SYNTHESIS OF N-(ω-[^{18}F]FLUOROALKYL)SPIPERONE DERIVATIVES: RAPID AND EFFICIENT METHODS FOR THE [^{18}F]FLUOROALKYLATION OF AMINES AND AMIDES

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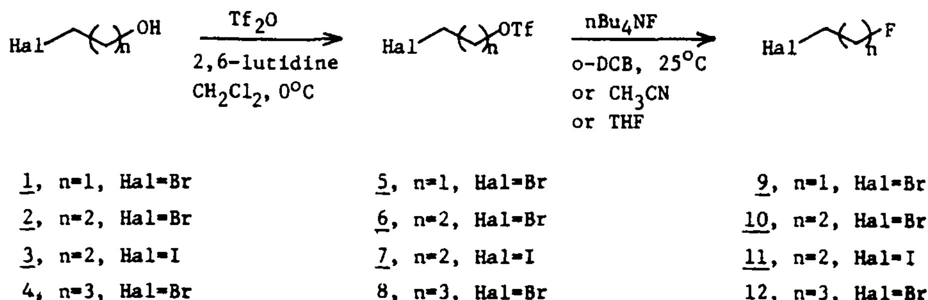
Recently, we reported that short-chain terminal olefins (propene, 1-butene, 1-pentene, 1-hexene) undergo rapid F-18 halofluorination, giving 2-fluoro-1-haloalkanes (1,2). These products are effective amine alkylating agents, and can be used to prepare N-2-fluoroalkyl amines.

In addition to this approach for the preparation of 2-fluoroalkyl compounds, we have found another rapid and efficient method for the introduction of terminal fluoroalkyl groups onto secondary amines or amides. As our first application of this fluoride displacement - N-alkylation sequence, we report here the facile synthesis of ω-[^{18}F]fluoroalkyl derivatives of the potent dopamine antagonist spiperone, described originally by Kiesewetter (3). One of these derivatives, N-(3-[^{18}F]fluoropropyl)spiperone appears to have uptake selectivity properties equal or superior to that of previously-prepared derivatives. We also report the preparation of ω-fluoroalkylated products of 1-phenylpiperazine as a secondary amine model compound.

Triflates 5 - 8 of 2-bromoethanol (1), 3-bromopropanol (2), 3-iodopropanol (3) and 4-bromobutanol (4) provide ω-fluoro-1-haloalkanes 9 - 12 by fluoride ion displacement with tetrabutylammonium fluoride, and these primary alkyl bromides and iodides are very good N-alkylation reagents (Scheme I).

Scheme I



F-19 fluoride ion displacement reactions of the triflates were performed in a 10 µmol scale (triflate:fluoride = 1:1), with various sources of fluoride ion -HF/pyridine (0%), $nBu_4NF \cdot 2HF$ (30%), and $nBu_4NF \cdot 3H_2O$ (quantitative yield, 2 min), in o-dichlorobenzene (o-DCB), CH_3CN and tetrahydrofuran (THF). The finding that the displacement reaction with nBu_4NF gives a high yield and is quite water tolerant, is encouraging for the prospects of F-18 labeling, since reasonably dry and reactive $nBu_4N^{18}F$ can be produced from F-18 fluoride ion generated in a water target (4). The fluoride displacement - N-alkylation sequence can be run without isolation of ω-fluoroalkylhalides 9 - 11, as a one pot reaction. So, for example 1-phenylpiperazine (20 µmol) in CH_3CN (Scheme II), can be fluoroalkylated in 53-83% yields.

Spiperone (5 mg, 12.7 µmol) can also be fluoroalkylated with triflates 5 - 8 (10 µmol) in o-DCB in a one pot reaction (Scheme III), giving quantitative yields based on triflates (determined by HPLC with a standardized curve). This procedure can be adapted for the preparation of 3-N-(ω-[^{18}F]fluoroalkyl) spiperone (13 - 15) under no-carrier-added (NCA) conditions.

Scheme II

Hal–(CH2)n–OTf → (nBu_4NF, CH_3CN, 25°C, 2 min) → Hal–(CH2)n–F → (2eq. phenylpiperazine, 120°C, 30 min) → phenylpiperazine–(CH2)n–F

5 - 8 9 - 12

Scheme III

Hal–(CH2)n–OTf → (nBu_4NF, o-DCB, 25°C, 2 min or THF) → Hal–(CH2)n–F → (spiperone, nBu_4NOH, 110°C, 10 min) → N-(fluoroalkyl)spiperone

5 - 8 9 - 12

13, n=1

14, n=2

15, n=3

Table 1. Selective Data of % Injected Dose in the Whole Brain and the Ratio of Striatum to Cerebellum in Rat Brain.

	2 minutes		1 hour	
	%ID/brain	Striatum/ Cerebellum	%ID/brain	Striatum/ Cerebellum
[^{18}F]SP	0.68 ± 0.12	1.03 ± 0.04	0.44 ± 0.14	4.47 ± 0.29
N-Me-[^{18}F]SP	1.51 ± 0.26	1.44 ± 0.05	0.61 ± 0.09	10.33 ± 1.50
N-(2-[^{18}F]-fluoroethyl)SP	0.99 ± 0.09	1.37 ± 0.12	0.72 ± 0.09	4.92 ± 0.30
N-(3-[^{18}F]-fluoropropyl)SP	0.90 ± 0.05	1.18 ± 0.07	0.36 ± 0.04	10.38 ± 1.53
N-(2-[^{18}F]-fluoropropyl)SP	0.80 ± 0.09	1.28 ± 0.35	0.70 ± 0.08	4.96 ± 0.88
N-(2-[^{18}F]-fluorobutyl)SP	0.53 ± 0.20	1.10 ± 0.22	0.46 ± 0.06	5.11 ± 1.21
N-(2-[^{18}F]-fluoropentyl)SP	0.35 ± 0.06	0.96 ± 0.22	0.11 ± 0.02	3.79 ± 1.84
N-(2-[^{18}F]-fluorohexyl)SP	0.28 ± 0.07	0.68 ± 0.06	0.12 ± 0.06	1.95 ± 0.68

We have optimized this procedure in more than 130 runs. Under NCA conditions (0.1-100 mCi), the F-18 fluoride ion displacement can now be done reproducibly in 62 ± 7% yield, the N-alkylation in 80% and the HPLC purification 75% yield. The average overall isolated yield is 32.6% (not corrected for decay, 41.8% corrected), and the overall time of synthesis including purification is 40 min.

This work indicates that NCA-N-(ω-[^{18}F]fluoroalkyl)spiperone derivatives can be prepared rapidly and in high yield by a very facile method. Since these compounds show good uptake in rat brain, selective for the striatum (Table 1), they may prove to be good agents for dopamine receptor mapping.

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APPLICATION OF THE NUCLEOPHILIC SUBSTITUTION REACTIONS TO THE SYNTHESIS OF NO-CARRIER-ADDED (NCA) ^{18}F-LABELED RADIOLIGANDS

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There is a growing interest in the application of positron labeled radioligands to map the neuroreceptors *in vivo* (1). Recently, we reported a general synthetic strategy for the preparation of a series of NCA ^{18}F-labeled butyrophenones (2,3) using the nucleophilic aromatic substitution reaction (4). Based on studies in animals (5,6), we found that [^{18}F]-N-methylspiroperidol ([^{18}F]NMS) is an ideal radioligand for studying the dopamine receptor in humans (7). However, the synthesis of [^{18}F]NMS is multistep and the starting materials are not commercially available. For this reason, we have searched for radioligands which will have similar kinetic behavior as [^{18}F]NMS but are more easier to prepare. We report here the application of nucleophilic aliphatic substitution reactions to the synthesis of [^{18}F]NMS analogs and other receptor radioligands for PET studies.

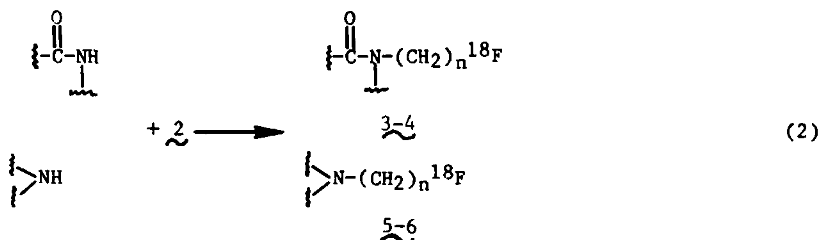
Reaction of compounds 1a-d with K[^{18}F]/Kryptofix 2.2.2 (8) in MeCN at 65-75°C for 10 min followed by purification gave the corresponding NCA [^{18}F]fluoroalkyl halides (2a-d) in 30-40% yield (equation 1).

$$(CH_2)_nX_2 \longrightarrow {}^{18}F(CH_2)_nX \qquad (1)$$

(a) n=2, X=Br; (b) n=2, X=I; (c) n=3, X=Br; (d) n=3, X=I

The yield of compound 2 depends on the solvent used, but is relatively insensitive to reaction temperature (e.g. the radiochemical yield of 2 is higher in MeCN than in DMSO, 30-40% vs. 5-10%). Increase in reaction temperature (60-90°C for MeCN and 60-140°C for DMSO) does not lead to an appreciable difference in the yield of 2. The nature of the substrates (X=Br or I) has little effect on the yield of 2, but Kryptofix 2.2.2 has a significant effect on the yield of 2.

N-Alkylations of amides and/or secondary amines with 2 gave a series of NCA ^{18}F-labeled N-[^{18}F]fluoroalkyl radioligands (3-6) in 20-60% yield (Table 1, equation 2).



The application of this method for the synthesis of other radioligands for PET studies is continuing.

Table 1. Radiochemical Yield of NCA ^{18}F-Labeled N-[^{18}F]Fluoroalkyl Radioligands[a]

Substrate	Product	Radiochemical Yield (%)[b]
Spiroperidol	N-(2-[^{18}F]fluoroethyl)-spiroperidol (3)[c]	10-20
Spiroperidol	N-(3-[^{18}F]fluoropropyl)-spiroperidol (4)[c]	30-50
(±)Normetazocine	(±)-N-(3-[^{18}F]fluoropropyl)-normetazocine (5)[d]	50-60
Lorazepam	N-(3-[^{18}F]fluoropropyl)-lorazepam (6)[e]	50-60

a. Reaction time: 10 min; reaction temperature: 110°C; substrate concentration: 10-15 μmole in DMF.

b. Products were isolated by a semi-preparative C_{18} column (10 x 250 mm) eluted with various solvents and were identified by comparison of retention times with those of authentic samples. The yield has been decay corrected.

c. The product was eluted with MeOH-H_2O-i-pr_2NH (70:30:0.1), 4 ml/min.

d. The product was eluted with MeOH-0.05N NH_4CO_2H (65:35), 4 ml/min.

e. The product was eluted with MeOH-H_2O-i-pr_2NH (60:40:0.1), 4 ml/min.

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RADIOCHEMICAL SYNTHESIS OF [^{18}F]-FLUOROETHYLSPIPERONE

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The development of a high affinity dopamine receptor ligand labeled with the positron emitting radionuclide ^{18}F ($t_{1/2}$ = 109.8 min) is of considerable interest for imaging and quantification of dopamine receptors in vivo. [^{11}C]-N-methyl spiperone has been of great utility in the study of dopamine receptor interactions.(1,2,3) However, the shorter half-life of ^{11}C ($t_{1/2}$ = 20.4 min) has limited the ability to gain understanding of receptor-ligand kinetics. Spiperone and various analogues have been prepared which are labeled with ^{18}F.(4,5,6,7) We have reported the chemical synthesis and D_2 dopamine receptor affinity of 3-N-(2-fluoroethyl)spiperone.(8) The receptor affinity of this compound, as measured by an in vitro assay, is nearly the same as spiperone (K_i = 292 pM for fluoroethyl spiperone vs. 309 pM for spiperone).

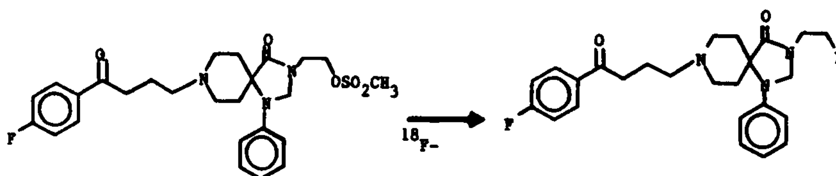

Figure 1. Radiochemical synthesis of 3-N-(2-fluoroethyl) spiperone.

The radiochemical synthesis was designed to employ the [^{18}F]fluoride displacement reaction in the final step (Figure 1). The precursor was prepared in three steps from the ethylene ketal of spiperone. Initially, the lactam nitrogen is alkylated with tetrahydropyranyl (THP) protected bromoethanol. The resulting THP ether was cleaved with methanol-HCl to yield 3-N-(2-hydroxyethyl) spiperone. The alcohol was activated toward displacement by conversion to the methanesulfonate ester. The resulting spiperone mesylate was purified by preparative HPLC to remove N-chloroethyl spiperone.

TABLE 1. OVERALL YIELDS FOR FLUORIDE DISPLACEMENT UTILIZING CYCLOTRON PRODUCED FLUORIDE.

Cation	OH- umol	F- umol	Activity uL	Substrate mg	Transfer %	Incorp. %	Overall %	N[a]
TMA[b]	1	0.01	50	1	63	42	27	7
TMA	1	0.01	200	1	54	42	23	5
TMA	1	NCA	200	1	51	36	16	3
TMA	1	NCA	50	1	46	16	7	2
K+[c]	0.2	0.2	100	0.2	93	33	31	1
K+	0.2	0.02	100	0.2	82	10	8	1
K+	0.2	NCA	100	0.2	77	5	4	1

[a] N is the number of trials in determining the means for percent transfer, incorporation, and overall.
[b] TMA = tetramethyl ammonium
[c] K = potassium--reaction employed 4 umol Kryptofix-[2.2.2]

TABLE 2. OVERALL YIELDS FOR FLUORIDE DISPLACEMENT UTILIZING REACTOR FLUORIDE.

K-222[a] umol	K_2CO_3 umol	KF umol	Activity uL	Substrate mg	Transfer %	Incorp %	Overall %
4.5	0.5	NCA	100	0.5	71	80	56
2.3	0.5	NCA	100	0.5	62	77	48
1.1	0.5	NCA	100	0.5	68	80	54
4	2	NCA	200	1.0	76	11	8
4	2	NCA	300	1.0	68	43	29
4	2	0.1	300	1.0	82	57	46

[a] K-222 = Kryptofix-[2.2.2]

The success of the radiochemical synthesis is dependent on the optimization of the [^{18}F]fluoride displacement reaction. Two sources of [^{18}F]fluoride were evaluated: a) reactor produced [^{18}F]fluoride from the National Bureau of Standards, and b) cycloton produced [^{18}F]fluoride generated at NIH. Numerous reaction parameters were studied in order to optimize the displacement reaction. The quantity of substrate, concentration of base, amount of carrier fluoride, initial activity, identity of cation, and radionuclidic purity have been investigated. Overall yields for the reactions were evaluated by considering the percent incorporation into the desired product and the percent of the total activity. The percent incorporation into desired product was determined by radio-TLC. A sample of the data is contained in Table 1 and Table 2.

Although work is still in progress, several trends are evident. On those occasions when visibly colored solutions from the cyclotron target were obtained, (analysis of the target water indicated high amounts (5-10 ppm) of metals (Ti^{+4},Fe^{+3})), the overall radiochemical yields were adversely affected. The use of approximately 1 mg of substrate provides more consistent overall yields. Also, the addition of a small amount of carrier (10 nmol) gives more consistant overall yields. This amount of carrier under our present operating conditions (6uA x 30 min) should result in a specific activity of 74 GBq/umol (2 Ci/umol) at E.O.B.

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N.C.A. [^{18}F]-FLUOROALKYLATION VIA NUCLEOPHILIC FLUORINATION OF DISUBSTITUTED ALKANES AND APPLICATION TO THE PREPARATION OF N-[^{18}F]-FLUOROETHYLSPIPERONE

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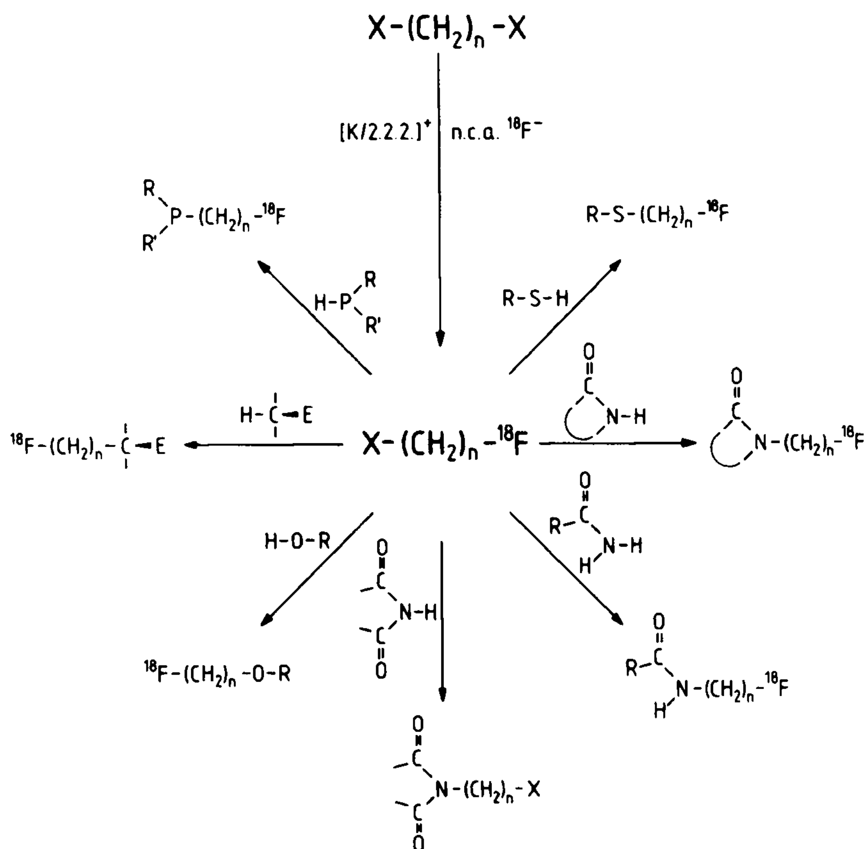
Direct nucleophilic substitution with n.c.a.[^{18}F]-fluoride is often limited, particularly in the case of H-acidic compounds. These molecules lend themselves to the condensation with small radiofluorine bearing alkanes as prosthetic groups as suggested for ^{18}F-fluoromethylation (1). Educts for this general method are alkanes with two nucleofugic groups (2). These bifunctional fluoroalkanes can be reacted with a variety of groups as indicated in Scheme 1. In contrast to the addition of fluoro-interhalogens to olefines (3) nucleophilic substitution leads to a wealth of bifunctional alkanes.



Scheme 1. Possibilities of [^{18}F]-fluoroalkylation of various H-acidic compounds via nucleophilic substitution of bifunctional alkanes with n.c.a. $^{18}F^-$.

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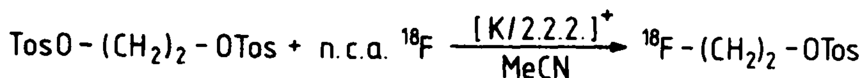

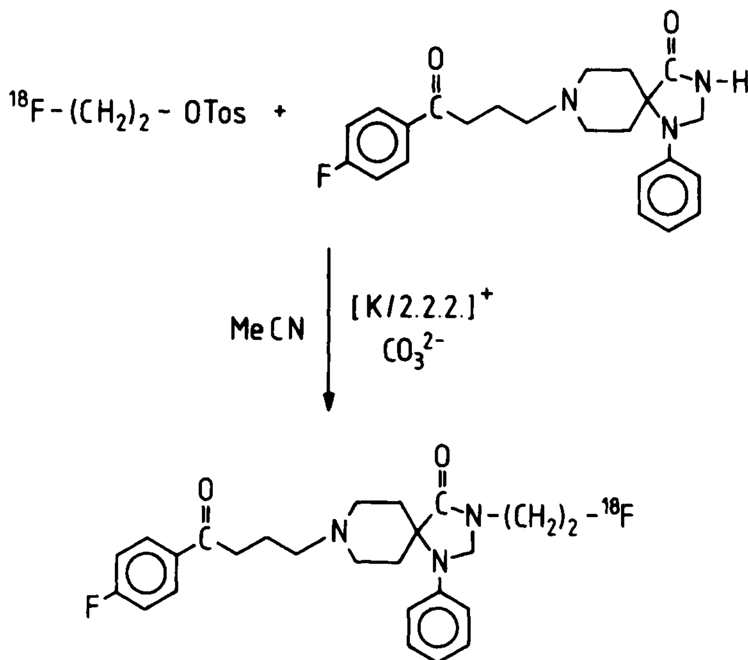

Scheme 2. N-Fluoroalkylation of spiperone with n.c.a. [^{18}F]-fluoroethyltosylate

ω-Disubstituted alkanes $X(CH_2)_nX$, where n = 1-3, X = Br, OMes, OTos, can be efficiently labeled with n.c.a. $^{18}F^-$ in the presence of the aminopolyether(2.2.2.)-K_2CO_3 complex (4). For n=1 the highest yield is obtained with bismesyloxymethane (85±9), while for n=2 and 3 the nucleophilic substitution with the bistosyloxy compounds is most effective (86±8 %). The reaction conditions have been systematically optimized for bistosyloxyethane with respect to temperature, time, substrate concentration and solvent. An almost linear increase of the substitution yield is observed with rising solvent polarity.

For condensation of the monofluorinated alkanes with C-H, N-H, and O-H acidic groups of simple model compounds the aminopolyether(2.2.2.)-K_2CO_3 complex conveniently be performed in a one-pot synthesis. The alkylation reaction has also been systematically optimized with respect to time, temperature, substrate and base concentration. The dependence of the alkylation yield on the solvent is widely determined by the solubility of the given substrate. From the three leaving groups TosO- proves to be most reactive. For example condensation of $^{18}FCH_2CH_2$-X with phenol yields 7, 25 and 51 % of the corresponding β-fluoroethylphenylether with X = MesO-, Br-, and TosO-, respectively. Whereas the fluoromethylated products are only stable in special cases, fluoroethylation and fluoropropylation is generally possible in good yields. The total synthesis of radiofluorinated products can be performed in less than 30 minutes.

As a longer lived alternative to 3-N-[^{11}C]-methylspiperone, spiperone has been fluoroalkylated using [^{18}F]-fluoroethyltosylate as optimal substituent for the 3-N position. The fluoroethylation proceeds with a corrected yield of 47+4 % in acetonitrile (Scheme 2). The 2-step labeling procedure is accomplished within about 2 hours. The 3-N-[^{18}F]-fluoroethylspiperone proves to be a potent antagonist for D_2-receptors in rodents and primates. A striatum-to-cerebellum ratio of about 6 is obtained in baboons 3 hours after injection.

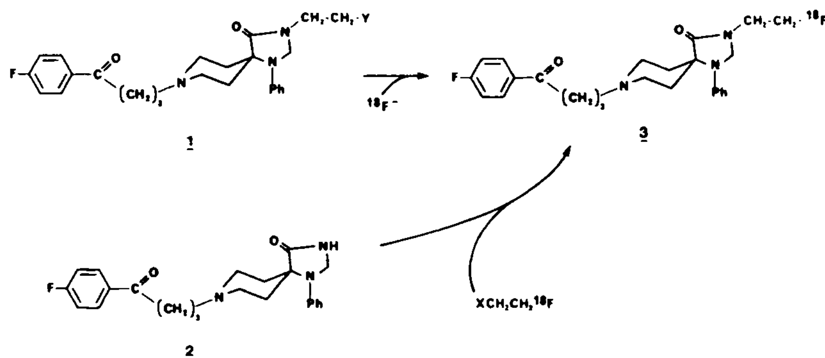

Figure 1. Reaction sequence for synthesis of FESP.

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SYNTHESES OF 3-(2'-[F-18]FLUOROETHYL)SPIPERONE, A DOPAMINE RECEPTOR-BINDING RADIOPHARMACEUTICAL FOR POSITRON EMISSION TOMOGRAPHY.

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It is increasingly clear that positron emission tomography (PET) is an excellent tool for the in vivo, non-invasive investigation of neurotransmitter systems. This possibility offers for the first time direct access to the biochemistry of neuropsychiatric diseases in humans. Of all neurotransmitter systems, dopamine has been the most intensively investigated with PET (1). In humans, for example, presynaptic neurotransmission was studied with 6-[F-18] fluorodopa (2) and postsynaptic receptor interactions with radiolabeled dopamine-D_2 receptor antagonists such as N-[^{11}C]methylspiperone (3,4) and [^{11}C]raclopride (5).

For the successful estimation of some kinetic parameters related to receptor binding (i.e. in vivo dissociation rates), it is generally recognized that the F-18 label should be preferred to C-11 (1). Therefore, considerable emphasis has been placed to date on the incorporation of the F-18 isotope at the para position on the phenyl ring of the butyrophenone moiety, but all these syntheses either involve multistep reactions (6) or give low radiochemical yields (7,8). Thus, the aim of the present work was to develop a simple, high yield synthesis of a F-18 labeled neuroleptic ligand. Since short chain 3-N-alkylations of spiperone and derivatives do not critically affect their potential as dopamine-D_2 receptor binding tracers (3,9), we decided to investigate 3-N-fluoroalkylspiperone derivatives as candidates for in vivo studies with PET. We report herein that this strategy has resulted in a new class of F-18 labeled neuroleptic ligands, as exemplified by 3-(2'-[F-18]fluoroethyl) spiperone (FESP), with attractive properties for dynamic characterization of dopamine receptor binding in living primates with PET (10,11).

FESP (3) was synthesized by alkylation of the amide in spiperone (2) by non-carrier added ^{18}F-labeled fluoroethyl derivatives (X = Br, I or OSO_2CH_3) in the presence of strong bases, in 15-20% radiochemical yield (EOS). Alternatively, fluoroethyl spiperone 3 could also be prepared from 1 (Y = Cl, Br, OSO_2CH_3, $OSO_2C_6H_4CH_3$, OSO_2CF_3 and quaternary ammonium salts) by the reaction of nca $Cs^{18}F$, $Ag^{18}F$ or $K^{18}F$/Kryptofix in acetonitrile or DMSO. For example, the reaction of 3-(2'-methanesulfonyloxyethyl)spiperone (1,Y = OSO_2CH_3) in CH_3CN with nca $K^{18}F$/Kryptofix followed by purification by preparative HPLC gave 3 in ~30% radiochemical yield with a specific activity of 500-1000 Ci/mmol (EOS) in less than 60 min. The radiochemical purity of the final product was determined by analytical HPLC and TLC and found to be >99%.

The intermediates 1 and the final product 3 (obtained by ^{19}F-carrier added syntheses) were characterized by IR, ^{1}H NMR, ^{19}NMR and high resolution mass spectroscopy. X-ray crystallographic analysis of a single crystal of 3 provided unequivocal evidence for its structure.

The salient features of FESP provided by this work are as follows: i) it can be easily and reproducibly generated in multimillicurie amounts (>50 mCi) for PET studies; ii) the simplicity of the synthesis (one step) and its high radiochemical yield facilitate tomographic studies including multiple studies/day; iii) the long-lived F-18 isotope (110 min) permits in vivo estimation of kinetic parameters with PET for more than 12 hours.

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N.C.A. RADIOFLUORINATION OF SPIPERONE AND N-METHYLSPIPERONE VIA AMINOPOLYETHER SUPPORTED DIRECT NUCLEOPHILIC SUBSTITUTION

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^{18}F-labeled spiperones are attractive radiotracers for mapping D_2 dopamine receptor-areas in brain using PET (1,2). The most efficient radiofluorination method reported so far involves a multistep synthesis (3), while in direct nucleophilic ^{18}F-for-NO_2 displacement only very small yields have been obtained (4,5). An efficient aminopolyether (APE) 2.2.2. mediated nucleophilic substitution with n.c.a. $^{18}F^-$ was recently applied to the preparation of other radiopharmaceuticals (6,7). A modified APE-system which widely avoids the destruction of the butyrophenones was now successfully used for the n.c.a. one-step radiofluorination of spiperone and its methylated analog. Both isotopic ^{18}F-for-^{19}F and ^{18}F-for-NO_2 exchange have been studied (see Table). High specific activity products (> $3 \cdot 10^5$ GBq/mmol) have been obtained by ^{18}F-for-NO_2 exchange on 4'-nitro-defluoro-spiperone and 4'-nitro-defluoro-3-N-methyl-spiperone, respectively. The reaction proceeds in anhydrous DMSO at about 140°C within 20 to 30 min, and the fluorine-18 labeled n.c.a. spiperones are separated from the reaction mixture by HPLC. The radiochemical yields obtained for the N-methylated butyrophenones (see Table) are considerably higher than those in the unmethylated substrate.

