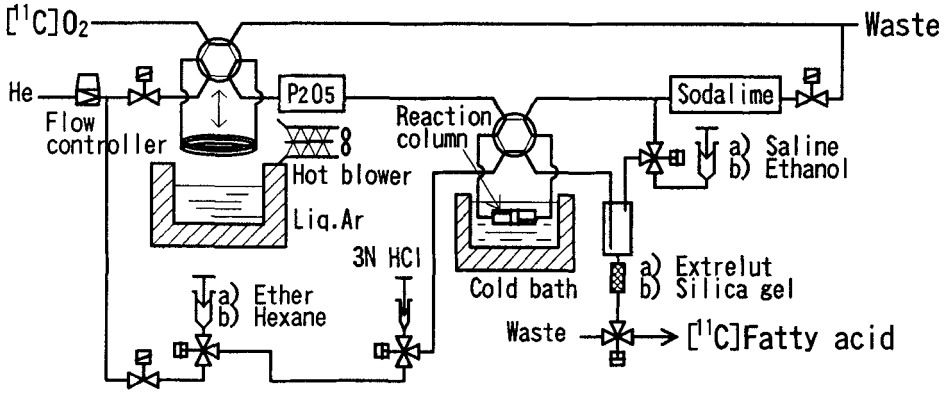

Figure 1. A flow chart of the system for the on-line preparation of [^{11}C]fatty acids

Reagents: (a) for [^{11}C]acetate, (b) for [^{11}C]palmitate

DEVELOPMENT OF AN AUTOMATED SYNTHESIS APPARATUS FOR 1-[^{11}C]-ALDOSES BY A MODIFICATION OF THE KILIANI-FISCHER METHOD.

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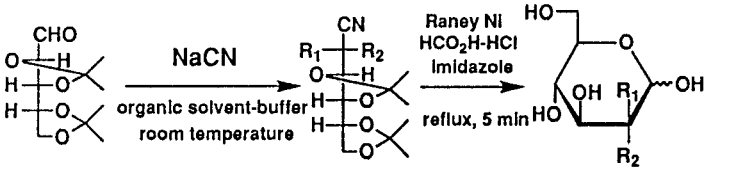
The purpose of our investigation was to develop a full automated synthesis apparatus for preparing a wide variety of 1-[^{11}C]-labelled aldoses, such as 1-[^{11}C]-D-glucose, D-galactose, 2-deoxy-D-glucose.(1, 2, and 3) As the first step, we investigated the stereoselective and rapid synthesis method for 1-[^{11}C]-D-glucose and then developed an automated apparatus which could also be used to produce other 1-[^{11}C]-labelled aldoses.

The well-known classical Kiliani-Fischer method is popular for preparing 1-[^{11}C]-D-glucose, however it is D-mannose (5) rather than D-glucose (4) selective.(4) Thus, in order to investigate the possibility to synthesize selectively either optical isomers by changing the reaction condition we further studied the reaction rate and the stereoselectivity of the cyanohydrin formation with the cold experiments. As a typical example, we chose the reaction of 2,3:4,5-di-*O*-isopropylidene-D-arabinose (1) with one equivalent of sodium cyanide in a mixture of organic solvent and alkali buffer as shown in Scheme 1.



Scheme 1.

The yields of the cyanohydrins, gluconononitrile 2 and mannononitrile 3, were determined by using HPLC. The total yield curve producing 2 and 3 versus reaction time is shown in Figure 1. The initial reaction rate calculated from the summation yield of 2 and 3, depended upon the organic solvent. However the total yield curves appeared to level off within 5 minutes. Interestingly, the formation ratio of 2 to 3 was found to be greatly dependent on the organic solvent and the pH of the buffer as shown in Figure 2. In these conditions, we found that the toluene-buffer condition was most suitable for preparing 2 which is a precursor of 4, the formation ratios of 2/3 increased with time and the pH of the buffer, and leveled off after 5 minutes. Furthermore, we focused on the toluene-buffer condition for preparing 2 and then the optimum condition was found to be 10.8 as shown in Fig.3.

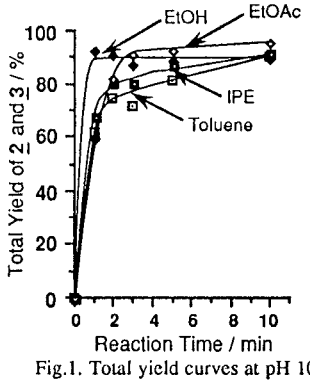


Fig.1. Total yield curves at pH 10.8.

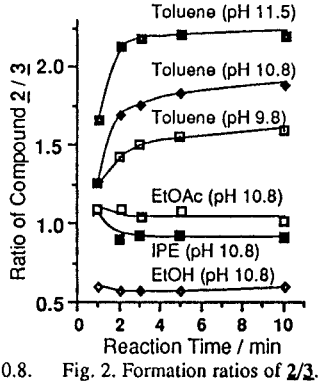


Fig. 2. Formation ratios of 2/3.

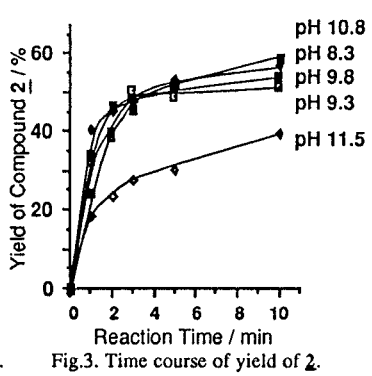
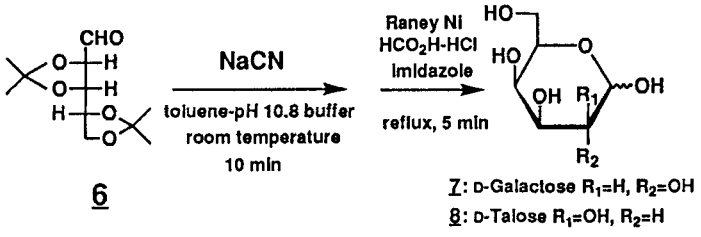


Fig.3. Time course of yield of 2.

This two phase reaction is practical and not moisture sensitive, so it is possible to apply to a microsynthesis of aldoses by combining it with a reductive hydrolysis step. Thus, a one-pot synthesis of 4 and 5 was carried out as shown in Scheme 1 to give them in 23.0% (from NaCN) and 13.5% (from NaCN) , respectively. This one-pot reaction was performed within 15 minutes. Furthermore, we tried the synthesis of D-galactose (7) and D-talose (8) by using the similar method, and succeeded in obtaining 7 (28.1% from NaCN) and 8 (11.9% from NaCN) from 2,3:4,5-di-*O*-isopropylidene-D-lyxose (6) as shown in Scheme 2.



Scheme 2.

We then constructed an automated synthesis apparatus on the basis of our result. The hardware consists of a synthesis system, an I/O box , and a 32 bit personal computer (PC-9801FA, NEC). The synthesis system consists of a series of units, which have the following functions: supplying reagents; performing reactions; purifying ^{11}C-labelled aldose; and preparing an injectable solution of ^{11}C-labelled aldose. The diagram of the apparatus is shown in Fig. 4. The reagent supply unit has eight reservoirs. Each reservoir is filled with the liquid reagent, the volume is measured and transferred to the corresponding reactor by the combination of three valves, a photosensor, and nitrogen gas pressure. The reaction unit contains two reactors. The cyanohydrin formation is carried out in reactor 1. The reaction mixture is then transfered to reactor 2 and treated with the reagents to give a mixture of the aldoses. The mixture is purified in the purification unit which contains three devices: first is a column for desalting, second is a vessel for evaporating (reactor 3), and last is a preparative HPLC system. The eluate of HPLC containing the produce is then transferred to reactor 4, adjusted to pH 6.5-7.5, diluted with saline, and filtered through a membrane filter to give an injectiable solution of 1-[^{11}C]-labelled aldose. These operations are performed by the personal computer.

Thus, we tried to a preliminary synthesis of 1-[^{11}C]-D-glucose by using our synthetic method and the apparatus. The optimization of the operation conditions and the synthesis of the other labelled aldoses are now in progress.

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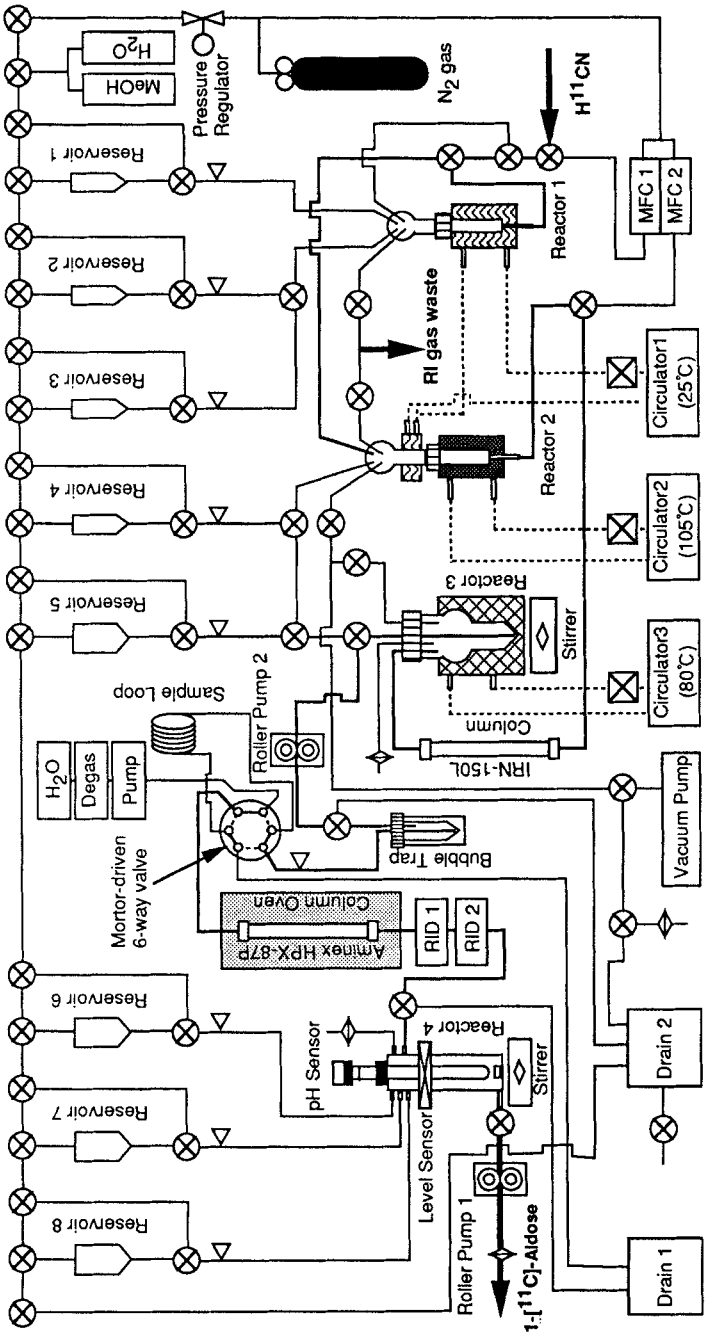

Fig.4. Diagram of the Automated Synthesis Apparatus for 1-[^{11}C]-Labelled Aldoses.

SIMPLE AND DIRECT SYNTHESIS APPARATUS FOR ^{11}C-ACETATE

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An automated synthesis apparatus for ^{11}C-acetate has been developed. ^{11}C-Acetate is a useful tracer for studying myocardial metabolism by positron emission tomography (PET). The first preparation of ^{11}C-acetate for clinical use is reported by Pike et al.[1].

We have developed a direct method employing a solid-phase extraction technique[2], instead of the liquid-liquid extraction[1)3)] and distillation method[4)5)], and have applied it to a fully automated synthesis apparatus using distinctive turntable techniques.
It is a simple and flexible synthesis apparatus without many valves and any complicated tube lines. A schematic diagram of the automated apparatus is shown in Fig.1.

The apparatus consists of two distinctive turntables which turn and move up and down. A purification column and several vials containing reagents and solvents are arranged on it. Another turn table supports syringes with disposable needles which carry $^{11}CO_2$ and helium gas, withdraw and inject reagents, and transfer the reaction mixture from a vessel to a column. By means of both a radiation sensor and a small CCD camera, the states in a reaction vessel is monitored numerically and visually during each process.

$^{11}CO_2$ produced by $^{14}N(p,a)^{11}C$ reaction is first condensed in a stainless steel coil and then swept out to Grignard reagent in a reaction vessel by helium flow.
$^{11}CO_2$ is trapped and simultaneously reacted with the 0.1M methylmagnesium bromide in 2 ml ether. A small portion of water is added to the reaction mixture to destroy the excess of Grignard reagent. The mixture is then evaporated to dryness with a hot blow and increased He flow. After cooling by the cooled N_2 flow blowing, the residue is hydrolysed with 2 ml of 0.4M hydrochloric acid and vigorous bubbling by helium flow. Then the hydrolysate is diluted with 2 ml of water and is transferred to the purification column.
The mixture is passed through an ion retardation resin (AG11A8, Bio-Rad) for neutralization, the Sep-pak C-18 cartridge (Waters) for elimination of labelled by-products, 0.22 μm membrane filter, and into a sterile vial. An additional 2ml of water is passed through in order to increase the recovery.

The radiochemical purity was over 98% and the radiochemical yield was 45 to 60 % (based on $^{11}CO_2$) with a synthesis time of 15 min. Over 100 mCi of the final product (EOS) are produced depending on 10 μA, 10 min irradiation condition.

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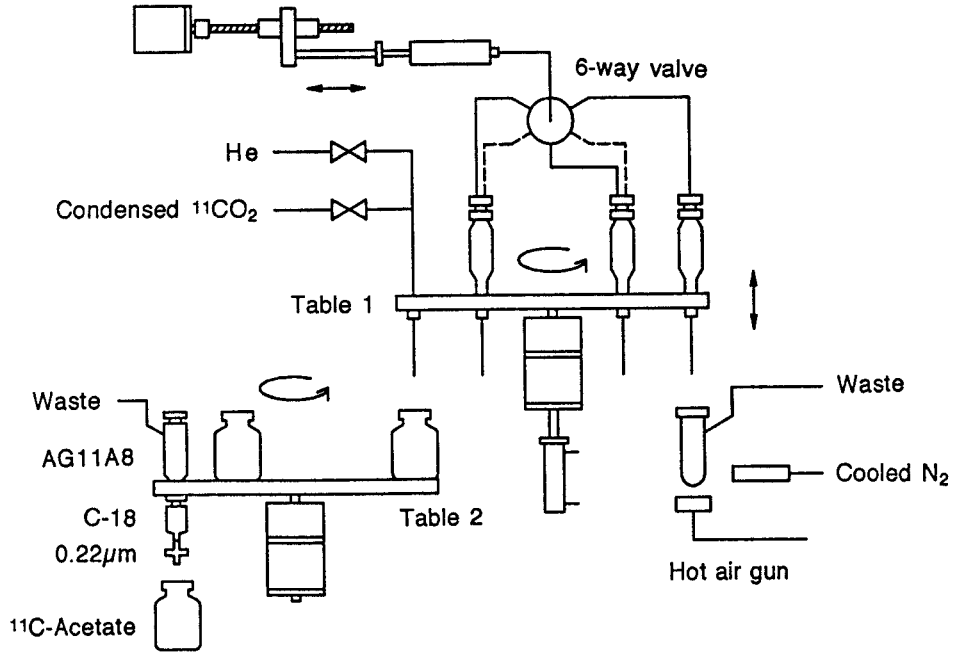
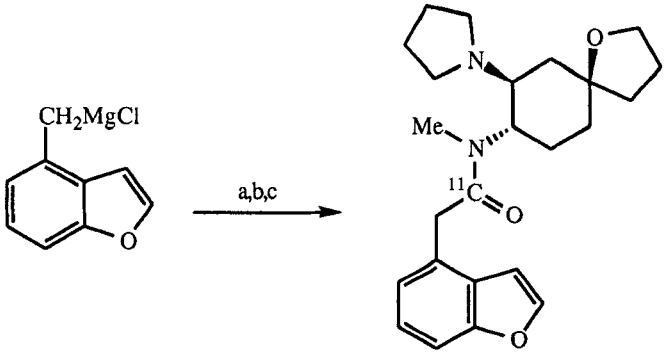


Fig. 1 Schematic diagram of the automated apparutus for ^{11}C-acetate

Synthesis of [Carbonyl-^{11}C]CI-977, a Potent κ-Opioid Receptor Agonist. SCRIPKO, J.G.; HUANG, C.C.; and KILBOURN#, M.R. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105 and #Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109.

CI-977 is a centrally active, highly selective κ-opioid receptor agonist of high potency. Because of the low phamacologically effective dose pharmacokinetic studies require radiolabeled drug of very high specific activities. Since neither carbon-14 nor tritium can achieve the required specific activity, the use of carbon-11 and application of positron emission tomography (PET) in pharmacokinetic studies of CI-977 in dogs offers a potentially useful technique for characterizing the disposition of the drug *in vivo*. While the presence of an N-methyl group suggests methylation with $^{11}CH_3I$, metabolic considerations led to the selection of a route incorporating the label in the carbonyl carbon. This is accomplished through a one-pot sequence of carboxylation of the appropriate Grignard reagent (4-benzofuranyl)methylmagnesium chloride, conversion to the acid chloride with thionyl chloride and amide formation, having a total synthesis time of 5 minutes. The only radiochemical impurity is the intermediate acid, which is removed by trapping the product on a C18-SepPak® and washing with base. Normal phase HPLC provides the final purification, with a total synthesis and purification time of less than 30 minutes.



[^{11}C] CI 977

a:[^{11}C]CO_2, THF/Et_2O, -40°; **b**: $SOCl_2$, THF; **c**: PD 130812, Et_3N.

The Synthesis of N-[methyl-^{11}C]-β,β-difluoromethamphetamine for the Investigation of the Transport and Binding Mechanisms of Biogenic Amines

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Chronic amphetamine abuse is known to cause symptoms resembling paranoid schizophrenia in laboratory animals and humans. In vivo measurement of the kinetics of amphetamine and related biogenic amines using positron emission tomography (PET) is therefore of considerable interest.

It has been suggested that the kinetics and binding of these amines may be influenced by both a polar and ionic interaction. Preliminary studies using dimethylbenzylamine and trifluorobenzylamine have been undertaken to investigate the contribution of pKa to the kinetics and binding of such amines[1]. We wish to extend this work by comparing the in vivo binding and kinetic characteristics of methamphetamine and β,β-difluoromethamphetamine by labelling with carbon-11. Methamphetamine has previously been labelled with carbon-11, by methylation of amphetamine with ^{11}C-methyl iodide, to give N-[methyl-^{11}C]-methamphetamine in good radiochemical yield[2].

