NEW DERIVATIVES OF SCH-23390 AS CNS D-1 DOPAMINE RECEPTOR LIGANDS

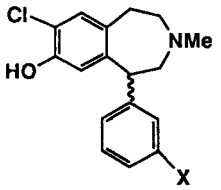
S. Chumpradit, M.P. Kung, H.F. Kung, University of Pennsylvania, Philadelphia, PA.

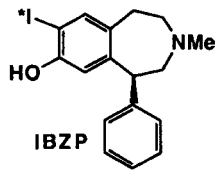
In developing CNS D-1 dopamine receptor SPECT imaging agents with improved specificity and longer brain retention, a series of new benzazepine derivatives, analogs of SCH-23390, were investigated. The synthesis of the TISCH, **1**, and aldehyde derivative, **2**, of this series of compounds was achieved by the schemes shown below (1) (Scheme 1). The amines, **3-5**, were prepared by condensation of the substituted amines and followed by the reduction of the imines (Scheme 2). The 3'-fluoro derivative, **6**, was prepared by a similar reaction scheme reaction starting with 3-fluorostyrene oxide.

To compare the affinity of the SCH-23390 derivatives to the D1 dopamine receptor, a competition binding study of the derivatives to the binding of a known D1 ligand, [^{125}I] IBZP, with rat striatum tissue preparation was performed. Structure-activity relationship study of this series of compounds suggests that substitution at 3'-phenyl group leads to compounds with highest affinity to the D-1 dopamine receptor (Table 1). Based on this data, it is reasonable to predict that there is a bulk tolerance of receptor active-site at this position and this tolerance offers an unique opportunity to investigate further on this molecule for development of new D1 dopamine receptor specific ligands.

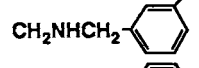
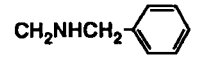
Reference: 1. Chumpradit S., Kung H.F., Billings J., Kung M.P., Pan S. J Med Chem 32:1431 (1989)

Table 1
Inhibition constant (Ki) of SCH-23390 derivatives on[^{125}I]IBZP binding to rat striatum membrane prepartion