TABLE Formation of [^{18}F]-Spiperone and [^{18}F]-3-Methylspiperone using Aminopolyether (2.2.2.) Supported Nucleophilic Substitution with n.c.a. [^{18}F]-Fluoride

Substrate[a]	Radiochem. Yield (%)[b]
Spiperone	33 ± 2
3-N-Methylspiperone	74 ± 8
4'-Nitro-spiperone	35 ± 6
4'-Nitro-3-N-methylspiperone	58 ± 3

[a] 10 mg in 1 ml DMSO containing APE(2.2.2.)/$K^{18}F$

[b] decay corrected; reaction time: 20-30 min, 140°C

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RADIOLYTIC SELF-DECOMPOSITION OF A HIGH SPECIFIC ACTIVITY POSITRON EMITTING RADIOTRACER, $[^{18}F]$-N-METHYLSPIROPERIDOL.

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Among the primary goals of those in the radiopharmaceutical field have been to provide more radioactivity, higher specific activities and to increase the chemical purity of their products. Progress in the fields of cyclotron targetry and radiotracer synthesis have made great advances in these directions; advances of sufficient magnitude, in fact, to raise new problems and concerns. An example of a chemically pure, high specific activity positron emitting radiotracer is $[^{18}F]$-N-methylspiroperidol ($[^{18}F]$NMS), which is purified by preparative high performance liquid chromatography (HPLC) and has a specific activity of 4-8 Ci/µmol (1). When high activity levels of this tracer were dissolved in saline (5-10 mCi/ml) analytical radio HPLC revealed that $[^{18}F]$NMS underwent significant decomposition resulting in a decrease of radiochemical purity of 10-25% during the first hour (Fig. 1). Since $[^{18}F]$NMS produced in low activity runs was thermally stable even at pH extremes, radiolytic decomposition appeared to be a plausible mechanism for the observed product instability at higher radiation levels. Because of the impact of such a radiolytic decomposition on the ability to transport or even store a batch of high-specific activity radiotracers for multiple PET studies during the day, a study of this phenomenon was undertaken.

$[^{18}F]$NMS was prepared and purified as usual (1). The product was dissolved in 2.9 ml of a sterile solution of saline:water (3:1) and the resulting solution was filtered through a 0.22 µm millipore filter into a multiinjection vial containing 0.1 ml of sterile $NaHCO_3$ solution (1 Meq/ml). This stock solution was used to make the experimental solutions for analysis. Aliquots of the experimental solutions at various times were quenched by addition to methanol and analyzed by radio HPLC.

In dilute aqueous solutions of organic materials, radiolytic decomposition of the water produces reactive species which can in turn react with the organic molecules in the solution and decompose them. This process follows pseudo first-order kinetics since the concentration of the reactive species in solution is nearly constant. The decomposition of $[^{18}F]$NMS was found to follow pseudo first-order kinetics when plotted as a function of the radiation dose to the solution. When $\ln(A_o/A)$ (where A_o = % $[^{18}F]$NMS at zero time and A = % $[^{18}F]$NMS at some later time) is plotted versus dose, an effective rate constant (k_{obs}) can be found. This constant will be the sum of the terms in the actual rate equation, i.e.,

$$\frac{\text{Rate of Decomposition}}{\text{Total Dose}} = k_{obs}[^{18}F]\text{NMS} = \frac{k_1' k_2 [^{18}F]\text{NMS}}{k_2[\text{NMS}] \text{ and } \sum k_i[S_i]}$$

where NMS is the unlabeled compound and $\sum k_i[S_i]$ is a sum over the other scavenger reactions. By plotting $1/k_{obs}$ versus each of the scavenger concentrations, it is possible to isolate the terms of the equation. For example, plotting $1/k_{obs}$ versus [NMS] gives a line whose slope will be $1/k_1'$ and with an intercept of $\sum k_i[Si]/k_1'k_2$. This reflects the fact that unlabeled NMS should compete effectively with $[^{18}F]$NMS for the reactive species generated from the radiolysis of water. Indeed, the reciprocal of the rate of decomposition was shown to be linearly dependent on the added carrier concentration in three solutions of equal radioactivity and pH (Fig. 2). Similar dependence was demonstrated for hydrogen ion concentration (Fig. 3), for carbon dioxide concentration and for an undefined scavenger(s) present in the stock $[^{18}F]$NMS solution.

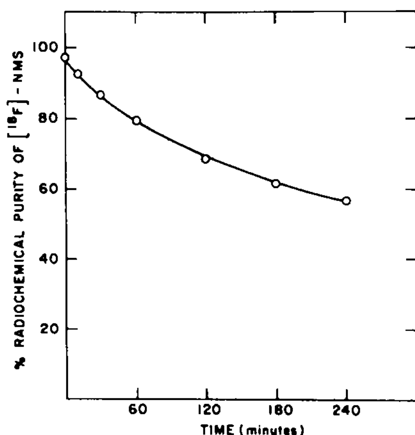

Fig. 1 Percent radiochemical purity of [^{18}F]NMS at time of preparation for injection (15.2 mCi/3 ml) as measured at different times after its preparation.

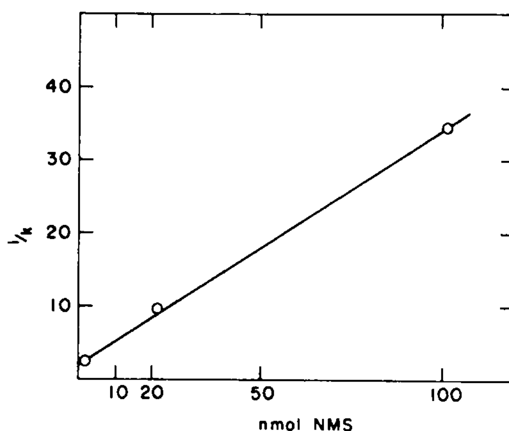

Fig. 2 A plot of the reciprocal of the observed rate constant (k in krad) for the decomposition of [^{18}F]NMS at three different specific activities.

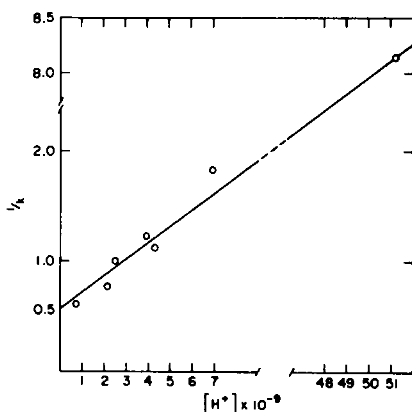

Fig. 3 A plot of the reciprocal of the rate constant (k in krads) for [^{18}F]NMS at different hydrogen ion concentrations.

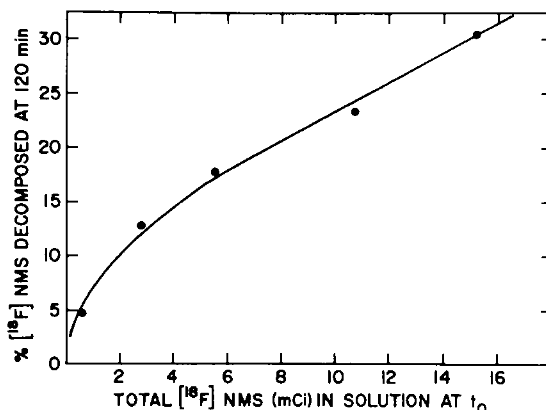

Fig. 4 Percent of [^{18}F]NMS decomposed at 120 minutes versus total mCi of [^{18}F]NMS at $\Delta t=0$.

To demonstrate that the rate of decomposition was proportional to total dose, a series of six solutions, each containing 9 nmole of NMS but varying in total activity over a 300-fold range was studied. A plot of percent [^{18}F]NMS decomposed at 120 min versus total mCi of [^{18}F]NMS at the start of the run shows a striking, although non-linear, dependence on total dose (Fig. 4).

To show independently that NMS was unstable in the presence of the products of the radiolysis of water, a solution containing 239 mCi of $^{18}F^{\ominus}$ and 23.3 nmol of unlabeled NMS was analyzed as a function of time. Decomposition under these conditions were similar to the self-decomposition shown in Fig. 1.

This study has demonstrated that radiolytic self-decomposition can occur with high specific activity tracers labeled with short-lived β^+ emitters on a time scale comparable to the physical half-life of ^{18}F. Whether radiolytic self-decomposition will present a problem with other high specific activity ^{18}F-labeled tracers or with ^{11}C-labeled compounds, and whether additives which are suitable for intravenous injection can be used to retard the rate of decomposition remains to be determined along with the effect of chemical structure on radiolytic stability.

In light of these observations, the stability of a chemically pure radiotracer of high specific activity should not only be determined immediately after its preparation but also at later time points especially if delay in use is anticipated. In the case of [^{18}F]NMS, if there is a delay in time between the time of preparation and the anticipated time of injection, or if shipment to a collaborating institution is required, the tracer is left in the HPLC solvent (CH_3OH:0.02 N NH_4HCO_2, 75:25) or in methanol (where it is stable throughout the useful half-life of fluorine-18) and processed for injection immediately before use.

This research was carried out at Brookhaven National Laboratory under contract DE-AC02-76CH00016 with the U. S. Department of Energy and supported by its Office of Health and Environmental Research and also supported by National Institutes of Health Grant NS-15380.

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^{11}C-DIAZOMETHANE : A NEW CARBON-11 PRECURSOR FOR THE LABELLING OF RADIOPHARMACEUTICALS

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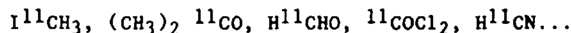
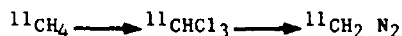
The synthesis of ^{11}C-labelled radiopharmaceuticals, used in positron emission tomography studies, requires, that the carbon-11 label at end of the nuclear bombardment, is incorporated into the skeleton of a molecule in less than 40 minutes.
Precursors that have been routinely used in the carbon-11 labelling of radiopharmaceuticals include :



O-methylation reactions are difficult to carry out with methyl iodide but are easily performed with diazomethane. Also, this precursor allows the methylation of alcohols and amides. For these reasons, a simple method to prepare ^{11}C-diazomethane with a high specific radioactivity has been developed.

The synthesis involves, formation of ^{11}C-methane, catalysis to ^{11}C-chloroform followed by direct treatment with hydrazine in alcohol, to yield ^{11}C-diazomethane.
^{11}C-Methane is produced by irradiation of a nitrogen-5 % hydrogen mixture with protons.

Catalysis of ^{11}C-methane at 310-320°C on pumice stone impregnated with cupric chloride (1) gives ^{11}C-chloroform and reaction with hydrazine in ethanol with potassium hydroxide at 60°C (2-4) results in the formation of ^{11}C-diazomethane.



The convertion of ^{11}C-methane to ^{11}C-chloroform was found to be 40-50 %, with ^{11}C-diazomethane having an overall yield of 30 % from ^{11}C-methane.
The specific radioactivity of ^{11}C-diazomethane was determined by HPLC analysis of the methyl-ester obtained by the action of ^{11}C-diazomethane on nitrobenzoic acid.

From, ^{11}C-methane with a specific radioactivity of 3-5 Ci/µMole (EO8), ^{11}C-diazomethane can be obtained in 10 minutes with a specific radioactivity of 2.5-3.5 Ci/µMole.
With a thirty minute irradiation of the nitrogen target by 20 MeV protons (30 µA), 300 mCi of ^{11}C-diazomethane has been synthesized.

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SYNTHESIS OF [^{11}C]METHYLCYCLOPROPYL IODIDE

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In our work on the synthesis of radiopharmaceuticals for PET studies, the availability of different labeled alkyl halides is of great importance. [1-^{11}C]Ethyl iodide, [1-^{11}C]propyl iodide, [1-^{11}C]butyl iodide, [1-^{11}C]isobutyl iodide and [1-^{11}C]benzyl iodide have all been prepared in fair yields and used in many applications (Figure 1)(1).

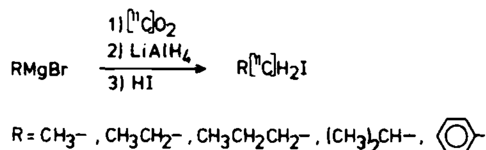
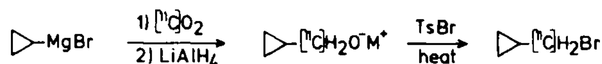


Figure 1. Synthesis of ^{11}C-labelled alkyl iodides

In this paper the synthesis of [^{11}C]methylcyclopropyl iodide in a four-step reaction from [^{11}C]carbon dioxide is reported. The cyclopropylmethyl group is a common structural element in many opioids.
The method used for the synthesis of ^{11}C-alkyl halides mentioned above could not be used in the preparation of [^{11}C]methylcyclopropyl iodide (2). In our experiments, the radiochemical purity of [^{11}C]methylcyclopropyl iodide never exceeded 40 percent (LC analysis after trapping). The main difficulty was to avoid the rearrangement of cyclopropyl methanol in the strong acidic medium used. Several other methods were tried, the most successful being the reaction of the alcoholate with tosyl bromide (3) at elevated temperature. This gave the desired alkyl bromide after five minutes reaction with good radiochemical purity. Attempts to prepare the alkyl iodide with tosyl iodide failed, probably due to the instability of the tosyl iodide.



TsBr= p-toluensulphonyl bromide

Figure 2. Synthesis of [^{11}C]methylcyclopropyl bromide.

Since cyclopropylmethyl bromide is a poor substrate in alkylation reactions compared to the corresponding iodide, the [^{11}C]methylcyclopropyl bromide was

trapped in a solution of sodium iodide in acetone (4) and heated at 100°C. This gave a complete conversion of the bromide to the corresponding iodide in one minute.

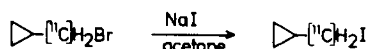


Figure 3. Halogen exchange of [^{11}C]methylcyclopropyl bromide.

Alkylation was performed by adding DMSO and the substrate and heating the mixture at 100°C for some minutes.
Using phenethylmethylamine as a model substance, the cyclopropylmethyl phenethylmethylamine (Figure 4) was obtained in 60% radiochemical yield with a reaction time of five minutes at 100° C.

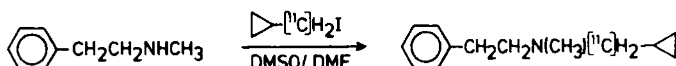


Figure 4. Alkylation with [^{11}C]methylcyclopropyl iodide.

Work is now in progress to prepare interesting opiate ligands, such as [N-[^{11}C]methylcyclopropyl]diprenorphine and [N-[^{11}C]methylcyclopropyl]-naltrexone.

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SYNTHESIS OF NCA ^{11}C-LABELLED AROMATICS USING ^{11}C-CYANIDE AND ARYL-CHROMIUM TRICARBONYL INTERMEDIATES

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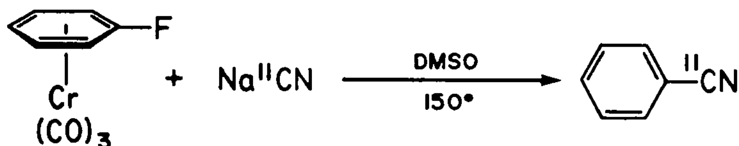
It has recently been shown (1) that arenechromium tricarbonyl complexes readily undergo nucleophilic displacement reactions on the aromatic ring This enhanced reactivity to nucleophiles can be attributed to the removal of electron density from the aromatic ring through coordination to chromium. The incorporation of the chromium tricarbonyl moiety into aromatic systems can in most cases be done more easily and under milder conditions than the synthesis of aromatic nitro derivatives; the nitro group having approximately the same magnitude of electron withdrawal as the chromium metal. We decided to explore this chemistry further with the aim of labelling aromatics with ^{11}C using $^{11}CN^-$ as shown below.



In a typical experiment $H^{11}CN$ was bubbled into a solution of NaOH (0.1N, 1 mL) and this solution was then evaporated to dryness. Under inert atmosphere, DMSO and η^6-fluorobenzenetricarbonylchromium was added. The mixture was heated to 150°C for 5 min, then cooled to room temperature and diluted with MeOH. ^{11}C-Benzonitrile was obtained in ~50% radiochemical yield after HPLC purification. It is interesting to note that under these conditions decomplexation also occurs so that the usual oxidative decomplexation step is not necessary. In a control experiment using fluorobenzene instead of the chromium complex no product was obtained under these same conditions.

Subsequent conversion of these aromatic nitrile compounds may provide a route to a variety of ^{11}C-labelled carbonyl containing compounds. We also plan to extend this method to the use of other positron labelled nucleophiles such as halides.

We wish to thank the Natural Science and Engineering Council of Canada for financial support. We are also grateful to Kenneth Krohn and Jean Link of the University of Washington in Seattle for providing us with $^{11}CN^-$ for our initial experiments.

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ENZYMATIC SYNTHESIS OF L-[^{13}N] TYROSINE

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It has been previously demonstrated that commercial preparations of glutamate oxalocetate transaminase (GOT) from pig heart can be used to catalyze the transfer of the ^{13}N amino moiety of glutamate to p-hydroxyphenylpyruvate to synthesize L-[^{13}N] tyrosine (1). However, separation of labeled tyrosine from glutamate and ammonia by cation or anion exchange columns failed to yield ^{13}N labeled tyrosine of sufficient radiochemical purity to initiate biological studies. We now report on the synthesis of ^{13}N-labeled tyrosine, with a radiochemical purity exceeding 98%, from [^{13}N] ammonia by coupling the glutamate dehydrogenase (GDH) and GOT reactions and by separating labeled tyrosine from glutamate and ammonia with a column composed of Poropak Q, a neutral polystyrene resin.

In this procedure, GDH and GOT are immobilized on 0.5 g and 2.0 g CNBr activated Sepharose, respectively, in methods previously described (2). After synthesis of L-[^{13}N] glutamate, 100 µmoles p-hydroxyphenylpyruvate are added to the reaction, and the mixture allowed to incubate on the GOT column. After 10 min, the reaction mixture contained 57% tyrosine, 39% glutamate and 4% NH_3. The pH of the solution is lowered to 2.0 and unreacted ^{13}N-ammonia and glutamate are eluted from a 10 g column of Poropak Q (100/200 mesh) with 10 ml 50 mM phosphate buffer (pH 2.0) and 10 ml 50 mM phosphate buffer (pH 12). L-[^{13}N] Tyrosine is then eluted with 6 ml of 50 mM phosphate buffer (pH 12) (3). The pH of the solution containing labeled tyrosine is lowered to neutral and made isotonic with NaCl. After passage through a Millex filter with 0.22 µm pore size, the solution is suitable for patient studies. A yield of 20 mCi of L-[^{13}N] tyrosine was produced from 400 mCi of [^{13}N] ammonia 25 min EOB.

Immobilized GOT can also utilize phenylpyruvate and indole-3-pyruvate as ^{13}N-amino acceptors for the preparation of L-[^{13}N] phenylalanine and L-[^{13}N] tryptophan, respectively. Under conditions where 59% of L-[^{13}N] glutamate is transaminated to p-hydroxyphenylpyruvate to form tyrosine, 28% and 15% of ^{13}N from glutamate can be converted to labeled phenylalanine and tryptophan.

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SYNTHESIS OF DL- AND L-[3-^{11}C]-AMINO ACIDS

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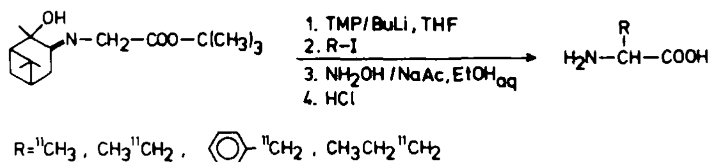
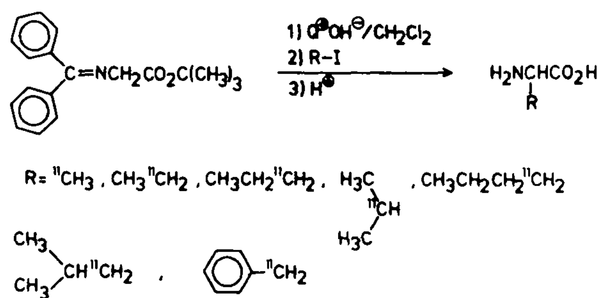
Three synthetic pathways leading to enantiomerically pure or enriched L-[3-^{11}C]-amino acids are presented. The L-[3-^{11}C]-amino acids were prepared either by asymmetric syntheses or by treatment of a racemic [3-^{11}C]-amino acid preparation with D-amino acid oxidase.

The reaction sequence, as shown in Scheme 1, has proved to be an efficient way of synthesizing L-[3-^{11}C]-amino acids. L-[3-^{11}C]Alanine has been obtained by this route in 89 % enantiomeric excess (e.e.), as reported earlier (1). In a preliminary study, L-2-amino[3-^{11}C]butyric acid, L-[3-^{11}C]norvaline and L-[3-^{11}C]phenylalanine were synthesized by this method in 10-25 % radiochemical yields. The enantiomeric excess of L-2-amino[3-^{11}C]butyric acid was determined to be 87 %. The optical purity of the other ^{11}C-amino acids has not yet been determined. Since the other enantiomer of the chiral substrate [(+)-2-hydroxypinanyl-3-idene]glycine tert-butyl ester is available (2), this synthesis also provides access to the D-form of the ^{11}C-amino acids presented here.



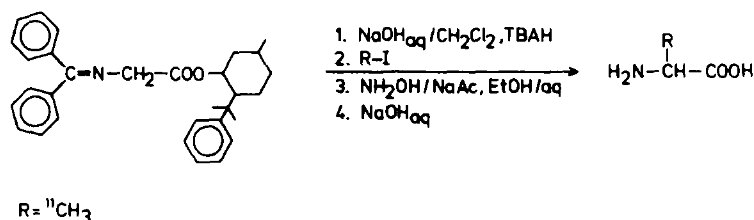
Scheme 1

A phase-transfer alkylation reaction on N-(diphenylmethylene)glycine tert-butyl ester with ^{11}C-alkyl iodides leading to racemic [3-^{11}C]-labeled amino acids is presented in Scheme 2. By this route, DL-[3-^{11}C]-labeled alanine, 2-aminobutyric acid, norvaline, valine, norleucine, leucine and phenylalanine have been obtained in 10-55 % radiochemical yields with radiochemical purities in the range 93-99 %. L-[3-^{11}C]Alanine, L-[3-^{11}C]valine and L-[3-^{11}C]phenylalanine were obtained in enantiomeric purities higher than 99 % on treatment with immobilized D-amino acid oxidase (3).



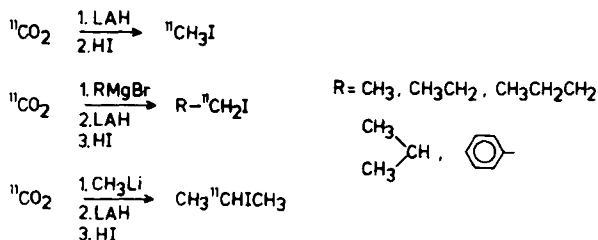
Scheme 2

L-[3-^{11}C]Alanine has also been prepared by an asymmetric phase-transfer alkylation reaction using N-(diphenylmethylene)glycine 8-phenylmenthyl ester as substrate (Scheme 3). The L-[3-^{11}C]alanine was obtained in 35-45 % enantiomeric excess and 30 % radiochemical yield with a radiochemical purity higher than 98 %.



Scheme 3

The ^{11}C-alkyl iodides were prepared according to Scheme 4 which is presented in detail elsewhere(4).



Scheme 4

This project was financially supported by grants K-KU 3464 and K-KU 2446 from the Swedish Natural Science Research Council.

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REAGENTS FOR FLUORINE-18 LABELING OF PROTEINS

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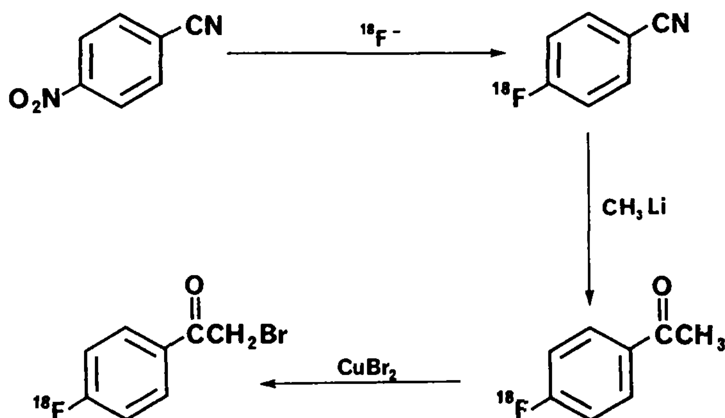
Although proteins have been radiolabeled with carbon-11, radioiodine and radiobromine, and various metal radioisotopes, little effort has been made in labeling with fluorine-18. The acylation of urokinase with [F-18]fluoroacetic acid by Muller-Platz et al (1) is the only published report.

Two approaches can be taken towards fluorine-18 labeling of proteins: (a) direct fluorination of amino acid residues or (b) modification with fluorine-18 labeled reagents. We have taken the second approach to protein labeling, and have prepared two fluorine-18 labeled reagents, 4-[F-18]fluorophenacyl bromide (I, α-bromo-4-[F-18]fluoroacetophenone) and methyl 3-[F-18]fluoro-5-nitrobenzimidate (II).

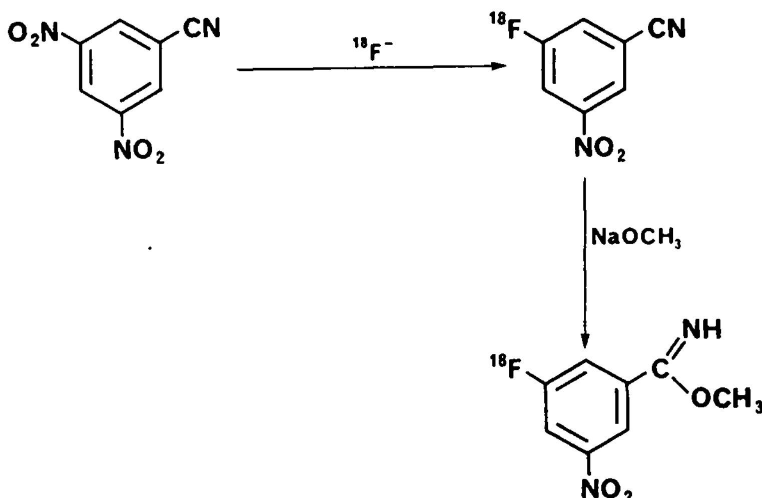
No-carrier-added [F-18]fluoride ion was prepared by proton bombardment of a small volume [O-18]water target (2). The target water was combined with 2-5 micromoles of tetrabutylammonium hydroxide and evaporated (glass or platinum vessel). The activity was then resolubolized as tetrabutylammonium fluoride in no-carrier-added form.



Scheme I

The preparation of 4-[F-18]fluorophenacyl bromide is shown in Scheme I. 4-[F-18]Fluorobenzonitrile was prepared by [F-18]fluoride for nitro substitution (75%) and the product isolated by C18 SEP-PAK chromatography. The nitrile was converted to 4-[F-18]fluoroacetophenone by treatment with excess methyllithium (75%) and isolated by C18 SEP-PAK. One step synthesis of 4-[F-18]fluoroacetophenone gave low yields (10%). Bromination of the methyl group was done using cupric bromide in refluxing chloroform:ethyl acetate in essentially quantitative yield, with the product purified using silica gel and Florisil SEP-PAKs.