Trans β-methylstyrene was brominated in good yield by refluxing in carbon tetrachloride with bromine (molar equivalent). The resulting dibromomethylstyrene was reacted with sodium azide in dimethyl sulphoxide followed by sodium hydroxide to give the vinyl azide, which was pyrolysed to yield 3-methyl-2-phenyl-1-azirine[3]. Fluorination with hydrogen fluoride-pyridine gave β,β-difluoroamphetamine (figure 1). Labelling with carbon-11 was achieved by reaction of this precursor with 11C-methyl iodide (figure 2). ^{11}C-methyl iodide was transferred in a stream of nitrogen to a solution of 6 mg β,β-difluoroamphetamine in dimethyl sulphoxide. The reaction mixture was analysed by reverse-phase HPLC using an Apex ODS column (250 x 4.6 mm), acetonitrile/water eluent, UV (254 nm) and radioactivity detectors. A decay corrected 32% radiochemical yield of N-[methyl-^{11}C]-β,β-difluoromethamphetamine (based on ^{11}C-methyl iodide) was recorded after 30m at room temperature. Optimised labelling conditions and purification procedures for in vivo studies will be presented.

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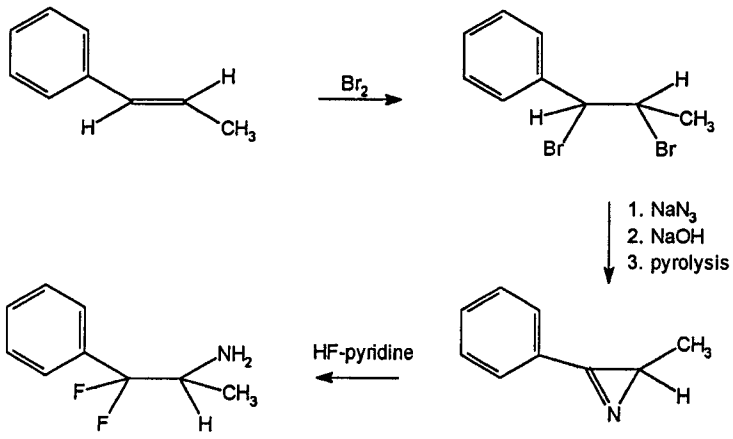
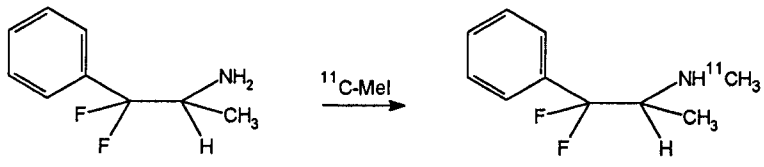
Figure 1



β,β-difluoroamphetamine

Figure 2



N-[methyl-^{11}C]-β,β-difluorometamphetamine

Synthesis of [^{18}F]- or [^{11}C]-1,2-diacylglycerol.

HAMMADI, A.; CROUZEL, C.; HINNEN, F.; and DEMPHEL, S. Service Hospitalier Frédéric Joliot, DRIPP, CEA, 91406 Orsay, France.

Diacylglycerols are the endogenous modulators of protein kinase C (PK. C) which is involved in many biological processes, including cellular signal transduction and proliferation[1]. A series of synthetic diacylglycerols with a variety of chain lengths are also able to bind to and activate PK. C[2]. Also, the free hydroxyl in the sn-3-position is required.

In order to study the PK. C activity in the brain by positron emission tomography, we have undertaken the synthesis of 1-palmitoyl-2-[1-^{11}C]acetylglycerol **1** and 1-[8-[^{18}F]-octanoyl]-2-[3-phenylpropanoyl]-glycerol **2**.

Synthesis of 1-palmitoyl-2-[1-^{11}C]acetylglycerol **1**

1-palmitoyl-2-[1-^{11}C]butyrylglycerol has been synthesized by Imahori et al[3] using [1-^{11}C]propyl ketene. In this paper, we report the synthesis of 1-palmitoyl-2-[^{11}C]acetylglycerol **1** by [^{11}C]acetylchloride as acylating agent.

The carbonation of methylmagnesium bromide in tetrahydrofuran with [^{11}C] carbon dioxide under nitrogen at room temperature and the addition of phtaloyl dichloride (PDC) affordes [^{11}C] acetylchloride[4]. The [^{11}C] acetylchloride released by heating (73°C) is carried by a slow stream of nitrogen into a methylene chloride solution of 1-palmitoyl-3-O-trityl-glycerol[5] and 4-dimethylaminopyridine (DMAP). 1-palmitoyl-2-[1-^{11}C]acetyl-3-O-tritylglycerol **3** is obtained after heating 10 min at 60°C.

The detritylation catalyst, boron trifluoride in methanol (BF_3-MeOH), is then added and the mixture is stirred 5 min at room temperature. Detritylation of **3** is carried out with BF_3-MeOH to prevent acyl migration[6]. The conversion of **3** to **1** is obtained with a yield of 90 %.

The mixture is then diluted with methylene chloride. HPLC separation of the product using a reversed phase C-18 column, eluted isooctane/isopropanol (90/10) yielded the labeled compound in 98% purity. Chemical purity is established by comparing the chromatograms of the [^{11}C] product with the chromatographic mobility of an authentic sample of **1** by refractometry. After collection of **1** by HPLC, the radiochemical yield is 10-15 % (corrected for decay). The total synthesis time from EOB is about 50 min.

Synthesis of 1-[8-[^{18}F]-octanoyl]-2-[3-phenylpropanoyl]-glycerol **2**

1-[8-(bromo)octanoyl]-2-[3-phenylpropanoyl]-glycerol **7** is synthesized by the steps illustrated in Scheme 1.

Fluorine-18 labeled **2** is obtained by nucleophilic aliphatic substitution of bromine with nca [^{18}F]fluoride. The reaction conditions are found to be 85°C for 15 min, using Kryptofix 2.2.2. and K_2CO_3 in acetonitrile. The mixture is then transferred onto a silica Sep-Pack cartridge. HPLC purification is performed on a C-18 μ-bondapack column with CH_3CN/H_2O (50/50 v/v) as elution solvent. The final product **2** is obtained with radiochemical yield of 10% (corrected for decay), total synthesis time of 80 min and with radiochemical purity > 98%.

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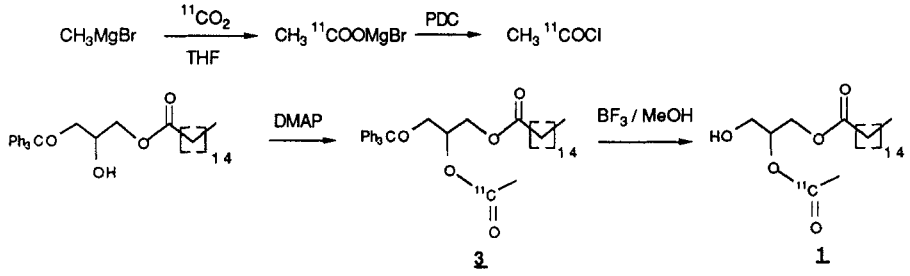
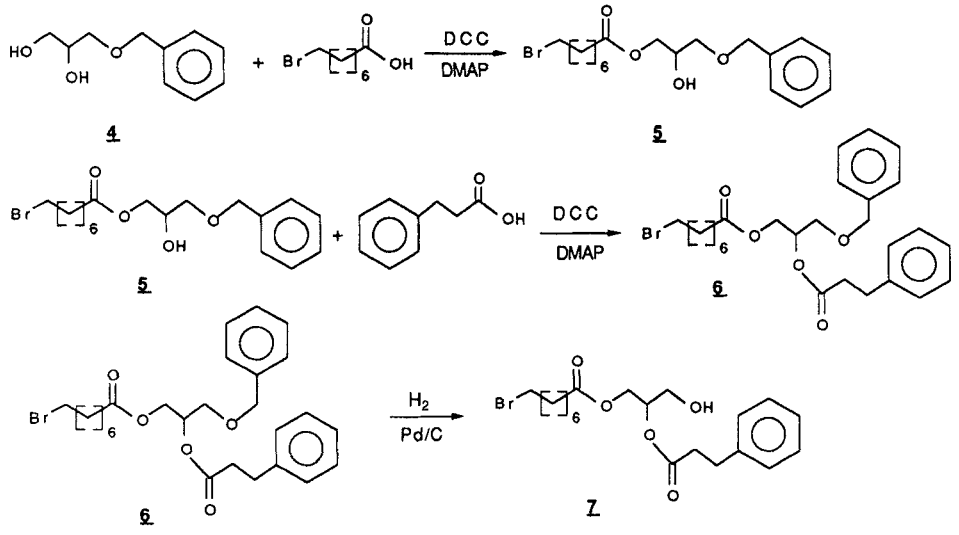
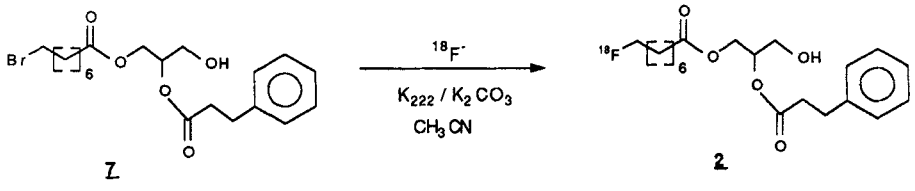
Synthesis of 1-palmitoyl-2-[1-^{11}C]acetylglycerol **1**



Synthesis of 1-[8-(bromo)octanoyl]-2-[3-(phenyl)propanoyl]-glycerol **7**



Synthesis of 1-[8-[^{18}F]-octanoyl]-2-[3-(phenyl)propanoyl]-glycerol **2**



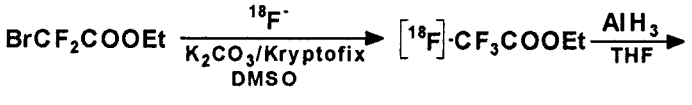
Synthesis of Fluorine-18 Labelled 2,2,2-Trifluoroethyl Triflate

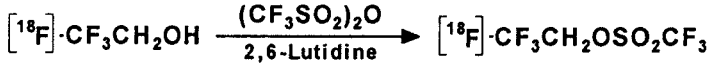
JOHNSTRÖM P.[1,3] and STONE-ELANDER S.[1,2] Karolinska Pharmacy[1], Box 60024, and Departments of Clinical Neurophysiology[2] and Psychiatry and Psychology[3], Karolinska Hospital and Institute, S-104 01 Stockholm, Sweden.

Alkyl halides and sulfonate esters have often been used as a means of introducing an alkyl group labelled with a positron-emitting nuclide in radiotracers for PET. To generate the ^{18}F-labelled analogs of these precursors, bifunctional substrates with the general structure X-$(CH_2)_n$-X (X= halide or sulfonate ester) have been used. The radiolabelling procedure thus contains two reaction step: first a nucleophilic incorporation of [^{18}F]F$^-$ and then alkylation of the desalkyl precursor (for examples see ref 1).

In an extension of this series of precursors, we have developed a method which enables the introduction of a ^{18}F-labelled 2,2,2-trifluoroethyl alkyl group. Since the alkyl group is unsymmetrical we could not use a bifunctional substrate of the type shown above. Instead a substrate was required with one end activated for the nucleophilic attack of [^{18}F]F$^-$ and the other end with a masked functionality which could be transformed into an appropriate leaving group for the subsequent alkylations. A method similar to those used in the preparation of ^{18}F-labelled esters previously reported (2-5) was chosen for this precursor synthesis. The appropriate haloester, ethyl bromodifluoroacetate, was available commercially. The ^{18}F-labelled product ester was subsequently reduced to the corresponding alcohol, which in this case we chose to transform to the corresponding triflate instead of the iodide due to its higher reactivity (6-8).

The synthetic method is shown in the scheme below. Ethyl bromodifluoroacetate was reacted with the Kryptofix/K$^+$ complex of [^{18}F]F$^-$ in DMSO. The ^{18}F-labelled ethyl trifluoroacetate was distilled into a THF solution of AlH_3 for almost instantaneous reduction to the corresponding alcohol. After hydrolysis and isolation by distillation, [^{18}F]-2,2,2-trifluoroethanol reacted with trifluoromethylsulfonic anhydride to generate the product triflate, which can readily be distilled from the reaction mixture for trapping in a solvent which is suitable for the alkylation reaction. Using this method, [^{18}F]-2,2,2-trifluoroethyl triflate has now been produced in a total radiochemical yield of 30-35%, decay-corrected with a total synthesis time of 40-45 minutes for the 3-step procedure. The radiochemical purity of the triflate isolated in this way is 90-95%.





In preliminary experiments to demonstrate the potential of [^{18}F]-2,2,2-trifluoroethyl triflate as a radiolabelling precursor, *ca.* 90% nearly quantitative conversions were obtained in reactions with benzylamine after 1 minute in acetonitrile using a prototype wave-guide microwave cavity (9). Investigations are now continuing on the use of this precursor to label more biologically interesting compounds for *in vivo* evaluation with PET.

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Improved synthesis and evaluation of (S,S)- and (S,R)-[^{18}F]-fluorocarazolol, ligands for the visualisation of ß-adrenergic receptors.

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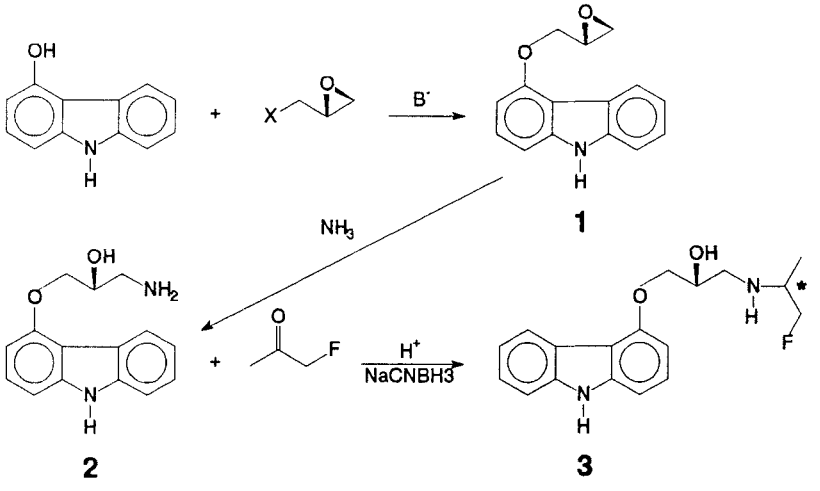
Myocardial ß-adrenoceptors are altered in hypertension and heart failure, while binding sites in the lung may be modified during airway infections, allergy and asthma. A suitable procedure for visualization and quantitation of cardiac and pulmonary ß-receptors by PET would therefore be of great clinical interest.

Our investigations was directed towards the synthesis of a [^{18}F]fluorinated ligand for the *in vivo* visualisation of ß-adrenoceptors[1,2].

Carazolol is a non-selective ß-blocking ligand with a high affinity for the ß-adrenergic receptors (K_D=0.01 nM). The compound has been labelled with ^{11}C and ^{18}F in the isopropyl group via a reductive alkylation by either ^{11}C-acetone[3] or ^{18}F-fluoroacetone[4,5] on the corresponding (S)-desisopropyl compound **2**. The synthesis of **2** has been described previously by Berridge *et al.*[3]. In the reaction step involving the coupling of (S)-glycidyl tosylate with 4-hydroxycarbazol[3], racemization can occur[6]. We developed an improved synthesis yielding the desisopropyl compound **2** with a higher enantiomeric excess. Changing to the better leaving group **X** = 3-nitrobenzenesulphonate yields the intermediate compound, epoxyde **1** with an e.e. of >97% compared to 88% using the tosylate. The optical purities were determined using a chiral HPLC-column, Chiralcel OD.



Two types of [^{18}F]fluoroalkylation can be applied for the synthesis of [^{18}F]fluorocarazolol **3**, i) with 1-[^{18}F]fluoroisopropyl tosylate[1,2] and ii) with [^{18}F]fluoroacetone[4,5].
The introduction of fluorine in the isopropyl group creates a new chiral centre resulting in the formation of two diastereomers, (S,S)- and (S,R)-[^{18}F]fluorocarazolol. Since receptor studies with PET have to be performed preferably with the pure stereoisomers, the diastereomers were separated using a Zorbax-NH_2 HPLC-column. Via this method both diastereomers could be obtained with a specific activity of 400-1200 Ci/mmol. In order to determine the binding properties of both diastereomers, tissue distribution studies have been performed in male Wistar rats. Both (S,S)- and (S,R)-diastereomers showed a high receptor mediated uptake in lung [DAR 12.0 $\pm$ 1.6 (unblocked), 1.5 $\pm$ 0.5 (blocked)] and heart [2.2 $\pm$ 0.4 (unblocked), 0.5 $\pm$ 0.1 (blocked)]. ß-Adrenoceptor uptake was also demonstrated in cerebellum, cortex, red blood cells, spleen and submandibular gland. However no significant differences were observed between the two diastereomers. These results suggest that the new created chiral center in the [^{18}F]fluoro-isopropyl group does not influence binding to the ß-receptor. Work is in progress to determine the K_D-values *in vitro* to confirm the before mentioned conclusion.

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An Efficient Synthesis of[*fluoroacetyl*-^{18}F]Fluoromelatonin and N^{ω}-[^{18}F]Fluoroacetylserotonin.

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The pineal gland hormone melatonin (N^{ω}-acetyl-5-methoxytryptamine) has recently received much attention because it is an important neuroendocrine component of animal physiology (1). Melatonin, a metabolite of serotonin,is produced *in vivo* by *N*-acetylation and *O*-methylation. Recently we reported the concise syntheses of [*carbonyl*-^{11}C]melatonin and *N*-[*carbonyl*-^{11}C]acetylserotonin as imaging agents (2). The introduction of ^{18}F (β^+ decay, $t_{\frac{1}{2}}$=110min) at the terminal position of melatonin or *N*-acectylserotonin is attractive for diagnostic imaging agents in positron emission tomography (PET) study.

As part of the investigation of the synthesis of positron emitting compounds for PET study, the rapid syntheses of [*fluoroacetyl*-^{18}F]fluoromelatonin (N^{ω}-[^{18}F]fluoroacetyl-5-methoxytryptamine)(1) and N^{ω}-[^{18}F]fluoroacetylserotonin(2) will be reported.

Unlabelled fluoromelatonin (N^{ω}-fluoroacetyl-5-methoxytryptamine) (3), substituted in the side-chain of melatonin, was prepared from 5-methoxytryptamine (4) with fluoroacetic acid by the ordinary method using dicyclohexylcarbodiimide (DCC). The yield of (3) based on (4) was 25%. N^{ω}-Fluoroacetylserotonin (N^{ω}-fluoroacetyl-5-hydroxytryptamine)(5) was also prepared from 5-hydroxytryptamine(6)(30% yield). These synthetic pathways are shown in Fig. 1. We recently established the one-pot synthetic method for the introduction of a [^{18}F]fluoroacetyl group into aminosugar (3,4). The method is a combination of halogen exchange, alkaline hydrolysis, and condensation. This was applied to the syntheses of (3) and (1) with some modifications. The one-pot synthesis from potassium fluoride and ethyl bromoacetate gave (3) in a 10% yield based on (4).