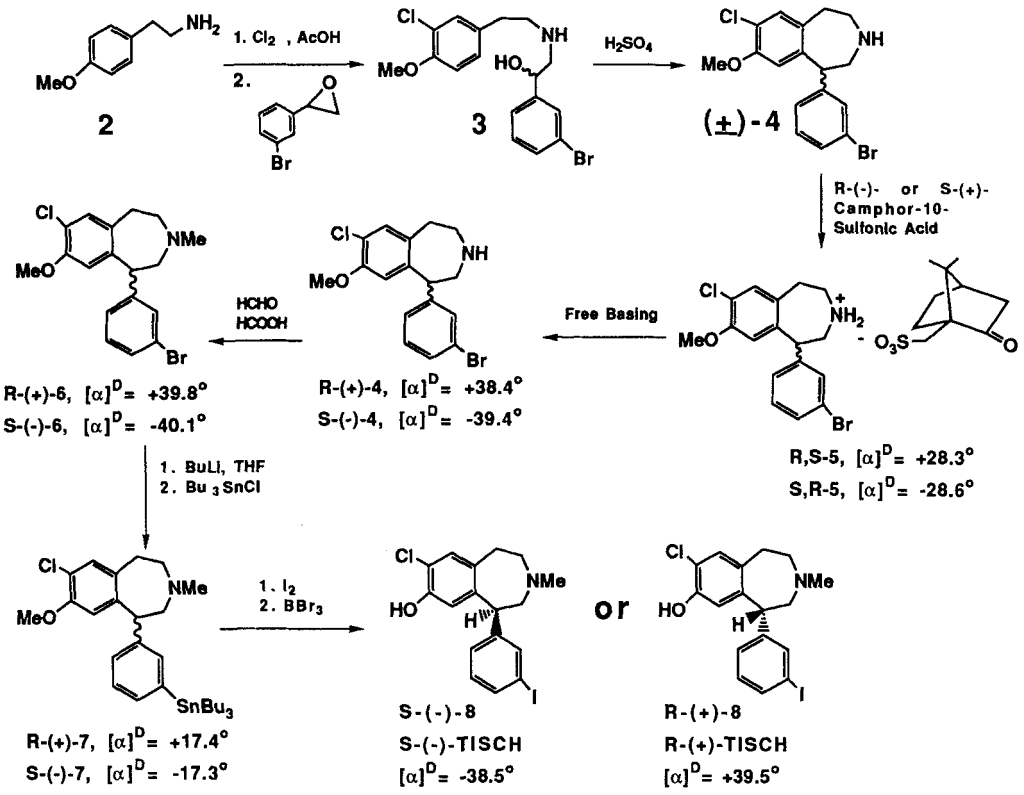




IBZP

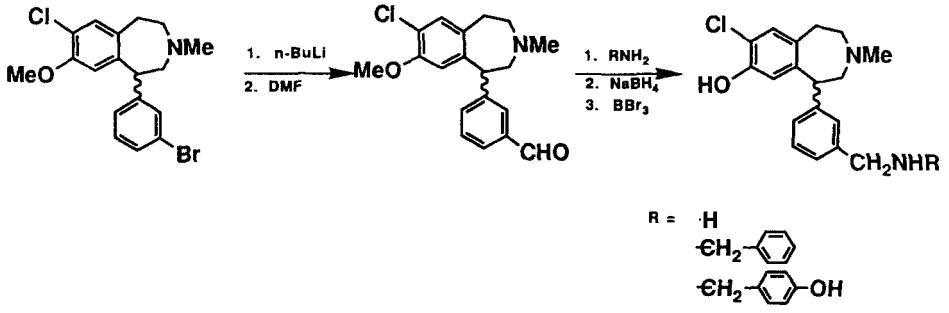
Compound	X	Ki (nM)
1	I	0.88±0.04
2	CHO	2.25±0.12
3		0.18±0.02
4		0.44±0.06
5	CH_2NH_2	1.51±0.19
6	F	0.34±0.09
SCH-23390	H	0.35±0.05

Scheme 1. Preparation of R-(+)- and S-(-)-8



Scheme 2

Synthesis of TISCH Analogs



SYNTHESIS OF [^{11}C]SCH 39166, A NEW SELECTIVE D-1 DOPAMINE RECEPTOR LIGAND FOR PET.

C. Halldin, L. Farde, A. Barnett and G. Sedvall. Department of Psychiatry and Psychology, Karolinska Institute, Stockholm, Sweden and Schering-Plough, Bloomfield, New Jersey, USA.

SCH 23390 is a potent D-1 dopamine receptor antagonist (1) which has been labelled with [^{11}C] and used as a radioligand for PET-analysis of central D-1 dopamine receptor binding in monkey and in man (2-4). However, [^{11}C]SCH 23990 has affinity also for $5HT_2$-receptors and is rapidly metabolised (80-90 %) during the time course of a PET-experiment (1 hour).

SCH 39166 ((-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo(d)naptho-(2,1-b)azepine) is a new potent and more selective D-1 dopamine receptor antagonist (5). The compound is considered for clinic trials as a potential antipsychotic drug. In animals SCH 39166 is more slowly metabolized than SCH 23990.

[^{11}C]SCH 39166 was labelled by N-alkylation of the free base of the secondary amine with [^{11}C]methyl iodide, as is shown in Scheme 1. Reaction in acetone with subsequent straight-phase semi-preparative HPLC purification resulted in 15-20 % radiochemical yield (from EOB and decay-corrected) with a total synthesis time of 35-40 min and a radiochemical purity >99 %. The specific activity obtained at EOS was about 1000 Ci/mmol.

[^{11}C]SCH 39166 was injected into a cynomolgus monkey. PET-analysis demonstrated accumulation in the caudate and putamen, areas known to have a high density of D-1 dopamine receptors. In a displacement experiment, radioactivity in the caudate and putamen was markedly reduced after injection of 6 mg unlabelled SCH 23390, thus demonstrating the specificity and reversibility of [^{11}C]SCH 39166 binding to D-1 dopamine receptors. [^{11}C]SCH 39166 should be a useful PET ligand for D-1 dopamine receptors in man.

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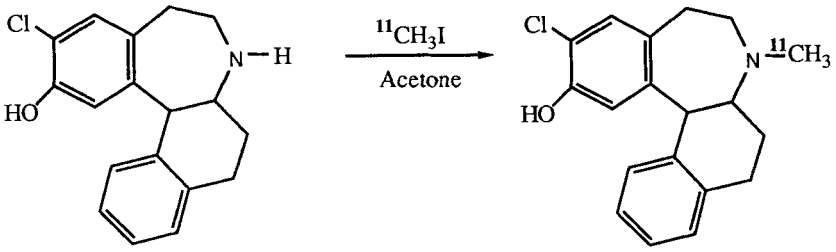

[^{11}C]SCH 39166

Scheme 1.

[C-11]-CARAZOLOL: A NEW LABELED BETA-ADRENERGIC LIGAND

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There has been an interest for many years in making measurements of beta-adrenergic receptors *in vivo* using labeled beta adrenergic antagonists. Several labeled ligands have been prepared in the past with less than optimal *in vivo* results. Among these are practolol, propranolol, and pindolol[1-3]. In each case either no specific binding was observable by PET, or the amount of binding which was observable was insufficient to allow application of a receptor model for quantitation. The results obtained with these ligands do allow one to postulate that a high affinity ligand, possessing a slow rate of dissociation from the receptor, may allow the use of compartmental modeling to measure receptor concentrations.