Methyl 3-[F-18]fluoro-5-nitrobenzimidate (II) was prepared as shown in Scheme II. The [F-18]fluoride for nitro substitution was used to prepare 3-[F-18]fluoro-5-nitrobenzonitrile (75%), which was reacted with sodium methoxide in dry methanol to yield the benzimidate II (90%). The product was purified via Florisil SEP-PAK.



Scheme II

Table 1. Concentration Dependence of Protein Labeling with 4-[F-18]Fluorophenacyl Bromide (I) and Methyl 3-[F-18]Fluro-5-nitrobenzimidate (II). Reaction conditions 100 microliters borate buffer, pH 8.0, 1 hour.

mg HSA	% protein labeled	
	I-HSA	II-HSA
0.1	18	--
0.2	51	--
0.5	75,82	15
1.0	85,100	30
5.0	95	65
10.0	--	73

Both reagents I and II can be used to successfully attach fluorine-18 to proteins. Conditions for labeling were studied using human serum albumin (HSA) as a model protein. Reaction of I or II with HSA was found to be essentially pH independent above pH 7.5, but labeling efficiency was very dependent on protein concentration, as shown in Table 1. Analysis of protein labeling efficiency was done using HPLC (Synchropax AX-300, 0.02M tris acetate, pH 8.0). The fluorine-18 labeled proteins could be isolated by size exclusion chromatography (Sephadex G25). The isolated [F-18]proteins were used for studies of the in vivo blood clearance rates (in rats) as compared to iodine-125 labeled HSA. The %ID/gram and clearance rates for the [F-18]proteins and the [I-125]HSA were essentially identical.

The fluorine-18 labeled reagents I and II have also been used to radiolabel fibrinogen. Uisng purified fibrinogen (95-97% clottable) and the following conditions (pH 8.0, 0.033M borate buffer, 36° C), we obtained yields of 27% for I-fibrinogen and 18% for II-fibrinogen. The radiolabeled fibrinogens could also be isolated by size exclusion chromatography, and the isolated proteins retained 65% of their ability to clot upon treatment with thrombin.

Finally, the potential for radiolabeling of antibodies with fluorine-18 was demonstrated by the successful attachment of either I or II to human IgA. Efforts in this area are continuing.

In conclusion, both 4-[F-18]fluorophenacyl bromide (I) and methyl 3-[F-18]fluoro-5-nitrobenzimidate (II) can be used to covalently attach fluorine-18 to protein molecules.

Acknowledgement. This work was supported by National Institutes of Health Grant HL 13851.

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A NEW METHOD FOR THE LABELLING OF PEPTIDES WITH CARBON-11.

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Peptides and proteins exhibit the largest structural and functional variation of all classes of biologically active macromolecules. They are of prime importance in the regulation and maintenance of all biological processes. The development of methods to prepare ^{11}C-labelled peptides and proteins provides new tool to investigate in man biological processes by PET. An approach for labelling is the reaction of $^{11}CH_3I$ with the corresponding demethyl-peptide as reported by Nagren [1]. However, this method is limited to methionine containing peptides. A more general approach is the ^{11}C-labelling by the addition of ^{11}C-amino acid to an appropriate precursor peptide. This

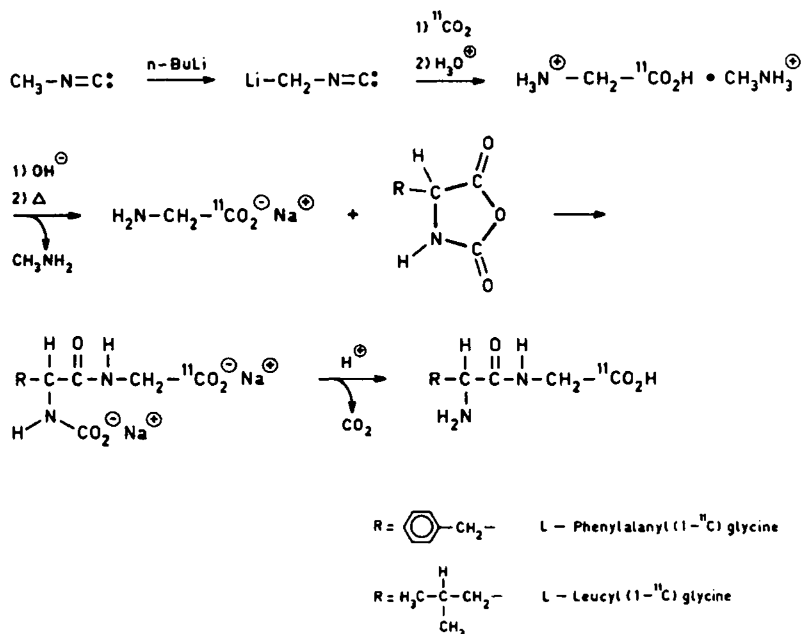

fig. 1

report is a first contribution to this approach. Carbon-11 labelled glycine has been synthesized and subsequently coupled with the activated amino acid substrates L-phenylalanine-NCA and L-leucine-NCA (NCA means N-carboxyanhydride) to the corresponding dipeptides. In fig.1 the reaction scheme for the

synthesis of L-phenylalanyl- and L-leucyl-(1-^{11}C)-glycine is shown. Carbon-11 labelled glycine has been prepared in 30 min by carboxylation of α-lithiomethylisocyanide with a radiochemical yield of 10-15%. After coupling with L-phenylalanine-N-carboxyanhydride and L-leucine-N-carboxyanhydride followed by HPLC purification, the corresponding dipeptides were obtained in 20 min with a radiochemical yield of 30-40%. Consequently, starting with $^{11}CO_2$, non carrier added L-phenylalanyl-(1-^{11}C)-glycine and L-leucyl-(1-^{11}C)-glycine in 0.1 N NaH_2PO_4 were obtained in 50 min with a radiochemical yield of 3-6%. The radiochemical yield figures are not corrected for decay.

This research is supported by the Dutch Cancer Foundation "Koningin Wilhelmina Fonds".

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A NEW SYNTHESIS OF NO-CARRIER-ADDED [1-^{11}C]DOPA

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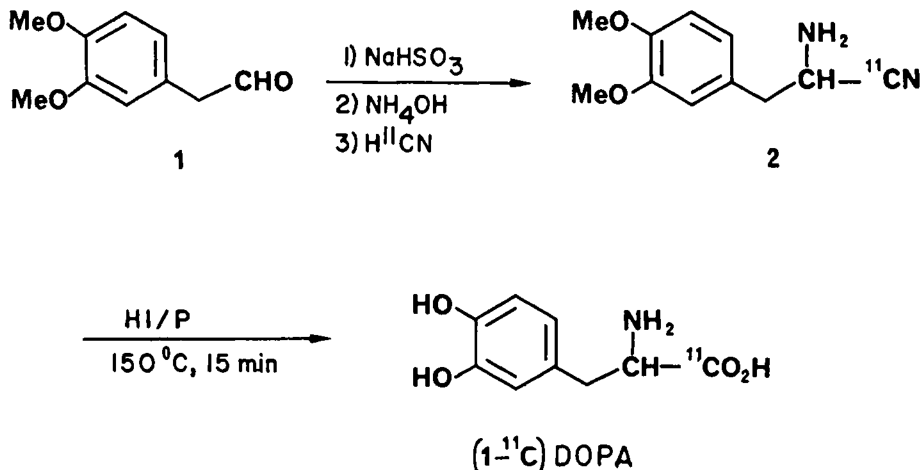
Carbon-11 labeled DOPA (3,4-dihydroxyphenylalanine) has been suggested as a suitable agent for kinetic studies of dopamine biosynthesis in the brain (1), detection of malignant melanoma (2), and detection of tumors (3). (3-[C-11])DOPA has been prepared by the reaction of [C-11]veratraldehyde and 2-phenyl-5-oxazolones (4), and (1-[C-11])DOPA has been prepared by the carboxylation of 3,4-dimethoxyphenethyl isocyanide (3). (1-[C-11])DOPA synthesis via the isocyanide involves difficult reaction conditions (low temperatures, inert atnosphere) and suffers from poor reproducibility. From our own experience we have not been able to reproduce the reported yields: the highest yield we have ever obtained was 5% (corrected) using dry ice as the source of CO_2. In no-carrier-added syntheses routine yields of less than 1% were obtained. Therefor, we felt a simpler and more reliable synthesis was needed.

We have successfully developed a new synthesis of (1-[C-11])DOPA based on the well known procedure for preparing α-aminonitriles (5). This reaction has been previously applied to the synthesis of other [C-11]amino acids, but a detailed procedure has yet to be published (6).

Our new synthesis involves two steps (Scheme I): formation of [C-11]α-aminonitrile 2, and acidic hydrolysis of the addition product 2 to (1-[C-11])DOPA. The formation of addition product 2 was achieved by bubbling no-carrier-added [C-11]HCN into a premixed solution of aldehyde 1 (6 mg in 1 ml water; prepared by oxidation of 3,4-dimethoxyphenethyl alcohol with chromium trioxide-pyridine (7)), $NaHSO_3$ (10 mg), and NH_4OH (0.5 ml), and heating the solution for 5 min at 80 °C. The solution was passed through a C18 SEP-PAK, and the labeled addition product eluted with diethyl ether (50-60%, corrected). For the hydrolysis step, the ether was gently boiled off, and a mixture of concentrated HI and red phosphorus added. The solution was heated at 150 °C for 15 minutes, cooled, neutralized, and the phosphorus removed by filtration. After HPLC purification on a C18 reverse phase column (0.1 M NaH_2PO_4), D,L-(1-[C-11])DOPA was obtained in an overall yield of 25% (corrected), or 4% at end of synthesis, in an overall reaction time of less than one hour.



Our initial studies indicate that this new synthesis is both simple and reproducible. The use of unstable organometallic reagents, low temperatures, and inert atmosphere are all avoided, a condition important for future development as a remote or automated synthesis. Experiments on the resolution of the D and L isomers using ligand exchange chromatography are currently in progress.

Acknowledgement. This work was supported by National Institutes of Health Grant HL 13851.

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REGIOSELECTIVE RADIOFLUORODEMERCURATION: A SIMPLE, HIGH YIELD SYNTHESIS OF 6-[F-18]FLUORODOPA

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Much attention has been recently focused on 6-[F-18]fluorodopa, an agent that permits the investigation of presynaptic dopamine neurotransmission in humans with positron emission tomography (1). Several syntheses of 6-[F-18]fluorodopa have been previously published. The widely used electrophilic radiofluorination of dopa and derivatives has been reported to produce regioisomeric mixtures and low yields (~1-4%) of 6-[F-18]fluorodopa after chromatographic purification (2-4). Also a synthesis based on radiofluorodesilylation has been proposed (5), but the difficulties in preparing the starting precursor seems to limit its applicability.

We describe herein a simple, high yield synthesis of 6-fluorodopa (3) based on regioselective fluorodemercuration (6) of the easily accessible intermediate 1. Acetyl hypofluorite (AcOF) prepared in the gas-phase (7) cleanly reacts with the organomercury derivative 1 in chloroform or a mixture of chloroform: freon (20:80) at room temperature to afford the regiospecifically pure 6-fluorodopa derivative 2 in good yields (36%). To minimize the formation of by-products, a 2:1 molar ratio of the organomercury derivative 1:AcOF was used. The intermediate 2 was easily separated from the starting material 1 by column chromatography (silicagel; dichloromethane: methyl acetate, 90:10) and fully characterized by NMR and mass spectroscopy. ^{1}H NMR ($DCCl_3$/TMS): 3.10-3.70 (m, 2H, CH_2), 3.78 and 3.82 (2s, 9H, 3- and 4-OCH_3 and CO_2CH_3), 4.70-4.90 (m, 1H, CH), 6.49 [d,1H,ArH(2),$J_{H(2),F^{19}}$ = 7.04 Hz], 6.58 [d,1H,ArH(5), $J_{H(5),F}$ = 11.20 Hz], 6.80-7.00 (m, 1H, NH); ^{19}F NMR ($DCCl_3$/ $CFCl_3$): -76.9 ppm ($NHCOCF_3$), -126.0 ppm (6-F, $J_{F,H(2)}$ = 7.0 Hz, $J_{F,H(5)}$ = 11.0 Hz); MS (70 eV), m/e: 353 (M^+). Acid deprotection with 47% HBr and purification (C-18 Sep-pak, water) afforded pure 3.

Extension of this procedure to radiofluorination with [F-18]AcOF yielded 6-[F-18]fluorodopa with >98% chemical and radiochemical purity as shown by ^{19}F NMR, radio TLC (silicagel; isopropanol: conc NH_4OH, 6:4; R_f = 0.45) and radio HPLC (Ultrasphere ODS, 5 µm, 4.6 x 250 mm; gradient run (20 min), 97% 100 mM potassium phosphate, pH 3.0, 3% acetonitrile to 80% 100 mM potassium phosphate, pH 3.0, 20% acetonitrile; flow rate: 1.3 ml/min; RT = 3.0 min). Typically the synthesis was accomplished 50 min after production of [F-18]AcOF with radiochemical yields of ~40%. Thus, multimillicurie amounts of pure 6-[F-18]-fluorodopa (>20 mCi) for tomographic studies are now routinely available. In addition, using the same approach, several radiofluorinated dopa derivatives (e.g. 6-fluoro-α-methyldopa) (8,9) can be easily synthesized.

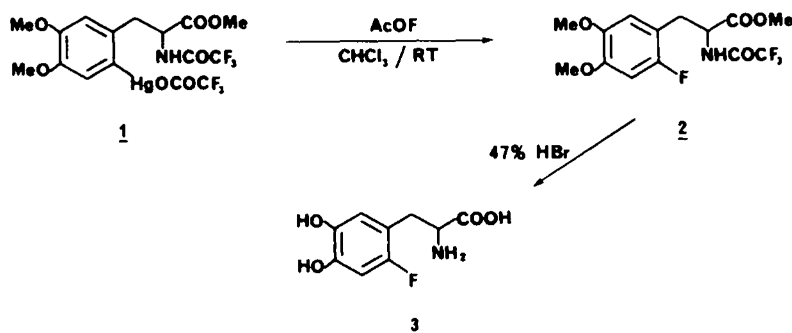

Figure 1. Reaction sequence for synthesis of 6-[F-18]fluorodopa.

This work was supported in part by DOE Contract DE-AC03-76-SF00012, NIH grants R01-NS-20867-08, P01-NS-15654, and NIMH grant R01-MH-37916-02.

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PREPARATION OF N-[^{11}C-METHYL]CHLORPHENTERMINE (^{11}C-NMCP) THROUGH METHYLATION OF CHLORPHENTERMINE TRIFLUOROACETAMIDE

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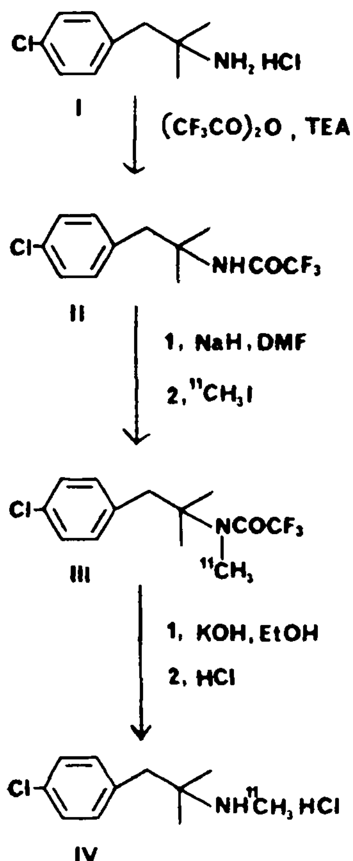
N-[^{11}C-Methyl]Chlorphentermine (^{11}C-NMCP) has recently been developed in our laboratory as a potential brain blood flow agent for PET studies (1). Initially, ^{11}C-NMCP was prepared by the reaction of chlorphentermine and $^{11}CH_3I$ in DMF with heating at 140°C for 5-10 min and followed by HPLC purification. The radiochemical yield of ^{11}C-NMCP was substantially low because the reaction gave a mixture of mono-, di- and quarternary amines. To improve the radiochemical yield of ^{11}C-NMCP, we investigated the Gabriel-like transformation of the primary to the secondary amine through a sequence of acylation, ionization, alkylation and hydrolysis (2).

Scheme 1 Synthesis of ^{11}C-NMCP via methylation of trifluoroacetamide derivative



N-trifluoroacetyl, N-acetyl and N-formyl derivatives were prepared and examined as possible intermediates for this study. Hydrolysis of the N-trifluoroacetamide was quantitative after 3 min of heating with KOH/EtOH whereas that of the other two derivatives was less than 10%. Thus the N-trifluoroacetamide was chosen for the radiolabeling experiment as shown in Scheme 1. The trifluoroacetyl derivative (II) was prepared from chlorphentermine HCl (I) and trifluoroacetyl anhydride in the presence of two equivalents of triethylamine in ether with a 90% yield. Ionization of II was carried out with NaH in DMF. $^{11}CH_3I$ was prepared from $^{11}CO_2$ according to the published procedure (3) and was distilled into the reaction vial containing the mixture of II and NaH. The reaction mixture was heated at 140°C for 7 min and the $^{11}CH_3$-labeled intermediate III was extracted with ether. The solvent was evaporated and 0.3 ml KOH/EtOH (10%) was added followed by heating at 100°C for 3 min. The resulting residue was diluted with H_2O and acidified with 1N HCl to pH 5. TLC analysis using a silica gel plate and HPLC showed that the radiochemical purity of ^{11}C-NMCP was > 98.5%. The radiochemical yield was approximately 20-30%.

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THE UTILITY OF $^{13}C/^{11}C$-CO-LABELLING AND SUBSEQUENT ^{13}C-NMR IN THE CHARACTERISATION OF ^{11}C-LABELLED PRODUCTS

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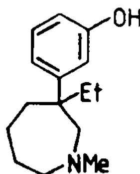
[2]City of London Polytechnic, 31 Jewry Street, London, EC3N 2EY, UK.
[3]North London Polytechnic, Holloway Road, London, N7 8PD, UK.
[4]Queen Mary College, Mile End Road, London, E1 4NS, UK.

With few exceptions, the vast majority of ^{11}C-labelled compounds reported in the literature have been characterised by chromatography only. This is not completely satisfactory. Even when several different chromatographic techniques are applied to identifying or measuring the radiochemical purity of a labelled compound there remains a risk that some impurity will be undetected and a false conclusion drawn.

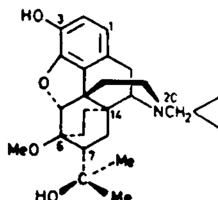
Recently the value of ^{19}F-NMR in the characterisation of ^{18}F-labelled products has become appreciated. Thus ^{19}F-NMR has been applied (1) to the measurement of D-2-[^{18}F]fluoro-2-deoxy-mannose (^{18}FDM) impurity in D-2-[^{18}F]fluoro-2-deoxy-glucose (^{18}FDG) prepared by various methods. Earlier the amount of ^{18}FDM impurity generated by many methods had been undetermined or underestimated. In the light of accurate ^{19}F-NMR measurements, improved radiochemistry (2) and analytical chromatography (3) have now been developed for ^{18}FDG production.

In our laboratory we have exploited $^{13}C/^{11}C$ co-labelling and subsequent broad-band proton-decoupled Fourier-transform ^{13}C-NMR spectroscopy (FT$^{13}C\{^1H\}$NMR) as an important aid to the characterisation of ^{11}C-labelled products. In principle the approach is to add a ^{13}C-enriched reactant (90 atom %) to the corresponding [^{11}C]reactant at an appropriate stage in the radiochemistry, to separate the $^{13}C/^{11}C$-labelled product of interest, and then to examine this product by FT$^{13}C\{^1H\}$NMR. Here we present practical examples of this approach in the development of carbon-11 radiochemistry.

1. [^{11}C]Meptazinol
We have developed a method (4) for labelling meptazinol (1) _via_ the _N_-[^{11}C]methylation of normeptazinol with [^{11}C]iodomethane. In order to validate this method we carried out a preparation in which ^{13}C-enriched iodomethane (90 atom %, 0.3μmol) was added to the [^{11}C]iodomethane. Analysis of the purified product by FT$^{13}C\{^1H\}$NMR (D_2O, 22.5 MHz) gave a single peak at δ 48.30. Analysis of a mixture of collected product and authentic meptazinol gave a single intense peak at δ 49.22, attributable to the _N_-methyl carbon in meptazinol, and the remainder of the spectrum of meptazinol at lower intensity. (The small variation in the chemical shift of the _N_-methyl carbon is probably pH-dependent). Thus FT$^{13}C\{^1H\}$NMR provides excellent assurance for the identity, radiochemical purity and position of carbon-11 in the product from our radiochemical procedure. The product from any reaction of [^{11}C]iodomethane could, of course, be investigated by a similar approach.



(1)



(2)

2. [^{11}C]Diprenorphine

Recently we have developed a method for preparing [^{11}C]diprenorphine (5). A novel labelling agent, [1-^{11}C]cyclopropanecarbonyl chloride, is prepared via the reaction of cyclotron-produced [^{11}C]carbon dioxide with cyclopropylmagnesium bromide and subsequent quenching of the radioactive adduct with phthaloyl dichloride. N-(de-cyclopropylmethyl)diprenorphine is then treated with the [^{11}C]acid chloride and the resultant [^{11}C]amide reduced with lithium aluminium hydride (Figure 1).

In one experiment ^{13}C-enriched carbon dioxide (90 atom %; 4.7 μmol) was co-included in the radiosynthesis and the purified product examined by FT^{13}C{^{1}H}NMR ($CDCl_3$, 22.5 MHz). The spectrum shows a single peak at δ 59.2 in accord with the chemical shift assigned (6) to C-20 in diprenorphine (2). This peak was also attributed to a CH_2 carbon by an appropriate spin-echo gated decoupling technique (7). Thus FT^{13}C{^{1}H}NMR assures the radiochemical purity of the product, and the position of the carbon-11.

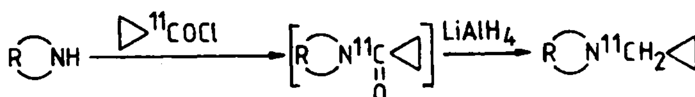


Figure 1. The radiosynthesis of [^{11}C]diprenorphine. R = diprenorphine (2) residue

In the same experiment a sample was taken before reduction, chromatographed and the main radioactive peak collected. FT^{13}C{^{1}H}NMR revealed a singe peak at δ172.0. This was attributed to the amido-carbon of the proposed intermediate amide (Figure 1) by reference to chemical shift data for analogous amides. Evidence for the identity of other ^{11}C-labelled by-products was similarly obtained. The acquisition of such evidence is particularly useful during the development of [^{11}C]radiochemistry if appropriate intermediates are unavailable to act as chromatographic standards.

3. [^{11}C]Glucose

Several groups now produce [^{11}C]glucose routinely by photosynthetic methods. We adapted the original procedure of Ehrin et al. (8) to make it more amenable to remote control (9) and were interested to obtain information on the distribution of label in our product.

In several preparations we used ^{13}C-enriched carbonate (90 atom %, 9 mol) as carrier for [^{11}C]carbonate. The products from these preparations were examined by high field (100 MHz) FT^{13}C{^{1}H}NMR (Figure 2). This spectrum shows all the peaks present in the spectrum of D-glucose with natural ^{13}C abundance (10). These peaks also show multiplicity indicative of ^{13}C-^{13}C coupling. Coupling constants, where measurable, are in agreement with those reported for ^{13}C-enriched glucose (60 atom %) (10). From the spectrum we conclude that our procedure for [^{11}C]glucose production introduces carbon-11 into each position. A small peak in the spectrum at δ62.4 indicates the presence of a minor radioactive impurity in the prepared [^{11}C]glucose. This impurity was undetected by chromatography.

In conclusion ^{13}C/^{11}C-co-labelling and subsequent FT^{13}C{^{1}H}NMR provides evidence of

(i) product identity
(ii) radiochemical purity
(ii) the position(s) of label
(iv) information on the identity of intermediates.

This approach, in combination with chromatography, provides more rigorous characterisation for ^{11}C-labelled products than may be achieved by chromatography alone; its application is therefore recommended wherever possible.

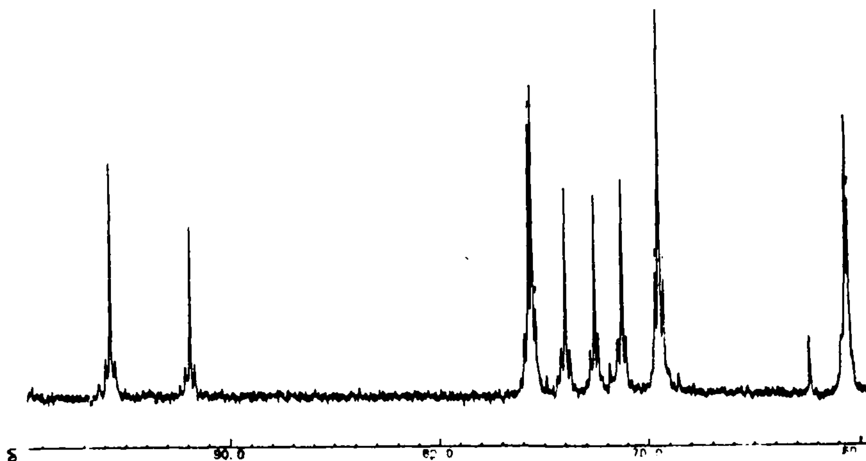


Figure 2. High field (D_2O, 100 MHz) FT^{13}C(^{1}H)NMR spectrum of [^{13}C]glucose produced photosynthetically.

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HIGH SPECIFIC RADIOACTIVITY PHOSGENE-^{11}C

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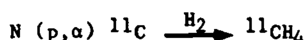
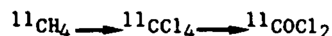
Phosgene-^{11}C is an important precursor in the carbon-11 labelling of certain medically useful molecules. Phosgene allows a carbonyl function to be inserted between two stereochemically close amino functions.
Until now, Phosgene-^{11}C synthesized from $^{11}CO_2$ cannot be obtained with a specific radioactivity higher than 400-500 mCi/µMole.
We propose a new method of phosgene synthesis in order to improve the specific radioactivity.
The method consist in a chlorination of ^{11}C-methane then oxidation of ^{11}C-CCl_4 to ^{11}C-Phosgene.
^{11}C-methane is produced directly by irradiation of a N_2, 5 % H_2 mixture with protons.



An irradiation of 30 minutes, with 30 µA of 20 MeV protons gives 1.5 Ci of $^{11}CH_4$ with a specific radioactivity of 3-5 Ci/µMole (EOB).
Then ^{11}C-methane is chlorinated on pumice stone impregnated with cupric chloride at 380°C-400°C.



The yield of the first stage is 60-65 %. Then ^{11}C-tetrachloride is oxydized to phosgene on steel fillings at 300°C (2-3).
The overall yield of ^{11}C-phosgene from ^{11}C-methane is 40-45 %. The specific radioactivity has been determined by labelling of ketanserine (4).
From 1.5 Ci $^{11}CH_4$, it is possible to obtain, 10 minutes after the end of bombardment, 400-500 mCi of ^{11}C-Phosgene with a specific radioactivity of 1.4-1.6 Ci/µMole.