[^{18}F]Fluoride was produced by the ^{18}O(p, n)^{18}F nuclear reaction from a circulating 20%-enriched [^{18}O]water target using the Tohoku University Cyclotron (5). The ^{18}F nuclide thereby formed was converted to potassium [^{18}F]fluoride with potassium carbonate. After addition of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosan (Kryptofix 222), the resulting mixture was submitted to the one-pot synthesis to afford the hormone (1) in a 6.9% radiochemical yield (decay-corrected, based on [^{18}F]fluoride). The total time required for synthesis of (1) is *ca.* 90 min. The radiochemical purity is >95%.

As the reproducibility of the yield was invariably poor, the improvement was obtained by change of the substrate. The reaction of [^{18}F]fluoride with ethyl *p*-toluensulfonyloxyacetate derived from ethyl bromoacetate with silver *p*-toluensulfonate afforded ethyl [^{18}F]fluoroacetate, which was then hydrolyzed with alkali and condensed with (4) in the presence of DCC to give (1) in a 14.2% radiochemical yield. The purity and the specific activity(EOB) are >98% and 540 mCi/μmol, respectively. The treatment of (6) in an analogous fashion gave 13.5% radiochemical yield of (2). The synthesis time and the radiochemical purity of (2) are *ca.* 90 min and >98%, respectively.

The present work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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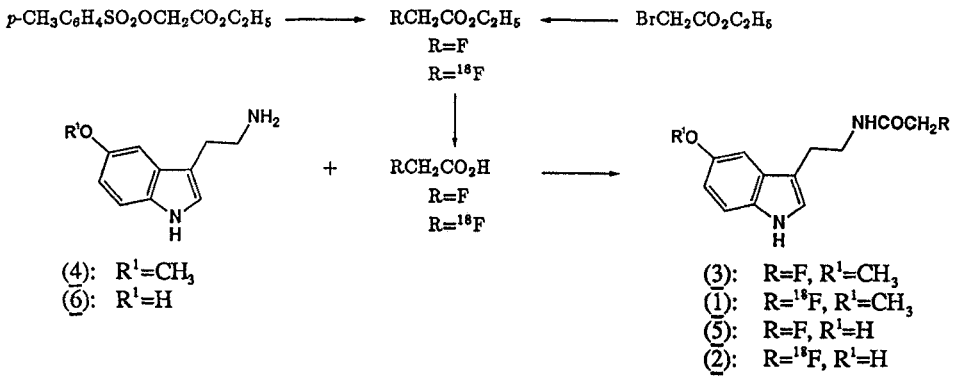

Fig. 1. The synthetic pathways of (1), (2), (3), and (5).

RADIOFLUORINATION OF ALKENE WITH NCA [^{18}F]FLUORIDE AND BENZENESELENENYL BROMIDE.

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Arylselenides are convenient precursor for alkenes. When selenides are oxidized under the mild condition (e.g., H_2O_2, $NaIO_4$, O_3 or peracids), slenoxide groups thus afforded will be generally cleaved to form double bond. Arylselenides can be prepared with either nucleophilic selenium reagent (PhSeNa) or electrophilic selenium reagent (PhSeBr or PhSeCl). Recently, several authors described the fluoroselenenylation of alkenes as shown in Table 1. All synthesis includes *in situ* formation of benzeneselenenyl fluoride equivalent followed by its electrophilic addition reaction across double bond. We tried to use this fluoroselenenylation in radiofluorination using no carrier added (NCA) [^{18}F]fluoride.

Table 1 Reported Methods for Fluoroselenenylation

Reference	Source of F	Se Reagent	Solvent	Reaction Condition
(1)	AgF	PhSeBr	CH_2Cl_2	Sonication at 5~10℃, 1h
(2)	AgF	PhSeCl	CH_3CN	R.T., 18h
(3)	Et_3N/3HF	N-Phenylselenophthalimide	CH_2Cl_2	25℃, 7~30h
(4)	XeF_2	PhSeSePh	CH_2Cl_2	-20~20℃, 5~30min

In this preliminary observation some salts of [^{18}F]fluoride were tested for [^{18}F]fluoroselenenylation of 4-allylanisole together with benzeneselenenyl bromide in CH_2Cl_2. Proton irradiation of $H_2{}^{18}O$ was carried out using a small medical cyclotron (MCY-1750, Shimadzu Corp., Japan) and circulating water target system (NKK Corp., Japan). Small amount of [^{18}F]fluoride was mixed with reagent shown in Table 2 in a pyrex test tube and dried. After cooled to room temperature, solution of benzeneselenenyl bromide (50μmol) in CH_2Cl_2 was added dropwise following 4-allylanisole (100μmol) in CH_2Cl_2. The reaction mixture was stirred at room temperature for 30 min and the formation of radioactive adduct was determined with radio TLC.

Results are summarized in Table 2. Uneyama and Kanai reported that PhSeF equivalent reacted with olefins immediately at low temperature[(4)]. The methods in the other reports, however, required longer reaction time or ultrasound activation. In our experiment with Ag[^{18}F], that was prepared from cold AgF and [^{18}F]fluoride solution, the reaction underwent rather fast and no progress was observed after 30 min. Similar radiochemical yield was obtained with the ultrasound irradiation of the same reaction mixture at ice temperature, but subsequent sonication at temperature up to 40℃ decomposed the product. For the preparation of Ag[^{18}F] of NCA state, [^{18}F]fluoride solution was mixed with $AgOCOCH_3$. As competition of the two nucleophiles, fluoride and acetate, toward the intermediate selenirenium cation is expected, excess amount of benzeneselenenyl bromide and alkene were used. But no reaction was observed. The same result was obtained for $KOCOCH_3$ with and without aminopolyether (APE) as a phase-transfer catalyst. When NCA [K/APE]$^+$[^{18}F]$^-$ was

used, a considerable amount of [^{18}F]fluoroselenenyl adduct was produced. This radiofluorinated product of high specific activity was suggested to be less stable than the compound of carrier added state, as TLC showed tailed radioactivity disribution other than the peak. Tomoda and Usuki also reported that fluoroalkyl selenides were unstable under the silica gel chromatographic condition(1). Although we tried for comparison another preparation of fluoroselenenyl adduct of carrier added state using KF as a carrier fluorine, the radiochemical yield was significantly reduced.

When H_2O_2 was added to the reaction mixture, more than 90% of the obtained radioactive product was decomposed rapidly to the more polar compound as it was after the addition of water.. McCarthy *et al*. reported that transformation of fluoroselenide to fluoroolefin was only achieved by ozone oxidation in CCl_4(2), although Uneyama and Kanai reported the clean oxidative deselenenylation with H_2O_2(4). When efficient method for this deselenenylation was established, the new method of radiofluorination described here would be a favorable route for the preparations of radiopharmaceuticals, as it would deliver fluoroalkenes of high specific activity from alkene precursors without any chemical modifications to them.

Table 2 Radiofluorination of Alkene with [^{18}F]Fluoride and Benzeneselenenyl Bromide

[^{18}F]Fluoride	Radiochemical Yield[a)]
Ag[^{18}F], AgF(10μmol)	29%
Ag[^{18}F], $AgOCOCH_3$(10μmol)	No Reaction
[K/APE[b)]][^{18}F], KF(10μmol)	Trace
[K/APE[b)]][^{18}F], K_2CO_3(5μmol)	14%
[K/APE[b)]][^{18}F], $KOCOCH_3$(10μmol)	No Reaction

[a)]Radiochemical yields of [^{18}F]fluoroselenenyl adducts were measured with radio TLC. [b)]Twenty μmol of Kryptofix 222 (Merck, Germany) was used.

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The Combined Use of Microwave Heating and Photolysis in the Synthesis of [^{18}F]-Substituted Benzamides.

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Benzamides have been labeled and utilized for imaging of dopamine D_2 receptors,[1,2] as inhibitors of acetyl-cholinesterase[3], imaging of sigma receptors[3] and as agents for imaging melanomas[4,5].

We have combined the short reaction times of microwave heating and photolytic reactions in the preparation of F-18 substituted benzamides. Our method was based on a report indicating the direct conversion of aldehydes to acid bromides using N-bromosuccinimide (NBS) in the presence of light.[6] The intermediate acid bromides were converted in situ to the benzamide by condensation with the desired amine. The chemical transformations involved are given in Figure 1.

The NO_2^- for $^{18}F^-$ nucleophilic displacement in several commercially available aromatic aldehydes has been previously reported[7-9]. Our standarized procedure involved the resolubilization of $^{18}F^-$ in the usual manner[10] (K 2.2.2/K_2CO_3/CH_3CN) using DMSO; the ^{18}F/Kriptofix was added to a Reactivial™ containing 0.01 mM of the desired nitro-substituted benzaldehyde. The mixture was heated in a microwave oven at 500W power for 3-5 minutes and the desired product isolated by C-18 SepPak as a dry CCl_4 solution. After the addition of about 0.11 mM of freshly recrystallized NBS, the flask was purged with nitrogen and a light source (150 watt flood lamp) was shined on the flask with stirring. The mixture was heated briefly (< 1 min) while continuing the illumination for a total of 4-7 minutes. Upon cooling, the intermediate acyl bromide was converted in situ to the desired benzamide. Typically, about 0.10 mM of the amine was quickly added and the mixture stirred for about 10 minutes at RT; formation of the benzamide was confirmed by HPLC and TLC vs co-elution with the authentic standard (see Table 1).

As an example, we have converted 4-NO_2-benzaldehyde to [^{18}F](2-piperidinylaminoethyl) 4-Fluorobenzamide (1A) and to [^{18}F](diethylaminoethyl) 4-Fluorobenzamide (1B) in a synthesis time of 40 min before HPLC purification with overall yields of 50-60%, and radiochemical purities >97%. The effect on the yield of different substituents in the benzaldehyde ring (CH_3, CH_3O, NO_2) will be discussed. We are presently investigating the use of photolysis to convert [^{18}F]-substituted heterocyclic aldehydes to the desired benzamide.

This work was supported by NIH Grant Number HL13851.

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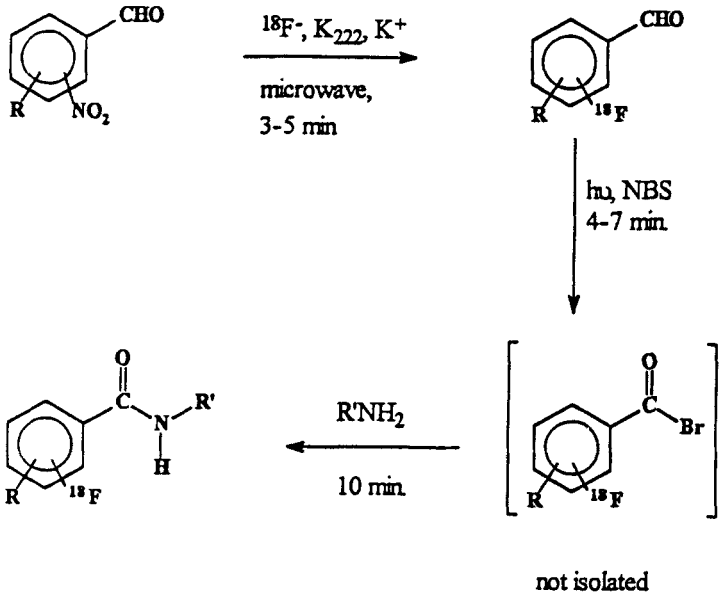
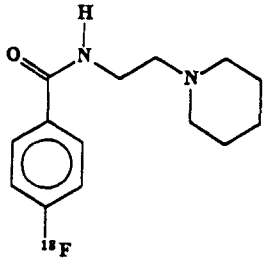


FIGURE 1.

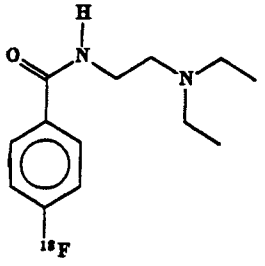
TABLE 1

Precursor	^{18}F-Product (%)[1]	hν (min)	Benzamide (%)[2] R'=A	R'=B
$4NO_2$-C_6H_4CHO	4^{18}F-C_6H_4CHO	4	80	85
	(60 - 70)	7	95	91
$2NO_2$-C_6H_4CHO	2^{18}F-C_6H_4CHO	4	80	83
	(70 - 80)	7	96	90
2,6diNO_2- C_6H_3CHO	2^{18}F, $6NO_2$-C_6H_3CHO	4	60	65
	(50 - 70)	7	84	82

[1]Reactions performed in a microwave oven at 500 Watts power for 4 minutes in DMSO; (%) are isolated yields.
[2]Yields (%) are based on TLC/HPLC analysis. A = 1-(2-Aminoethyl) piperidine and B = N,N - Diethylethylenediamine



1A



1B

A Safety Interlocked Remote System for the Synthesis and Reactions of [^{18}F]Fluorobenzyl Iodide. MACH, R.H.; MORTON, T.E.; EHRENKAUFER, R.L.E. Department of Radiology/PET Center, Bowman Gray School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157, USA.

[^{18}F]Fluorobenzyl Iodide has proven to be a valuable new prosthetic group for the labeling of PET agents *via* N-alkylation [1,2]. Specifically, we have developed the synthesis of several new, selective, high afinity [^{18}F]labeled postsynaptic dopamine ligands for potential use in PET quantitation of the D_2 receptor [3,4,5]. One of these ligands, [^{18}F]MBP, a benzamide, is currently in routine use in our laboratories for animal studies. Thus, we have developed a remote system to accommodate the demands of routine production and have incorporated safety features to ensure "containment" of isotope release in the case of system failure (Figure).

[^{18}F]Fluorobenzyl Iodide is prepared by nucleophilic [^{18}F]fluoride displacement of 4-trimethylammonium benzaldehyde triflate (RV1). The resulting [^{18}F]fluorobenzaldehyde is reduced to the [^{18}F]fluorobenzyl alcohol (RV2) and treated with hydriodic yielding the [^{18}F]fluorobenzyl iodide (RV2). Benzylation reactions with isolated [^{18}F]fluorobenzyl iodide are carried out in RV3.

Table. [^{18}F]Fluorobenzyl iodide and [^{18}F]MBP Yields

% [^{18}F]Fluoride incorporation[1]	% Conversion to [^{18}F]FBI[2]	% Yield [^{18}F]FBI[3]	% Yield [^{18}F]MBP[4]
73.9±12.4 (n=5)	52.4±5.6 (n=4)	40.5±10.0 (n=16)	16.2±7.8 (n=5)

1 % [^{18}F]Fluoride incorporation yielding [^{18}F]Fluorobenzaldehyde (EOB).
2 % [^{18}F]Fluorobenzaldehyde conversion to [^{18}F]Fluorobenzyl iodide.
3 % [^{18}F]FBI from [^{18}F]Fluoride (EOB).
4 % [^{18}F]MBP from [^{18}F]Fluoride (EOB). Specific activity (EOB) of [^{18}F]MBP was variable and averaged about 800mCi/μmol (range 284-2054mCi/μmol).

Attributes of the system are: 1) it is compact (45 cm high x 60 cm wide x 45 cm deep) allowing for complete lead enclosure in less than 1/2 the area of a 5 ft. fume hood. 2) The system integrity is maintained with charcoal traps on all vents to contain volatile components and minimize releases. 3) Due to the minimization of releases, the accountability of the starting radioactivity is about 95%. 4) The system is configured to fail in a "safe" or containment mode. 5) Valving sequences for synthesis steps are controlled by interlock switches. System performance yields of [^{18}F]MBP and intermediate [^{18}F]Fluorobenzaldehyde and [^{18}F]Fluorobenzyl iodide are shown above in the Table.

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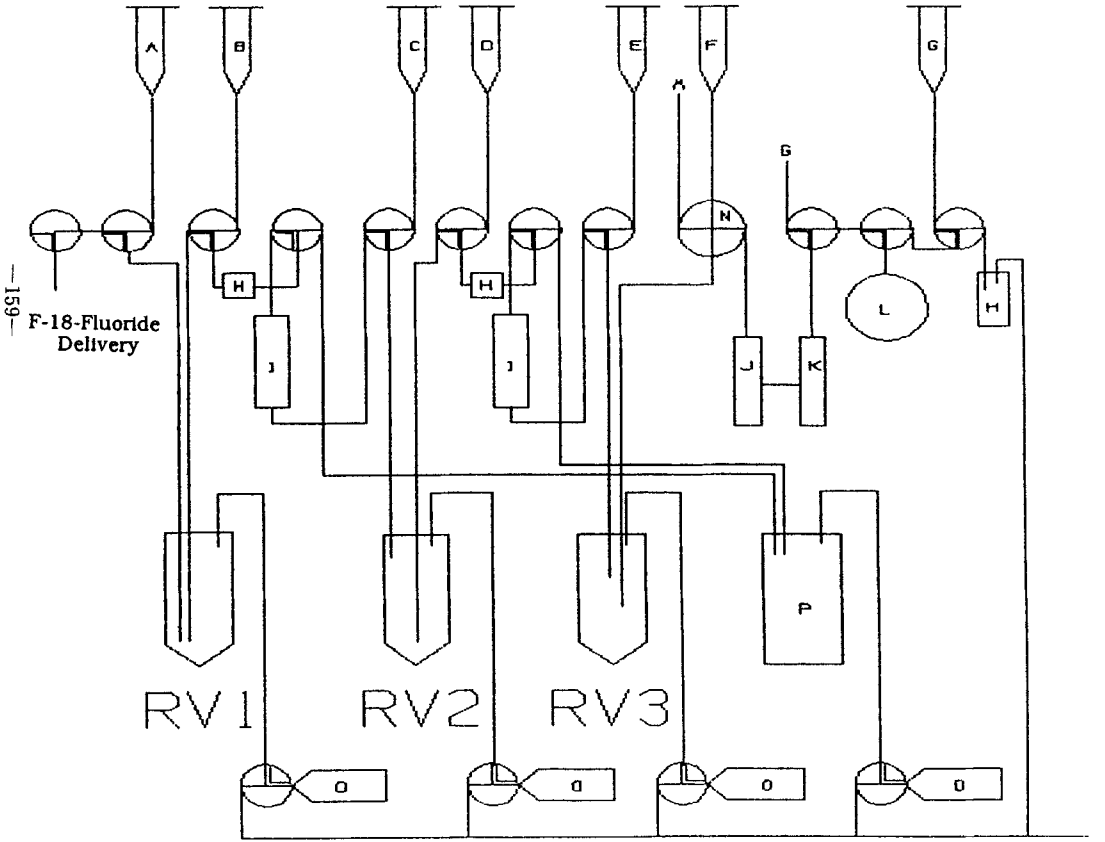
F-18-FLUOROBENZYL IODIDE REMOTE CHEMISTRY SYSTEM



KEY

A-Solvent to RV1
B-Solvent to SepPak1
C-Solvent to RV2
D-Solvent to SepPak2
E-Solvent to RV3
F-HPLC Loop Loading Syringe
G-Solvent to Rotovapor
H-SepPak
I-Drying Tube
J-UV Detector (HPLC)
K-Radiation Detector (HPLC)
L-Rotovapor
M-HPLC Pump
N-HPLC Injector
O-Charcoal Trap
P-Chemistry Waste
Q-HPLC Waste
R-Product Vial

Three Way Valve

Robot-Assisted Synthesis of [^{18}F]Altanserin, 4-[^{18}F]Fluorotropapride, 6-[^{18}F]Fluoro-L-Dopa and 2-[^{18}F]Fluoro-L-Tyrosine

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For high-level routine production and development of new molecules, a robotic system that is operated automatically is more desirable than remote control synthesis methods since it appears to be more versatile (1). For that reason, a robotic system has been installed in our Cyclotron Research Center of the University of Liège. It has been configurated and programmed to synthesize a large variety of ^{18}F-radiopharmaceuticals such as [^{18}F]altanserin, 4-[^{18}F]fluorotropapride, 6-[^{18}F]fluoro-L-dopa and 2-[^{18}F]fluoro-L-tyrosine by n.c.a. nucleophilic fluorination. [^{18}F]Altanserin has been labelled by nucleophilic substitution in the corresponding nitro compound and purified by HPLC (2). Tropapride has been labelled with fluorine-18 at the position 4 of its benzylic group by reductive amination in presence of 4-[^{18}F]fluorobenzaldehyde and norbenzyl precursor (3). The n.c.a. enantioselective synthesis of 6-[^{18}F]fluoro-L-dopa and 2-[^{18}F]fluoro-L-tyrosine is based on the nucleophilic substitution in the corresponding trifluoromethane benzaldehyde triflate (4). The other steps of this multi-step synthesis require the conversion of the ^{18}F-fluorinated aldehydes to the corresponding benzyl iodide [by treatment with diiodosilane (DIS)], alkylation of (S)-Boc-BMI with this electrophilic agent, hydrolysis and HPLC purification (5).