One such ligand is carazolol, which has a dissociation constant of 10 pM, and a dissociation half-time of at least one hour. The ligand is also relatively subtype non-specific, a feature which would allow greater experimental flexibility. We expect that it will prove to be a useful ligand for PET measurements of beta-adrenergic receptors.

We have labeled carazolol by the reaction of [C-11]-acetone with the free amine precursor[1-3] (4-(2-hydroxy-3-aminopropoxy)-carbazole), as shown. A 10-minute reaction at 80°C in acetonitrile/water solution in the presence of sodium cyanoborohydride and acetic acid gives a 30% chemical yield of carazolol from acetone. The product is purified by HPLC using an analytical silica column eluted with $CHCl_3$ containing 7% ethanol, 0.1%water, and 0.1% piperidine. The carazolol product is then dissolved in warm saline and filtered for injection. The procedure requires 45 min from EOB to produce injectable carazolol, at a specific activity on injection of 500 Ci/mmole. The precursor was in turn synthesized from readily available materials, as shown. The authors gratefully acknowledge the generous cooperation of Drs. Bartsch, Strein, and Leinert of Boehringer Mannheim GmbH and their gift of authentic samples of carazolol and several intermediate compounds.

Preliminary biodistribution experiments have been completed with the labeled carazolol. While data concerning specific binding is not available at this writing, tissue uptake in mice at 40 minutes after injection have shown uptake of 1-3% of the injected dose per gram in heart and brain, 20% in lung. Tissue to blood ratios for these tissues were 3-4 , 29 for lung. This is a sufficient degree of uptake for PET imaging, and it is clear that the label enters the brain. Additional biodistribution, metabolism and specific binding studies will be reported.

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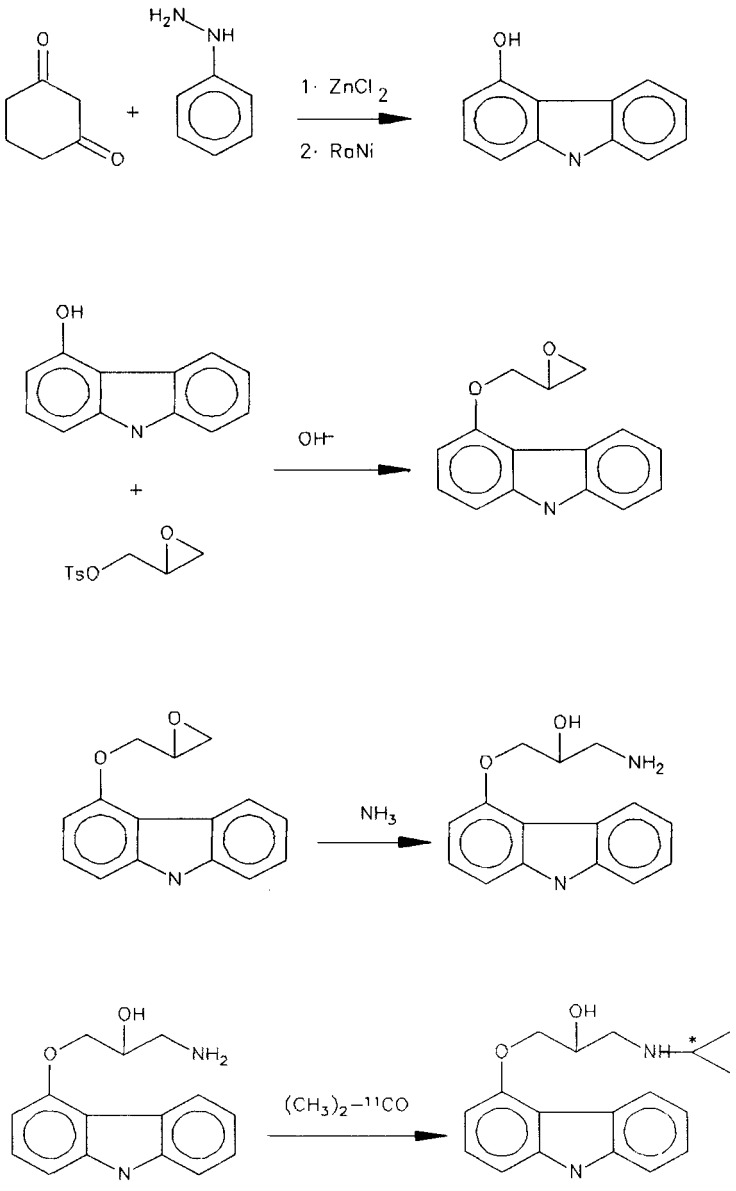
Synthesis and Labeling of ^{11}C-Carazolol



[^{18}F]N-METHYLSPIPERONE: DIRECT N.C.A. NUCLEOPHILIC [^{18}F]FLUORINATION OF N-METHYL-4-NITROSPIPERONE FOR REMOTE CONTROLLED ROUTINE PRODUCTION OF N.C.A.[^{18}F]MSP.