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FLUOROMETHANE LABELLED WITH CARBON-11 FOR PET STUDIES OF REGIONAL CEREBRAL BLOOD FLOW

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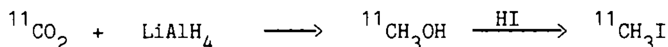
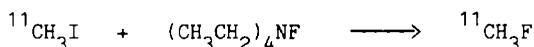
The use of fluoromethane labelled with ^{18}F has previously been shown to provide an accurate measurement of regional cerebral blood flow using positron emission tomography (1,2). The tracer is inert _in vivo_, freely diffusible, has a high blood-brain partition coefficient and the biological half-life is short due to the effective clearance of the tracer by the lungs. Fluoromethane has been labelled with ^{18}F-fluoride ion produced from neutron-irradiated lithium carbonate (2,3), proton-irradiated $^{18}O_2$ (4), as well as with $^{18}F_2$ from deuteron-irradiated neon gas (5). Since we were interested in using fluoromethane as a blood flow tracer in combination with other ^{11}C- and ^{15}O-labelled tracers, we investigated the possibility of labelling with ^{11}C instead of ^{18}F to minimize the number of target changes during the series of PET investigations. Herein we report an alternative labelling method for fluoromethane starting from $^{11}CO_2$, according to the reactions below.



$$^{11}CO_2 + LiAlH_4 \longrightarrow {}^{11}CH_3OH \xrightarrow{HI} {}^{11}CH_3I$$



$$^{11}CH_3I + (CH_3CH_2)_4NF \longrightarrow {}^{11}CH_3F$$

$^{11}CH_3I$, produced from $^{11}CO_2$ by a one-pot method described previously (6) and trapped in 1.5 mL dry acetonitrile, was added to a solution of tetraethylammonium fluoride (25 mg) in 0.5 mL dry acetonitrile. The reaction vessel was closed and the contents were heated with stirring at 110°C for 10 min. The reaction vessel was air-cooled and the ^{11}C-fluoromethane gas was removed by suction into a 50 mL syringe. The gas was analyzed by radio-gas chromatography on a Porapak P column. The average radiochemical purity was $\geq$ 99%, with an occasional impurity ($\leq$1%) of $^{11}CO_2$. Use of the Ag_2O catalyst which was previously reported to increase the ^{18}F labelling yields caused extensive chemical degradation of $^{11}CH_3I$ to $^{11}CO_2$ with very little effect on the radiochemical yield. In the absence of this catalyst, 60-80% yields were obtained based on the ^{11}C-methyl iodide used. In a typical run, nitrogen gas was proton-irradiated for 15 min with a beam intensity of 40 μA. The total synthesis time was about 25 min from end-of-bombardment, yielding 3.3 - 5.5

GBq (90 - 150 mCi) ^{11}C-fluoromethane suitable for immediate administration in PET studies.

PET evaluations of rCBF with $^{11}CH_3F$ have now been performed in more than 50 studies on healthy volunteers and patients at the Karolinska Hospital. Typically a subject inhales 1.8 - 2.2 GBq (50 - 60 mCi) in a single 10 sec inhalation from a short tube. Sequential scans are performed with the PET camera and the radioactivity in arterial blood is measured continuously by an automatic sampling system. After 25 min less than 2% of the administered $^{11}CH_3F$ remains in the brain. Multiple rCBF measurements have therefore been performed on the same individual 30-40 min apart, enabling studies of the effects of different types of neurological activation on regional cerebral blood flow.

This work has been supported by grants from the Swedish Medical Research Council, the Danish Medical Research Council, the Nordic Council of Secretaries of State, the Bank of Sweden Tercentenary fund and the Karolinska Institute, which is gratefully acknowledged. The authors would also like to thank Mr. Göran Printz for assistance with the radionuclide production.

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SYNTHESIS OF S-[METHYL-^{11}C]SUBSTANCE P

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The preparation of ^{11}C-labeled peptides by the route originally developed for the synthesis of ^{11}C-methionine(1,2,3) has now been extended to the synthesis of the undecapeptide Substance P.
Problems in the preparation of the substrate, mainly in the 4-11 and the 1-11 coupling steps, have now been solved. The syntheses shown in Figure 1 thus demonstrate a convenient route for the synthesis of the substrate.

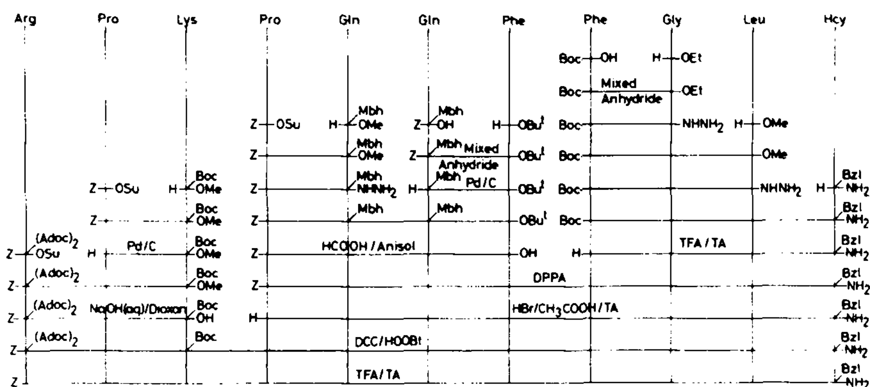

Figure 1

This route also gives a suitable substrate which can be labeled with ^{11}C to produce the C-terminal octapeptide of Substance P. This peptide retains some of the physiological properties of Substance P.
Both peptides were labeled with ^{11}C by using sodium in liquid ammonia to generate the appropriate sulfide anions and by using ^{11}C-methyl iodide in the subsequent alkylation reactions as illustrated in Figure 2 for the synthesis of S-[methyl-^{11}C]Substance P.
Following LC purification, the labeled Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met was obtained in 55-60 % radiochemical yield, decay corrected, within 43-50 min counted from start of the ^{11}C-methyl iodide synthesis. The radiochemical purity was higher than 99 %.

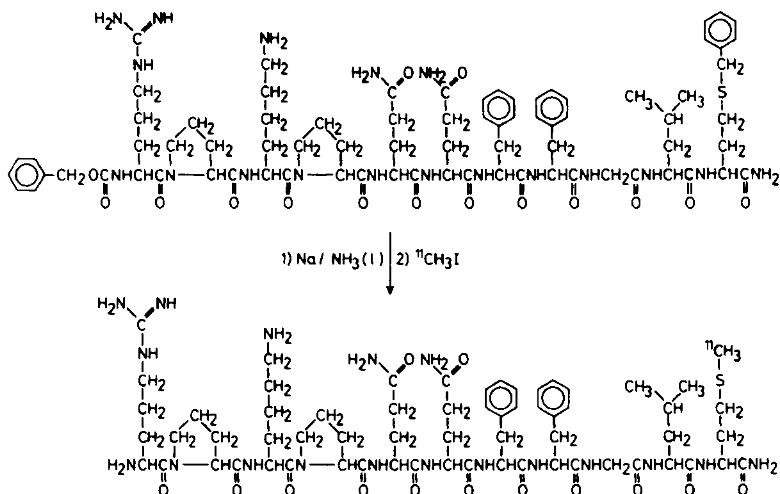

Figure 2

Preliminary experiments with small amounts of the substrate for Substance P show 20-60 % radiochemical yield in the alkylation reaction. Work is now in progress to increase this yield by using larger amounts of the substrate.

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SYNTHESES OF ^{11}C-LABELED PROSTAGLANDIN ESTERS FOR PET STUDIES

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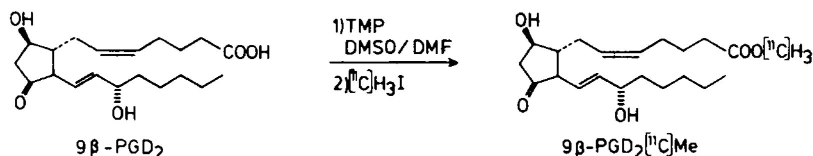
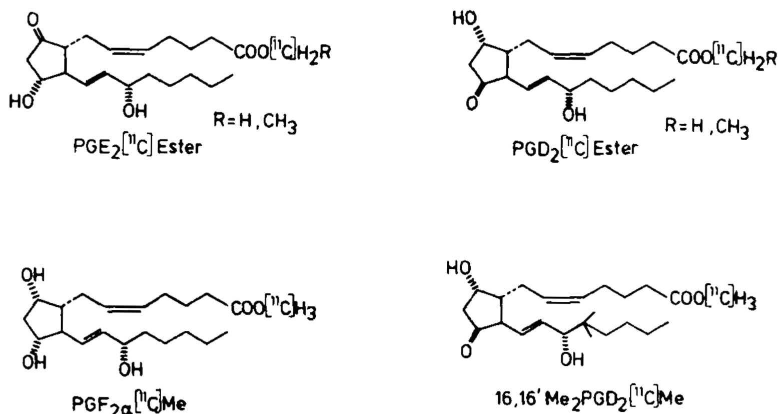
Since they were discovered in the 1930s, prostaglandins have been the subject of extensive research. It was recently shown by ^{3}H-autoradiography that prostaglandins and prostaglandin analogues bind specifically to certain brain areas in vitro (1). To permit PET studies of brain binding in vivo, prostaglandins or prostaglandin analogues were subjected to labeling syntheses with ^{11}C.
Our first approach was to synthesize prostaglandin methyl esters according to scheme 1, that is, labeling the alcoholic part of the ester.



Scheme 1

The prostaglandin 9β PGD_2, which is highly susceptible to isomerization or dehydratization, was dissolved in a polar aprotic solvent mixture with a certain amount of tetramethylpiperidine (TMP) as base just before the condensation of [^{11}C]methyl iodide (2) from a carrier gas stream. Gentle heating then yielded 9β PGD_2 [^{11}C]methyl ester in high yield (95 % in 5 min) (3).
Plasma studies showed that the methyl esters were hydrolyzed relatively fast in vivo. With a view of decreasing the hydrolysis rate, larger alkyl iodides in the alkylation reaction were investigated. The ethylations, carried out analogously to the methylations, were substantially slower and thus required elevated temperatures. This, in turn, led to severe side-reaction problems. The temperature, amount of base and solvent composition were carefully determined by an optimization procedure (Simplex) to minimize the side-products and good yields of the prostaglandin [1-^{11}C]ethyl esters were obtained in a relatively short time (75 % in 10 min) (4).

The various labeled prostaglandin esters prepared are shown in scheme 2.



Scheme 2

With [^{11}C]isopropyl iodide, the esterification rate is still too low to compete with the side-reactions.

This project was financially supported by grants K-KU 3464, K-GF 3464 from the Swedish Natural Science Research Council.

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THE SYNTHESIS OF NO-CARRIER-ADDED α [^{11}C-METHYL]-L-TRYPTOPHAN

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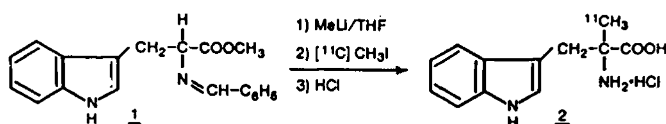
The metabolic pathway of α -methyl-L-tryptophan (α-MeTry) and its influence on tryptophan metabolism was studied by Sourkes' group[1] who found that α -MeTry follows at least partially the metabolic pathway of tryptophan. The end product of α -methyl-L-tryptophan is α -methyl-serotonin.

We report here the synthesis of no-carrier added α -[^{11}C]MeTry, a potential tracer for the measurement of in vivo synthesis of the neurotransmitter serotonin[1]. The starting material (1) was prepared by reacting tryptophan methyl ester with a mixture of benzaldehide and triethylamine at about -10°C[2]. After treating the reaction mixture with solid KOH and filtration, the solvent was evaporated to dryness and residual oil triturated with diethylether. The Schiff's base (1) was prepared in an overall yield of 40%, and identified by M.p. 124-125°C (lit. 124-126°C[2]), ^{1}H-NMR, and MS m/e 306 (10%, M^+).

The resultant Schiff's base (1) (∿ 1 mmol) was reacted with CH_3Li (∿1 mmol) in THF to obtain anion. The anion was reacted with no-carrier added [^{11}C]CH_3I dissolved in THF while the reaction mixture was kept in a dry ice-acetone bath. The mixture was then warmed to room temperature, filtered, and the solvent evaporated under reduced pressure. The protecting groups were removed by hydrolysis with 2M HCl for 5 min at 135°C (bath). After hydrolysis, the solution was extracted with ethyl ether to remove bensaldehyde. The aqueous layer was concentrated and passed through a reverse phase Sep-Pak column. The synthesis required 25 to 30 min to complete and had a radiochemical yield of about 30%. The specific activity assessed by HPLC was in excess of 1500 Ci/mmol. TLRC on silica gel using CH_3OH - NH_4OH (3.5:0.05) as the solvent showed one spot with R_f = 0.85, the same as an authentic sample identified by MS and ^{1}H-NMR. The L-configuration of the final product was confirmed by HPLC using reverse phase column and chiral eluent as described by Gil-Av et al.[3] The elution volume was identifical to that of an authentic sample prepared by a standard procedure and identified by ^{1}H-NMR, MS, and M.p.

The work described here was supported by the Medical Reseach Council of Canada, SP-5, and the Killam Scholarship Fund of the MNI.



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THE PREPARATION OF ^{11}C-CARBOXYLIC LABELLED L-METHIONINE FOR MEASURING PROTEIN SYNTHESIS RATES.

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A number of carbon-11 labelled amino acids has been synthesized and used for studying the metabolic rate of tissue both for normal and pathological states. Up to now most attention has been paid to L-[S-methyl-^{11}C]methionine. Accumulation of this amino acid was observed in several types of tumors with high tumor to non-tumor ratios and a simple three compartment kinetic model has been developped to estimate the protein synthesis rate in brain. For the applicability of the model it is essential to assume that transfer of the methyl group via S-adenosyl-L-methionine to various non-protein substrates occurs to a very minor extend. The validity of this assumption has been checked by quantitative autoradiography studies in rat brain and values of 4 and 36% for the transmethylation pathway have been reported [1,2]. By using carboxylic labelled methionine potential problems with the transmethylation pathway would be circumvented. The label is expected to be incorporated in protein or diluted in the CO_2-bicarbonate pool and removed from the tissue rapidly. Furthermore by comparing PET studies with either methyl or carboxylic labelled ^{11}C-L-methionine, it might be possible to differentiate between protein synthesis and transmethylation pathways. We prepared DL-[1-^{11}C]-methionine and separated the labelled amino acid in its enantiomers. The reaction scheme is given in figure 1. The starting methylthio-3-propyl-isocyanide was prepared by dehydration of the corresponding formamide

$CH_3-\ddot{S}-CH_2-CH_2-CH_2-N{=}C:$ $\xrightarrow{n\text{-BuLi}}$ (cyclic Li chelate: $CH_3-\ddot{S}$, H_2C-CH_2, $C(H)-N{=}C:$, Li) $\xrightarrow{^{11}CO_2}$

$CH_3-\ddot{S}-CH_2-CH_2-CH(^{11}CO_2H)-N{=}C:$ $\xrightarrow{H^+, H_2O}$ $CH_3-\ddot{S}-CH_2-CH_2-CH(^{11}CO_2H)-NH_2$

DL-(1-^{11}C)-methionine

fig. 1

according a literature procedure [3]. The lithiation of the isocyanide was achieved in THF with n-butyllithium at -90 °C. The $^{11}CO_2$ is produced by the $^{14}N(p,\alpha)^{11}C$ reaction.

After introduction of the $^{11}CO_2$ into the reaction flask, the mixture was stirred for a minute. Next 2 ml of $HCl/H_2O/THF$ solution (prepared from 1 ml conc HCl and 4 ml THF) was added followed by 1.5 ml H_2O. The resulting solution was evaporated under reduced pressure and 2 ml of 3 N HCl was added to the residue. After heating this solution at 120 °C for 8 minutes the solution was cooled to room temperature and applied onto a Bio-Rad Aminex-A7 column (10x0.42 cm). The column was washed with water till pH 4 and next the amino acid was eluted with 0.1 N NaH_2PO_4 (pH=4.6). The HPLC purification was achieved in 13 min and DL-(1-^{11}C)methionine was obtained with a radiochemical purity of better than 94% at 35 min after EOB. The radiochemical yield of ten preparations varied between 30 and 50% (corrected for decay). The enantiomeric separation was achieved by HPLC using a Waters RCM-100 module, a C-18 analytical column and a chiral buffer (8mM $Cu(OAc)_2$, 17 mM L-proline and 30 mM NaOAc, pH=5). To switch the solvent from the chiral buffer to physiological saline, a Waters C-18 analytical column was used. The final L-[1-^{11}C]methionine was obtained at 55 min after EOB with a radiochemical yield between 13 and 22% (corrected for decay).

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AN EFFICIENT, REMOTE CONTROL SYNTHESIS OF C-11 LABELED BRANCHED AMINO ACIDS.

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We have developed a general process for the routine production of several C-11 labeled branched-chain amino acids and analogues, which (a) yields sterile and pyrogen-free preparations, (b) gives consistent and reproducible radiochemical yields,(c) affords preparations of high specific activity and radiochemical purity as required by a given experimental application and in vivo studies, (d) is suitable for succesive, batch, preparation of the same or different amino acids within a single (4 Hr.) operating session, and (e) offers improved laboratory safety by eliminating any unnecessary exposure of personnel to radiation.

Based on the Bucherer-Strecker synthesis we developed preparative procedures for the following amino acids: α-amino isobutyric acid (AIB), leucine, isoleucine, valine and 1-amino-cyclopentane-1-carboxylic acid (ACPC). They provide any one of these materials in an average 60% yield. The purification and isolation of each of these amino acids from the respective crude reaction mixture has been simplified and accelerated through the introduction of a novel, streamlined, chromatographic treatment. This treatment employs four small ion-exchange columns (approx. 3 ml of wet resin each) connected in series. Only one solvent system (NaOH, 0.5 N) is required, in addition to water, to give a final, neutral and salt free, preparation of any given amino acid. Such preparations can be, readily, made isotonic through the addition of a measured volume of a prepared, sterile, 20% aqueous stock solution of NaCl.

A given reaction mixture, containing approximately 6 mmoles of NaOH, is directed first through an Amberlite CG-50 column (H^+) which is washed with 20-30 ml of water. The resulting neutral eluate and washings are then passed,directly and in a continuous stream, through the second column (Dowex AG 1, CH_3COO^-) which retains the inorganic anions present in the reaction mixture, i.e., Cl^- and excess CN^- (in a carrier added synthesis) and then through a Dowex AG 50W (H^+) column that concentrates the amino acid. Neutral, organic, byproducts of the rection (e.g. unhydrolyzed hydantoin, an intermediate in the Bucherer-Strecker synthesis) are not retained and are eluted from the AG 50W column. The product is conveniently eluted with aqueous NaOH solution (0.5 N). The fraction of the eluate containing the product is neutralized by passing through a fourth column,Amberlite CG 50 (H^+), sterilized by filtration through a Millipore Millex-GS, 0.22 um filter, collected in a capped serum bottle and made isotonic with the addition of a small volume of 20% NaCl solution.

The over all process yields, routinely, small volume preparations (from approx. 4.0 ml for α-amino-isobutyric acid to approx. 15 ml for leucine)with an average specific activity of 2.5 Ci/mmole. For α-amino-isobutyric acid, which is most often prepared in our laboratory, specific activities as high as 4.0 Ci/mmole Carrier added preparations) have been obtained. Average, total yields of 40-70 mCi per preparation have been common for all the amino acids.

We have constructed an apparatus for the remote control application of the process just described. It is designed to be easily adaptable to fully automated operation. The basic feature of the apparatus are the twin sets of four ion-exchange columns which provide the capability for two consecutive, uninterupted, runs.The switch over between these sets of columns at the completion of the first run is controlled by four six-port rotary valves operated in tandem by a single activating pneumatic switch.

To demonstrate the synthetic versatility of the developed methodology and apparatus, we have performed numerous succesful, carrier or no carrier added, experiments involving the sequential preparation of two or more amino acids. In the most complex of these experiments we synthesized four different amino acids, i.e.,

valine, leucine, isoleucine and 1-amino-cyclopentane-1-carboxylic acid, within seven hours form the initiation of the production (EOB) of the first batch of C-11 CO_2, an early intermediate in the preparation of C-11 hydrogen cyanide which we use in the Bucherer-Strecker synthesis. This seven hour session included a two and one-quarter hour "cooling off" period after the first two runs.

Using these techniques we have provided, within a six month period in 1985, preparations of AIB labeled with C-11 at the carboxyl group for seventeen in vivo investigations in human subjects and in animals (dogs, rabbits). Some of the experiments in animals required two consecutive preparations within two hours.

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NEW SYNTHESIS OF (^{11}C)LABELED FATTY ACID FROM $H^{11}CN$

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Fatty acids are the primary substrates for aerobic myocardial metabolism and radio-labeled fatty acids have been used to study myocardial metabolism in vitro and in vivo in animals and man. (^{11}C)labeled palmitic acid has been used for the quantitative measurement of both regional myocardial perfusion and regional fatty acid metabolism with a PET imaging system (1-5). 3-Methyl-(1-^{11}C)heptadecanoic acid, a fatty acid analog designed to inhibit the β-oxidation process by preventing the formation of corresponding β-ketoacyl SCoA has also been a useful myocardial metabolic tracer for PET (6). These (^{11}C)labeled fatty acids have been synthesized by the use of the Grignard reaction. However this method has disadvantages such as the need for absolutely anhydrous solvent and the requirement for a lot of starting material (alkyl bromide). Therefore we proposed a new synthesis of (^{11}C)labeled fatty acid by the use of $H^{11}CN$.

Figure 1. Synthetic scheme for (1-^{11}C)labeled fatty acids

$$\underset{\text{(I)}}{R_1\underset{\displaystyle R_2}{CH}CH_2\text{-}Br} \xrightarrow[\text{120 °C, 10 min}]{H^{11}CN \atop NaOH/DMSO} \underset{\text{(II)}}{R_1\underset{\displaystyle R_2}{CH}CH_2\text{-}^{11}CN} \xrightarrow{\text{hydrolysis}} \underset{\text{(III)}}{R_1\underset{\displaystyle R_2}{CH}CH_2\text{-}^{11}COOH}$$

a. $R_1=C_{12}H_{25}$, $R_2=H$
b. $R_1=C_{13}H_{27}$, $R_2=H$
c. $R_1=C_{14}H_{29}$, $R_2=H$
d. $R_1=C_{15}H_{31}$, $R_2=H$
e. $R_1=C_{14}H_{29}$, $R_2=Me$

Figure 2. Synthetic method for (1-^{11}C)labeled fatty acids

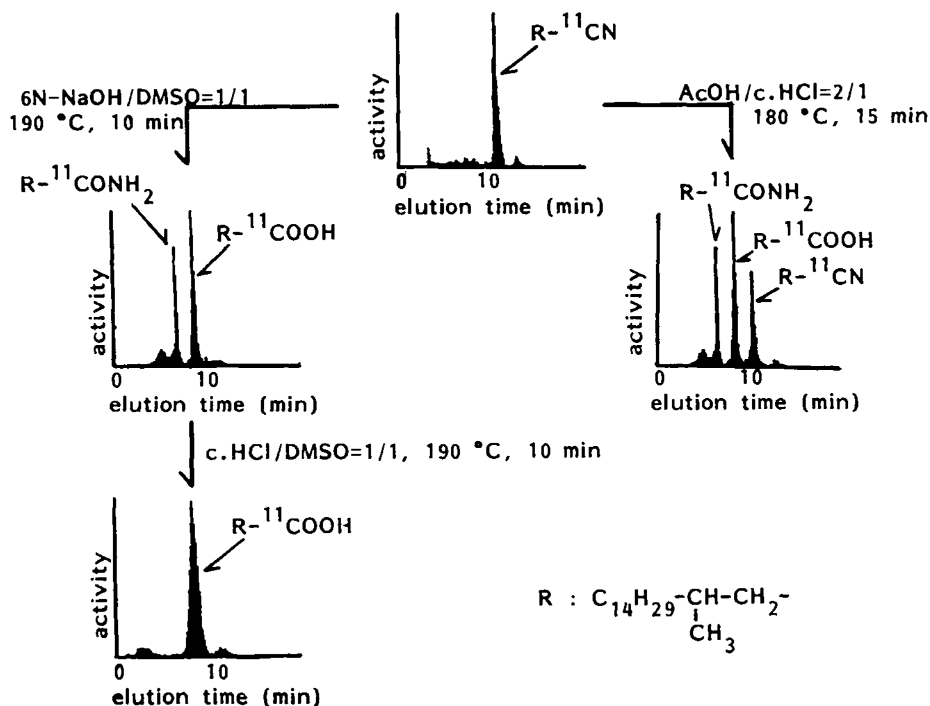
0.1M NaOH in DMSO (suspension) 300 - 500 μl
↓ alkyl bromide (10 μl) was added
↓ $H^{11}CN$ was introduced (room temperature)
reaction mixture
↓ reaction mixture was heated (120 °C) for 10 min with stirring
reaction mixture
↓ hydrolysis solution was added
hydrolysis (160 - 190 °C, 15 - 20 min)
↓
reaction mixture
↓ excess H_2O was added
↓ water solution was extracted with ether
etheral solution
↓ solvent was evaporated in vacuo
(1-^{11}C)labeled fatty acids

Table 1. Radiochemical yields, radiochemical purities and preparation times for the preparation of (C-11)labeled fatty acids

Compounds	Method[a]	Radiochemical yield (%)	Radiochemical[d] purity (%)	Time required for synthesis (min)
IIIa	A	30 [b]	76	120 [e]
IIIb	A	16 [b]	80	76 [e]
	G	17 - 67 [c]	> 99	34 - 48 [f]
IIIc	B	83 [b]	96	73 [e]
IIId	B	70 [b]	93	59 [e]
IIIe	BA	33 - 42 [b]	> 94	60 - 86 [e]
	G	2 - 8 [c]	> 96	35 - 52 [f]

a) A : acid hydrolysis (AcOH/c.HCl=2/1, 160 °C, 15 min)
B : alkali hydrolysis (6N-NaOH/DMSO=1/1, 180 °C, 15 min)
BA : alkali hydrolysis (6N-NaOH/DMSO=1/1, 190 °C, 10 min), followed by acid hydrolysis (c.HCl/DMSO=1/1, 190 °C, 10 min)
G : Grignard reaction
b) based on recovered (C-11)HCN
c) based on recovered (C-11)CO_2
d) determined by HPLC analysis (column : Fatty acid analysis (WATERS), mobile phase : THF/MeCN/H_2O=5/9/7)
e) from the end of (C-11)HCN trapping
f) from the starting of (C-11)CO_2 introducing

Figure 3. HPLC radiochromatogram of hydrolysis of IIe in acid or alkali solution system



A series of straight chain fatty acids and a branched chain fatty acid were prepared. The straight chain compounds synthesized were (1-^{11}C)pentadecanoic acid (IIIa), (1-^{11}C)hexadecanoic acid (IIIb), (1-^{11}C)heptadecanoic acid (IIIc), (1-^{11}C)octadecanoic acid (IIId). The branched chain fatty acid was 3-methyl-(1-^{11}C)heptadecanoic acid (IIIe). The synthetic scheme for this series is as follows; i) replacement of Br in alkyl bromide by $^{11}CN^-$ ii) hydrolysis of (^{11}C)labeled nitrile (Fig. 1, Fig. 2). The radiochemical yields, the radiochemical purities and the time required for the synthesis are summarized in Table 1.

Na^{11}CN easily reacted with alkyl bromides to furnish the corresponding (^{11}C)labeled nitriles. Hydrolysis of straight chain nitriles easily proceeded in c.HCl/AcOH or 6N-NaOH/DMSO solution. AcOH or DMSO was added to increase the solubility of the nitriles. Hydrolysis in alkali solution was more advantageous than in acid solution for radiochemical yield and radiochemical purity (Table 1). A hydrolysis time of 15 min was sufficient to obtain (^{11}C)labeled straight chain fatty acids. The (^{11}C)labeled 3-methyl nitrile (IIe) was not hydrolized sufficiently under the same hydrolysis conditions because of steric hindrance by the methyl group at the 3 position. The hydrolysis of IIe in c.HCl/AcOH solution at 180°C for 15 min gave three radioactive compounds (nitrile, amide, carboxylic acid), however the nitrile was still present even after hydrolysis for longer than 40 min. But, the hydrolysis in 6N-NaOH/DMSO solution at 190°C for 10 min led to the disappearance of the nitrile and gave two radioactive compounds (amide, carboxylic acid) (Fig. 3). Furthermore, the branched chain amide was easily hydrolized in c.HCl/DMSO solution at 190°C for 10 min.
Consequently, IIe was converted completely to IIIe by using a combination of both of the above-mentioned hydrolysis conditions, that is, hydrolysis at 190°C in 6N-NaOH/DMSO solution for 10 min, followed by in c.HCl/DMSO solution for 10 min.
This new method is more useful for the synthesis of (^{11}C)labeled branched chain fatty acids than the Grignard reactin because of better radiochemical yield and reproducibility.