Components of the system.

The robot, used for the synthesis of the previously cited molecules is a Zymate II Laboratory Robot (Zymark Corp.). The software controlling the system is written in the Easylab Plus language, and for the home-made modules, routines were written using this language. Several components were acquired from the Zymark firm, and other modules, more specific to the synthesis of the cited radiopharmaceuticals were fabricated "in house". The components were arranged in a 360° arc around the robot arm and were mounted in sectors positioned around the Zymate II Core System. All the system was set up on a shielded bench top (150 x 175 cm). Located beneath the robot are the Power and Event Controller as well as the multiplexer also developed " in house" which allows the control of 64 outputs for the control of valves and other modules. The controller and the HPLC system are also located beneath the bench top. A block diagram of the Zymate system is shown in Figure 1. In the legend, the modules marked Zymark were purchased from Zymark Corporation while the modules developed in Liège are marked CRC. The different steps of the syntheses as well as their location in the various sectors are shown in Figure 2.

Labelling of [^{18}F]altanserin as an example.

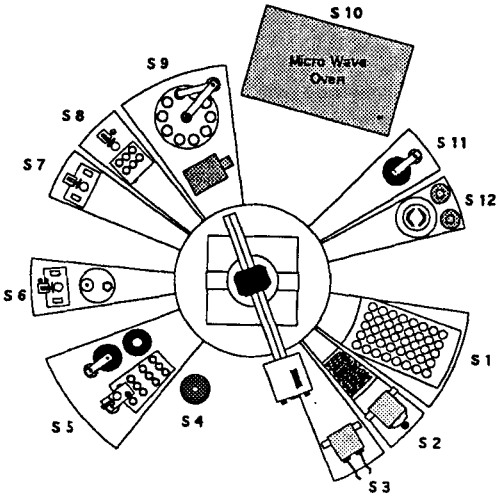
As an example of robotic synthesis, the preparation of [^{18}F]altanserin is described since this radiochemical synthesis requires many of the chemicals steps needed to produce the other radiopharmaceuticals previously cited. During the irradiation, the robot initialisation program is loaded into the robot controller and executed. It allows to prepare the modules of the robotic system with all the necessary components (vials, tips, tubes,..), solvent and reagents needed for the production of the [^{18}F]radiopharmaceutical. To continue, manual validation of each step of the program with the keyboard is required. At the end of bombardement, the main program is loaded. No-carrier-added ^{18}F$^-$ is produced in a Ni target by the ^{18}O(p, n)^{18}F nuclear reaction on ^{18}O-enriched water (45%). At the end of bombardement (1 h, 10 μA), the ^{18}F-activity (300-350 mCi) is transferred under nitrogen gas pressure (10 psi) through 30 m of Teflon^{TM} tubing to the robot room (Sector 5, Figure 1). The activity is trapped on a Dowex 1 X 8 anion exchange resin and the ^{18}O-enriched water recovered in a receiving vial placed in Sector 5. A conical glass vessel containing kryptofix and potassium carbonate is then substituted for the ^{18}O-enriched water vial which is capped in the capping station (Sector 12). [^{18}F]fluoride is then eluted from the resin by K_2CO_3 solution. Conversion of the ^{18}F-fluoride ion to its potassium/kryptofix salt in an anhydrous organic solvent (DMSO) is achieved in the oven in the Sector 5. For that purpose, the ^{16}O-water is evaporated to dryness under nitrogen (120°C). The end of evaporation is detected automatically with an optical probe and a feedback signal is sent to the robot's

computer which then allows the synthesis to continue. The Pipetting Hand is then selected and the $[K/222]^{+}{}^{18}F^{-}$ complex is dried by three successive additions of acetonitrile and evaporation. The nitro precursor of altanserin in DMSO, previously placed in the rack of Sector 5, is aspirated in the tip and added to the dry residue. The probe is then moved up. The vial is capped in the capping sector with the General Purpose Hand and introduced into the microwave oven. After labelling, the door of the microwave is opened and the vial placed either in a lead pig (Sector 4, assessment of the activity), or uncapped for subsequent Sep-Pak^{TM} pre-purification. By using the Pipetting Hand, the DMSO solution is diluted in the glass tube n°1 in the rack in Sector 1 which contains 0.5 N HCl. The tube containing the activity is poured into a 50 ml seringue (previously filled with 0.5 N HCl) whose lid is pneumatically actuated. The solution is then pushed through the C-18 Sep-Pak cartridge with a slight flow of nitrogen. The level of liquid is detected automatically with two Pt electrodes. Before dryness, the lid is moved up and the support washed with water (tube 2, Sector 1). [^{18}F]altanserin is finally eluted with a CH_3OH/THF (75/25) solution. The organic solution is collected in a conical vial and injected onto the HPLC column. A home-made fraction collector has been developed (Sector 9). For formulating the injectable solution, the solvent containing the radioactive peak is diluted with water and passed through a C-18 Sep-Pak^{TM} cartridge (Sector 7). After washing with water, [^{18}F]altanserin is eluted with ethanol and filtered through a Millex GV to insure sterility. Saline solution is then added. The robotic preparation of [^{18}F]altanserin takes a similar time (120 min.) to a remotely control system and gives a similar radiochemical yield (25 % decay corrected to E.O.B.).

Others radiopharmaceuticals.

The software written for the [^{18}F]altanserin synthesis can be used for labelling other ^{18}F-radiopharmaceuticals with only minor modifications. Indeed, the flexibility of the Easilab Plus programming language easily allows any modification needed. For the synthesis of 6-[^{18}F]fluoro-L-dopa and 2-[^{18}F]fluoro-L-tyrosine other specific home-made workstations are required such as halogenation, evaporation, alkylation and hydrolysis racks. Moreover, the HPLC system can be modified to accept an additional C-18 column. All the modifications specific to the new radiosynthesis can easily be added to the main program to adapt it to the new preparation. Nowadays, the application of robotics is not limited to the routine preparation of these four radiopharmaceuticals for PET studies. The set up of the labelling reaction and of the purification steps of several new other[^{18}F]fluorinated compounds can be easily performed the same day with all the hardware presently available on our robotic system.

Figure 1. Description of the differents sectors around the Zymate II Core System.



S1. Rack containing disposable glass tubes (Zymark)
S2. Pipetting Hand and tips (Zymark)
S3. General Purpose Seizing Hand (Zymark)
S4. Lead pig (CRC)
S5. Column for the separation of ^{18}F from the irradiated water, a rack with capped reagents vials, two ovens, one equipped with an optical level probe (CRC)
S6. C-18 Sep-Pak column chromatography system (CRC)
S7. Sector for formulating the injectable solution (CRC)
S8. Home made silica chromatography system and rack for the preparation of DIS and benzyl iodide derivatives (Dopa and Tyrosine)(CRC)
S9. HPLC injector and fraction collector (CRC)
S10. Microwave oven (CRC)
S11. Oven for the hydrolysis step of fluorodopa and tyrosine (CRC)
S12. Capping Pysection providing capping and uncapping of round, screw cap containers (Zymark)

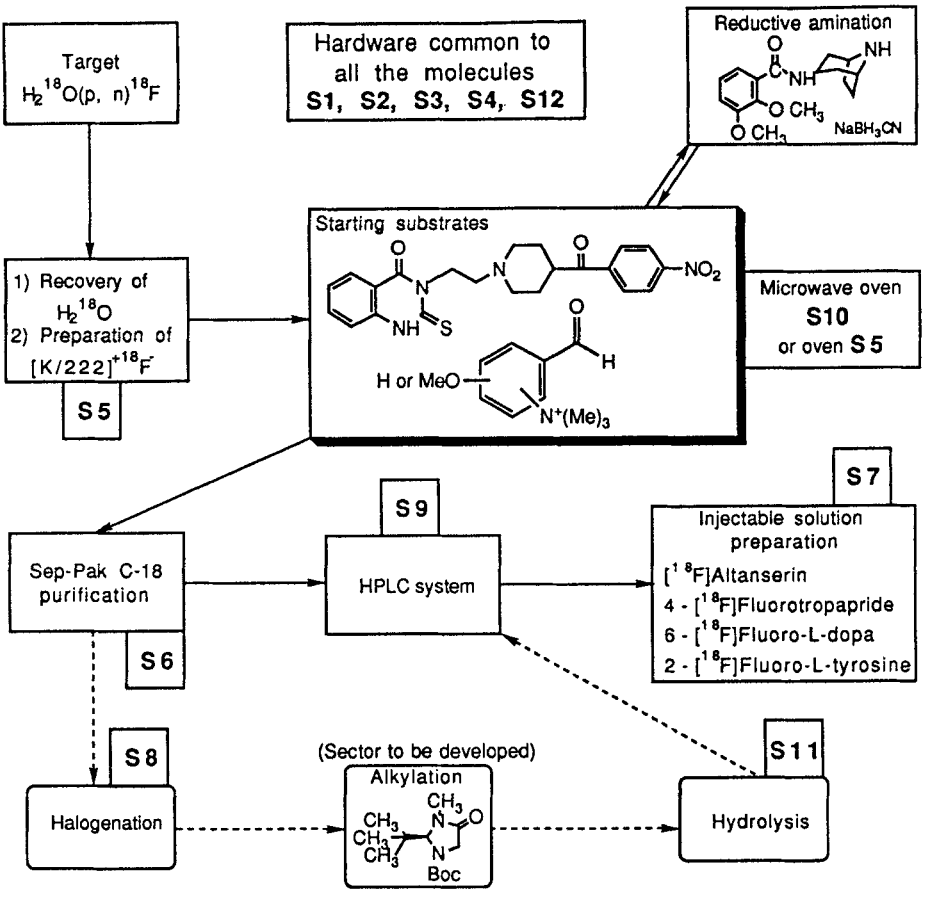


Figure 2. Different steps of the syntheses with their location in the various sectors

Common steps to all the syntheses

Additional steps for the preparation of the amino acids (6-[18F]fluoro-L-dopa and 2-[18F]fluoro-L-tyrosine)

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Nucleophilic [^{18}F]Radiofluorination Using Microwave Cavity: Application to [^{18}F]FDG and Aromatic [^{18}F]Fluoro Amino Acids Synthesis.

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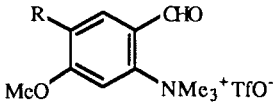
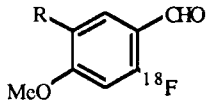
During the past decade, microwave technology has received considerable attention in organic chemistry[1] and radiochemistry[2,3] primarily due to substantial rate increase and time gain. Most of the examples described are reactions done with domestic microwave ovens. Space and shielding requirements, difficulties in integrating them in an automated synthesis system and poor reproducibility were the major concerns for their use in radiochemistry. We here describe a system in which the microwave cavity (10 cm diameter) is connected to the magnetron (2.45 GHz, 0-500 W variable intensity) thanks to a coaxial cable (Figure 1). The cavity can accommodate vials up to diameters of 20 mm. The main feature of the microwave heating system is the possibility to heat different solvents without changing the parameters of the cavity.
For [^{18}F]FDG production, the three major steps (drying K[^{18}F]F/K$_{222}$, labelling of the mannose triflate and HCl hydrolysis)[4] are done in the microwave cavity. Higher radiochemical yields (65-70%, EOB corrected) and shorter synthesis time (25-30 min) are the major advantages compared to thermal heating.
The microwave heating system has been used for the synthesis of 6-[^{18}F]fluoroveratraldehyde (2a) and 2-[^{18}F]fluoro-4-methoxy benzaldehyde (2b), two important intermediates in the asymmetric synthesis of 6-[^{18}F]fluorodopa and 2-[^{18}F]fluorotyrosine described recently by Lemaire.[5,6] Starting from the quaternary amines 1a and 1b, 6-[^{18}F]fluoroveratraldehyde (2a) and 2-[^{18}F]fluoro-4-methoxy benzaldehyde (2b) have been obtained in 60% and 70 % radiochemical yield respectively (corrected to EOB) in 20 min and with a chemical purity higher than 85 %.





1a: R = OMe
1b: R = H

2a: R = OMe
2b: R = H

Reduction-iodination of the aldehydes 2a and 2b with DIS, reaction of the iodinated intermediates with the Boc-BMI enolate, hydrolysis with HI and HPLC purification provide 6-[^{18}F]fluorodopa and 2-[^{18}F]fluorotyrosine respectively in less than 70 min (from the EOB) in 10-15 % radiochemical yield. A remote, semiautomated system including the microwave system has been developed for the production of these two amino acids.
In conclusion, the microwave cavity described, which can be easily integrated in an automated synthesis system, provides several advantages in terms of shielding, space requirement, radiochemical yield, synthesis time and control of the reaction parameters.

Acknowledgments: The authors thank the FNRS for financial support (grant # FRSM-LN 9.4519.91).

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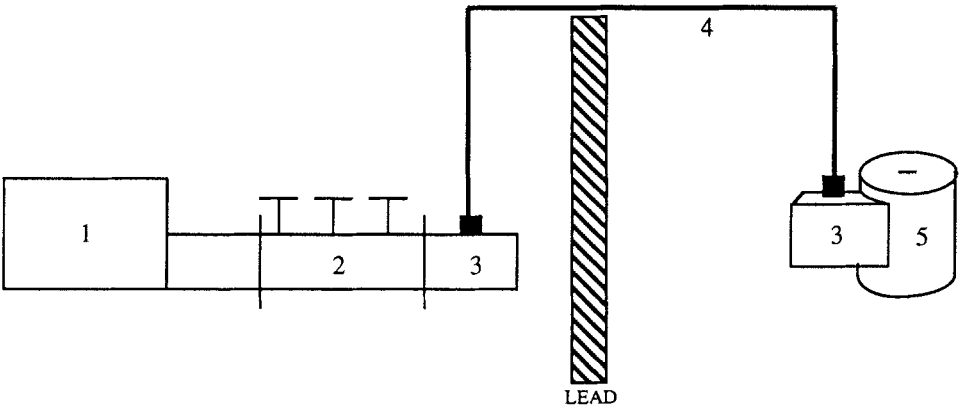

1. microwave generator
2. 3 stub tuner
3. WR340-coaxial adaptors
4. high power coaxial cable
5. cavity with a flat characteristic

Figure 1. Microwave heating system.

Automated Synthesis of [^{18}F]-FDG using Tetrabutylammonium Bicarbonate. M.J Adam, P.A. Culbert, T. Hurtado, J. Huser, G.A. Gschwandtner, S. Jivan, D. Morris, T.J. Ruth, and S.K. Zeisler, TRIUMF/UBC PET Program, University of British Columbia, Vancouver, B.C. Canada.

The synthesis of [^{18}F]-FDG reported by Johnson[1] et al has been successfully modified to accomodate automation using the Optomux® control system. The most noteworthy change is the addition of a small ion exchange column (Bio-Rad AG 1X8, 100-200 mesh, HCO_3^- form, 10 mg) which serves to quantitatively (>99%) trap [^{18}F]-F^- from the target water. The [^{18}F]-fluoride can then be quantitatively eluted using tetrabutylammonium bicarbonate (93 mM in 20% aqueous actonitrile, 1 mL). This column can be reused again after washing without loss of function. The tetrabutylammonium ion then serves as a phase transfer catalyst for the reaction of [^{18}F]-fluoride with the manno-triflate precursor. After fluorination the solvent was evaporated and HCl added to the same vessel and heated to remove the acetate groups. The crude product was then filtered through a small cation exchange column (Amberlite IR 120, H^+ form, 1 g), an ion retardation column (AG 11 A8), C-18 and Alumina Sep Paks, and finally through a membrane filter. The tetrabutylammonium phase transfer catalyst is easily removed with the first cation exchange column.

In order to monitor the trapping of ^{18}F-fluoride on the ion exchange resin and its elution into the reaction vessel, a small radiation detector located approximately 1 cm from the column is used. It consists mainly of a large area photodiode connected to a dual FET op-amp circuit which delivers a signal suitable fo processing in the Optomux system[2]. When the aqueous solution containg the [^{18}F]-fluoride passes through the ion exchange resin, the detector signal steadily rises until the collection of the activity is finished. The display screen gives a reliable reading of one unit per mCi which is sufficient for process control and yield estimation. After eluting the resin the detector signal drops quickly to the initial value indicating complete elution of the fluoride. These detectors are also located at the reaction vessel and the product vial.

Yields to date have been excellent (30-35% uncorrected, 60 minutes synthesis time). The recycle time for multiple syntheses is on the order of 30 minutes with no additional radiation exposure to the operator. The primary advantages of this new method are the reproducible yields, elimination of the toxic Kryptofix® 2.2.2., the ease of recovery of [^{18}O]-water and the avoidance of on-column heating of ion-exchange resins.