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Institut für Chemie 1 (Nuklearchemie), Forschungszentrum Jülich GmbH, Jülich, FRG

Among the labeled butyrophenone neuroleptics used as D_2-receptor ligands for PET methylspiperone (MSP) and N-fluoroethylspiperone (FESP) show highest uptake and specificity with respect to nonspecific binding (see [1] and refs. therein). Due to its relatively slow clearance fluorine-18 is the tracer of choice. Multi step reactions starting with the nucleophilic ^{18}F-labeling of small precursors of butyrophenones lead to useful radiochemical yields of [^{18}F]spiperones [2] but a direct nucleophilic aromatic substitution method would certainly be a simpler labeling procedure. First attempts, however, gave only small radiochemical yields [3]. Despite our previous report [4] it was presumed that nucleophilic substitution in nitrospiperone would not be possible in presence of Kryptofix 222 due to enolization of the educt [5]. In fact, although the ^{18}F-containing cryptate $[222/K]_2CO_3$ has been successfully applied to the preparation of various n.c.a. F-18 radiopharmaceuticals, it can not be used for the [^{18}F]labeling of N-methylspiperone, since the strong alkaline conditions lead to a rapid destruction of N-methyl-4-nitrospiperone.

To increase the stability of the educt in presence of a cryptate, the less basic potassium oxalate was used instead of the carbonate. Only a very low amount of K_2CO_3 (0.1 μmol) was added to the oxalate to prevent the loss of ^{18}F activity as $H^{18}F$ in the course of the drying process. The nucleophilic fluorination is conducted in DMSO at 140-150 °C in about 15 min. A two-step purification procedure by reversed phase chromatography using different types of columns yields n.c.a. [^{18}F]N-methylspiperone with a specific activity of > 10^3 Ci/mmol. The radiochemical yield is 20 $\pm$ 5% in a total time of 70 min.

This remotely controlled one-step [^{18}F]MSP synthesis can be carried out in the same apparatus as used for FDG-synthesis [6]. The single unit reactor is connected on-line to a device for HPLC purification.

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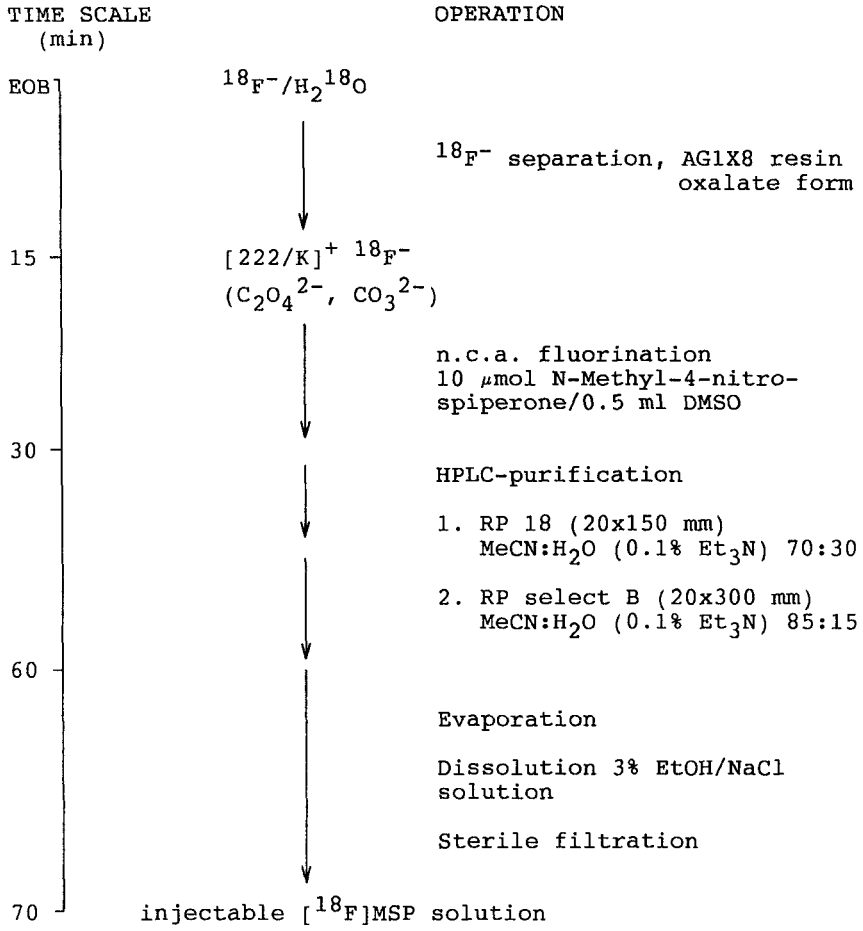

Flow scheme of direct nucleophilic [^{18}F]MSP synthesis

SYNTHESIS OF [^{18}F]NCQ 115, A NEW SELECTIVE REVERSIBLE D-2 DOPAMINE RECEPTOR LIGAND FOR PET.