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SYNTHESES OF ^{11}C-LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS

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Carbon-11($T_{1/2}$=20.4 min. E_{β^+}=0.968 MeV) was the only radioactive carbon can be used widely to in-vivo nuclear medicine, because of carbon was the foundation composite element of organisms, thus it can be used to label pharmaceutical molecular without altering molecular structure as well as physiological character. In the meantime, it is a positron emitter and its positron annihilation radiation of two photons (0.511 MeV) can be measured by coincidence circuit with high resolution. These unique features attract more and more attentions in the fields biomedical studies and clinical diagnosis.

Table 1

^{11}C-labelled Carboxylic acid

compound	yield(%)	time(min)
^{11}C-propionic acid	99	30
^{11}C-benzoic acid	89	40

Table 2

^{11}C-labelled amino acid

compound	yield(%)	time(min)
^{11}C-valine	73.8	50
^{11}C-leucine	51.7	50
^{11}C-amino cyclopentane carboxylic acid	66.2	50

The crux in syntheses of ^{11}C-labelled compounds and radiopharmaceuticals is rapidity, that is the total time of the preparation must be less than three half lives, in other words it must be completed

within an hour. Moreover its micro-amount makes the organic syntheses technique often having to be modified to meet the new situations. We have synthesized ^{11}C-Carbon dioxide, ^{11}C-Sodium carbonate, ^{11}C-Potassium bicarbonate, ^{11}C-Cyanide, ^{11}C-Methanol and ^{11}C-Methyl iodide, which are very important precursors for further preparations of radiopharmaceutials. With these starting materials and useing Grignard reaction or Bücherer-strecker method the carboxylic acids (Table 1)[1] and amino acids (Table 2)[2] were obtained. The ^{11}C-labelled glucose was prepared by photosynthesis.

The products were separated and purified by extraction and ion exchange. The chemical and radiochemical purity were determineded by paper chromatography and radio-gaschromatography, both had efficiencies of over 95%. They were studied radiopharmacoloically in mice and rabbits. ^{11}C-benzoic acid may be a useful radiopharmaceutical for detecting renal disease[3]. ^{11}C-Valine might be used as a patential pancreas imaging[4] and ^{11}C-glucose is useful for in-vivo dynamic studies of metabolism.

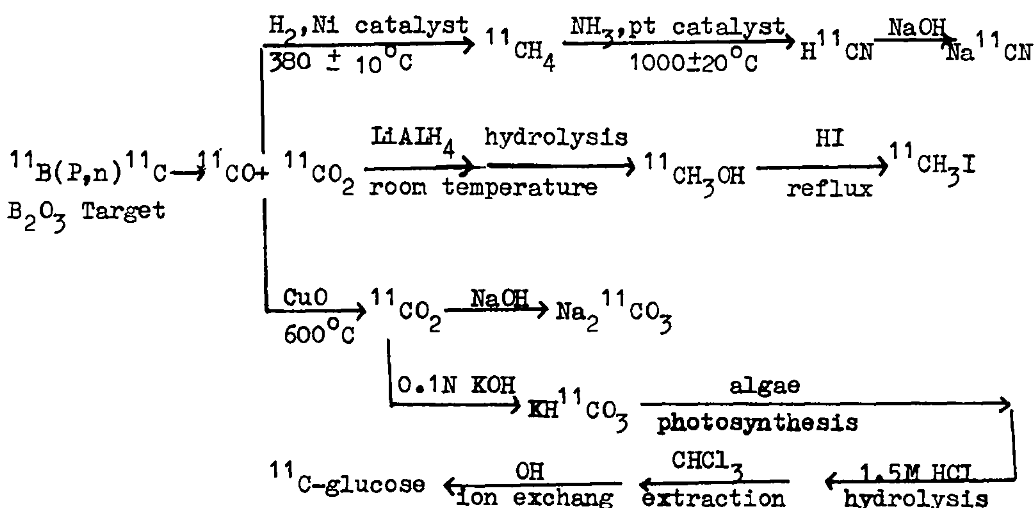

Fig.1 Chemical syntheses of important precursors and photosynthesis of glucose

The syntheses of some important intermediates were shown in figure 1; The use of boron oxide as target was bombarded with protons produced ^{11}CO and $^{11}CO_2$. The mixture gas coming out of the target was carried through a cupric oxide furnace kept at 600°C using a

continuously purging heliun gas stream. It was converted immediately to pure ^{11}C-Carbon dioxide with a rate of 99.84%, which was absorbed with an efficiency of 99.96% in sodium hydroxide solution forming ^{11}C-sodium carbonate, and absorbed in potassium hydroxide solution forming ^{11}C-potassium bicarbonate which was used in glucose synthesis. We have prepared cyanide the mixture gas was carried by 99% hydrogen to pass through the nickel catalyst furnace combining into ^{11}C-methane, Subseguently the mathane mixed with ammonia passed the platium catalyst furnace and converted almost quantitively to cyanic acid which was absorbed in sodiun hydroxide solution as ^{11}C-sodium cyanide. In preparation of methanol and methyl iodide the mixture gas was reduced with lithium aluminum hydride (in tetrahydrofuran). The reductive complex was hydrohyzed to form ^{11}C-methanol. The methanol refluxed with hydroiodic acid to obtain ^{11}C-methyl iodide in a atmosphere of nitrogen. We are going to use methyl iodide to prepare methionine.

All chemical reactions were proceeded in mini-glass system which designed by ourself. The operation and proceduce are very simple and easy. The synthetic time required 1-2 minutes for $^{11}CO_2$, 20 minutes for Na^{11} CN and 10 minutes for $^{11}CH_3I$ respectively. The photo-synthesis by an algae---scenedesmus obliquas of ^{11}C-glucose and its extraction and purification took approximately 25 minutes with a yield up to 67%.

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AN IMPROVED SYNTHESIS OF [1-^{11}C]PUTRESCINE

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Recently we reported a synthesis of no-carrier-added [1-^{11}C]putrescine, a potential probe for cerebral malignancy (1). The product was obtained in a state of high radiochemical purity by the use of preparative high performance liquid chromatography (HPLC) utilizing a Bio-Rad Aminex column and a mobile phase of 0.03 M sodium hydroxide. While the synthesis was straight forward and gave [^{11}C]putrescine in good yield (20%) and acceptable synthesis time (50 min), this procedure required separate vessels for displacement and reduction steps and the time and effort required to maintain this particular HPLC system made an alternative method of purification desirable.

In the course of developing a rapid analytical method for determining the amount of unmetabolized [^{11}C]putrescine in human plasma, we investigated the use of small cellulose phosphate ion exchange columns which have been reported (2) to separate polyamines of different base strengths from a variety of other compounds including monoamines and amino acids by elution with buffers of increasing pH and ionic strength. The effectiveness of this technique led us to modify it for use in the purification of [1-^{11}C]putrescine. We report here a simplified one-pot synthesis of [1-^{11}C]putrescine based on the use of cellulose phosphate in the purification step.

[1-^{11}C]Succinonitrile was prepared by the Michael addition of potassium [^{11}C]cyanide to acrylonitrile in aqueous THF at 65° for 5 min (1). The solvent was evaporated under vacuum. To the residue was added borane-methyl sulfide in THF and the resulting solution was refluxed at 140° for 5 min. Anhydrous methanol-hydrogen chloride was added to decompose the amine-borane complex and the resulting mixture evaporated to dryness. The residue was dissolved in 0.03 M sodium phosphate buffer and then transferred to a 1.5 cm x 3 cm column of cellulose phosphate (Bio-Rad Cellex P). The column was eluted with 0.06 M phosphate to remove the less basic impurities and then with 4.2% sodium bicarbonate into fraction collectors in an ionization chamber. After a small forecut of the sodium bicarbonate, [1-^{11}C]putrescne was collected in the following 2.0 ml. To this was added 3.6 ml of water and the resulting solution was sterilized by millipore filtration. The reaction time was 35 min, the radiochemical yield was 35-40% (EOB) and the radiochemical purity was > 97%.

This procedure is a significant improvement over the previous one in terms of convenience, synthesis time and radiochemical yield.

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2-^{18}F-FLUOROPUTRESCINE, PREPARATION AND BIODISTRIBUTION.

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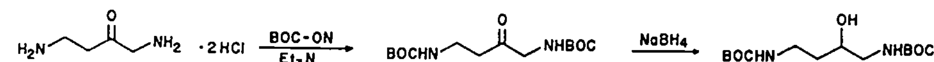
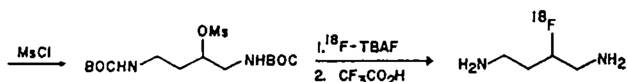
Natural aliphatic polyamines have been a subject of interest because of their association with the proliferation and differtiation of rapidly growing cells in both normal and neoplastic tissues (1,2). Using ^{3}H-labeled putrescine Calrk demonstrated the selective in vivo incorporation of putrescine into rat prostate (3). These results suggested the possibility of using putrescine radiolabeled with a suitable γ-emitting radionuclide as an imaging agent for prostate or other tissues containing high polyamine concentratons in human (4,5). This potential has prompted efforts over the past several years to radiolabel putrescine with a positron-emitting radionuclide for in vivo imaging of prostate, prostate derived tumors, and brain tumors by positron emission tomography (PET). To date, the preparation and biological studies of carrier-added (CA) and no carrier-added (NCA) 1-^{11}C-putrescine (6-8), and a putrescine analog, N-^{11}C-methyl-putrescine (9-11), has been reported.

Recently it has been shown that the in vivo uptake of ^{14}C-putrescine into rat prostate 3h after administration is greatly enhanced following pretratement with the enzyme inhibitor difluoromethylornithine (DFMO) and androgen (12). Similarly, enhanced ^{14}C-putrescine uptake was also shown to occur in rat prostate derived tumors (13). These results suggest that for the purpose of imaging prostate and prostate derived tumors by PET, putrescine radiolabeled with fluorine-18 ($t_{1/2}$= 110 min) would be preferable to carbon-11 ($t_{1/2}$= 20 min).

We have developed the radiosynthesis of a fluorine-18 labeled putrescine analog, 2-^{18}F-fluoroputrescine I. The title compound I was prepared in a two-step synthesis: ^{18}F-fluoride displacement of a mesylate II (prepared from 1,4-diamino-2-butanone in 3 steps), followed by amine deprotection, in a synthesis time of 2h. Radiochemical yields were 1-3% at end-of-synthesis, and specific activities ranged from 0.85 to 4.3 Ci/mmol.





Yield: 1-3 % (EOS)

Specific Activity: 0.85-4.5 mCi/μmol

TBAF(μmol)	Solvent	Temp(°C)	h	Yield
20	THF	80	1	15*
10	THF	80	1	10*
2	THF	80	1	3
0	THF	80	1	0

* TLC yield of N-protected product.

The in vivo biodistribution of I in mature male rats showed the uptake by the ventral (0.22 %ID/g) and dorsal (0.4 %ID/g) prostate 1h after administration was considerably lower than the uptake of NCA 1-^{11}C-putrescine (1.8 %ID/g and 0.96 %ID/g respectively); however when the effect of specific activity was taken into consideration, the prostate uptake of the title compound was not so different from previous studies. The most notable result, however, was the significant uptake in the bone (25% and 40% ID/organ at 1h and 3h respectively), indicating defluorination of the compound. In animals pretreated with aminoguanidine, an inhibitor of diamine oxidase, defluorination was markedly reduced (6% and 3% ID/organ at 1h and 3h respectively), but the uptake by the prostate did not improve.

Our proposed mechanism of defluorination is based on the known mechanisms for putrescine metabolism and the deactivation of ornithine decarboxylase by the enzyme-activated inhibior DFMO (14). That is the formation of an imine via the aldehyde group of pyridoxal phosphate (the prosthetic group of diamine oxidase) and 2-fluoroputrescine substrate generates a stabilized carbanion intermediate which facilitates the fluoride displacement.

These results suggest the need for development of a F-18 labeled analog of putrescine which does not defluorinate in vivo, and which has biological properties similar to those of putrescine.

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COMPARATIVE EVALUATION OF SYNTHETIC ROUTES TO 2-FLUORO-(^{18}F)-2-DEOXY-D-GLUCOSE

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The increasing importance of 2-fluoro-(^{18}F)-2-deoxy-D-glucose as the principal radiopharmaceutical agent for the measurement of glucose metabolic rate as well as for other behavioral investigations, has prompted numerous synthetic routes to this compound. The paramount concern at our institution is the concurrent establishment of the Cyclotron Facility and PET Installation within the department. Therefore, we are evaluating the kinetic and synthetic parameters of three reported routes (1-3) including electrophilic and nucleophilic displacement for the preparation of 2FDG to ascertain the most suitable procedure for our clinical needs.

Variables included in this evaluation have been substrate concentration, amounts of carrier fluorine/fluoride, reaction time and temperature as well as source of fluorine-18 activity (reactor-produced versus cyclotron-produced) and target chemical impurities. These results are being evaluated using radio-TLC, HPLC and wet chemical analyses. An abbreviated example of the data is given for the cyclic sulfate reaction in Table 1.

Table 1. Fluoride Reactions With Methyl-4,6-O-benzylidine-2,3-O-Cyclic Sulfato-B-D-Mannopyranoside*

Concentration of Reagents (umole)			% Incorporation
Cyclic Sulfate	Base[a]	Fluoride[b]	
2.9	2.5	2.5	95
5.8	5.0	5.0	88
2.9	2.5	1.2	96
2.9	2.0	NCA (K_{222})	87
1.5	2.0	1.0	95
1.5	2.0	NCA	20

*Reaction times and temperature were 15-20 minutes at 95°C for this table. [a]-tetramethylammonium hydroxide or K_2CO_3; [b]-tetramethylammonium fluoride; (K222)-Kryptofix-(2.2.2);

Technical refinements resulting in improvements for both the chemical and radiochemical yields will be presented.

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STEREOSPECIFIC SYNTHESIS OF N.C.A. 2-[^{18}F]-FLUORO-2-DEOXY-D-MANNOSE AND 2-[^{18}F]-FLUORO-2-DEOXY-D-GLUCOSE AND THE INFLUENCE OF ADDED CARRIER (KF) ON FDG-SYNTHESIS

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A stereospecific synthesis of ^{18}F-labeled n.c.a. 2-FDM, similar to the aminopolyether (APE) supported synthesis of n.c.a. 2-FDG was developed (1) to study the physiological behaviour in comparison to 2-FDG. Starting with 1-O-methyl-4,6-O-benzylidene-α-D-glucopyranoside, the precursor 1-O-methyl-2-O-trifluoromethanesulfonyl-3-O-benzoyl-4.6-di-O-acetyl-α-D-glucopyranosid was synthesized via the corresponding 4.6-O-benzylidene-derivative (2) in a 4-step reaction. The APE mediated nucleophilic fluorination at the no-carrier-added level yielded 45 % acylated 1-O-methyl-2-[^{18}F]-fluoro-2-deoxy-α-D-mannopyranoside. The removal of the protecting groups and further purification of 2-FDM can be carried out as described in the literature (3). The epimerical purity of FDM was confirmed by TLC on monosodium phosphate impregnated silica plates (4) and isocratic HPLC.

The synthesis of 2-FDG via nucleophilic substitution starting from tetra-acetylated mannose (1), has shown that the uncorrected radiochemical yield of FDG at the no-carrier-added level is 50 %. To investigate the influence of KF-carrier on the yield of 2-[^{18}F]-FDG the ratio of the $^{19}F^-$-concentration to the concentration of the substrate (1.3.4.6-tetra-O-acetyl-2-O-trifluor-methanesulfonyl-ß-D-mannopyranose) was varied in the range of 10^{-3} to 1. It was shown that below a molar ratio (fluoride-to-triflate) of $5 \cdot 10^{-3}$ (^{19}F-content <5 μg) the uncorrected radiochemical yield of FDG remained unchanged. With increasing F-concentration the yield of 2-^{18}FDG decreased significantly to about 6 % when the amount of ^{19}F was 100 to 900 μg (Figure). The results indicate that at higher fluoride concentration the elimination reaction is dominant. This effect is even stronger in the case of 2-FDM.

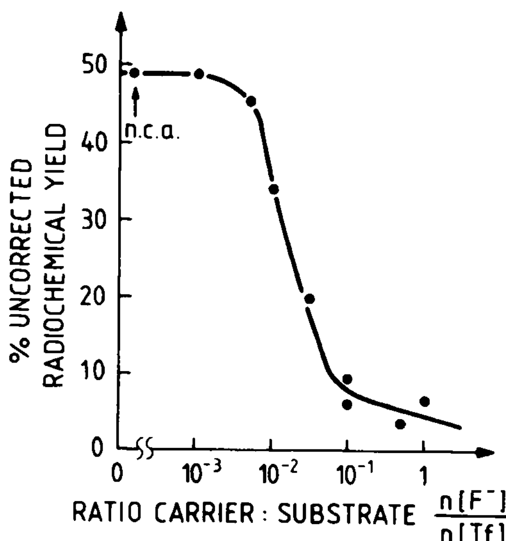

Figure. Dependence of radiochemical yield of 2-[^{18}F]-FDG on the amount of added fluoride carrier

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THE ROUTINE PRODUCTION OF 2-[F-18] FLUORO-2-DEOXY-D-GLUCOSE FROM REACTOR PRODUCED F-18

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F-18 Labelled 2-deoxy-2-fluoro-D-glucose, 2-[F-18] FDG continues to be a widely used radiopharmaceutical in Positron Emission Tomography. Work continues on a variety of production methods for 2-[F-18] FDG.

F-18 can also be produced in a nuclear reactor any time during its operation without problems of scheduling. Thus, [F-18] fluoride is readily available any time for the radiopharmaceutical synthesis. We have implemented a method for the preparation of 2 [F-18] FDG that uses [F-18] flouride from a small research reactor [MRT, swimming pool type, 2 MW]. F-18 labelled tetraethylammonium fluoride, TEAF, was produced by a modification of the procedure by Gatley et al (1), Scheme 1. It was then reacted with the cyclic sulfur ester of the carbohydrate substrate developed by Tewson (2,3) to form 2-[F-18] FDG, Scheme 1.

Using a remotely operated and shielded apparatus (Figure 1) we are able to synthesize routinely 2-[F-18] FDG in millicurie quantities to support a neurological PET programme.

On average we prepare 8.2 mCi (n=30) of 2-[F-18] FDG starting from 37 mCi (n=30), radiochemical yield 22% EOS (n=29). The procedure takes 2 hours. The final solution of 2-[F-18] FDG does not contain [F-18] fluoro-deoxy-mannase. The contamination with H-3 is only 50 nanocuries per preparation. F-19 NMR Spectroscopy of the final solution shows up to 15% fluoro-deoxy-altrose. It can be removed by preparative HPLC (4).

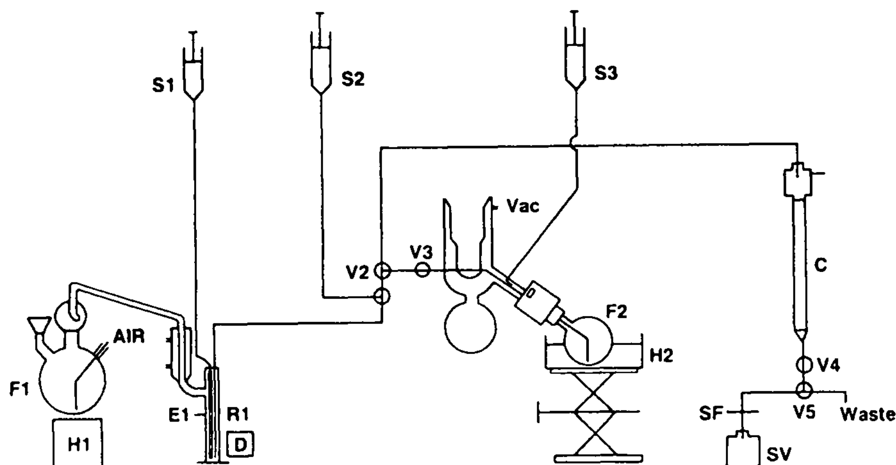

Figure 1.

This work demonstrates that a nuclear reactor can provide 2-[F-18] FDG for a clinical programme. At present there are in the United States more nuclear research reactors in use than medical cyclotrons (Fig. 2). It should therefore be possible to make 2-[F-18] FDG and other F-18 radiopharmaceuticals (F-18-fluoromethane and F-18-N-methylspiperone) available to a larger number of medical centres that are presently supplied from medical cyclotrons.

Figure 2.

Geographic distribution of medically used cyclotrons and nuclear research reactors with power greater than 1 megawatt in the United States.

● reactors

◎ cyclotrons

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PREPARATION OF 2-^{18}F-FLUORO-2-DEOXY-D-GLUCOSE BY NUCLEOPHILIC SUBSTITUTION WITH TANDEM ACCELERATOR PRODUCED ^{18}F-FLUORIDE

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The use of the nuclear reaction $^{18}O(p,n)^{18}F$ and ^{18}O-water targets has recently drawn an increased interest in the production of ^{18}F-fluoride to be used in labelling radiopharmaceuticals for positron tomography (1).

We developed a small volume target system of nickel (Figure 1.) for effective production of ^{18}F-fluoride (Table 1.) (2).

A remote controlled apparatus (3) was constructed for the production of 2-^{18}F-fluoro-2-deoxy-D-glucose (2-^{18}F-FDG). The synthesis was based on the method described by Tewson (4). Activated water was introduced into a flask containing 7.26 mg of tetramethylammonium hydroxide and 3.7 mg of tetramethylammonium fluoride and evaporated to dryness at 130°C. The white residue was dried by addition and intermittant evaporation of three 5 ml aliquots of dry acetonitrile and 13.76 mg of 4,6-benzylidene-1-β-methyl-D-mannopyranoside-2,3-cyclic sulphate in 10 ml of dry acetonitrile was added. The solution was heated for 12 min at 80°C and the solvent evaporated. The protective groups were removed by treatment with 5 ml of 1 M boron tris (trifluoroacetate) in trifluoroacetic acid for 15 min at 55°C. Then 2 ml of water was added, the reaction continued for 2 min and the solvents evaporated. Borate was removed by the addition and evaporation of two 5 ml aliquots of methanol followed by a 3 ml portion of water (5). The residue was dissolved in 5 ml of water. Fluoride as well as coloured impurities were removed by passage through a neutral alumina column (0.9 x 10 cm) using water as eluent at a flow rate 1.5 ml per minute. 2-^{18}F-FDG-containing fractions were used in experiments with human tumor implanted nude mice.

The overall yield ranged from 15 - 40 %, with a synthesis time of about 90 min. 2-^{18}F-FDG was the main product as analysed by thin layer chromatography (TLC) (silica gel, acetonitrile/water 95:5, n-butanol/acetic acid/water 15:7:8, methanol/chloroform/acetic acid 15:85:1) and autoradiography, allthrough several impurities were also present. When radiochemical purity was determined by liquid chromatography (HPLC), using NH_2-column with 70 % acetonitrile as eluent, no impurities were observed. There appears to be a poor correlation between TLC and HPLC results, which emphasized the need for careful checking of radiochemical purity.

TABLE 1. Yields of ^{18}F-Fluoride at End of Bombardment with a 9.6 MeV Proton Beam from a EPG-10-II Tandem Accelerator

Activation time min	Current μA	Gained activity[a] mCi	Saturation activity mCi	Efficiency %
60	3.0	108	108	100
60	3.6	135	135	100
60	4.4	150	158	95[b]
60	5.4	181	194	94[b]
110	3.5	170	195	87[b]

a) ^{18}O-enriched water (98 %)

b) Loss of efficiency was caused by radiolysis (highly focused proton beam)

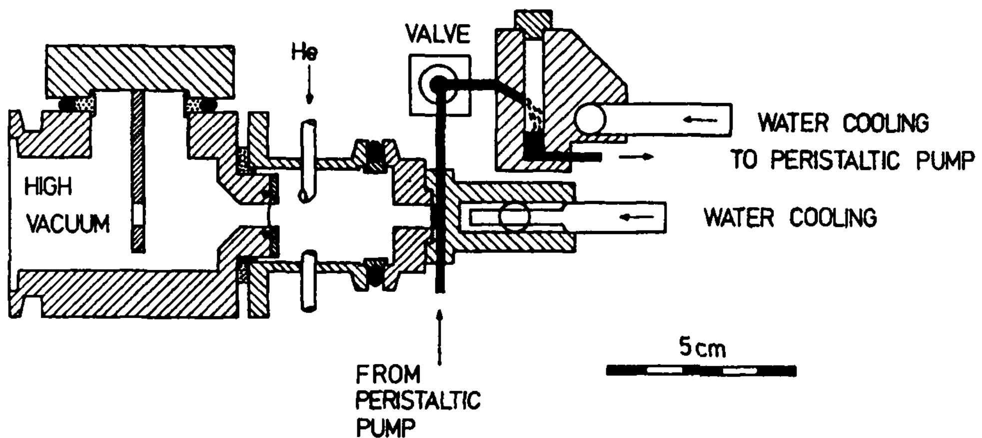

Figure 1. Side-view of the target arrangement. The proton beam goes through a tantalum collimator, the Havar exit window, helium atmosphere and the Havar entrance foil before entering the target.

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SYNTHESIS OF ^{18}F-LABELED 4-FLUOROANTIPYRINE VIA GASEOUS ACETYLHYPOFLUORITE: OPTIMIZATION OF REACTION PARAMETERS AND REMOTE CONTROLLED PRODUCTION

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4-^{18}F-Fluoroantipyrine (4-FAP) has recently been validated as a cerebral blood flow tracer by Sako et al. (1). Since the synthetic methods published up to now (2-4) have not employed gaseous acetylhypofluorite it was undertaken to simplify the reaction by using different solvent systems and substrate concentrations. Seven different solvents wre tested: CH_2Cl_2, $CHCl_3$, CH_3CN, $CFCl_3$, H_2O, acetic acid and ethylacetate. Methylene chloride, water, CH_3CN and acetic acid proved equally successful, whereas freon did not dissolve the substrate antipyrine, and the other solvents led to higher proportions of difluorinated products.

Since methylene chloride is most readily removed and replaced by a chromatographic solvent, optimization of substrate concentration was done in this solvent. At low substrate concentration, four different radioactive species were found in thin layer chromatography of the crude reaction mixture. Three of these could be identified as fluoride, 4-FAP and 4,4-difluoro-3-acetoxy-2,3-dimethyl-1-phenylpyrazolidine-5-one as described by Diksic and Di Raddo (3). The fourth unidentified product, having a slightly higher R_f-value than 4-FAP, is only formed at low substrate concentrations.
A tenfold molar substrate excess over acetylhypofluorite was found to give optimal results (yields were 8 % fluoride, 80 % 4-FAP and 12 % difluoro compound). The fourth compound was no longer detectable.
Flash chromatography using a SiO_2-column and ethylacetate as eluent yields a radiochemically pure product, which is free from the starting compound. A remote controlled system was set up for synthesis of higher activities. First test runs showed, that ^{18}F4-FAP can be produced with a 25 % radiochemical yield (based on recovered F_2, corrected for decay), giving an injectable solution within 35 min after EOB.
As an alternative, reaction in acetonitrile followed by hydrolysis of the difluorocompound with $NaOCH_3$ may be employed. This requires a shorter synthesis time by omitting extraction and evaporation procedures from the original method of Diksic and Di Raddo (3). The yield is comparable to the method described above. The injectable product however still contains the full amount of the substrate antipyrine because only Al_2O_3-Sep-Pak purification is used to remove the fluoride.

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DIRECT FLUORINATION OF MELATONIN AND 5-HYDROXY-L-TRYPTOPHAN WITH [^{18}F]F_2

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The neurohormone melatonin is the chemical manifestation of the biological clock. It is known to cue cyclic events of life, like sleep and ovulation. There are specific receptors for melatonin in the brain that change with the cycle. To investigate these receptors during life in health and in depressive disorders with positron emission tomography melatonin labelled with a positron emitting isotope is needed.