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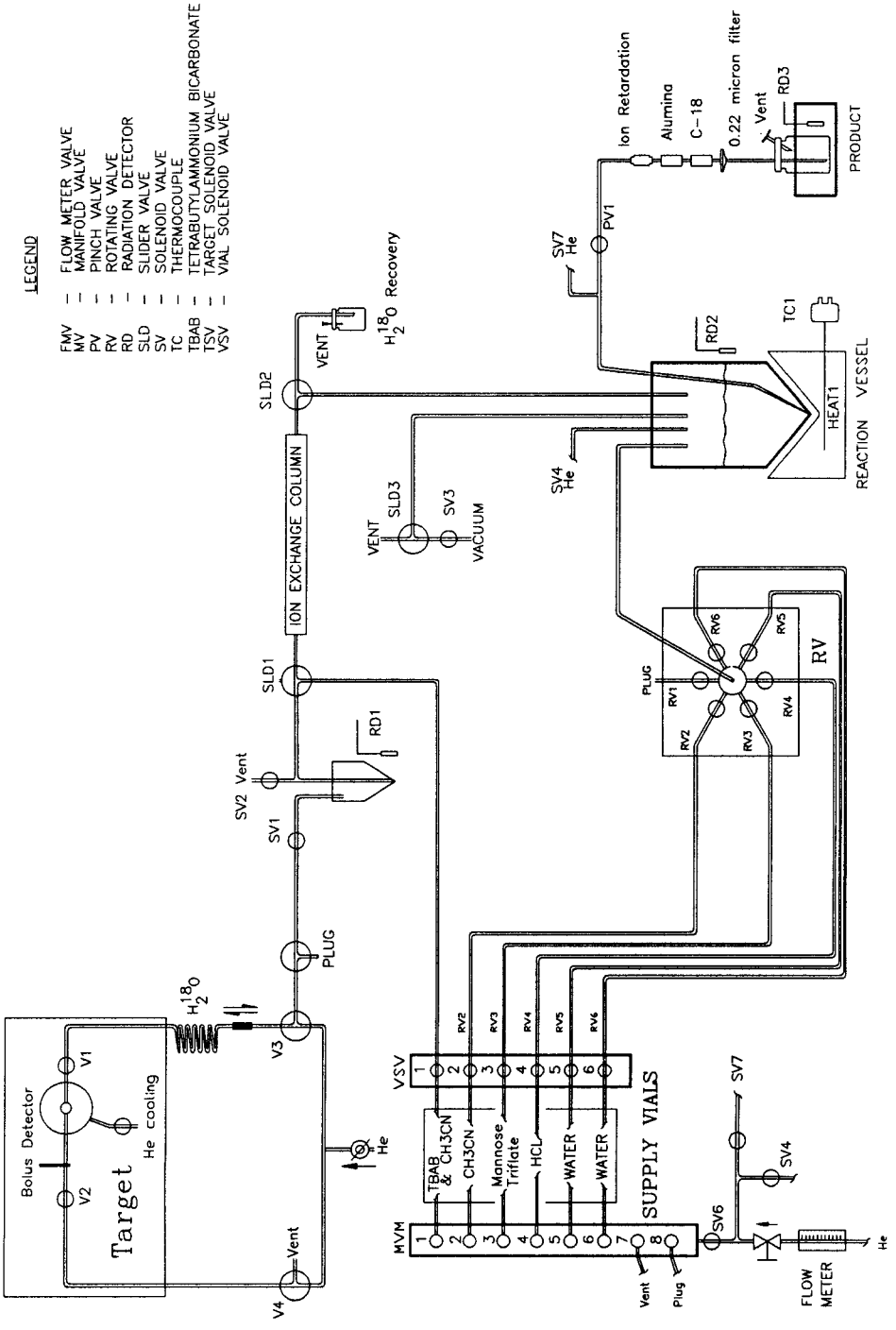

FDG PROCESS SCHEMATIC

INVESTIGATION OF POSSIBLE ROUTES TO NO-CARRIER-ADDED 4-[^{18}F]FLUOROPHENOL

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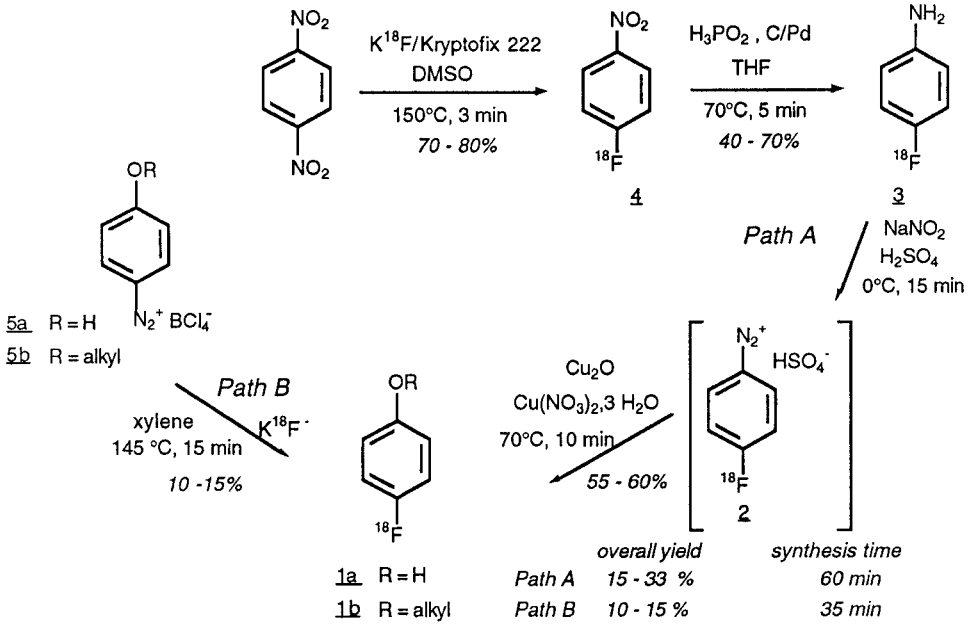
Fluorophenols are valuable intermediates for the preparation of bioactive compounds[1]. In radiolabelled form, using the positron emitting fluorine-18 ($t_{1/2}$ = 110 min), such compounds have applications in the *in vivo* imaging of biological processes using positron emission tomography. The preparation of fluorophenols is well documented in the literature. The strategy includes either the introduction of the fluorine atom at the first step of the synthesis (hydrolysis of chlorofluorobenzenes[2], thermal decomposition of a diazonium ion in a highly acidic aqueous medium[3]) or in the last one (diazotization and fluorodediazoniation of 4-aminophenols in pyridine-HF[4]). To our knowledge, three different methods (using a [^{18}F] monofluoride) have been used to synthesize [^{18}F] fluorinated phenol derivatives : a direct nucleophilic substitution for the preparation of ring fluorinated biogenic amines[5], a Baeyer Villiger oxydation of [^{18}F]fluorohydroxybenzaldehydes[6] and a decarbonylation of methoxysubstituted [^{18}F] phenols[7]. We report here two different approaches for the synthesis of 4-[^{18}F]fluorophenol 1. The main results are summarized in the scheme 1 and the two methods compared. (scheme 1) The first route (path A) involves the hydrolysis of the [^{18}F] diazonium salt 2, a reaction that was suggested in the late sixties[8]. The trapping of the salt 2 with an appropriate reagent allowed the synthesis of [^{18}F]fluorobenzenesulfonylchloride[9] or [^{18}F]fluorophenylhydrazine[10] but was never used as a [^{18}F] fluorophenol source. The second approach is based on the thermal decomposition of a [^{18}F] fluorotetrahaloborate. If this modified Balz-Schiemann reaction has been successfully applied to the no carrier added synthesis of 4-[^{18}F]fluorotoluene[11,12] its feasibility with a fonctionalized benzene ring was not investigated.

Path A : Hydrolysis of the diazonium salt :
[^{18}F]Fluoroaniline 3 was prepared in 35-60 % yield (decay corrected, synthesis time : 25-30 min) from [^{18}F^{-}] according to a previously described method[13] for which we have optimized both reaction times and reagents. The diazotization was carried out in sulfuric acid with sodium nitrite and the hydroxydediazoniation with an aqueous mixture of cuprous oxide and cupric nitrate, a reaction known to process in a few minutes and in the presence of a large excess of reagent[14]. The best conditions (scheme 1) were firstly determined using the stable isotope. 4-[^{18}F]Fluorophenol 1a was obtained after purification by HPLC [μ Porasil, retention time : 6 min, λ : 254 nm, flow rate : 2 ml.min^{-1}, eluent: CH_2Cl_2 and 2 % of EtOH: H_2O: $EtNH_2$ (96: 2: 2, v/v/v) in 55-60% yield from 4-[^{18}F]fluoroaniline (20 % from ^{18}F fluoride) in less than 60 min. All the yields are decay corrected and 1a was identified by comparison of its retention time in HPLC and its Rf in TLC with an authentic sample.

Path B : Modified Balz-Schiemann reaction :
The thermal decompositon of the diazonium tetrachloroborate 5a in the presence of a fluorinating agent was carried out under different conditions. Parameters studied included a) the labeling agent ($nBu_4N^{18}F$, $K^{18}F$, $K^{18}F$/Kryptofix 222, $Cs^{18}F$), b) the solvent (heptane, DMF, DMSO, xylene), c) the reaction time. The highest yields (13-15%) were obtained when the tetrachloroborate 5a (prepared by a described method[15]) was stirred with $K^{18}F$ in acetonitrile for 10 min at 80°C, then heated in xylene at 145°C for 15 min. These results will be compared with those obtained with alkyl protected phenols .

Scheme 1: preparations of 4-[^{18}F]fluorophenol



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FLUORINE-18 LABELLED INDANONES AND INDANDIONES: INTERMEDIATES FOR THE PRODUCTION OF INDANE RADIOPHARMACEUTICALS

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Recently, we have explored potential synthetic routes towards fluorine-18 labelled atipamezole **1**, a potent α_2-adrenoceptor antagonist (1,2), for imaging brain adrenoceptors with PET. As part of this effort, we synthesized several model indanones and indandiones as substrates for nucleophilic aromatic substitution reactions with [^{18}F]fluoride ion (3). In this report, we describe the synthesis of NCA 5-[^{18}F]fluoro-2,2-dimethyl-1-indanone **5**, 6-[^{18}F]fluoro-2,2-dimethyl-1-indanone **6** and 5-[^{18}F]fluoro-2,2-dimethyl-1,3-indandione **7**.

The nitro precursors, shown in Table 1, were synthesized from 1-indanone as previously described (3). Proton NMR and crystal structures confirmed the 5- and 6- nitro positional isomers on the indanone precursors. The [^{18}F]fluoride was azeotropically dried in the presence of K_2CO_3/Kryptofix 2.2.2, resolubilized in 100 µL of anhydrous DMSO and added to the nitro substrate. The reaction mixture was heated for 30 min at 145 °C. Upon cooling, the mixture was partitioned between water and ether. The decay corrected incorporation yields for the [^{18}F]fluoro-for-nitro exchange reactions are given in Table 1.

The 5-nitro-1-indanone **2** with the electron withdrawing ketone in an activating position para to the nitro group gave the corresponding 5-[^{18}F]fluoro compound in high yield. The 5-nitro-1,3-indanedione **4** gave the corresponding fluoro compound in 54% yield. The presence of a second electron withdrawing ketone moiety meta to the nitro group did not significantly alter the reactivity of this molecule. We unexpectedly found that the 6-nitro-1-indanone **3** with the ketone in a non-resonance activating position meta to the nitro group also fluorinated quite well. While the fluoro-for-nitro exchange reaction has been demonstrated on m-dinitrobenzene (4) and 3,5 dinitrobenzonitrile (5), a molecule possessing two electron withdrawing groups meta to a good leaving group, we believe this is the first evidence of a high yield fluorodenitration meta to a ketone moiety. This surprising finding may prove useful in the production of benzocyclanone radiopharmaceuticals where the para position is blocked by necessary substituents.

There are many pharmaceuticals, like atipamezole, that possess indane and indanone substructures. The ability to label these model compounds demonstrates a promising route for the production of indane PET radiopharmaceuticals.

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Supported by NIH Grants HL07367, HL95840 and NS2886 and the U.S. DOE (DE-AC0376SF00098). We would like to acknowledge CTI and the Northern California PET Imaging Center for producing the fluorine-18 used in this study.

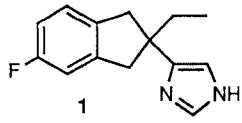

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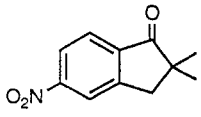
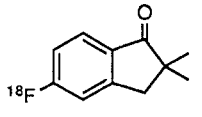
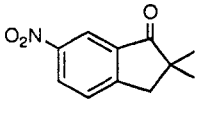
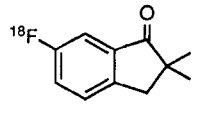
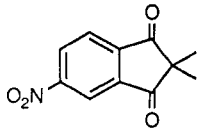
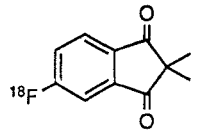
TABLE 1. Decay Corrected Yields of the [^{18}F]fluoro-indanones and -indandiones

Substrate	Product	Yield
 2	 5	67.5 ± 9.2 (n=2)
 3	 6	49.3 ± 10.4 (n=4)
 4	 7	54.0 ± 8.2 (n=3)

Site-Selective ^{18}F-Labelling of the CFC Alternative, 1,1,1,2-Tetrafluoroethane, for Biodistribution Studies in Man

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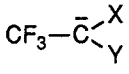
1,1,1,2-Tetrafluoroethane is now produced on a large scale as the main replacement for ozone-depleting chlorofluorocarbons (CFCs) in their many applications, including their use as refrigerants and coolants.[1-3] A further potential application is as a drug propellant. It is therefore important to understand the absorption, distribution and retention of this compound in humans after administration by inhalation. Such information might be gained by using a simple whole-body counting technique[4] to monitor the regional distribution of radioactivity for several hours after the inhalation of 1,1,1,2-tetrafluoroethane labelled with fluorine-18 ($t_{1/2}$ = 109.7 min; β^+ = 96.9%). By developing regioselective labelling techniques the metabolism of the compound as well as its biodistribution in man might be elucidated. Here we report on the use of [^{18}F]fluoride for labelling 1,1,1,2-tetrafluoroethane efficiently, site-selectively and in exceedingly high radiochemical and chemical purity for such studies.

Labelling in the 1-position.

The exchange of fluorine-18 in 2-substituted 1,1,1-trifluoroalkanes has been studied previously.[5,6] Successful exchange was observed only in those compounds having a 2-substituent capable of stabilising an intermediate carbanion (I) by a strong inductive effect.



(I)

Exceptionally, exchange was not observed when the 2-substituent was fluoro.[5] However, our attempts at direct exchange in 1,1,1,2-tetrafluoroethane with [^{18}F]fluoride gave low but significant radiochemical yields (*ca* 7%). It is supposed that the exchange mechanism involves a carbanion intermediate in a complex equilibrium (Scheme 1). Assuming this mechanism to operate we considered that higher radiochemical yields of [1-^{18}F]1,1,1,2-tetrafluoroethane might be obtained by [^{18}F]fluoride addition to trifluoroethylene. This was confirmed experimentally as follows.

Cyclotron-produced 'no-carrier-added' [^{18}F]fluoride in ^{18}O-enriched water (*ca* 20%)[7] was adsorbed onto an anion exchange resin and eluted with potassium carbonate solution (0.3M).[7,8] A portion of this solution (0.2 mL; *ca* 150 MBq: 4 mCi) was added to a solution of aminopolyether 2.2.2. (5.2 mg) in acetonitrile (0.2 mL) in a glassy carbon vessel and heated to dryness while drawing a slow stream of nitrogen through the vessel by vacuum. Then the vessel (internal volume, 2.5 mL) was pressurised to 50 p.s.i. with trifluoroethylene, sealed and heated to 150 ^{0}C. After 25 min, the vessel was vented to a GC injection valve (3 mL loop). The collected radioactive product was found by GC and by GC-MS to contain a mixture of [^{18}F]1,1,1,2-tetrafluoroethane (*ca* 50–75%), [^{18}F]trifluoroethylene (*ca* 25–50%) and sometimes a minor unknown radioactive impurity (0–10%). Separation by GC on a 60/80 Carboxen 1000 packed column gave [^{18}F]1,1,1,2-tetrafluoroethane in 45% radiochemical yield (decay-corrected) at 80 min from radioisotope production. GC analysis of the separated product on a PLOT fused silica capillary column revealed 99.995% radiochemical purity and 99.7% chemical purity. The specific radioactivity was estimated to be 37 MBq (1 mCi)/μmol (corrected to EOB). Scission of the carbon-carbon bond in the ionisation source of an 'isotope

separator'[9] gave $[^{18}F]CF_3^{+\cdot}$ (m/z = 68) and $[^{18}F]CH_2F^{+\cdot}$ (m/z = 32) fragments in the ratio of 35:1, demonstrating 97.2 % selectivity for labelling in the 1-position.

This process is carried out as a gas-solid reaction for convenience. The reaction may also be carried out as a solution reaction for higher radiochemical purity and higher selectivity for labelling 1,1,1,2-tetrafluoroethane versus trifluoroethylene.[10] These reactions demonstrate the use of the powerfully nucleophilic [^{18}F]fluoride-K$^+$-aminopolyether 2.2.2 system to achieve the equivalent of the addition of anhydrous hydrogen [^{18}F]fluoride, which is a far less accessible and manageable reagent. Hence, the scope of the reaction is being explored further.

This radiochemistry is now fully automated up to collection of the GC-purified product. The apparatus features a multi-ported reaction vessel with a glassy carbon insert, on-line GC separation and facility for the cryogenic trapping of purified product, as depicted in Scheme 2.

Labelling in the 2-position.

The method developed for labelling 1,1,1,2-tetrafluoroethane in the 2-position is based on nucleophilic substitution with [^{18}F]fluoride in 2,2,2-trifluoroethyl-*p*-toluenesulphonate. Nca [^{18}F]fluoride–K$^+$-aminopolyether 2.2.2. is prepared in a glassy carbon vessel as described above. The vessel is then loaded with the tosylate (25 mg) in acetonitrile (0.4 mL), sealed and heated at 95^{0}C for 25 min. The reaction vessel is cooled to 75 ^{0}C and the volatile product (58% radiochemical yield) is collected in a GC injection valve (loop size, 3 mL). GC and GC-MS show this product to be > 99% radiochemically pure and > 99% chemically pure. This may be further purified by GC to give a product free of any detectable radiochemical or chemical impurities. The preparation may be performed in the automated apparatus depicted in Scheme 2, with only minor modification for loading the substrate. Scission of the carbon-carbon bond in the labelled product in the ionisation source of an 'isotope separator' gave $[^{18}F]CF_3^{+\cdot}$ (m/z = 68) and $[^{18}F]CH_2F^{+\cdot}$ (m/z = 32) fragments in the ratio of 1:10.4, demonstrating 91.2% selectivity for labelling in the 2-position.[9]

The easy availability of selectively radiofluorinated 1,1,1,2-tetrafluoroethanes now enables the biodistribution and metabolism of this CFC alternative to be studied in man. The results of these studies, which are now in progress, will be published elsewhere.

Acknowledgement. The authors are grateful to Dr R.L. Powell and colleagues (ICI Chemicals and Polymers Ltd) for useful discussions and to Mr K.J. Dowsett for assistance on automation.