C. Halldin, T. Högberg, S. Bengtsson, H. Hall and L. Farde. Department of Psychiatry and Psychology, Karolinska Institute, Stockholm and Astra Research Centre AB, CNS2, Södertälje, Sweden.

Several benzamides bind with high affinity and selectivity to central D-2 dopamine receptors, and have been labelled with [^{11}C] for the *in vivo* examination of receptor binding in man (1). To study the binding during a longer time than possible with [^{11}C]-labelled ligands, both raclopride and eticlopride have been labelled with [^{18}F] in the N-alkyl position using [^{18}F]fluoroethyl bromide and [^{18}F]fluoropropyl bromide. The finding of no marked specific uptake of these analogues in the monkey brain indicates that labelling with [^{18}F] on the N-ethyl or N-propyl of substituted benzamides strongly changes ligand affinity to D-2 dopamine receptors (2). NCQ 115 ((+)-(R)-5-bromo-N-((1-(4-fluorobenzyl)-2-pyrrolidinyl)-methyl)-2,3-dimethoxybenzamide) is a selective D-2 dopamine receptor antagonist. The compound inhibits potently the binding of [^{3}H]raclopride (Ki = 147 pM) and has a F in the benzyl group. It is therefore suggested as a potential [^{18}F]-labelled ligand for PET.

[^{18}F]Fluoride ion was produced from [^{18}O]water and reacted with the trifluoromethansulfonate salt of 4-(dimethylamino)-benzaldehyde in DMSO to produce the [^{18}F]-4-fluorobenz-aldehyde (70 % isolated yield, SEP-PAK). The aldehyde was quantitatively reduced to alcohol with LAH and converted to [^{18}F]-4-fluorobenzyl iodide (40-50 % isolated yield) by use of HI followed by an extraction procedure (3). The quantitative N-alkylation of the secondary pyrrolidine precursor (prepared in a 3-step synthesis) was performed in DMF followed by a semi-preparative HPLC. The total radiochemical yield (EOB and decay-corrected) was 15-20 % with a total synthesis time of 90 min and a radiochemical purity >99 % (Scheme 1).

For binding studies *in vitro* [^{3}H]-labelled NCQ 115 was also prepared. Binding with high affinity and high selectivity to D-2 dopamine receptors in both rat striatum and in human caudate was demonstrated. [^{18}F]NCQ 115 was used in PET visualization of the dopamine receptor rich areas of the monkey brain. The results shows a conspicuous uptake of radioactivity in the monkey striatum and a possible equilibrium after 60-90 min. [^{18}F]NCQ 115 should be a useful PET ligand for D-2 dopamine receptors in man.

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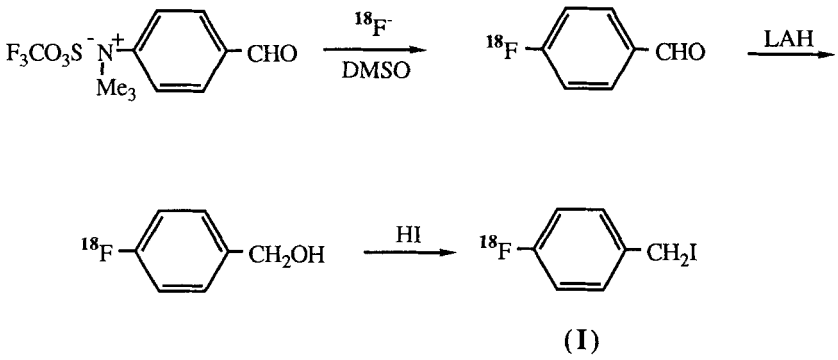

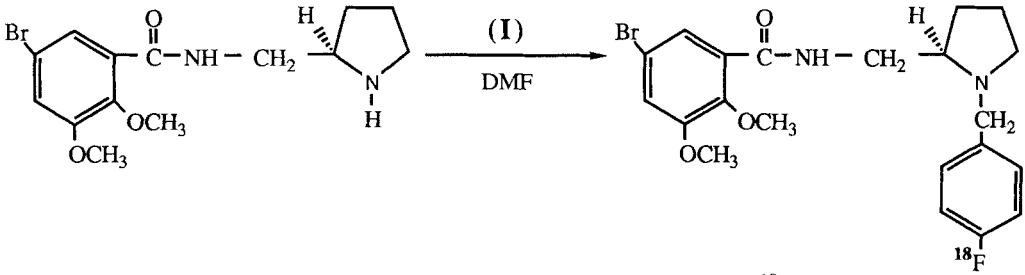

[18F]NCQ 115

Scheme 1.

SYNTHESIS OF ARYL-SUBSTITUTED 5-[^{18}F]FLUOROALKYLBENZAMIDES: HIGH AFFINITY LIGANDS FOR DOPAMINE D-2 STUDIES.