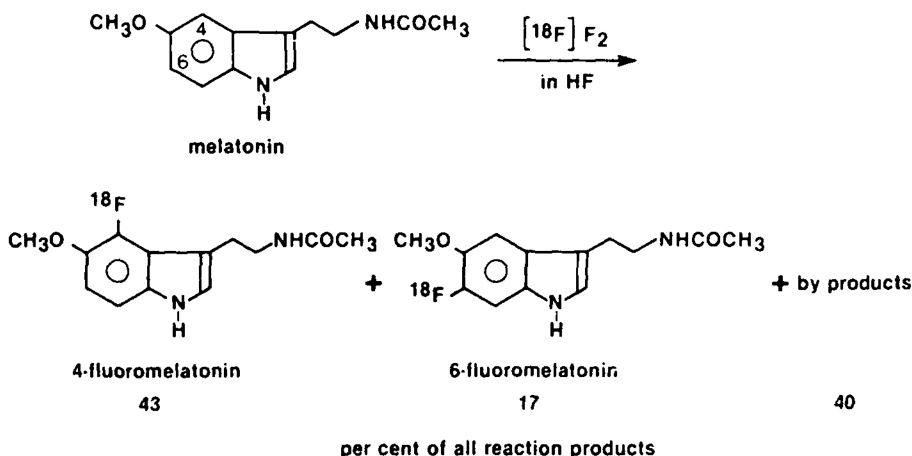

FIG. I REACTION SCHEME

We have chosen to approach the problem with 6-fluoro-melatonin labelled with F-18. "The fluorine in the 6-position of the indole structure does not interfere with the activation of the pituitary melatonin receptor" (1). We applied our general method for the fluorination of catecholes (2) to radiofluorinate melatonin (Fig. 1). Melatonin (540 umol) was dissolved in HF (5ml). At -70°C [F-18] F (230 umol; 0.5% F in neon) was passed through the solution. The HF was evaporated and the residue was taken up in ethanol/water. The reaction products were separated on a semipreparative reverse phase C-18 column which was eluted with 20% methanol in water (Fig.2).

The two F-18 carrying melatonins eluted were found to be 6-fluoro-melatonin (17%) and 4-fluoro-Melatonin (43%). They were characterized by their molecular ion using high resolution mass spectroscopy with chemical ionination by H-1 and F-19NMR spectroscopy. 4-Fluoro-melatonin is a new compound. Millicure quantities of either fluoro-melatonin (radiochemical yield 5% EOS) can be produced with a specific activity of 235 mCi/mmol.

The data above illustrate that our fluorination method, originally developed for catechole derivatives, is also applicable to fluorinate indoles. Not only melatonin but also the especially acid-sensitive 5-hydroxy-tryptophan was fluorinated to form 5-hydroxy-6-[F-18]fluoro-tryptophan and 4-[F-18]fluro-5-hydroxy-tryptophan in millicure quantities. These tracers will be used to develop a PET-method with which to study the neurotransmitter serotonin in the brain of man.

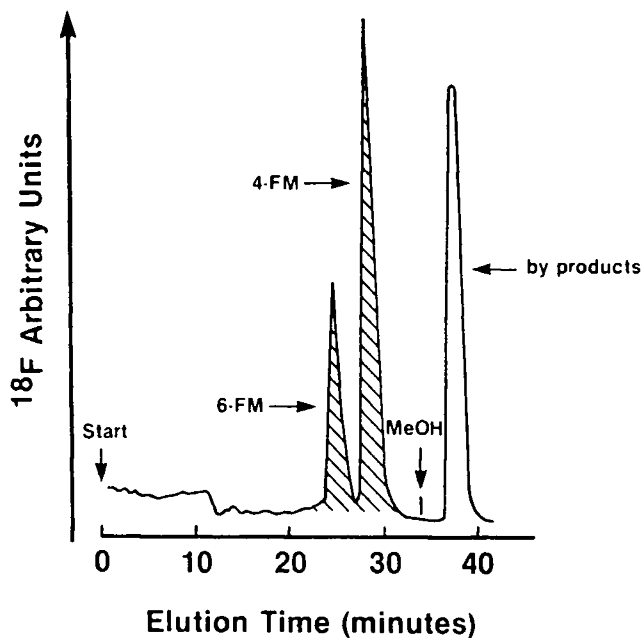

FIG. 2 HIGH PRESSURE LIQUID CHROMATOGRAPHIC ISOLATION OF 4-FLUOROMELATONIN (4-FM) and 6-FLUOROMELATONIN (6-FM)

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2-[F-18] FLUORO-L-DOPA

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Two fluoro-analogs of L-dopa, 5- and 6-fluoro-L-dopa, have been found to behave in vitro and in vivo similar to L-dopa. Positron Emission Tomography with 6-[F-18] fluoro-L-dopa as tracer has been able to visualize the cerebral dopaminergic pathways in the human brain (1). This PET-technique is currently used to measure the dopaminergic abnormalities in disorders of locomotion and mood (2,3).

Dramatic differences in adrenergic activity have been found among the isomers of fluoro-norepinephrine (4). In the light of this fact we suspected that differences of biological behaviour may also exist between the isomers of fluoro-dopa. For this reason we synthesized and investigated in vivo and third ring-substituted fluoro-analog of L-dopa, 2-fluoro-L-dopa.

2-[F-18] Fluoro-L-dopa was synthesized by a modification of the direct fluorination method for catecholes (5). L-Dopa [225 u mol] was dissolved in trifluoroacetic acid TFA (5 ml) and [F-18] fluorine gas in neon [90 u mol, 0.2% fluorine in neon] was passed through the solution at -10 C. After the reaction TFA was evaporated and the residue was taken up in dilute HCl, F-19 NMR Spectroscopy of the reaction mixture showed that only 2 and 5-fluoro-dopa were formed among other fluorinated products. 2-[F-18] Fluoro-L-dopa was isolated from the reaction products and 5-fluoro-L-dopa by reverse phase high pressure liquid chromatography using two semipreparative C-18 columns in series. Radiochemical yield was 3% EOS.

2-[F-18] Fluoro-L-dopa was injected into normal volunteers and the F-18 distribution evenly within the brain. The cerebral dopaminergic structures did not accumulate F-18; this is in stark contrast to 6-[F-18] fluoro-L-dopa. To explain these findings we suspect that the enzyme dopadecarboxylase may discriminate against 2-fluoro-L-dopa while 5-and 6-fluoro-L-dopa are accepted as substrates (6).

The study illustrates the importance of isometric considerations in radiopharmaceutical design.

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NUCLEOPHILIC DISPLACEMENT OF ACTIVATED AROMATIC DIMETHYLSULFONIUM GROUPS BY [^{18}F]FLUORIDE ION

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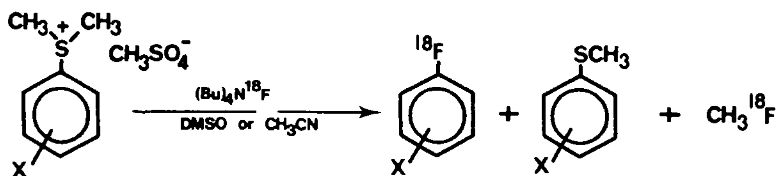
Considerable success has been achieved in the synthesis of ^{18}F-labeled aryl fluorides with high specific activity, based on the nucleophilic displacement of nitro and trimethylammonium groups in activated aromatic rings by [^{18}F]fluoride ion (1-6). A recent report by Angelini et al. showed that the reaction of aryldimethylsulfonium perchlorates with cesium [^{18}F]fluoride underwent no radiofluoride incorporation into the aromatic ring (4). Our previous investigation, however, has shown that activated phenyldimethylsulfonium methylsulfates undergo nucleophilic aromatic substitution with stable fluoride ion (7). The availability of large quantities of [^{18}F]fluoride ion by the $^{18}O(p,n)^{18}F$ reaction has recently been demonstrated (8) and ^{18}F-labeled tetraalkylammonium fluorides are gaining increasing importance as the precursor of choice in the preparation of ^{18}F-labeled radiopharmaceuticals (9). The utility of the dimethylsulfonium group as a leaving group in aromatic radiofluorination using tetra-n-butylammonium [^{18}F]fluoride has been investigated.

Fluorine-18 was produced using the $^{18}O(p,n)^{18}F$ reaction and a 6% enriched water target. An aliquot of the irradiated water was placed in a small TPX (polymethylpentene) tube or a pyrex vial containing a 20µl of 10% tetra-n-butylammonium hydroxide in water. The TPX was chosen as a suitable vessel to minimize the losses of the activity by adsorption to the vessel wall when dissolved in organic solvents. The water was evaporated using a 100°C oil bath and a stream of argon. The residue was again dried by adding dry acetonitrile and by further evaporation with a stream of argon. Ion chromatographic analysis (conductivity detector) of the irradiated water showed that carrier fluoride ion is present at a level of 2-3 ppm.

To a dry acetonitrile or dimethyl sulfoxide (DMSO) solution containing the tetra-n-butylammonium [^{18}F]fluoride in TPX or Pyrex vial was added p-nitrophenyldimethylsulfonium methylsulfate (1). The solution was heated at 100°C for 10 min. The reaction mixture was analyzed by radio HPLC and p-[^{18}F]fluoronitrobenzene (2) was isolated in radiochemical yields shown in Table 1. The p-cyano and p-acetyl substituted substrates, however, gave only few % or less of the ^{18}F-labeled aromatics. Our result shows that [^{18}F]fluoride ion can displace effectively the sulfonium group of (1), although the demethylation process leading to the formation of thioether is predominant reaction. Furthermore, it was found that the displacement yield with (1) is significantly higher using a TPX vessel than that using a Pyrex vessel. Nonetheless, the dimethylsulfonium group is not a good leaving substituent for aromatic radiofluorination by [^{18}F]fluoride ion, because of its thermal instability. The use of the TPX as a reaction vessel to other radiofluorinations is also under investigation.



(1) X = p-NO$_2$ (2) X = p-NO$_2$

(3) X = o-NO$_2$ (4) X = o-NO$_2$

(5) X = p-CN (6) X = p-CN

(7) X = P-COCH$_3$ (8) X = p-COCH$_3$

Table 1. Radiochemical yields of aryl [^{18}F]fluorides from the reaction of aromatic dimethylsulfonium salts with tetra-n-butylammonium [^{18}F]fluoride*

Substrate	Reaction vessel	Solvent	Aryl[^{18}F]fluoride (%)**
(1)	Pyrex	CH_3CN	(2) (27)
(1)	Pyrex	DMSO	(2) (40)
(1)	TPX	CH_3CN	(2) (59)
(1)	TPX	DMSO	(2) (54-68)
(3)	TPX	DMSO	(4) (30)
(5)	Pyrex	DMSO	(6) (3)
(5)	TPX	DMSO	(6) (5)
(5)	TPX	CH_3CN	(6) (10)
(7)	TPX	DMSO	(8) (0.5)

* All reactions were carried out at 100°C for 10 min.
**Percentage of activity isolated in the product (not corrected for decay) relative to the activity available in the reaction step.

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AROMATIC RADIOFLUORINATION WITH [F-18]F_2 IN ANHYDROUS HYDROGEN FLUORIDE

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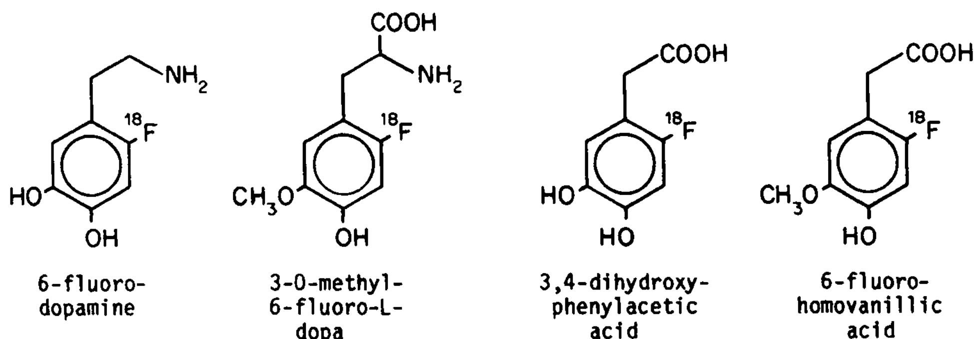
Anhydrous hydrogen fluoride, HF is an excellent solvent for organic compounds. The fluorine in HF does not exchange isotopically with that in fluorine gas F_2 (1). Therefore, HF can be used as a solvent for radiofluorinations with [F-18]F_2, especially at low temperature. The method has been applied first to the fluorination of L-dopa (2,3). As a result, the tracer 6-[F-18]fluoro-L-dopa with positron emission tomography has made it possible to investigate the neurotransmitter dopamine in Parkinson's disease, dystonia and disorders of affect and mood (4-6).

The method is versatile. Recently we used it to synthesize a variety of fluoro-cathechols of biological interest.



6-fluoro-dopamine | 3-O-methyl-6-fluoro-L-dopa | 3,4-dihydroxy-phenylacetic acid | 6-fluoro-homovanillic acid

These compounds are metabolites of 6-[F-18]fluoro-L-dopa and are needed as reference material during radiopharmacological investigation in vitro.

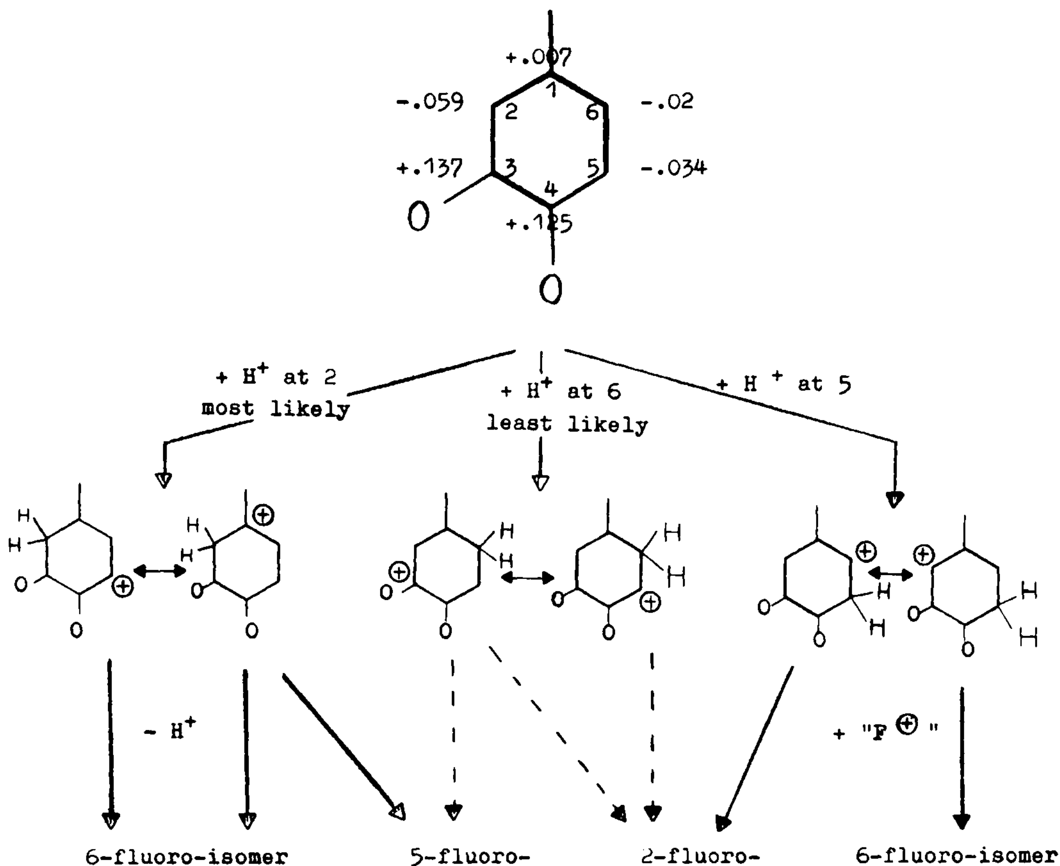
The substrate (400 µmol) was dissolved in a few ml of HF and BF_3 (4mmol) was added. [F-18]F_2 (190µmol, 0.5% F_2 in neon was passed through the solution at -70°C. After HF was evaporated, the remainder was taken up in dilute HCl and chromatographed to isolate the desired 6-fluoro isomer (2). The radiochemical yield was 9-15% EOS. All products were characterized by their molecular ion using high resolution mass spectroscopy with chemical ionization and by their F-19 NMR spectra.

TABLE 1

EFFECT OF SOLVENT ON THE ISOMER DISTRIBUTION DURING THE FLUORINATION OF L-DOPA

Solvent	Isomer distribution in fluoro-L-dopa per cent			Relative Yield of all fluoro-dopas
	2-	5-	6-	
H_2O/.1M HCl	56	44	0	0.05
CF_3COOH	75	25	0	0.3
HF	35	5	60	1
HF/BF_3	35	5	60	2.3
CH_3CN/BF_3	47	39	14	0.4

The fluorination of L-dopa produced all structural isomers of fluoro-L-dopa (Table 1). Their distribution depends upon the solvent used (Table 1). In solvents that do not protonate, fluorine attacks the ring carbons according to their relative electron density (7). Thus, 2- and 5-, but little or no 6-fluoro-L-dopa is produced. In HF and in HF/BF_3 the ring carbons are protonated according to the relative electron density in catechole (Scheme 1). The most likely cation structures are considered in Scheme 1. The



Scheme 1: Protonation of Catechol followed by Electrophilic Fluorination

electrophilic fluorine then can be thought to attack the aryl cations according to their relative electron density. The most likely position for the attack is 6, however fluorinations at other positions is also allowed according to Scheme 1, though less likely. The isomer distribution found experimentally when the reaction was run in the strongly protic solvent HF (Table 1) agrees with the trend derived in Scheme 1. The proposed cation structures are known to be stabilized by BF_4^- (8). Stabilized cations promote electrophilic fluorination over other reactions of fluorine. As a result, addition of BF_3 increases the yield of the fluoro-L-dopas (Table 1).
Our results support the notion that fluorination of catechols is an electrophilic reaction.

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A NEW ROUTE FOR THE SYNTHESIS OF ^{18}F-FLUOROAROMATIC SUBSTITUTED AMINO ACIDS SUCH AS p-FLUOROPHENYLALANINE.

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Radiofluorination by nucleophilic substitution with $^{18}F^-$ is increasingly considered to be the state of the art method for the NCA synthesis of ^{18}F radiopharmaceuticals. A new method of ^{18}F-labeling has been successfully demonstrated in the preparation of ^{18}F-p-fluorophenylalanine. This labelled amino acid analog is a potential marker for probing protein synthesis in the human brain by positron emission tomography and has an incorporation into protein similar to phenylalanine (1). In the NCA state, this tracer avoids any problem of toxicity.

The method of synthesis based on the nucleophilic displacement of the activated nitro group (2) of nitrobenzaldehyde by $^{18}F^-$ is shown in scheme 1.

Step 1 : $^{18}F^-$ was obtained from the $^{18}O(p,n)$ reaction on ^{18}O-enriched water with 18 MeV protons as previously described (3). A typical production run for the tracer studies requires a 10 µA beam for 65 min on a 5% ^{18}O-enriched water target and leads to a total activity removed from the target averaging 30 mCi.
The conversion of the NCA ^{18}F-fluoride activity into a reactive nucleophilic species was carried out by a method similar to that reported by Hamacher for the preparation of 2FDG (4).

Step 2 : A solution of p-nitrobenzaldehyde in DMSO was added to the dry ^{18}F-KF and the substitution reaction was carried out at 90–110° C for 20 minutes. After fixation on a C_{18} sep-pak column, ^{18}F-fluorobenzaldehyde (FBA) was recovered in EtOH with a radiochemical yield of 60–70 % at EOB.

Step 3 : (FBA) was then reacted with 2-phenyl-oxazol-5 one and DABCO according the method of Halldin and Langström (5). The chemical yield was 80–85 % with reaction time of 20 min under reflux at 140° C.

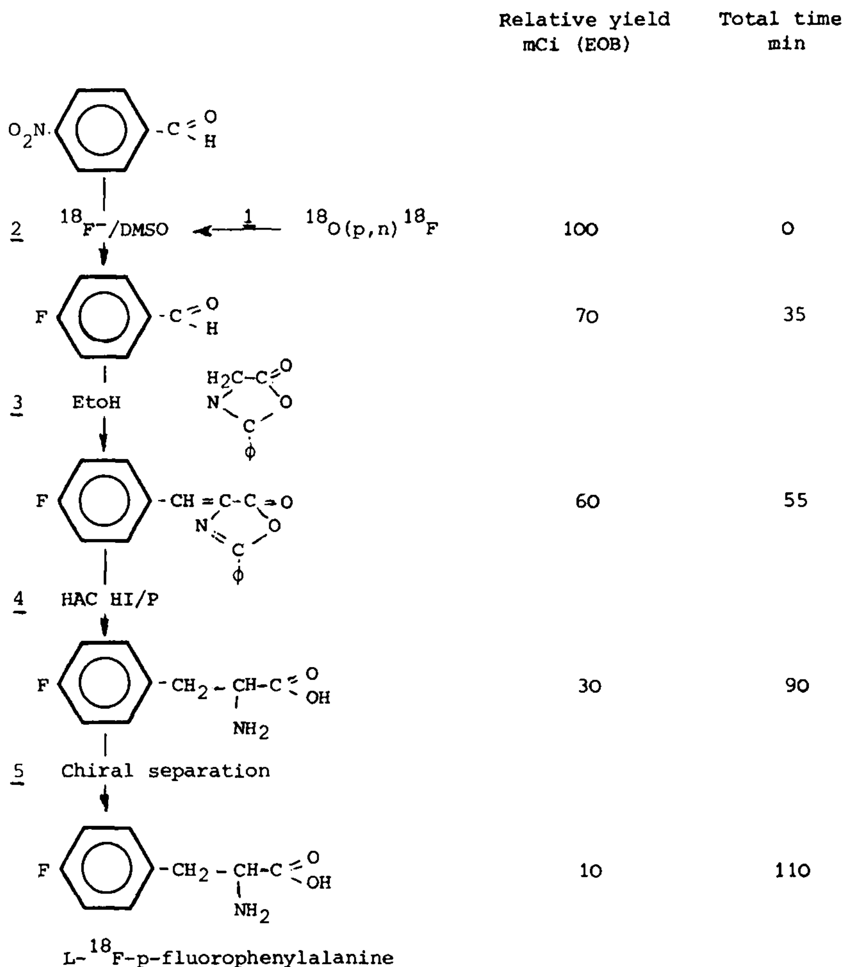
Step 4 : The reduction of the azlactone was carried out using hydroiodic acid and phosphorus, the mixture being refluxed for 10 min for complete hydrolysis.
A final purification procedure by cation exchange chromatography yielded the racemic p-^{18}F-fluorophenylalanine in 25–30 % yield in a total time of 90 min. The different sequential yields are given in scheme 1.
Identification of each compound was carried out by analytical HPLC by comparison with an authentic inactive sample.

Step 5 : The D and L-forms of the NCA ^{18}F-fluorophenylalanine have been separated on an analytical scale chiral column (6). A preparative column is now under development. At present, before optimisation of the different steps, a cyclotron production run of 10 µA.h of 18 MeV protons on 98 % ^{18}O-enriched water (600 mCi of ^{18}F from the target) would allow the synthesis of a batch of 30–40 mCi of L-^{18}F-p-fluorophenylalanine after a time of 110 min.

The main advantages of this synthesis in comparison with the presently available ones are :

- the NCA state of the nucleophilic radiotracer $^{18}F^-$ is easily produced at very high levels of radioactivity;
- a better radiochemical yield at the NCA level is possible within an equivalent time for chemistry including the separation of the enantiomeric forms.

This method has been extended to the preparation of other labelled aminoacids. Preliminary work has shown that NCA ^{18}F-DL-6-fluorodopa can be obtained by a similar procedure with consistent yield at present better than 5 %. This work is in progress.



Preparation scheme 1.

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RADIOSYNTHESIS OF ^{15}O-LABELED BUTANOL AVAILABLE FOR CLINICAL USE.

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[^{15}O]H_2O is commonly used for measuring cerebral blood flow (CBF) by positron emission tomography (PET). The method using [^{15}O]H_2O is useful, but it leads an underestimation of CBF in areas of high flow rate (1) because of a limitation of water extraction (2) in the brain tissue. [^{14}C]Butanol was reported (3, 4) to be a freely diffusible tracer over a wide range of CBF, therefore, [^{11}C]butanol was recommended (5) as a reference tracer for CBF measurement with PET. In addition, [^{15}O]butanol has been regarded to be more suitable from the standpoint of radiation dosimetry and possibility for repeating studies on the same patient with a short time interval. The basic chemistry of [^{15}O]butanol synthesis from tributylborane was first reported by Kabalka et al (6, 7). Recently, the synthesis of [^{15}O]butanol for clinical use was shown by Berridge et al (8), but its purity and radiochemical yield seems to be insufficient for clinical use. In this paper, we report the modified procedure to obtain [^{15}O]butanol with higher yield and higher purity within three minutes after the end of bombardment.

Studies for radiochemical yield: In this synthesis radiochemical yield depends on the trapping yield of [^{15}O]O_2 by tributylborane. In our method [$^{15}O_2$]O was trapped on the surface of packed agent in the micro column (Sep-Pak cartridge) by tributylborane. This method increased the trapping yield of [^{15}O]O_2 by twofold of that of the original gas bubbling method (8) either in the presence of THF solvent or not (Table 1). This trapping method using micro column had following advantages. The chromatographical purification was available with a simple operation. The [^{15}O]O_2 trapping time was able to be shortened by increase of the [^{15}O]O_2 flow rate, hence resulting in the rapid radiosynthesis.

TABLE 1. Yield§ of [^{15}O]O_2 Trapping† (%)

	THF(+)	THF(-)
vial bottle⁂	53.1	41.8
Sep-Pak silica	97.0	92.3
Sep-Pak C-18	—¥	92.3

§ with time decay correction.
† [^{15}O]O_2 flow rate was 500ml/min, at room temperature, 1mmol of tributylborane was used.
⁂ gas bubbling method.
¥ the micro column could not retain the tributylborane THF solution.

TABLE 2. Radiochemical Purity and Yield by Two Purification Method

	Method A	Method B
radiochemical purity(%)	45.2	93.0
radiochemical yield(%)	36.0	48.3

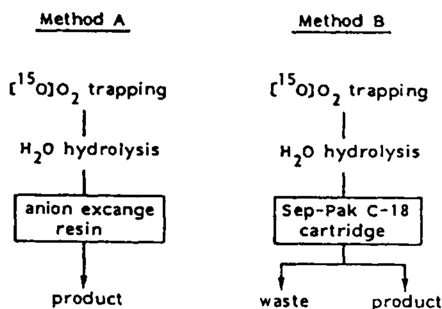

Figure 1. Procedures of the two purification methods.

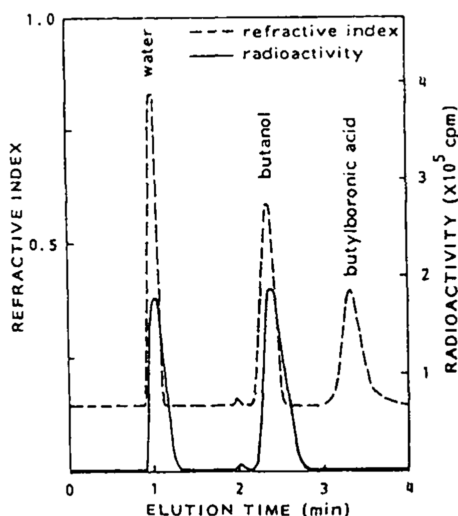

Figure 2. Profiles of radioactive and chemical detection of the reaction mixture on HPLC (Radial PAK C-18, EtOH:H_2O=20:80, 2ml/min).