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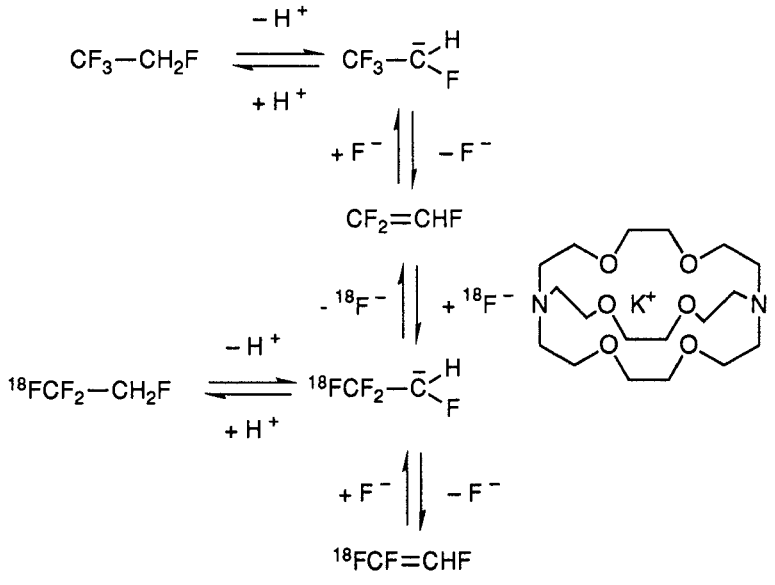
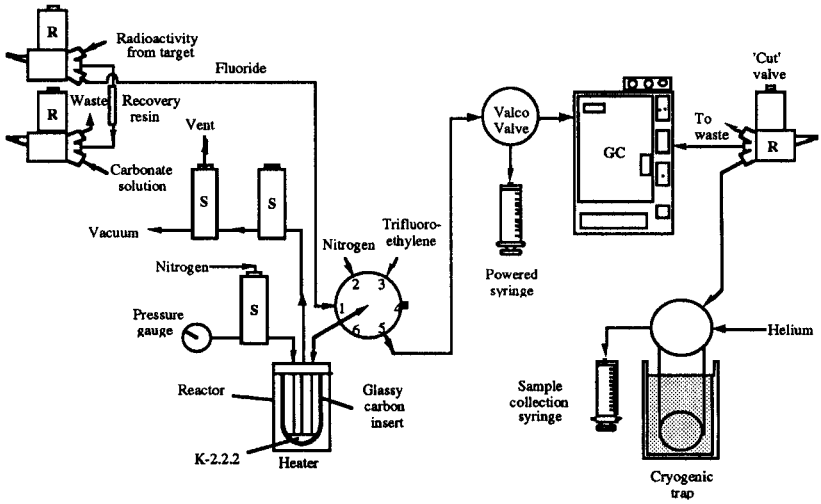


Scheme 1. The labelling of 1,1,1,2-tetrafluoroethane by treating trifluoroethylene with [^{18}F]fluoride in the presence of K+-aminopolyether 2.2.2.



Scheme 2. Scheme of the automated apparatus used to produce [1-^{18}F]1,1,1,2-tetrafluoroethane for human administration. R and S are pneumatically operated Rheodyne and solenoid valves, respectively.

Automated synthesis of [^{18}F]-fluoromethane ([^{18}F]CH_3F).

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[^{18}F]CH_3F has been shown to be useful both as a blood flow tracer for PET investigations (1) and as a synthon in the production of [^{18}F]F_2 (2, 3). Several methods have been described for the synthesis of [^{18}F]CH_3F, both ^{11}C- and ^{18}F-labelled (4, 5). We have developed a fast, computer controlled method for the production of chemically and radiochemically very pure [^{18}F]CH_3F.

[^{18}F]-fluoride, produced in a 0.19 cm^3 water target chamber with the nuclear rection $^{18}O(p,n)^{18}F$ from ^{18}O-enriched water (6), is transferred into a reaction vessel containing potassium carbonate (2 mg), kryptofix.2.2.2 (12 mg) and 1 mL acetontrile. Evaporation with helium flow under reduced pressure (teflon membrane pump) is carried on for 90 s. During the evaporation the vessel is heated to a temperature of 80 °C in a sonic bath. One mL of acetonitrile is added and the evaporation is continued for a further 90 s. The vessel is evacuated to a pressure of ~ 10 mbar. To the dry residue is added 100 µL of CH_3I in 1 mL of acetonitrile, and the vessel is again lowered into the sonic bath. Then [^{18}F]CH_3F is formed, and released from the solvent. The reaction is complete in less than 60 s. The product is drawn off into a 60 mL syringe. After this the product is injected onto a Haysep preparative gas chromatography column (length 30 cm, ID 0.8 cm), eluted with a carrier gas, and trapped into a stainless steel trap at LN2 temperature (-190 °C). From the trap the product can be transferred into a syringe and used as a gas, or dissolved into saline and used as an injectable solution. Alternatively the product can be used for further chemistry.

The synthesis is computer-controlled (Interface: Data Translation Inc., USA, I/O-card). Three radioactivity detectors are used for monitoring and controlling the synthesis (see figure 1).

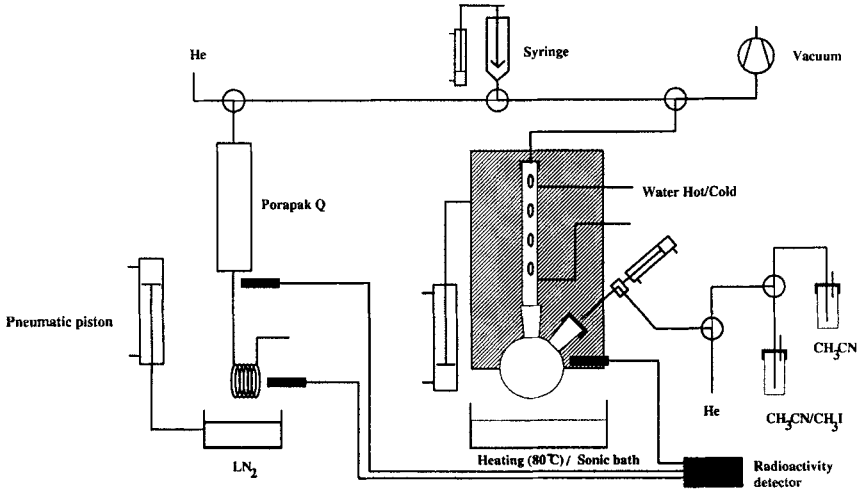

Fig. 1. Schematic outline of system for production of [^{18}F]CH_3F.

The synthesis time for fluoromethane is about 5 min and the chromatographic purification and trapping takes a further 7 min. The radiochemical yield has been 76 ± 10% (n=26). The

radiochemical yield is a function of the target chamber condition, see figure 2. A target chamber overhaul (change of foil and removal of solid residues) dramatically increases both the radioactivity yield from the target and the fluoromethane synthesis yield. In standard "good" runs we produce 187 ± 13 mCi [^{18}F]-fluoromethane, starting from 234 ± 17 mCi [^{18}F]-fluoride.

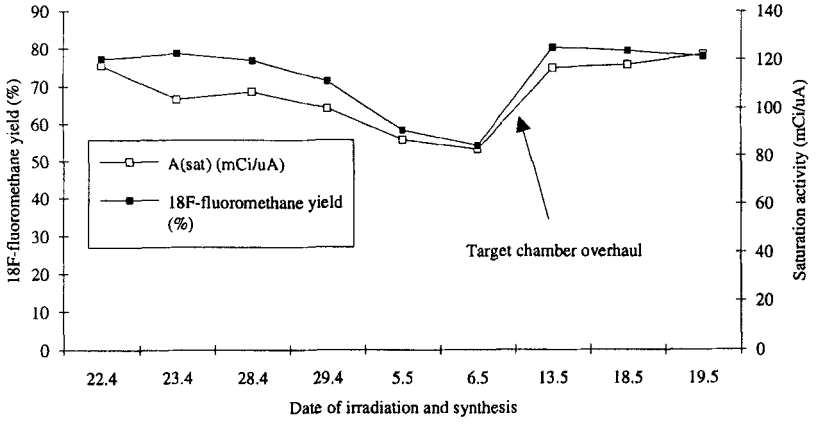

Fig. 2. Radioactivity yield from target and synthesis yield in nine consecutive runs.

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Preparation of [^{18}F]16α-Fluoro-17β-Estradiol by Selective Nucleophilic Substitution.

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Departments of Radiology and Chemistry, Case Western Reserve University and University Hospitals of Cleveland, Cleveland Ohio 44106 USA
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Steroid receptor based imaging agents have marked potential for application in the diagnosis and treatment of cancer. In recent years many of these agents have been developed. A variety of compounds have been labeled with ^{3}H, ^{123}I, ^{77}Br, ^{18}F, and even with ^{99m}Tc. Several ^{18}F-labeled estrogen receptor ligands have been prepared and tested as potential PET imaging agents for estrogen receptor-rich tissue such as tumors of the breast.[1] Among these, 16α-fluoro-17β-estradiol[2] was one of the earliest prepared, and remains perhaps the best for the imaging of tumors based on its binding affinity and low non-specific uptake in target tissues. The use of [^{18}F]16α-fluoro-17β-estradiol in conjunction with PET has been demonstrated to represent a useful non-invasive approach to tumor assessment in relation to hormone therapy and for the identification of tumor metastases.[3]

Now that [^{18}F]16α-fluoro-17β-estradiol has proven to be valuable for PET as a clinical modality its development for widespread daily clinical and research applications depends on its ready availability in the clinical setting. We propose a one-step labeling approach to prepare [^{18}F]16α-fluoro-17β-estradiol by selective nucleophilic substitution of [^{18}F]fluoride on a cyclic sulfate derivative. The use of a cyclic sulfate in this case served the dual role of activating a hydroxy position to nucleophilic substitution and simultaneously protecting the vicinal hydroxyl group.

The cyclic sulfate used as a synthetic precursor was prepared easily in two steps from commercially available 16-epiestriol (Scheme). The phenol at C_3 was first protected by reaction of epiestriol 1 with chloromethyl methyl ether (50-60% after chromatography on silica using 30-50% EtOAc/Petroleum ether, mp 139-140°C). Then, the resulting diol 2 was reacted with sulfuryl chloride[4] or diimidazolyl sulfate[5] to produce the cyclic sulfate 3 (60%, mp 129-131°C). Compound 3 was characterized by IR, NMR and mass spectroscopic analysis.

Authentic fluoroestradiol was prepared by reaction of the same cyclic sulfate 3 with stoichiometric anhydrous tetramethylammonium fluoride, followed by hydrolysis (2 N HCl, 20% EtOH) to give 16α-fluoro-17β-estradiol in 65% yield (mp 178-181°C, lit mp[6] 183-187°C). Its ^{1}H-NMR spectrum was consistent with the literature report. The favored approach of the attacking nucleophile is from the back side of the plane (α) and both positions 16 and 17 present the same probability. However, a nucleophile attacking at the 17-position experiences three 1,3-diaxial interactions (with positions 12, 14 and 15), while at the 16-position it encounters only one (with position 14). This is apparently a sufficient steric effect to account for the selective substitution at the 16-position, as no other fluorinated products were detected in either the stoichiometric unlabeled or no-carrier-added ^{18}F reactions.

The results of the radiosynthesis of [^{18}F]16α-fluoro-17β-estradiol will be presented. The overall radiochemical yield was 50-60%, obtained within 60 min. The reaction used 1-2 mg of cyclic sulfate precursor in anhydrous acetonitrile, catalyzed by kryptofix 2.2.2 and potassium carbonate.

Compared to the current reported procedure, this approach offers several advantages. First the cyclic sulfate is considerably more stable than the ditriflate estrone derivative. Precursor **3** when kept dry in the cold is stable. Then the one-step procedure did not required any additional chemistry followed the labeling step which increases the overall yield and allowed simpler purification method. Finally, this procedure can be carried out in any existing FDG set-up apparatus.

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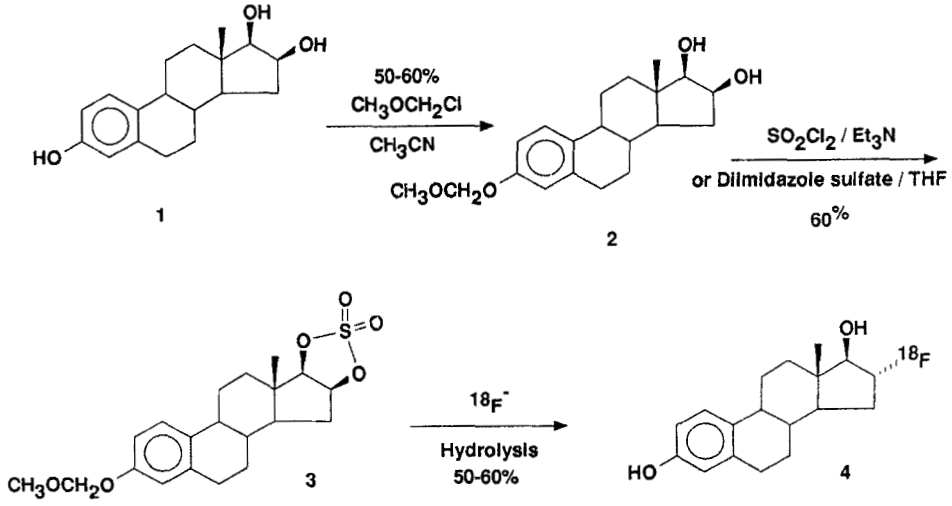

N.C.A. Asymmetric Syntheses of α-Methyl and β-Hydroxy α-Amino Acid Labelled with Fluorine-18 for Probing Enzymatic Function.

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Many amino acid derivatives are biological active molecules. Among these, α-methyl and β-hydroxy α-amino acids exhibit interesting enzymatic inhibitor properties (1,2).

α-Methyl α-amino acids

α-Methyl tyrosine is known as a competitive inhibitor of tyrosine hydroxylase, which is the rate limiting enzyme for the conversion of L-tyrosine into L-dopa (3). This compound was previously labelled with ^{14}C, ^{11}C (4,5) and ^{123}I (6), positron and gamma emitters. Among the different chemical methods available for the preparation of enantiomericaly pure amino acids, asymmetric synthesis is certainly the most adequate technique. This approach was used for the preparation of α-methyl-2-[^{18}F]fluoro-L-tyrosine (4d), a potential radiopharmaceutical for probing the dopaminergic system with Positron Emission Tomography (PET). The synthesis pathway was extended to the preparation of other α-methyl amino acids. These compounds and starting substrates for the radioactive syntheses are presented in Table 1.

The method required in the first step the preparation of a methylated asymmetric auxiliary: the (2S,5S)-1-(*tert*-Boc)-2-(*tert*-butyl)-3,5-dimethyl-4-imidazolidinone [(S)-BocBMI-CH_3] (2). This compound was prepared by alkylation of the chiral auxiliary of Seebach (1)(7) using lithium diisopropylamine (LDA) and methyl iodide. The dialkylated product (3), obtained after reaction with substituted ^{19}F-fluorobenzyl bromide and LDA was purified on a Silica Gel column and identified by ^{1}H-NMR [i.e.: the precursor of α-methyl-2-fluoro-L-tyrosine: $CDCl_3$, δ (ppm)(mixture of two rotamers): 0.88/0.90 (s, 9H, ^{t}Bu), 1.43/1.51 (s, 9H, ^{t}Boc), 1.63/1.68 (s, 3H, α-CH_3), 2.56/2.88 (s, 3H, N-CH_3), 2.88/3.43 (d, 2H, J_{H-H}=14,3Hz, -CH_2-Bz), 3.71/3.73 (s, 3H, -OCH_3), 4.45/4.60 (s, 1H, $C_{(2)}$-H), 6.40-7.00 (m, $3H_{arom}$)]. The α-methyl-L-amino acid was obtained after hydrolysis (HCl 6N, 180°C, in a sealed vial) [^{1}H-NMR of α-methyl-2-fluoro-L-tyrosine: D_2O/DCl δ (ppm): 1.64 (s, 3H, α-Me), 3.23 (m, 2H, -CH_2-), 6.60-7.20 (m, $3H_{arom}$)].

The n.c.a. method of preparation of α-methyl-[^{18}F]fluoro-L-amino acids is a multistep synthesis based on the nucleophilic displacement of substituted trimethylammonium benzaldehyde triflate with the kryptofix/K_2CO_3 activated [^{18}F]fluoride. The labelling conditions were optimized for each precursor (radiochemical yield ranging from 10 to 70%). Reductive iodination of the aldehyde group with diiodosilane (DIS)(8) affords the [^{18}F]fluoro-electrophilic agents. Alkylation of (S)-BocBMI-CH_3, hydrolysis and HPLC purification (Figure 2) lead to the α-methyl-L-amino acids.

The radiochemical yield for all these radiopharmaceuticals (except 4e) was of 10% (decay corrected) within 120 min. The enantiomeric excesses were higher than 97%.

First animal studies are currently under investigation.

β-Hydroxy α-amino acids

The β-hydroxy amino acids were obtained through a similar approach using direct alkylation of the enolate of (S)-BocBMI (generated with LDA) and various [^{18}F]fluorobenzaldehyde. The dialkylated product were identified by ^{1}H-NMR [i.e.: the precursor of β-hydroxy-6-fluoro-L-dopa: $CDCl_3$, δ (ppm): 1.01 (s, 9H, ^{t}Bu), 1.08 (s, 9H, ^{t}Boc), 2.99 (s, 3H, N-CH_3), 5.79 (s, 3H, -OCH_3), 5.82 (s, 3H, -OCH_3), 4.14 (d, 1H, J_{H-H}=8.72Hz), 4.95 (s, 1H, $C_{(2)}$-H), 5.13 (d, 1H, J_{H-H}=8.76Hz), 6.50 (d, $1H_{arom}$, J=11.34Hz), 7.14 (d, $1H_{arom}$, J=6.7Hz)]. This chiral compound was hydrolysed in the same conditions as described above.