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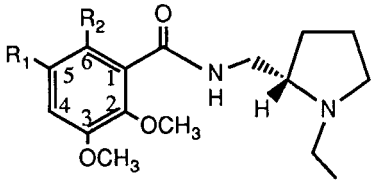
†University of North Carolina School of Medicine, Chapel Hill, NC 27599.

Benzamides, such as ^{11}C-raclopride, have the advantages of high selectivity for dopamine D-2 receptors and low non-specific binding compared to butyrophenones such as spiperone (1). An ^{18}F-labelled benzamide would be useful for PET studies because the longer half-life of the ^{18}F-radionuclide will permit extended kinetic evaluations and facile analysis of blood metabolites compared to a ^{11}C-labelled compound. Previously, investigators have synthesized fluorine containing benzamides using pyrrolidine substituted N-fluoroalkyl analogues of raclopride (2,3). However, these compounds display a much lower affinity for the D-2 receptor than raclopride.

Recent studies of the structure-activity relationship of benzamides have shown that the 2,3-dimethoxy substitution pattern of (S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxy-5-iodobenzamide (compound **1**) resulted in a potent D-2 antagonist (4). Based upon these results and the concept that the potency of receptor ligands can be preserved when aromatic halogen substituents are replaced by fluoroalkyl functional groups (5), we synthesized a series of aryl-substituted (S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxy-5-(fluoroalkyl)benzamides (compounds **2** and **3**) and salicylamides (compounds **4** and **5**) (6). The tetra- and penta-substituted aromatic nuclei of compounds **2**-**5** were constructed using Claisen rearrangements of the appropriate methylsalicylate allyl ethers. The relative binding affinities of compounds **2**-**5** for the dopamine D-2 receptor were determined by their ability to inhibit ^{3}H-spiperone binding in rat striatal membranes, and they displayed greater affinity for the D-2 receptor than raclopride (IC_{50} values ranged from 1 to 9 nM).



Compd	R_1	R_2
1	I	H
2	CH_2CH_2F	H
3	$CH_2CH_2CH_2F$	H
4	CH_2CH_2F	OH
5	$CH_2CH_2CH_2F$	OH
6	$CH_2CH_2CH_2OTs$	H
7	$CH_2CH_2CH_2OTs$	OMEM
8	$CH_2CH_2CH_2F$	OMEM

The radiolabelled forms of compounds **3** and **5** were synthesized with high specific activity (500-2000 Ci/mmole) by the routes shown in the scheme. Striatal:cerebellar activity ratios following injection of ^{18}**F-3** and ^{18}**F-5** in rats increased steadily to >100:1 at 5 h indicating the potential of these compounds as ligands for dopamine D-2 studies using positron emission tomography.

6 $\xrightarrow{a}$ ^{18}**F-3** (20% EOS Yield)

7 $\xrightarrow{a}$ ^{18}**F-8** $\xrightarrow{b}$ ^{18}**F-5** (7% EOS Yield)

a. $^{18}F^-$, K_2CO_3, Kryptofix [2.2.2], CH_3CN, 80°C, 30 min b. $TiCl_4$, 25°C, 10 min; H_2O

This work was supported by funding from the U.S. Department of Energy (DE-AC0376SF00098) and NIH (NS22899, HL07367 and HL25840).

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SUPPORTING DATA FOR --- SYNTHESIS OF ARYL-SUBSTITUTED 5-[^{18}F]FLUORO-ALKYLBENZAMIDES: HIGH AFFINITY LIGANDS FOR DOPAMINE D-2 STUDIES

(Authors: C. Mathis, J. Bishop, J. Gerdes, B. Faggin, R. Mailman)

The syntheses of the S-enantiomers of N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxy-5-[(3'-[^{18}F]fluoro)propyl]benzamide (termed **3**) and the 5-[(3'-[^{18}F]fluoro)propyl]salicylamide derivative (termed **5**) were accomplished in a manner exemplified below for compound **3**.

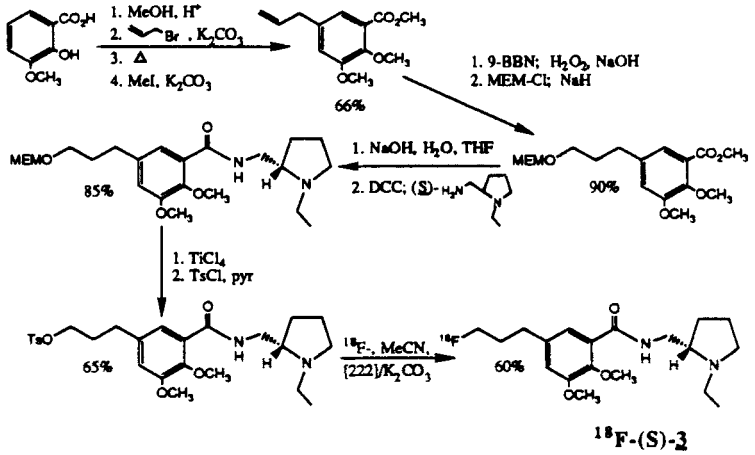

Fig. A. Synthesis of (S)-(N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxy-5-[(3'-(^{18}F)fluoro)propyl]benzamide (^{18}F-3).