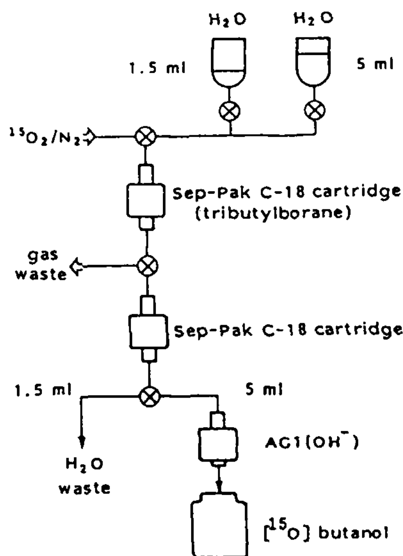

Figure 3. Schema of [^{15}O]butanol synthetic procedure for clinical use.

Studies for radiochemical and chemical purity: The THF solvent is a toxic chemical and it must be removed in this synthesis. We used the [^{15}O]O_2 trapping reaction in the absence of THF in the micro column. Under this condition the reaction equation was (9, 10)

$$Bu_3B + O_2 + 2H_2O \longrightarrow 2BuOH + BuB(OH)_2$$

We examined the two purification method as shown in Fig. 1. Method A was the original procedure (8) using a strong anion exchange resin (OH^- type) which adsorbed boric acid derivatives (butylboronic acid). Method B was our procedure using Sep-Pak C-18 cartridge which performed a reverse phase liquid chromatography. The purity of the products were determined by HPLC. As a result, the method A removed the nonradioactive impurity, butylboronic acid, but not the radioactive impurity which had the same retention time as that of water (Fig. 2). The method B removed the radioactive impurity by taking off the first eluate, but not the nonradioactive impurity. In the original report (8), the radioactive impurity was thought to be [^{15}O] boric acid. However, in our study the radioactive impurity was not adsorbed to a strong anion exchange resin (OH^- type) and showed the same retention time as that of H_2O in HPLC analysis. Therefore, it might not be [^{15}O] boric acid, but probably [^{15}O]H_2O. In the method A [^{15}O]butanol was thought to be adsorbed to the resin from its low radiochemical purity and radiochemical yield (Table 2).

[^{15}O]butanol synthetic procedure for clinical use (Fig. 3): We designed the synthetic procedure based on above two experiments, as follows. [^{15}O]O_2 was produced by deuteron irradiation of 0.5 % O_2 in N_2. The target gas was purified by passing through active charcoal and soda lime columns, and was sent by He flow to the Sep-Pak C-18 cartridge which contained solvent free tributylborane (1 mmol). After the trapping of [^{15}O]O_2 (0.2 mmol), another Sep-Pak C-18 cartridge was connected at the outlet. Then 1.5 ml of water was passed through the two columns to hydrolyze the tributylborane-O_2 complex. At this step, the radioactive impurity was eluted, and the unreacted tributylborane was retained in the cartridges. Subsequently, the strong anion exchange resin

column (OH^- type, 0.2 ml) was connected under the two Sep-Pak cartridges, and [^{15}O]butanol fraction was eluted by additional 5 ml of water through the three columns. At this step the nonradioactive impurity was adsorbed to the resion column. The eluate was collected and passed through a sterile 0.22 µm filter into a sterile syringe. By our method, [^{15}O]butanol was obtained in a radiochemical yield of 43 % within three minutes. The radiochemical purity was 93 %, and other chemical contaminants were undetectable.

As a conclusion, our [^{15}O]butanol synthetic method was suitable for clinical use, and was easily applicable automatic synthetic system. Furthermore, the gas trapping method using the micro column we used here was applicable to other RI gas (e.g. $^{11}CO_2$, $H^{11}CN$) for the purpose of high radiochemical yield.

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ENZYMATIC SYNTHESIS OF ^{13}N-LABELED γ-AMINO-BUTYRIC ACID

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In the field of positron emission tomography (PET), a continuous demand exists for new tracer molecules designed to fullfill the needs of applied and fundamental medical research. γ-Aminobutyric acid (gaba) is an important neurotransmitter which has an inhibitory effect on the postsynaptic membrane. The existence of gaba receptors is well known, as well as their role in the mechanism of action of the benzodiazepines. Recently, the synthesis of ^{11}C-labeled benzodiazepines has been reported (1). By labeling the physiological neurotransmitter gaba itself, we offer new possibilities for investigations in vivo on this complex neuroreceptor system.

[^{13}N]-Gaba can easily be obtained from cyclotron-produced [^{13}N]-ammonia by two subsequent enzymatic reactions : (i) the production of [^{13}N]-L-glutamate by incorporation of [^{13}N]-NH_3 into α-oxoglutaric acid, by means of glutamate dehydrogenase (L-Glutamate : $NAD(P)^+$oxidoreductase (deaminating) E.C. 1.4.1.3) and (ii) the decarboxylation of [^{13}N]-L-glutamate by glutamate decarboxylase (L-Glutamate 1-carboxy-lyase E.C. 4.1.1.15).

The enzyme glutamate dehydrogenase is immobilized on γ-aminopropylsilane-coated controlled pore silica beads (Daltosil 500) by N-hydroxysuccinimide ester coupling. Glutamic acid decarboxylase is immobilized on γ-aminopropylsilane-coated controlled pore silica beads by activation with glutaraldehyde. A detailed study on the immobilization of glutamate dehydrogenase was performed earlier (2). For glutamic acid decarboxylase, a screening of immobilization techniques was done, which will be published elsewhere (3).

The enzymes glutamate dehydrogenase and glutamic acid decarboxylase have different pH optima (7.6 and 5.0 respectively) and therefore, have to be packed into separate columns, which are each optimized to gain the highest possible yield of [^{13}N]-gaba (column dimensions, enzyme loading, flow rate).

The set up for the production of [^{13}N]-gaba is shown in Fig. 1. After 10 minutes irradiation of water with 18 MeV protons at an intensity of 20 μA, the water is pumped to a hot cell where it reacts with Devarda's alloy and sodium hydroxide pellets (4). The $^{13}NH_3$ is distilled and trapped in 0.5 ml ice-cold water containing 60 μmoles of HCl (Fig. 1, A1). Substrates and cofactors are added in sodium phosphate buffer of pH 7.6. Via a 1 ml loop, and by activating pumps P3 and P1 in sequence, the substrate mixture is pumped over column C1 containing the immobilized glutamate dehydrogenase. The unreacted $^{13}NH_3$ is retained immediately on a cationexchanger in column C2. About 4.5 ml eluens from C2 containing the [^{13}N]-L-glutamate is collected into A2. The collection is controlled by a Geiger-Müller tube (GM1). 0.5 ml of 1 M acetic acid, containing 1 mM pyridoxalphosphate is added to A2 to decrease the pH of this mixture to 5.0. By activating pumps P4 and P2 in sequence, the [^{13}N]-L-glutamate solution in 0.1 mM pyridoxalphosphate and 0.1 M sodium acetate buffer of pH 5.0 is sucked into a 5 ml loop and pumped over the immobilized glutamic acid decarboxylase in column C3. The anionexchanger in column C4 is equilibrated at pH 7.0 to retain the unreacted [^{13}N]-L-glutamate and to let the [^{13}N]-gaba elute in the first fraction. The elution of the latter is monitored by GM2. The [^{13}N]-gaba solution is pumped over a bacterial filter F and collected into a sterile flask. A total activity of about 40 mCi is obtained in a volume of 7-10 ml at EOB + 15 min.

The collected activity contains radiochemically pure [^{13}N]-γ-aminobutyric acid. This is demonstrated by HPLC radiochromatography on an aminopropyl column eluted with an acetonitrile-phosphate buffer mixture.

The carrier amount is about 0.45 μmole, determined enzymatically by the gabase reaction (5). The specific activity is thus calculated to be $\pm$ 200 mCi/μmole at EOB.

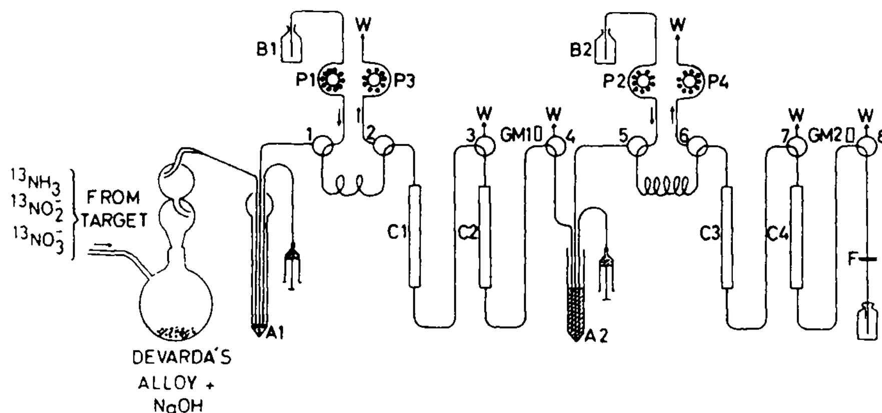

Fig. 1. Set up of the [^{13}N]-gaba production.

A1-2 : collection vials for [^{13}N]-ammonia and [^{13}N]-L-glutamate, respectively.
B1 : buffer for [^{13}N]-L-glutamate production ; 75 mM sodiumphosphate buffer of pH 7.6.
B2 : buffer for [^{13}N]-gaba production ; 100 mM sodium acetate buffer of pH 5.0.
C1 : column 0.6 x 10 cm ; immobilized glutamate dehydrogenase.
C2 : column 0.6 x 8 cm ; cation exchange resin AG50WX8.
C3 : column 0.6 x 8 cm ; immobilized glutamic acid decarboxylase.
C4 : column 0.8 x 12 cm ; anion exchange resin, AG1X8.
F : bacterial filter 0.22 µm pore size.
GM1-2 : Geiger-Müller tubes.
P1-4 : peristaltic pumps.
W : Waste
1-8 : Hamilton Mininert® valves.

The radiopharmaceutical quality of the [^{13}N]-gaba solution is guaranteed by sterility and pyrogen tests, and by checking the pH and osmolality, which are close to physiological values without any adjustment, so that the [^{13}N]-gaba solution is immediately suitable for human intravenous administration.

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PREPARATION OF ^{13}N-AMINES BY REDUCTION OF ^{13}N-AMIDES

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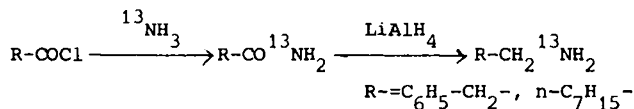
Since amines play very important roles in organisms as drugs, neurotransmitters, and neuromodulators, etc., synthesizing ^{13}N-amines is of great significance for visualization of their biodisposition in live animals or human. Especially, the combination use of ^{11}C- and ^{13}N-amines is thought to contribute greatly to clarify their metabolism.

We have already reported the preparation of ^{13}N-amines by Hofmann rearrngement of ^{13}N-amides(1). This labelling method was far better than the method reported before(2) in the respects of the radiochemical yield and the specific activity. However, there remained some room for improvement in the labelling yield in a non-carrier added state. We recently developed an organic solution of ^{13}N-ammonia as a labelling precursor. Taking advantages of this precursor, we investigated the ^{13}N-labelling of amines by $LiAlH_4$ reduction of ^{13}N-amides as follows;



$$R\text{-COCl} \xrightarrow{^{13}NH_3} R\text{-CO}^{13}NH_2 \xrightarrow{LiAlH_4} R\text{-CH}_2{}^{13}NH_2$$

$R\text{-}=C_6H_5\text{-}CH_2\text{-},\ n\text{-}C_7H_{15}\text{-}$

^{13}N-ammonia was distilled in a stream of nitrogen and introduced into a reaction vessel containing diethyl ether solution of the acid chloride (phenylacetyl chloride or n-octanoyl chloride) and powder Na_2CO_3. The mixture was stirred vigorously for 1 min to complete ammonolysis. The radiochemical yield of this reaction determined by thin-layer chromatography (TLC, silica gel, acetone) was almost 100%. Then the filtrate of the mixture was poured into the reduction vessel containing diethyl ether refluxing over $LiAlH_4$. This mixture was allowed to reflux for 5-20 min. The radiochemical yield of the ^{13}N-amine was determined by TLC(silica gel, chloform:methanol (2:1)) and summarized in Table 1. ^{13}N-amines were synthesized in a fair yield even in a non-carrier added state. After the reduction, excess $LiAlH_4$ was destroyed by aq.NaOH and the product ^{13}N-amine was isolated by extraction. Typically, from 20-30 mCi of $^{13}NH_3$ distilled into ether, 1-2 mCi of the product was obtained. The radiochemical purity of the product was more than 90%(TLC). Total preparation time was 25-30 min.

Table 1 Labelling Efficiencies of ^{13}N-Amines

carrier (μmol)	reduction time (min)	yield(%) ^{13}N-PEA	yield(%) ^{13}N-OA
0	5	52	59
0	10	62	75
0	15	65	72
0	20	67	76
5	10	56	71
5	20	63	77
10	10	63	66
10	20	75	75

PEA: 2-phenylethylamine, OA: n-octylamine
Reaction Conditions; acid chloride 30μl, Na_2CO_3 excess, $LiAlH_4$ excess, diethyl ether 5-10ml

Table 2 Organ Distribution of ^{13}N-PEA in Mice (male C3H)

(%dose/g)

	Time after i.v. (min)			
Organ	1	5	15	30
Blood	2.95±0.11	1.83±0.12	1.43±0.16	1.35±0.13
Brain	3.78±0.32	3.92±0.33	3.86±0.57	4.01±0.10
Heart	12.75±1.62	12.74±1.42	11.54±1.74	9.26±0.46
Lung	11.23±0.85	6.16±0.57	3.38±0.69	2.70±0.51
Liver	2.88±0.45	5.90±0.30	6.48±0.39	5.24±0.37

average±1 st. S.D.

Table 3 Organ Distribution of ^{13}N-OA in Mice (male C3H)

(%dose/g)

	Time after i.v. (min)			
Organ	1	5	15	30
Blood	2.46±0.21	1.59±0.17	1.26±0.13	1.10±0.25
Brain	7.49±1.66	7.05±1.40	6.91±1.62	5.97±1.34
Heart	19.42±4.34	14.74±1.04	14.94±2.90	13.73±4.95
Lung	28.56±4.87	12.36±2.04	6.63±1.25	2.91±0.17
Liver	4.72±0.91	4.47±0.56	6.02±1.25	3.02±0.78

average±1 st. S.D.

The organ distribution study of ^{13}N-amines (prepared in a non-carrier added state) was also performed (Table 2,3). The results suggest the metabolic trapping of the radioactivity in the brain and the heart as described in the previous paper(1); briefly, ^{13}N-amine is oxidized by monoamine oxidase (MAO) into $^{13}NH_3$, which is converted into ^{13}N-glutamine and other ^{13}N-amino acids and trapped in the tissue.

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NEW TECHNETIUM RADIOPHARMACEUTICALS: BORONIC ACID ADDUCTS OF VICINAL DIOXIME COMPLEXES

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A new class of technetium(III) compounds has been prepared by template synthesis from vicinal dioximes. These are seven coordinate complexes which are capped at one end with a boronic acid and have an axial ligand which may also be varied, giving a large number of compounds which are stable, neutral and lipid soluble. Biodistribution studies of one of the complexes, Technetium-(chloro[tris((1,2cyclohexanedione-dioximato)(1-)-0,0')-methylborato(1-)N,N',N'',N''',N'''',N'''''], ^{99m}Tc-SQ 30217, indicate that it is a promising myocardial imaging agent.

As with the similar iron dioxime-boron clathrochelates described in the literature (1-4), the preparations of the technetium complexes are simple. NH_4TcO_4, reduced with $SnCl_2$, as well as Tc(IV) and Tc(V) compounds can be used as starting materials. In one instance SQ 30217 was synthesized from stoichiometric amounts of the reagents by combining CDO and $CH_3B(OH)_2$ in 95% EtOH and adding pertechnetate in 3N HCl. Addition of a solution of $SnCl_2$ in 6N HCl immediately gave a solution of the red-orange product which was precipitated by adding H_2O. Dissolution in acetonitrile, centrifugation to remove traces of insoluble residue and reprecipitation with EtOH and 1N HCl gave the final product.

After recrystallization from hot EtOH a number of techniques including mass spectrometry, IR, conductivity measurements, x-ray photoelectron spectroscopy and x-ray crystallography of the bromo analog were used to identify and characterize the complex shown in Figure 1. HPLC and paper chromatography were used to show that the ^{99m}Tc and ^{99}Tc complexes are the same.

Conductivity measurements were made on 10^{-3}M solutions in acetonitrile. A value of 1.1-2.3 $ohm^{-1}cm^2mol^{-1}$ was obtained for SQ 30217, indicating that the complex is neutral.

The positive and negative FAB spectra showed both $(M+H)^+$ $583^+/585^+$ (Cl isotope) and $(M^{+\cdot})$ $582^+/584^+$ (Cl isotope), $(M+H)^+$-HCl 547^+, and $(M-H)^-$ $581^-/583^-$ Cl isotope. The bromo analog gave the same 547^+ $(M+H^+$-HBr) as well as both the protonated and molecular parent ions. After exposure to ~ 1N DCl in D_2O, FAB showed the presence of only two exchangeable protons (rather than three). Results from desorption chemical ionization (DCI), thermospray (TSP) ionization and field desorption (FD) mass spectra support the FAB data.

The IR spectrum (KBr pellet) was similar to that reported (5,6) for other iron dioxime-boronic acid complexes with dominant bands at 1550-1590 cm^{-1} (C=N stretch) 1220 and 1070 cm^{-1} (N-O stretch), 1200 and 815 cm^{-1} (B-O stretch). A band at 1735 cm^{-1} is assigned to O-H····O in agreement with previous data on similar cobalt DMG complexes (7).

With x-ray photoelectron spectroscopy the range of Cl 2p3/2 binding energies in Tc compounds is about 195.7 (ionic Cl) to 198.2 (coordinated Cl). The value for SQ 30217, 197.1, is between the coordinated and anionic Cl implying a partial charge separation in the Tc-Cl bond (8). This charge separation is reflected by the ease of changing the axial ligand.

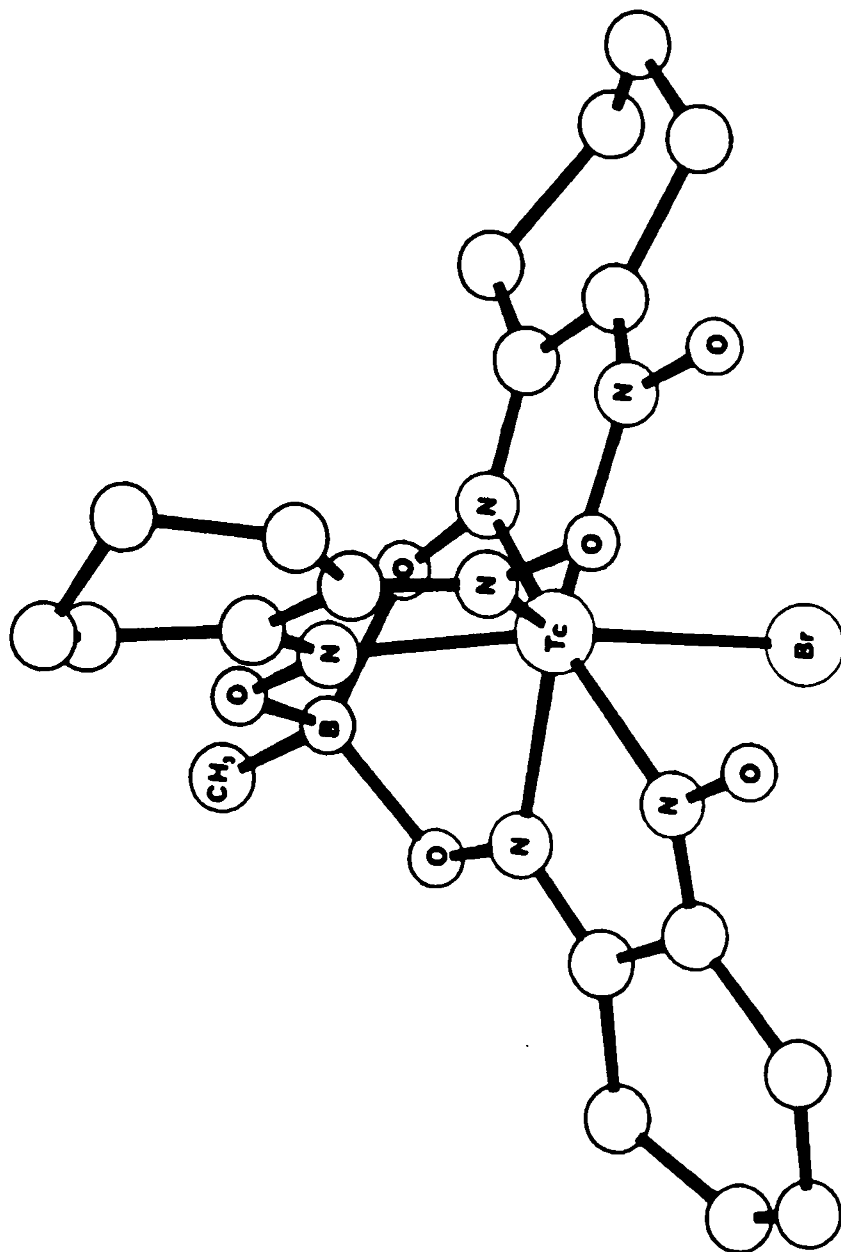

Figure 1. SQ 30216, TcBr(cyclohexanedione dioxime)$_3$(BCH_3)

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MASS SPECTRAL ANALYSIS OF CATIONIC AND NEUTRAL TECHNETIUM COMPLEXES

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Cationic and neutral technetium compounds have been characterized by mass spectrometry using a variety of ionization methods. These compounds include octahedral cationic complexes containing phosphorous and arsenic ligands such as DIPHOS and DIARS (1) and neutral complexes containing PnAO (2) and dimethylglyoxime, DMG, or cyclohexanedione dioxime, CDO, ligands. Boronate esters incorporating methyl and butyl derivatives of the DMG and CDO dioximes represent a new class of seven-coordinate Tc radiopharmaceuticals whose characterization by mass spectrometry has not previously been described. These complexes show promise as myocardial imaging agents (3).

Previous work has shown the utility of fast atom bombardment (FAB) and field desorption (FD) mass spectrometry to the characterization of charged technetium complexes (4,5). Field desorption spectra of cationic Tc complexes exhibit the intact cation and may be obtained from either activated or unactivated emitters, serving to distinguish cationic from neutral complexes as neutral species yield ions only from activated field emitters (6). FAB mass spectra of cationic complexes yield intense ions which are readily characterized using high resolution and MS/MS analysis (7).

Thermospray (TSP) ionization (8) has shown limited success in generating representative mass spectra of cationic technetium compounds due to extensive destabilization of the complex upon oxidation of the chelate. Thermospray and, in some cases, desorption chemical ionization (DCI) may be applied to neutral Tc compounds including TcOPnAO and the dioxime-containing complexes as their monoboronate esters. Figure 1 illustrates the DCI MS/MS spectrum of the protonated molecular ion of TcOPnAO. FAB and FD

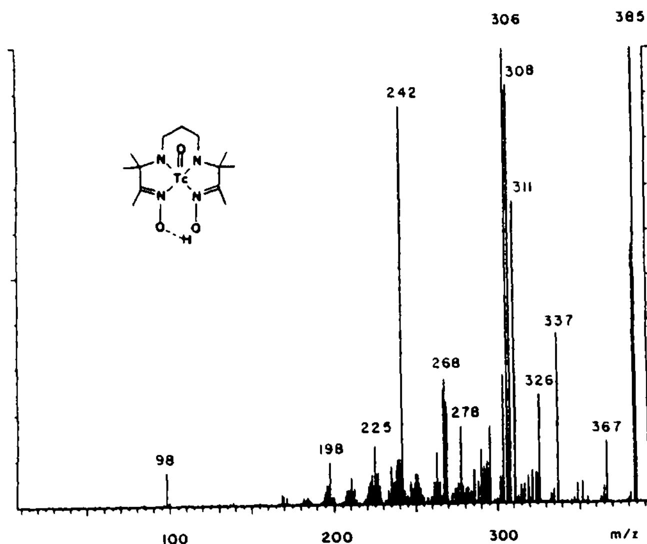

Figure 1. (Ammonia) DCI MS/MS Spectrum of the Protonated Molecular Ion of TcOPnAO.

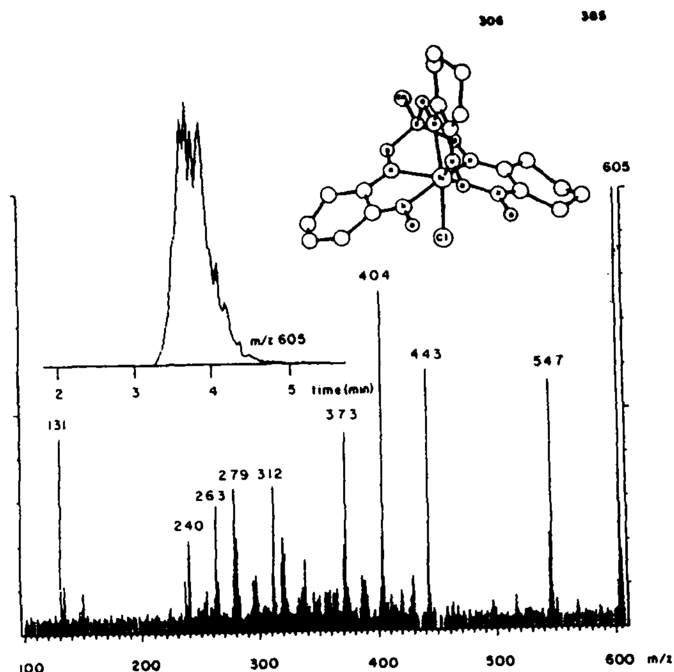

Figure 2. Thermospray MS/MS Spectrum of the $(M+Na)^{+}$ Ion of $TcCl(CDO)_3(B\text{-}me)$ and its Reconstructed Ion Profile.

Table 1 *

Positive Ions in Mass Spectra of Seven-Coordinate Tc(III) Compounds

Compound	FD-MS	FAB-MS	TSP-MS	DCI-MS
A	590/592(100) 295/296(22)	590/592(19) 591/593(23) 511(100)	591/593(100) 511(23)	591/593(100) 511(32)
B	546/548(100)	546/548(15) 547/549(13) 511(100)	547/549(100) 511(33)	547/549(100) 511(54)
C	626/628(100)	626/628(22) 627/629(28) 547(100)	649/651(100)Na 547(22)	627/629(100) 547(89)
D	582/584(100)	582/584(24) 583/585(18) 547(100)	583/585(100) 547(21)	583/585(100) 547(44)
E	668/670(100)	668/670(23) 669/671(26) 589(100)	691/693(100)Na 589(89)	669/671(100) 589(55)

A = $TcBr(DMG)_3(B\text{-}bu)$ B = $TcCl(DMG)_3(B\text{-}bu)$ C = $TcBr(CDO)_3(B\text{-}me)$
D = $TcCl(CDO)_3(B\text{-}me)$ E = $TcBr(CDO)_3(B\text{-}bu)$

* For Br and Cl containing compounds, intensity is given as the first isotope only.

mass spectrometry are also amenable to the analysis of these complexes but detection sensitivity and speed of analysis favors either DCI or TSP ionization. Thermospray ionization offers the advantage of on-line LC/MS and LC/MS/MS analysis as demonstrated for the sodiated molecular ion of $TcCl(CDO)_3(B\text{-}me)$ in Figure 2.

In general, these methods produce mass spectra containing either or both the molecular and protonated molecular ions, a fragment corresponding to the loss of HX (where X=axial ligand), and the deprotonated molecular ion. Table I compares the mass spectral results for these four ionization techniques from a series of related seven-coordinate technetium compounds.

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SYNTHESIS AND CHARACTERIZATION OF Re AND Tc COMPLEXES OF N2S2 AND N3S LIGANDS.