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Table 1 : Starting substrates for the preparation of various α-methyl-[^{18}F]fluoro-L-amino acids

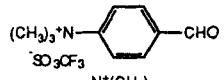
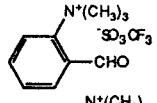
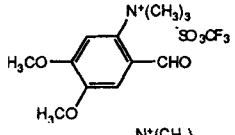
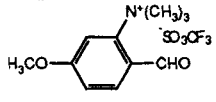
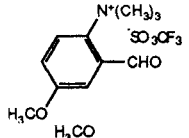
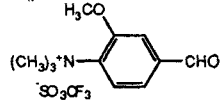
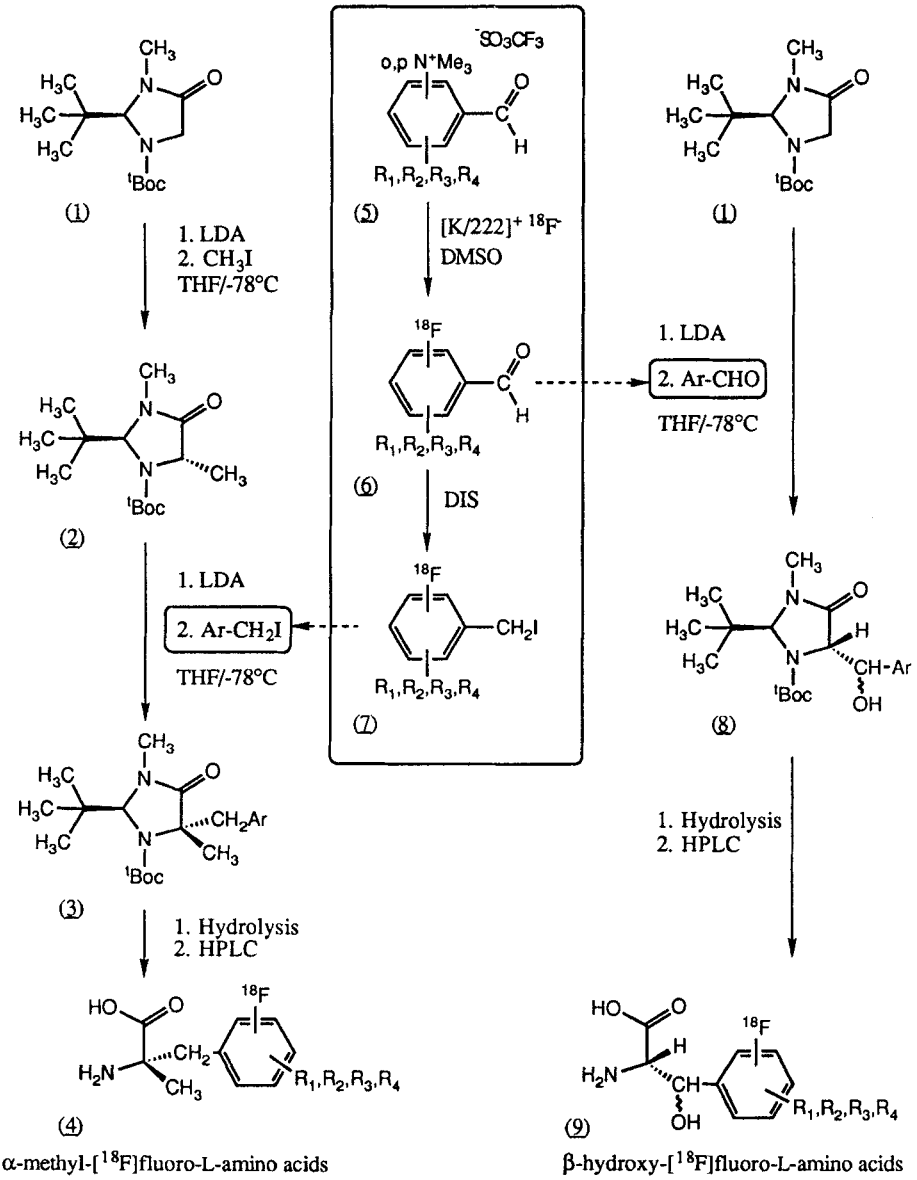
entry	Starting substrate	amino acid
a		α-methyl-4-[^{18}F]fluoro-L-phenylalanine
b		α-methyl-2-[^{18}F]fluoro-L-phenylalanine
c		α-methyl-6-[^{18}F]fluoro-L-dopa
d		α-methyl-2-[^{18}F]fluoro-L-tyrosine
e		α-methyl-6-[^{18}F]fluoro-L-m-tyrosine
f		α-methyl-4-[^{18}F]fluoro-L-m-tyrosine

Figure 2 : General synthesis pathway for the preparation of α-methyl-[^{18}F]fluoro-L-amino acids and β-hydroxy-[^{18}F]fluoro-L-amino acids



The application of LC-MS to establish the specific activity and chemical purity of ^{18}FDG

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Because of the short half-lifes of PET-radiopharmaceuticals, the quality control of these compounds requires special attention (1). A thorough and regular validation of the absence of possible impurities is required. Impurities may include the starting material as well as unintended reaction products. Consequently, the validation of (HPLC)-purification procedures is essential to assure the required specific activity and chemical purity of these radiotracers.

In our laboratory, 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}FDG) is routinely produced by a robotic RB86 system (Anatech) as described by Hamacher et al. (2). In principle, several impurities may be formed during this procedure (3). These compounds may include the partially deprotected precursor, glucose and polymerized sugar products. The final step of the ^{18}FDG production is a purification by HPLC. This procedure was validated by liquid chromatography combined with mass-spectrometry (LC-MS) via an ion-spray device (4) using the same separation conditions as applied for routine ^{18}FDG purification (Fig. 1).

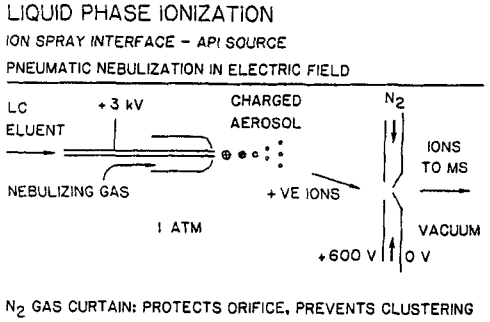

Fig. 1 LC-MS with an ion spray device (3).

Chemical-ionization mass-spectrometry of ^{18}FDG batches revealed principle peaks of m/e 203, corresponding to the molecular weights of glucose and/or mannose (180 + 23 (Na^+)). In addition, mass-spectrometry did not reveal any other potential reaction products, like furfural or polymerized sugar derivatives in the final product. Subsequently, glucose, mannose and FDG in ^{18}FDG batches were separated, identified and quantified using HPLC (Fig. 2) and TLC (n=81). The specific activity of HPLC-purified ^{18}FDG was >> 1 Ci/mmol. The concentration of glucose was 0.5-1.0 mg/ml and the concentration of mannose was less than 10 μg/ml.

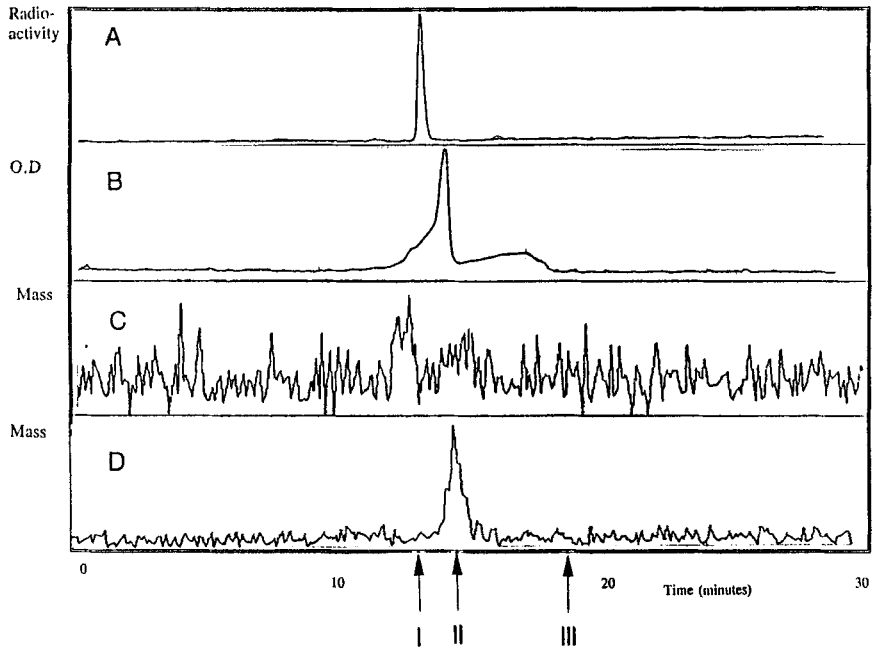

Fig. 2 HPLC-profiles of ^{18}FDG. Arrow I denotes FDG, arrow II denotes glucose and arrow III represents mannose. On-line detection was performed by radioactivity (A) differential diffraction (B) and positive ion mass-spectrometry (C and D). C, detection at m/e= 203 (FDG + Na^+). D, detection at m/e= 205 (glucose + Na^+ or mannose + Na^+).

In conclusion, LC-MS is a valuable tool for the identification of side-reaction-products and the validation of HPLC purification procedures for the pharmaceutical quality control of PET-radiopharmaceuticals.

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ON-LINE ANION EXCHANGE PURIFICATION OF [^{13}N]-NH_3 PRODUCED BY 10-MeV PROTON IRRADIATION OF DILUTE AQUEOUS ETHANOL.

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[^{13}N]-NH_3 is used for the measurement of myocardial blood flow by PET. [^{13}N]-NH_3 can be produced by irradiation of pure water followed by the reduction of [^{13}N]-NO_3^-/NO_2^- over DeVarda's alloy[1,2]. A more convenient method is the recently reported in-target production of [^{13}N]-NH_3 by irradiation of dilute (5 mM) aqueous ethanol[3]. In the latter procedure ethanol serves as a scavenger to eliminate hydroxyl radicals that are generated during irradiation in order to prevent the oxidation of [^{13}N]-NH_3 to [^{13}N]-NO_3^-/NO_2^-. Small amounts (< 1%) of [^{13}N]-NO_3^-/NO_2^- are nevertheless still present as radiochemical impurities beside the radionuclidic impurity [^{18}F]-fluoride generated by the (p,n) reaction on ^{18}O, the abundancy of which amounts to about 0.2% in natural water.

A production of [^{13}N]-NH_3 by irradiation with 10-MeV protons (I.B.A. Cyclone 10/5 cyclotron) at a beam current of 15 μA during 15 min of a 12-mm thick target containing 5 mM aqueous ethanol yields about 0.5 % of ^{18}F-fluoride and about 0.6 % of [^{13}N]-NO_3^-/NO_2^- at EOB. The cross section for the ^{16}O (p,α) ^{13}N reaction is low at 10 MeV, resulting in a relatively high ^{18}F-fluoride content of [^{13}N]-NH_3 preparations. The presence of [^{18}F]-fluoride is negligible at EOB and at time of injection but due to the faster decay of ^{13}N the relative amount of ^{18}F rises as a function of time. Especially if additional manipulations (e.g. determination of metabolites in plasma) are required, the [^{18}F]-fluoride content can amount to 10% of the total activity (45 min after EOB) implicating that initial countings need to be corrected for ^{18}F by recounting of the samples after a waiting period (120 min) during which ^{13}N has sufficiently decayed (Fig. 1). The latter procedure is, however, time consuming and suffers from amplified noise introduced by the double counting procedure.

We have therefore developed a simple on-line purification method in which the irradiated water (with 5 mM EtOH) leaving the target directly passes through a small (5 mm diameter, length 10 mm) ion exchange column (Dowex AG 1-X8 100-200 mesh, hydroxide form). ^{18}F-fluoride and [^{13}N]-NO_3^-/NO_2^- are anionic and are retained on the resin whereas [^{13}N]-NH_4^+ passes through the anion exchange column. The column effluent is filtered through a vented sterile 0.22-μm membrane filter (Cathivex, Millipore) and is collected in a sterile vial containing a hypertonic NaCl solution to make the final preparation isotonic. The final solution does not contain detectable amounts of [^{18}F]-fluoride or [^{13}N]-NO_3^-/NO_2^- (Fig. 2).

The use of the anion exchange resin causes an unexplained rise from 10% to 30% of activity retained on the 0.22-μm membrane filter. The retained activity can be rinsed from the membrane filter by flushing it with water. Analysis of the flushing water revealed that the retained ^{13}N activity is under the form of ammonia. The retention of [^{13}N]-NH_3 on the membrane filter is however reduced to 15% if the ion-exchange column is flushed extensively with water (50 ml) prior to usage.

The proposed purification method can be applied for the in-target production with a low energy cyclotron (10-MeV protons) of [^{13}N]-NH_3 used for studies requiring the assessment of ^{13}N-radioactivity during prolonged periods (e.g. quantification of

myocardial blood flow requiring a correction of the input function for the presence of metabolites of [^{13}N]-NH_3).

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This study was supported by grant nr 3.0080.90 from the Belgian National Fund for Scientific Research.

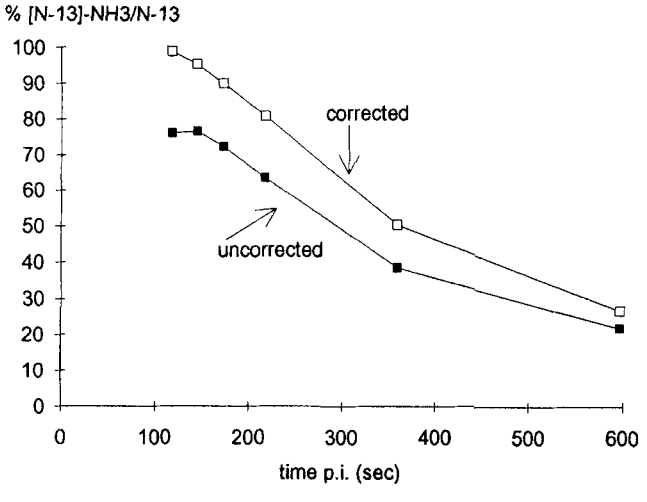

Fig. 1. ^{18}F-corrected and uncorrected values of the percentage of [^{13}N]-NH_3 relative to the total amount of ^{13}N in plasma as a function of time p.i.

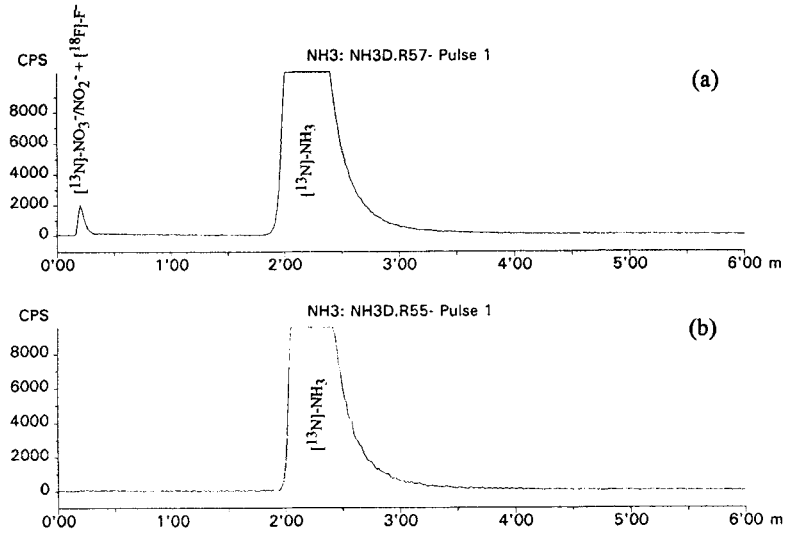

Fig. 2. HPLC chromatograms (Vydac 400IC, 50 mm x 4.6 mm, eluted with 2 mM HNO_3, flow rate 3.5 ml/min) of $[^{13}N]$-NH_3 before (a) and after (b) purification on ion exchange resin.

The Synthesis of Oxygen-15 Butanol Via the Oxidation of Tributylborane Adsorbed on Solid Surfaces.

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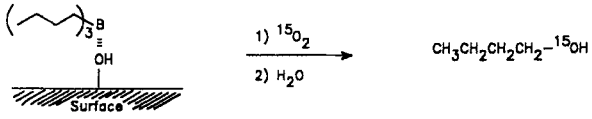
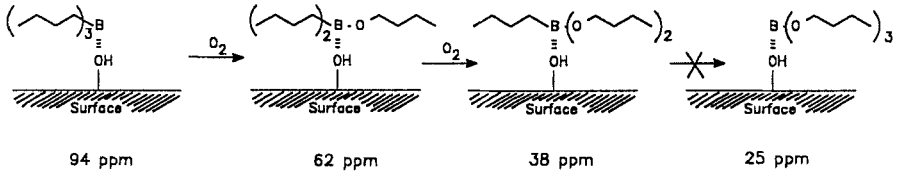
Organoboranes are versatile reagents in rapid radiolabeled syntheses.(1) In addition, solid-supported reagents offer the flexibility of rapid synthesis and purification.(2,3) Combining these two synthetic tools offers unique versatility for the efficient production and purification of radiopharmaceuticals in a clinical positron emission tomographic (PET) imaging center.



Oxidation of Bound Tributylborane to Labeled Butanol

We have been investigating the use of solid state reagents for the production of radiopharmaceuticals such as oxygen-15 labeled n-butanol. Oxygen-15 labeled n-butanol has been called the platinum standard of blood flow agents.(1) The development of a rapid synthesis of labeled n-butanol from the oxidation of tributylborane with $^{15}O_2$ in solution was first developed in this laboratory as well as the first butanol synthesis based on a polystyrene support.(1) Inorganic supports have also been utilized for this oxidation and offer advantages over the previous organic based solid supports.(4-7) The current support of choice is γ-

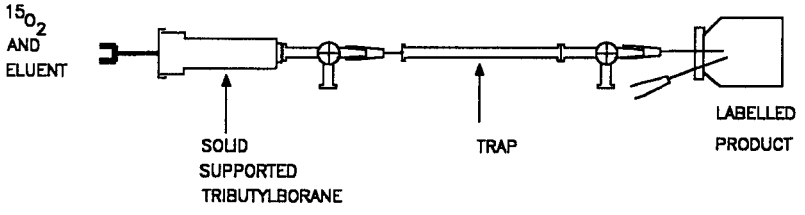


Relative to Sodium Tetraphenylboron 0ppm

alumina. We have performed boron-11 solid state NMR (SSNMR) studies on the γ-alumina system using unlabeled oxygen and have discovered that incomplete oxidation occurs with the oxidative process stopping at the butyl dibutylboronate stage.

Other supports, both organic and inorganic, exist that have the chelating properties of γ-alumina, but not the reactive Lewis acidic and basic sites characteristic of alumina. A series

of these supports were compared as to their efficiency in the production of radiolabeled butanol. The organic supports investigated were cotton and IR 743A (a polystyrene supported amino-glycitol). The inorganic supports were glass wool, diatomaceous earth (Celite 545) and diatomaceous earth coated with polyvinyl alcohol (PVA). We also re-evaluated alumina and silica gel. These supports all have hydroxyl groups which coordinate the tributylborane used in the oxidation studies.



Each of the solid supports was packed into reactor cartridges (see Figure) and degassed with dry argon. Tributylborane (150 μls, 0.6 mmol, Aldrich) was then injected into the center of the solid support via syringe and the cartridge sealed in a large test tube under dry argon for storage until exposure to $^{15}O_2$. After exposure to $^{15}O_2$, the labelled product was isolated by washing the cartridge with 6 mls of distilled water into a sterile vial. The product was purified by radio-HPLC using a C-18 column and utilizing 60/40 water/methanol as eluant.

The results of our experiments are summarized in Table 1. It is clear that oxygen-15 butanol can be prepared on a wide variety of surfaces indicating that the surface is simply serving as a dispersion medium. Both diatomaceous earth (celite) amd cellulose (cotton) provide excellent yields of butanol, but the celite-borane mixture is more stable than mixtures utilizing alumina or cotton. These observations support the mechanism put forth in the early borane-oxidation studies which emphasize the need for a Lewis base (normally an oxygen atom) to initiate the migration of the alkyl group from boron to oxygen.(8) Thus alumina is not unique in the preparation of oxygen-15 butanol in that it is simply provides the nucleophilic oxygen atoms required for rearrangement reactions. In fact, diatomaceous earth (celite) possesses a number of advantages in these oxidation reactions: efficient utilization of $^{15}O_2$ (trapping efficiency); excellent radiochemical yields, and enhanced stability of the tributylborane-celite mixture. The coproduction of oxygen-15 water is apparently due to free radical side reactions such as radiolysis and radical chain terminating reactions and occurs in all alkylborane oxidations whether or not a solid support is used. Fortunately the water byproduct is easily removed. Control reactions demonstrated that oxygen-15 labeled water does not arise via transhydroxylation between butanol and water; rinsing a reaction cartridge containing partially oxidized tributylborane adsorbed on celite with oxygen-15 water does not produce oxygen-15 butanol.