Small quantities (10-30 μCi) of compounds (S)-**3** and (S)-**5** were injected into the tail veins of rats, and the animals were sacrificed at various times post injection (3-5 animals per time point per compound). The body organs, including the brains, of these animals were excised, counted in a gamma well-counter and weighed. The time variation of the ^{18}F activity concentrations in several brain regions of interest are plotted in Figs. B and C.

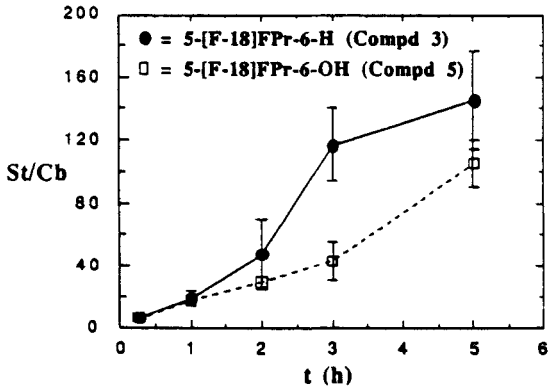

Fig. B. Striatal-to-cerebellar ratios of activity in rats following iv injection of [F-18]-**3** and [F-18]-**5** (3-5 rats per data point).

SUPPORTING DATA FOR --- SYNTHESIS OF ARYL-SUBSTITUTED 5-[^{18}F]FLUORO-ALKYLBENZAMIDES: HIGH AFFINITY LIGANDS FOR DOPAMINE D-2 STUDIES

(Authors: C. Mathis, J. Bishop, J. Gerdes, B. Faggin, R. Mailman)

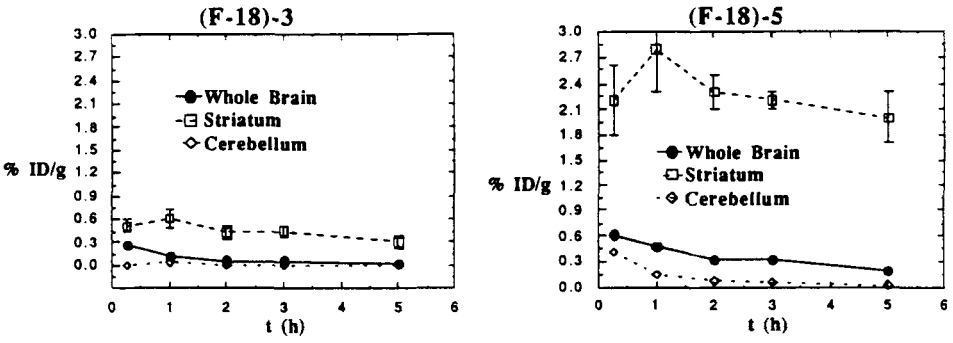

Fig. C. Brain uptake of activity following injection of compounds [F-18]-3 and [F-18]-5 in rats.

SYNTHESIS AND PRELIMINARY ANIMAL STUDIES OF [^{131}I]IODO-TROPAPRIDE : A CEREBRAL DOPAMINE D_2 RECEPTOR LIGAND.

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During the past several years, iodinated receptor ligands have been developed for the study of the neurotransmission system of the living human brain by single photon emission tomography (SPECT) (1,2,3). Recently, we have focused our interest on the development of a new iodinated radioligand : [^{131}I]iodotropapride. Tropapride 1, iodotropapride 2 and bromotropapride 3, are substituted benzamide antagonists which have shown high affinity and selectivity to dopamine D_2 receptors (4). [^{131}I]Iodotropapride was synthesized at the no-carrier-added (n.c.a.) level by nucleophilic substitution on the corresponding brominated analog.

The labeling was based on a nucleophilic exchange Br-I in the presence of Cu I and an excess of reducing agent (gentisinic acid - citric acid - tin sulfate) (5). The reaction was conducted in a sealed vial under N_2 atmosphere for 30 minutes at 160 °C. Free iodine was removed from the reaction mixture by the use of Sep Pak cartridges. [^{131}I]Iodotropapride was isolated from the unreacted bromoderivative by HPLC since the bromo-compound shows significant affinity for dopamine receptor sites. The chromatographic separation was carried out on a semi-preparative Bondapack C_{18} column with the following eluent : methanol / acetonitrile / water / triethylamine / acetic acid - 16/16/66/1/1. [^{131}I]Iodotropapride was obtained at a no-carrier-added level (no uv detection of the bromoderivative by HPLC) with an overall yield of 50%.