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The ^{99m}Tc complexes of diamide dimercaptide chelating agents have been of interest as potential renal function agents for a number of years (1). Improvement on the parent ligand ^{99m}Tc-1,2-dimercaptoacetamidoethane (^{99m}Tc-DADS) (2,3), resulted from addition of a carboxylate group to the ethylene bridge of the center chelate ring, yielding ^{99m}Tc-2,3-dimercaptoacetamidopropanoate (^{99m}Tc-CO_2DADS) (4). In fact, two complexes resulting from the carboxylate lying on the same side of the chelate ring as Tc=O group (syn isomer) or on the opposite side (anti isomer) were formed and separable by reversed phase HPLC. Differences in renal handling were apparent in mice and rabbits (5), but dramatic in humans; 58% of the dose was excreted in the urine at 30 minutes for the early HPLC component (called isomer A) and 19% for the latter (isomer B) (6). Since renal handling was sensitive to the stereochemistry of groups added to the chelate rings, it has been of interest to determine the structures of the carboxylate chelate ring epimers. The rhenium complex was initially prepared as a model since many examples show nearly identical structural chemistry. The oxo[2,3-dimercaptoacetamidopropanoate] rhenate (V) complexes (Re-CO_2DADS), as a mixture of epimers, were readily prepared by the exchange reaction of $ReO_2(en)_2Cl$ with the N_2S_2 ligand at pH = 8 to 11 (Fig. 1). Pure isomer B was obtained by preparative HPLC (Fig. 2) and isolated as orange crystals of $ReO(N_2S_2)$ Ph_4As. The complex was characterized by single crystal X-ray diffraction, IR, NMR, and UV/Vis spectroscopy. As can be seen from the ORTEP diagram (Fig. 3), the rhenium is bound to one oxygen atom and to the two sulphurs and nitrogen atoms of the ligand, forming a distorted square pyramid with the oxygen at the apex. The Re-O bond length is 1.669 (11) Å, almost identical to 1.674 (7) Å observed for $ReO(S_2C_2O_2)_2$ (7). The Re atom is ca. 0.758 Å above the square plane (N_2S_2), thus similar to other oxo Re (V) and Tc (V) complexes of square (pyramidal) geometry. The Re-S and Re-N bond lengths are in the same range as observed for a number of Re (V) complexes and for Tc-DADS (2). The carboxylate is not coordinated to the Re atom, and is on the same side of the chelate ring (N_2S_2) as the Re=O group, and therefore, isomer B is the syn isomer.

The oxo(mercaptoacetylglycylglycylglycine) rhenate (V) complex ($ReOMAG_3$) was also prepared by the exchange reaction of $ReO_2(en)_2Cl$ and MAG_3. The yield of the complex was, however, dependent on the pH and maximum yields were obtained between pH 8 and 10 at 90°C. The Re-(N_3S) reaction was slower compared to the Re-(N_2S_2) reactions. Since the carboxylate is attached to a planar nitrogen atom as an acetate, only the one expected product was observed. The complex was isolated as $ReO(MAG_3)X$, where X = Ph_4As^+, Bu_4N^+ or Ph_4P^+. The complex was characterized by elemental analysis, UV/Vis and IR spectroscopy. Single crystals of ReO (MAG_3) Bu_4N were of high quality for X-ray structure determination and work on the structure is in progress.

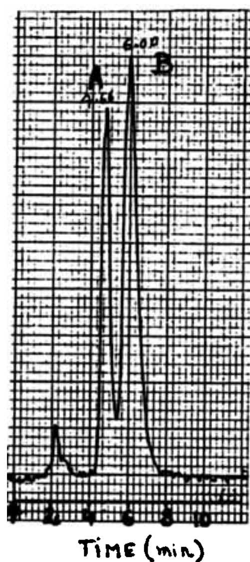

Figure 1.

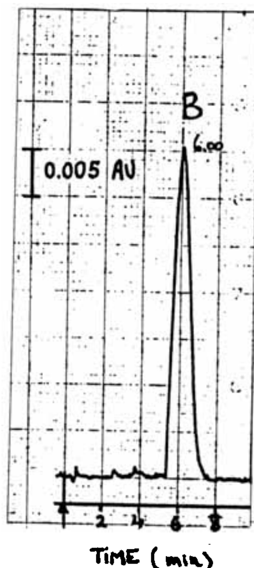

Figure 2.

Figure 1. HPLC chromatogram of crude Re-CO_2DADS preparation as showing the mixture of epimers A and B.
HPLC Conditions: Ultrasphere C_{18} 5 um, 2% CH_3CN/0.01 M phosphate buffer pH 7; 1.0 ml/min; UV detection at 254 mm; 0.05 AUFS.

Figure 2. HPLC chromatogram of purified Re-CO_2DADS isomer B after preparative HPLC.
HPLC Conditions: Same as Figure 1.

Figure 3. ORTEP representation of the Re-CO_2DADS isomer B anion with hydrogen atoms omitted for clarity.

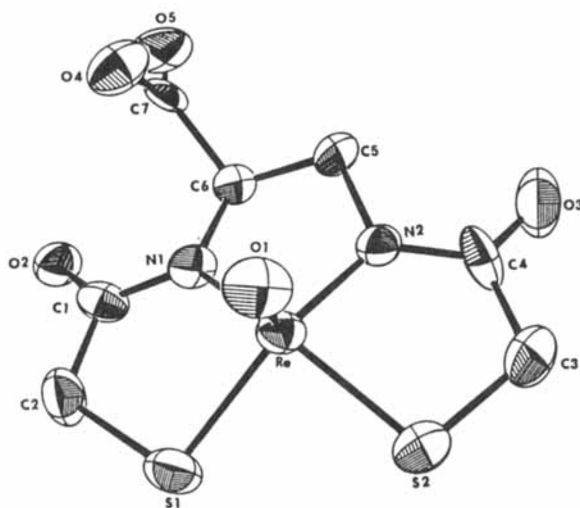

Figure 3.

The oxo[2,3-dimercaptoacetamidopropanoate] technetate (V) complexes ($Tc-CO_2DADS$) were prepared directly from pertechnetate via dithionite reduction in basic medium. The A and B isomers were separated by preparative HPLC and pure isomer A was isolated as yellow crystals of $TcO(N_2S_2)$ Ph_4As. The UV/Vis spectroscopic data of the complexes show that they are electronically similar to the Tc-DADS complex. Further characterization of isomer A is in progress.

The crystal structure of $Re-CO_2DADS$ confirms the structure indicated by mass spectrometry (4). In addition, it correlates structure with order of reversed phase HPLC elution and rate of renal tubular excretion.

$Re-CO_2DADS$ CHARACTERIZATION

1. Single crystal X-ray diffraction
2. IR spectroscopy (in KBr): ν Re-O = 980 cm^{-1}
3. UV/Vis spectroscopy (λ max, ε) in CH_3CN: 480 (40); 400 (200); 230 (13100)

$Re-MAG_3$ CHARACTERIZATION

1. IR spectroscopy (in KBr): ν Re-O : 975 cm^{-1}
2. Vis spectroscopy (λmax, ε) in CH_3CN: 480 (45); 402 (170)
3. Elemental analysis:

	C	H	N
Calc	45.50	3.78	4.96
F	45.61	3.71	4.92

4. Single crystal X-ray diffraction: in process

$Tc-CO_2DADS$ CHARACTERIZATION

1. UV/Vis spectroscopy (λ max, ε) in CH_3CN: 440 (sh); 366 (3500); 280 (sh)
2. Single crystal X-ray diffraction: in process

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CHEMISTRY OF A NOVEL Tc(III) THIOLATE COMPOUND: $Tc(SC_{10}H_{13})_3(CH_3CN)_2$

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The title compound is produced upon the reaction of the lithium salt of 2,3,5,6,-tetramethylbenzenethiol (TMBT) with $^{99}TcCl_6{}^{2-}$ in acetonitrile and has been characterized by IR, NMR, FDMS, and single crystal X-ray diffraction. It is a diamagnetic d^4 compound having trigonal bipyramidal geometry (see Fig. 1) and is conformationally rigid in solution at room temperature. The axial acetonitrile ligands are labile and are readily displaced by alkyl isocyanides, yielding the more robust series of compounds, $Tc(TMBT)_3(CNR)_2$ (R = i-Pr, cyclohexyl and t-Bu). Alternatively, $Tc(TMBT)_3(CH_3CN)_2$ can be oxidized by the addition of an oxo or nitrido group and one equivalent of TMBT to produce $TcO(TMBT)_4{}^{1-}$ and $TcN(TMBT)_4{}^{2-}$, respectively. If an oxo group is added in the absence of excess TMBT, a thiolate bridged dimer, $Tc_2O_2(TMBT)_6$ is isolated. The use of the title compound as a synthetic intermediate in technetium thiolate chemistry will be discussed.

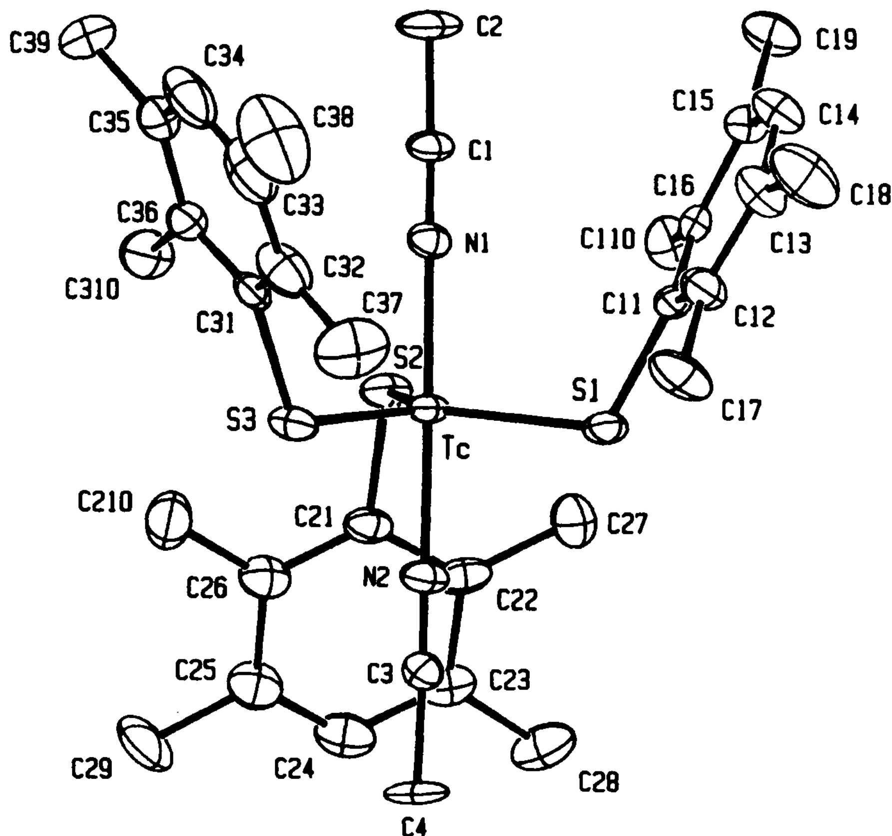

Figure 1.

TECHNETIUM DTS-BIFUNCTIONAL RADIOPHARMACEUTICALS : ROLE OF AMINO CONTAINING SIDE CHAIN ON BIODISTRIBUTION.

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In recent years, development of bifunctional radiopharmaceutical (BR), that is, a ligand containing a biologically interesting functional group and a technetium chelating site, the dithiosemicarbazone (DTS) spaced by a non charged molecule has attracted our interest. The ability of attached functional groups to display the provided specific chemical character has been already achieved (1, 2).

Since various amino derivatives have been recently reported as potential radiopharmaceuticals (3-5), attachment of amino functional group to the matrix of the BR was considered of interest as a mean to understand the chemical and biological significance of the designed DTS derivative. In the present paper, synthesis of various primary, tertiary an quaternary amino DTS derivatives of diverse basicity was carried out and the role conveyed by the amino containing side chain on the preferential biodistribution discussed. Synthesis of primary amino derivative, a p-PA-DTS (3-(p-aminoethyl)phenylpropane-2,3-dione-di(N-methylthiosemicarbazone), the tertiary amino derivatives, p-DPA-DTS (3-(p-N,N-dimethylaminoethyl) phenylpropane-2,3-dione-di(N-methylthiosemicarbazone), p-DPA-h-DTS (3-(p-N,N-dimethylaminoethyl) phenylheptane-2,3-dione-di(N-methyl thiosemicarbazone), p-DBA-DTS (3-(p-N,N-dimethylaminomethyl)phenyl propane-2,3-dione-di(N-methylthiosemicarbazone); finally a quaternary amino derivative, p-TPA-DTS (3-(p-N,N,N-trimethylaminoethyl) phenylpropane-2,3-dione-di(N-methylthiosemicarbazone). For preparation of those DTS-derivatives, β-phenylethylamine or its dimethylated intermediate were used as the starting material. Then through the acylation with propionyl chloride, the introduction of N-methyl-thiosemicarbazide group was carried via isonitrosoketone. As for p-DPA-h-DTS, acylation was performed with heptanoyl chloride. For p-DBA-DTS synthesis, benzylamine used as initial material was dimethylated before performing the described synthesis. In the quaternary amine derivative preparation, practically the method were those of dimethylation, acylation, isonitrosoketone preparation, followed by introduction of methyl iodide on the latter step. Every synthesized derivative was analyzed by elemental analysis, NMR, mass spectra and its composition assessed.

For the 99m-Tc labeling, synthesized derivatives were dissolved in 95% EtOH (10-3 M) and the reaction performed by the stannous chloride reduction as described (1). The Thin Layer Chromatography (TLC) in 10% ammonium acetate:ethanol (1:1), ElecroPhoresis (EP) (0.8 mA/cm) in cellulose acetate (0.06 M phosphate buffer, pH 7) and n-octanol Partition Coefficient (PC) in buffer phosphate were carried out. Biodistribution studies performed in ddy mice.

The various technetium labeled amino-DTS bifunctional radiopharmaceutical were tested in vitro as well as in vivo as for their chemical and biological functionality. Gathered data indicated very interesting conveyance of amino group functionality reflected in the mice biodistribution studies. Particularly, a pH dependent PC distribution offered good basis for considering the pH shift as a plausible amino relevant feature related to tissue localization; also, tissue having high content of MAO, like lung, myocardium appears as diplaying preferential distribution. Thus, the role conveyed by the various amino containing side chain in the biodistribution and the significance of the designed DTS derivatives as BR are discussed.

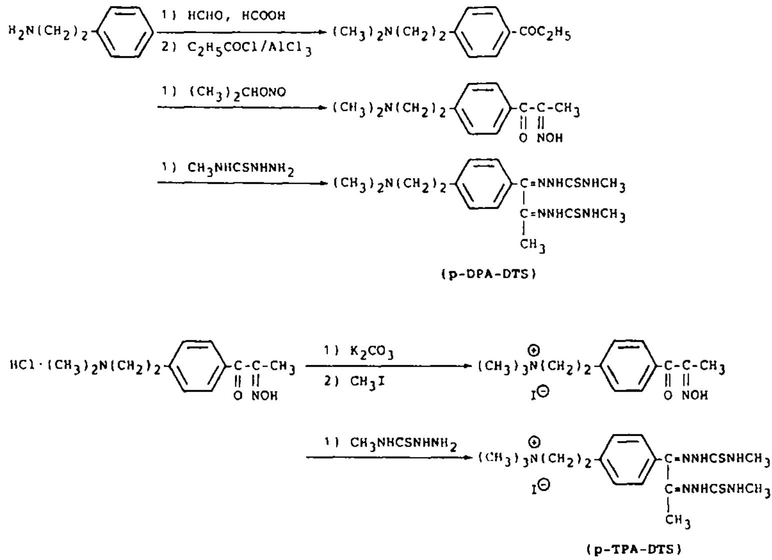
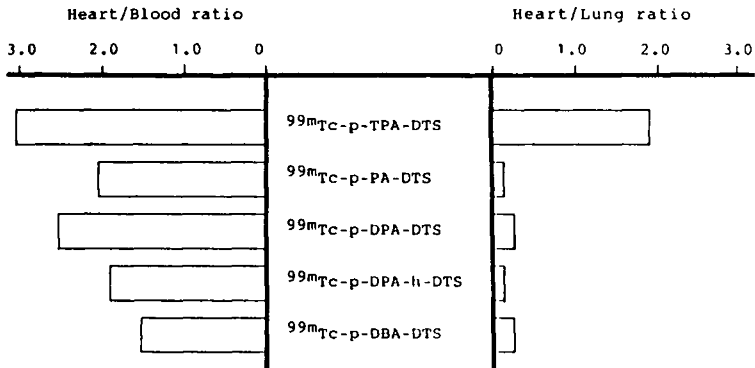


Fig.1. Scheme of amino-DTS derivatives synthesis.



* Calculated from mean % dose/g of Heart /mean % dose/g of Blood and Lung.

Fig.2. Target/non-target ratio of amino-DTS derivatives at 60 min.

Table 1. PARTITION COEFFICIENT OF ^{99m}Tc-AMINO DTS COMPOUNDS AT VARIOUS pH*

	pH			
	3.4	5.0	7.0	8.6
^{99m}Tc-p-PA-DTS	0.99±0.08	0.81±0.04	0.85±0.06	2.06±0.25
^{99m}Tc-p-DBA-DTS	0.57±0.08	0.88±0.06	0.95±0.08	4.09±0.85
^{99m}Tc-p-DPA-h-DTS	2.72±0.35	4.03±0.82	6.87±1.21	4.93±1.27
^{99m}Tc-p-DPA-DTS	0.77±0.03	0.81±0.04	0.96±0.05	4.50±1.03
^{99m}Tc-p-TPA-DTS	0.19±0.01	0.51±0.07	0.44±0.04	0.95±0.03

* Solvent extraction in octanol-buffer at various pH. Calculated from cpm/ml octanol / cpm/ml buffer. Mean ± 1 s.d. for triplicate experiments.

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TECHNETIUM METALLOTHIONEINS

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Metallothionein (MT) is a sulfur-rich, low MW protein (7000) that binds as many as twenty atoms of a wide variety of metals (1). Hence, MT offers unique potential as a bifunctional chelator (BFC) for radiolabeling biologically active molecules (BAM's)(2). In vivo, mamallian MT's typically sequester Class IB and IIB metals. However, MT's have been observed to bind other metals including the transition elements Co, Ni, and Pt. The association constants for MT depend upon the metal's affinity for thiolate ligands, i.e., Hg^{2+}, Ag^{+}, $Cu^{+}>Cd^{2+}>Zn^{2+}$. In general, metal ions in MT can be displaced by exchange with metals having higher stability constants. For radiolabeling, we have exploited the displacement of Zn^{2+} cations in Zn_7-MT by radionuclides of Cd, Hg, Cu, Ag, Au, and Tc. Since technetium(V) has a high affinity for sulfhydryl groups, the labile $TcO(glucoheptonate)_2$ complex (Tc-GH) was used to exchange the $^{99m}TcO^{3+}$ core for Zn^{2+}.

For MT to function as an effective BFC, radionuclide exchange must be efficient, i.e. >90% incorporation of the radionuclide in <1 hr, at concentrations appropriate for the particular BAM. Transchelation of $^{99m}TcO^{3+}$ from $^{99m}TcO(glucoheptonate)_2$ for Zn^{2+} in MT was evaluated as a function of MT concentration, pH, buffer, and reaction time. The percent of technetium incorporated in these exchange reactions was determined using ITLC-silica eluted with saline. Incorporation of >90% of the ^{99m}Tc presented was achieved <1 hr at a $[MT] = 7 \times 10^{-6}$ M in 0.01 M Na Phosphate (pH 6.5). Since this concentration of MT is well within the range of intrinsic solubility of most BAM's, e.g. 1 mg/mL= 6.7×10^{-6} M antibody, MT-BAM conjugates will prove to be excellent candidates for radiolabeling in the clinical setting.

To further understand the chemical nature of ^{99m}Tc-MT binding, the long-lived ^{99}Tc (β avg = 120 KeV, $t_{1/2} = 2.1 \times 10^5$ yr) was utilized to prepare macroscopic quantities of Tc-MT for conventional chemical and spectroscopic analysis. The ^{99}Tc-GH complex was made by the method of Kieviet (3). Various $^{99}Tc_x$,Zn-MT species were prepared by exchange labeling with ^{99}Tc-GH, in 0.01 M Na Phosphate (pH 7). These ^{99}Tc, Zn-MT species were analyzed for protein content by the Lowry method and for ^{99}Tc by LSC. The incorporation of Tc into Zn_7-MT is almost quantative at low ^{99}Tc-GH/MT ratios. As many as 7 Tc atoms could be incorporated into MT. The UV-visible spectra of $^{99}Tc_x$,Zn-MT species where x = 0.8 - 6.9 are shown in Figure 1. The absorbance at 405 nm has an extinction coefficient of 2500 M^{-1} cm^{-1} per Tc atom which is characteristic of a Tc-thiolate charge transfer transition. The Raman spectrum of $^{99}Tc_{6.9}$,Zn-MT (Figure 2) shows a band in the 940-960 cm^{-1} region indicating that the TcO^{3+} core is bound in square-base pyramidal geometry (4). Further evidence of technetium binding was obtained using the 2,2'-dithiodipyridine reagent at pH 4 to determine the number of thiol groups not ligated by $^{99}TcO^{3+}$. The data indicate that initially ^{99}Tc atoms bind with stoichiometry of $^{99}TcO(CYS)_4$. As more ^{99}Tc atoms (3-5) are bound, ligation of additional sulfhyryl groups is not observed suggesting that CYS residues are bridging TcO^{3+} cores as in $[(^{99}TcO)_2(SCH_2CH_2S)_3]$ (5). The chemical and spectroscopic data indicate that carrier-free ^{99m}Tc (1 Tc atom/mole MT) is bound to MT in a very stable configuration with the $^{99m}TcO^{3+}$ core in a square-base pyramidal environment having stoichiometry $^{99m}TcO(CYS)_4$.

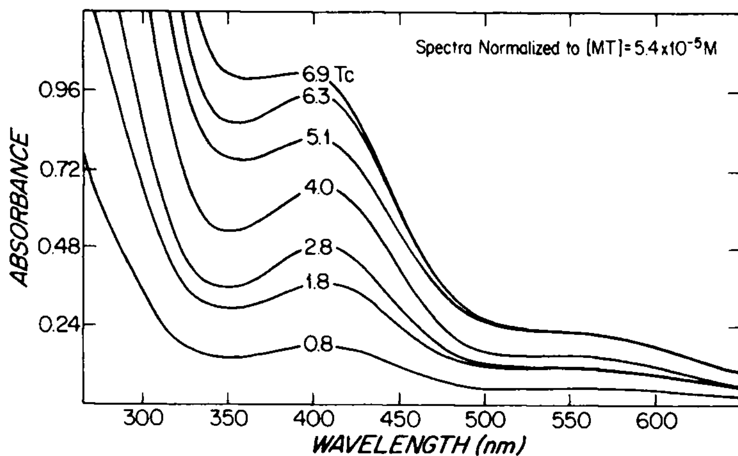

Figure 1. UV-Visible spectra of various ^{99}Tc,Zn-MT species containing 0.8 to 6.9 atoms to ^{99}Tc per molecule MT. The spectra are normalized to [MT] = 5.4 X 10^{-5} M.

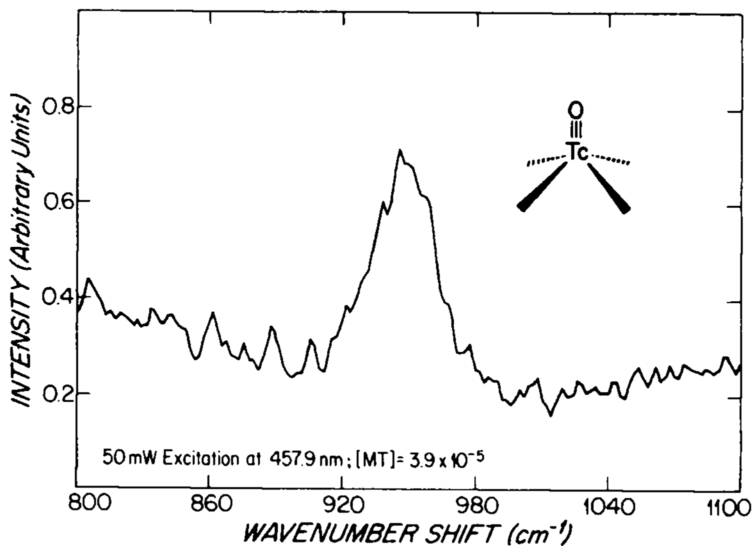

Figure 2. Laser Raman spectrum of the ^{99}Tc,Zn-MT species containing 6.9 g-atoms of ^{99}Tc. Excitation = 457.9 nm; [MT] = 3.9 X 10^{-5} M.

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PREPARATION AND EVALUATION OF ^{99m}Tc LABELLED SULFONAMIDES AS POTENTIAL RED CELL LABELING AGENTS

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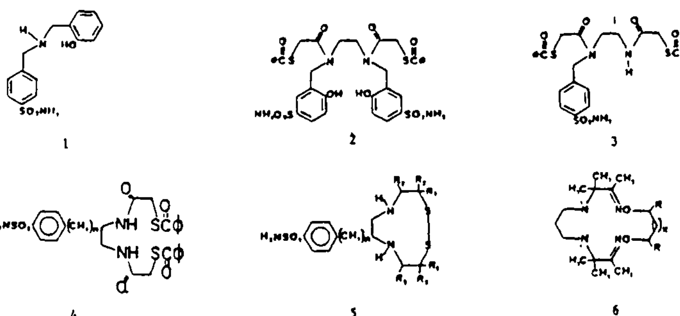
Maren and others[1] have shown that sulfonamide derivatives are taken up by erythrocytes via binding to their carbonic anhydrase enzyme system. This finding suggests the use of sulfonamide based ^{99m}Tc radiopharmaceuticals as erythrocyte labelling agents.

Accordingly we have synthesized and evaluated as erythrocyte labelling agents a number of ^{99m}Tc complexes in which the ligand bound to the technetium contains the sulfonamide moiety. These are shown below as compunds 1-6.

The synthetic routes to the ligands will be presented. One of the routes involves the use of a template procedure with which to synthesize the final complex. This "template" approach has been used by others to prepare a variety of transition metal complexes[2]. This approach using ^{99m}Tc has also been suggested by Deutsch[3].

The results of erythrocyte labelling studies carried out with these compounds will also be presented and discussed.

Ligands Synthesized



All ligands were characterized by their infrared spectra, melting points and elemental analysis.

The 99mTc complexes were formed by stannous ion reduction and were characterized by their octanol/buffer coefficients and by electrophoresis.

In vitro erythrocyte binding studies were carried out and, depending upon the complex, the binding was found to be 0 to 85% after two washings.

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SYNTHESIS OF Tc-99m(Sn)-N-PYRIDOXYLTRYPTOPHAN. Tc-99m(Sn)-N-PYRIDOXYL-PHENYLALANINE AND Tc-99m(Sn)-N-PYRIDOXYL-5-METHYLTRYPTOPHAN AND THEIR BIODISTRIBUTIONS IN RATS.

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Tc-99m(Sn)-N-Pyridoxyl(DL,L)tryptophan (PHT), -N-pyridoxylphenylalanine (PHP) and -N-pyridoxyl-5-methyltryptophan (PMT) have been synthesized and evaluated as the hepatobiliary imaging agents. Reaction of pyridoxal with the potassium salt of the amino acid gives the corresponding Schiff base. Reduction of the Schiff base with either sodium borohydride or H_2/Pd-C gives the final product in 65-85% yield.

The Tc-99m-labeled compounds (Tc-99m-PHT, Tc-99m-PHP and Tc-99m-PMT) were prepared by adding Tc-99m sodium pertechnetate into the vial containing the ligand, stannous chloride and vitamin C in greater than 96% yield. The radiochemical purity of the Tc-99m labeled products were checked by TLC with two solvent systems and showed to be greater than 96%. The labelling efficiency depends on the concentrations of the ligand, vitamin C and stannous chloride and also on the pH of the reaction medium.

The biodistributions of Tc-99m-PMT, Tc-99m-PHT and Tc-99m-EHIDA in rats showed that Tc-99m-PMT had the most rapid blood clearance, the fastest hepatobiliary transit and the lowest urinary excretion. In clinical studies, Tc-99m-PMT had more resistance to serum bilirubin than Tc-99m-EHIDA in patients and can be used as a hepatobiliary imaging agent.