Table 1. PRODUCTION OF ^{15}O-BUTANOL VIA OXIDATION OF TRIBUTYLBORANE ON SELECTED SOLID SUPPORTS.

Solid Support	Trapping Efficiency (%)[a]	Radiochemical Yield of butanol (%)[b]
IR-743A	99	10
Cotton	92	40
Cellulose acetate	90	20
PVA on celite[c]	68	24
Alumina[d]	75	37
Silica gel[e]	92	43
Celite (545)	89	44

[a]% of activity trapped by tributylborane supported on the surface. [b]Corrected to EOB and based on quantity of $^{15}O_2$ used. [c]PVA = Polyvinyl Alcohol (1 g PVA / 2 g Celite). [d]See Goodman, M. M., ref. 3.[e]See Takahashi et. al, ref. 3.

Acknowledgement

We wish to thank the Department of Energy for support of this research.

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A Simple System For Remote Processing of $H_2[^{15}O]$ Produced From a N2/H2 Gas Target

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$H_2[^{15}O]$ can be prepared by a variety of methods including reduction of $[^{15}O]O_2$ with H_2 over Pd (1) or Pt (2) catalysts, exchange between $[^{15}O]CO_2$ and water (3), recoil production via in-target reaction of ^{15}O atoms generated by the $^{16}O(p,pn)^{15}O$ reaction on natural abundance water (4), and recoil production via in-target reaction of ^{15}O atoms with H_2 generated by the $^{14}N(d,n)^{15}O$ reaction on a N_2/H_2 gas target (5). We report here a simple apparatus for trapping and processing $H_2[^{15}O]$ produced from the N_2/H_2 target, and for delivering that radiotracer in a purified injectable form.

As seen in Figure 1, the system operates through the actions of two Rheodyne 7010 injector valves which, along with peripheral traps and plumbing, fit inside a shielded Capintec Dose Calibrator for direct activity measurement. The valves are operated remotely from behind the shield through extension handles. When both valves are placed in the "Load" position, target gas is allowed to flow through a sterile water bubbler containing 6 mL of water, and exit to a shielded containment bag thus preventing radioactivity release to the atmosphere. Typically, 300 mCi of $H_2[^{15}O]$ accumulate in the bubbler within 5 minutes from the start of beam after transferring through a 120 m x 3.18 mm o.d. Impolene line enroute to the PET facility. Irradiations generally last for 3 minutes using 8 MeV deuterons of 15 μA intensity, while the target is operated in a dynamic mode at 4 L min^{-1} using 60 psi of N_2 + 5% H_2 gas. After accumulation is complete, valve A is repositioned to "Inject", and 60 mL of air from a syringe is used to displace the water through cation exchange resin (BioRad AG50W-X8 Polyprep column) and into a saline collector containing 1.6 mL of 5% sterile saline. This amount and strength of saline is sufficient to make the preparation isotonic. Passage through the cation resin is essential for removal of ammonium ions produced as a consequence of in-target radiolysis of the target gas. If left untreated,s the $H_2[^{15}O]$ preparation has a pH of 11.5. After treatment, however, the pH falls within a range of 5.5-6.0 that is acceptable for patient injection. Valve B is then repositioned to "Inject" and another 60 mL charge of air from a second syringe displaces the saline solution through a sterile L50 line, vented millipore filter and into a 10 mL syringe fixed within a Biodex Pro-Tec II titanium syringe holder. This assembly is allowed to rest at the bottom of a second shielded Capintec chamber, and is pulled up and locked into a shielded portable cannister only after filling (see Figure 2). The cannister is then removed from the Capintec chamber and lowered into additional shielding of the isotope delivery cart (see Figure 3) for safe transport of the radiotracer to the patient's side. Once connection to the intravenous line is made, the radiotracer can be injected as a bolus by depressing the plunger of a remote syringe filled with oil. This action drives the stem of a hydraulic piston upward thus displacing the radiotracer from the sterile syringe.

The system can process and make available for injection 100 mCi of $H_2[^{15}O]$ (>99% radiochemically pure), starting with 300 mCi of the radiotracer in the water bubbler, although no more than 40 mCi are injected in human studies at BNL. The machine is

easily prepped for subsequent deliveries by sliding it out of the Capintec chamber, recharging the water and saline bubblers and replacing the cation resin column. Additional doses of radiotracer can be made available within 12 minutes of the previous injection.

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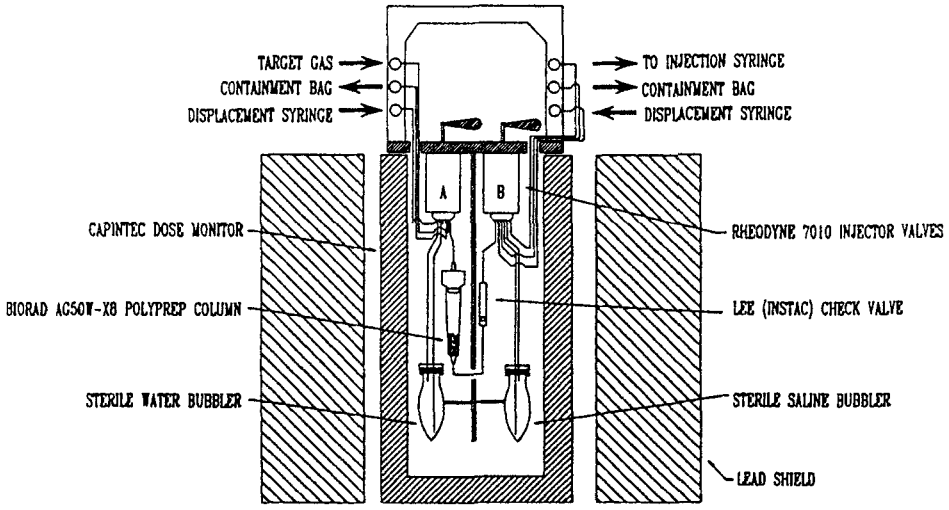

Figure 1. Schematic of $H_2[^{15}O]$ collection and processing system.

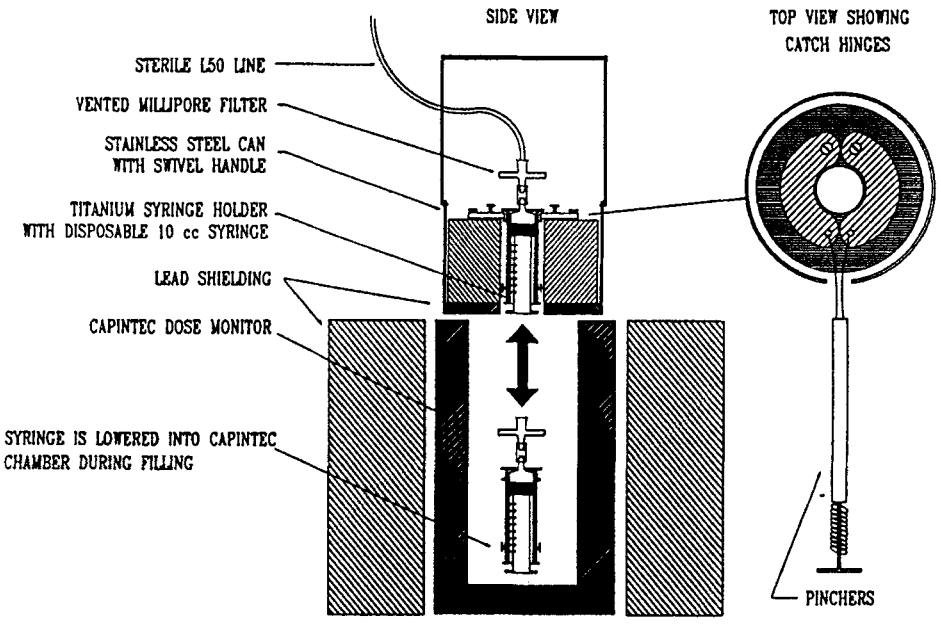

Figure 2. Schematic of injection syringe assembly in portable shielded cannister.

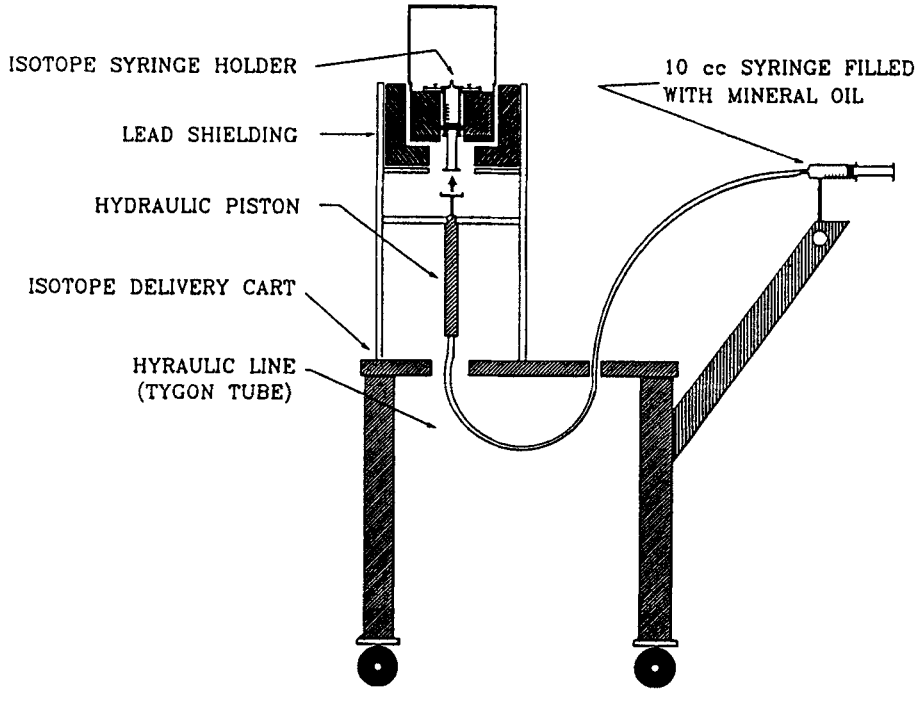

Figure 3. Schematic of shielded isotope delivery cart and remote hydraulic injector.

Radiosynthesis of ^{18}F-5-fluoro-azidouridine using ^{18}F-acetyl hypofluorite.

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Treatment of AIDS patients with antiviral drugs has many concerns mainly due to the high toxicity and side effects caused by these agents. Prolonged administration of the drug without assessing the biochemical changes will worsen the condition of the patients, especially the pediatric population infected with the disease. Azidouridine, a nucleoside analog which is undergoing phase II clinical trial as an antiviral drug for the treatment of AIDS, has great potential due to its less toxicity compared to AZT (1,2,3). Positron Emission Tomography (PET) has been proved to be an effective tool to study the disease processes at the biochemical level. It is therefore likely that azidouridine labelled with a positron emitting radionuclide may be an ideal drug for mapping the infected region of the brain. In this study, we describe the radiolabeling of azidouridine with ^{18}F-acetyl hypofluorite, an agent commonly used for electrophilic fluorination. A schematic description

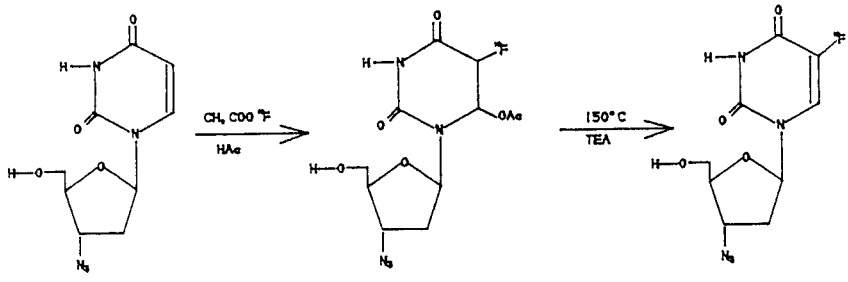

Fig. 1

of the synthesis is given in Fig.1. 10 mg of azidouridine was dissolved in acetic acid (6 ml) in a syringe setup consisting of a sterile 3-way valve and a 10 ml wheaton reaction vial. ^{18}F- acetyl hypofluorite was produced by passing $^{18}F_2$ through a column of potassium acetate (recrystallized from glacial acetic acid). The gaseous acetyl hypofluorite was bubbled through the solution of azidouridine in acetic acid until the target pressure was reduced to 5 psig. The target was flushed with neon, passed through the potassium acetate column and finally bubbled through the reaction mixture. When the fluorination was

completed, the intermediate product was transferred to the wheaton reaction vial. Excess acetic acid was removed by evaporation under reduced pressure at 150°C. 1 ml triethylamine was added to the reaction vial and the mixture was refluxed for 10 min. Excess triethylamine was removed under vacuum. The crude product was extracted with 1% acetic acid (1 ml x 4). The solution was passed through a florisil sep-pak cartridge. The product was again purified by preparative HPLC using a Phenomenex column (10 ODS 3, 250 x 9.4 mm) using 1% acetic acid as eluent at a flow rate of 2.5 ml/min. Total synthesis time was 65 min. and the radioactivity recovered from the HPLC column was 10.5 mCi. Chemical purity was >98% with radiochemical yield 15%.

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Simplified Synthesis of N.c.a. ^{18}F-Fluoroacylation Agents via 2-[^{18}F]Fluoropropionic Acid Chloride in View of Remote Controlled Labeling of Peptides and Proteins

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The synthesis of ^{18}F-fluoroacylation agents for labeling peptides and proteins has found increased interest for quantitative radioimmuno- and receptor imaging with PET. Especially the Iodine-123 and Indium-111 labeled analogs of Octreotide (1,2) have recently shown the great potency of radiolabeled peptides. Consequently Octreotide was also labeled with the positron emitting radionuclides Gallium-68 ($T_{1/2}$ = 68 min) and Fluorine-18 ($T_{1/2}$ = 110 min) (3,4). The usefulness of ^{18}F-fluoroacylation in protein labeling was recently also shown for monoclonal antibody fragments (5,6).

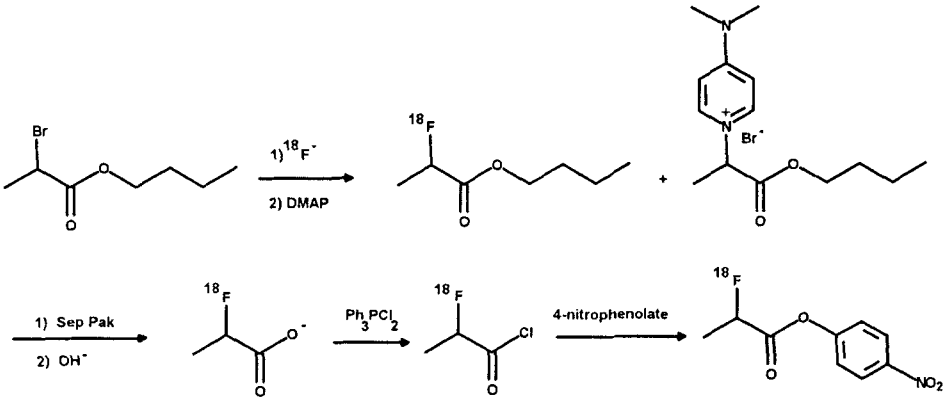
However, in contrast to iodinated acylation agents all known methods for the preparation of fluoroacylation agents require several radioactive synthesis steps and additional HPLC-purifications. Thus they are difficult to realize in automated or remote controlled systems. We describe here a simplified synthesis of our previously (7) described 2-[^{18}F]fluoropropionic acid p-nitrophenyl ester (^{18}F-PNE) for a remote controlled production of acylation agents. The synthesis is solely based on purifications via small Sep-Pak cartridges and distillation avoiding HPLC.

The first step of the radiosynthesis outlined in Scheme 1 is a conventional Kryptofix supported nucleophilic substitution on 2-bromopropionic acid n-butylester. Separation of the bromo precursor is necessary to prevent the formation of bromopropionic acid chloride in the subsequent synthesis steps. For this purpose addition of dimethylamino pyridine (DMAP) is used to react specifically with the excess of bromo precursor under formation of a quarternary pyridinium salt. This allows the separation of the ^{18}F-fluorinated ester by a simple Sep-Pak filtration as the reaction was found to be completed at quantities (20 mg) suitable for purification on Sep-Pak cartridges while no attack on the ^{18}F-fluorinated ester was observed.

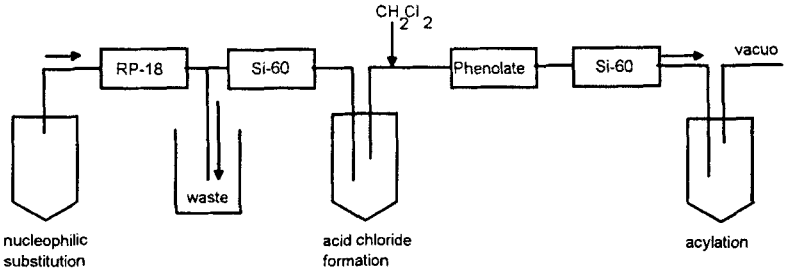
After hydrolysis followed by azeotropic drying, the 2-[^{18}F]fluoropropionate is quantitatively converted to the extremely reactive n.c.a. acid chloride by reaction with triphenylphosphine dichloride at room temperature within 3 minutes. This could be shown by derivatization reactions without separation from the reagent. Other reagents are also suitable for the formation of n.c.a. acid chloride, as was shown for [1-^{11}C]acetyl chloride (8), however, Ph_3PCl_2 with a melting point of 85°C is ideally suited in this case since the formed ^{18}F-acid chloride is very volatile and can be removed by distillation together with acetonitrile and small amounts of HCl.

The n.c.a. acid chloride can be used directly for fluoroacylation in organic solvents by trapping the activity in a solution of the amine at -78°C. Overall RCY with 0.01 N benzylamine solutions were 50±10% based on ^{18}F-fluoride.

Another useful application is the formation of activated esters needed for acylations in aqueous solutions. This can be accomplished by the use of an additional small column system containing salts of acidic hydroxy compounds for the formation of activated esters and an additional Si-60 column for puification from the free hydroxy compound liberated by HCl (Scheme 2). First results using salts of p-nitrophenol with formation of ^{18}F-PNE show that trapping of the distilled acid chloride on the column is quantitative. However, significant amounts were hydrolysed on the column and thus overall radiochemical yields of only about 35% (not optimized) for ^{18}F-PNE are lower than those obtained for direct amide formation via the acid chloride.



Scheme 1: Synthesis of n.c.a 2-[^{18}F]fluoropropionic acid p-nitrophenyl ester via 2-[^{18}F]fluoropropionic acid chloride



Scheme 2: Apparatus used for the synthesis of ^{18}F-fluoroacylation agents

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