Preliminary animal studies were carried out to evaluate the binding properties of [^{131}I]iodotropapride as an vivo dopaminergic tracer.
The general biodistribution in rats indicated that 0.45% of the injected dose localized rapidly in the total brain and remained constant during the first two hours indicating a significant retention by brain tissue. High initial uptake was identified in the lungs, liver and kidneys one hour after injection. The thyroid uptake was low suggesting a good in vivo stability of the compound.
Regional brain biodistribution showed a very high uptake in the striatum. The activities in the frontal cortex and the cerebellum were relatively low demonstrating an in vivo dopaminergic specificity of the radioligand for D_2 receptor sites. The striatum/cerebellum ratio increased with time and reached values of 11 and 21 at 60 and 120 minutes respectively (Table 1). No plateau appeared even 4 hours after injection. Uptake in the striatum was saturable (Table 2) and could be blocked by pretreatment with known D_2 antagonists such as spiperone (Table 3).

Human studies will be performed using [^{123}I]iodotropapride and SPECT.

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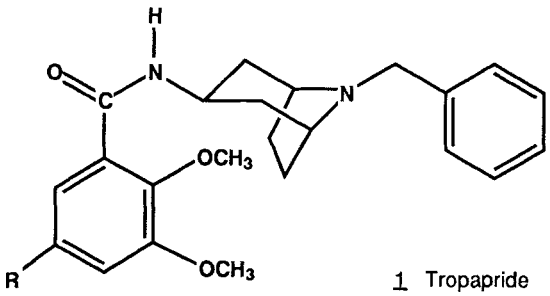

1 Tropapride R = H
2 Iodotropapride R = I
3 Bromotropapride R = Br

Table 1

Striatum / cerebellum ratio versus time

Time (min.)	Str/Cb ratio
5	2
30	9
60	11
120	21
240	35

Table 2

Saturability

Cold tropapride (ug)	Str/Cb ratio
0	16
0.5	16
1	16
5	16
10	11
25	7
50	4
250	1.4

Injected activity per rat : 15 uCi.

Table 3

Blocking experiment of the striatum uptake.

	Str/Cb ratio
Control	12.5
Spiperone	1.6
Ketanserine	12.5
Iodotropapride	1.4

Pretreatement with 1 mg/Kg of cold ligand 1h. before radiotracer injection.

SYNTHESIS AND EVALUATION OF (S)-5-[^{125}I]IODO-2,3-METHYLENEDIOXYBENZAMIDE.

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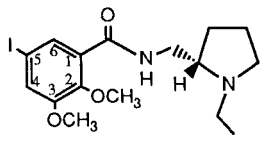
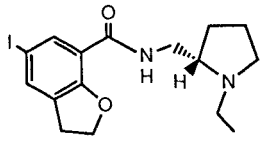
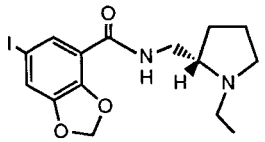
Recent reports have demonstrated that benzamides possessing 2,3-dimethoxy-5-iodo- and 2,3-dihydrobenzofuran-5-iodo-substitution patterns (termed epidepride and IBF, respectively) are potent dopamine D-2 antagonists (IC_{50}'s for ^{3}H-spiperone inhibition <1nM). These agents are potentially valuable as SPECT CNS imaging agents since they attain extremely high striatal:cerebellar ratios (>50:1 in rats) and show stable striatal uptake and retention for many hours post injection (1,2). A closely related benzamide ((S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-methylenedioxy-5-iodo-benzamide, termed IMD) was synthesized and its activity compared to that of epidepride and IBF.



Epidepride

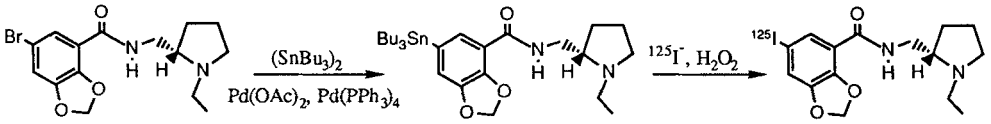


IBF



IMD

The syntheses of [^{125}I]IMD was accomplished as outlined in the scheme. High specific activity radioiodination (2200 Ci/mmole) of the tributyltin derivative (3) was achieved in 60% EOS yield using HPLC purification of the crude radioiodination reaction mixture.



The *in vitro* IC_{50} of IMD for the dopamine D-2 receptor as determined by its ability to inhibit the binding of ^{3}H-spiperone in rat striatal membranes was 40 nM. This value is 40 times greater than the IC_{50}'s of epidepride and IBF, but approximately equal to that of raclopride (32 nM). The *in vivo* uptake of [^{125}I]IMD in the rat striatum was 1.1% ID/g at 15 min, but fell to less than 0.05% ID/g at 3 h. Over this same time period, the striatal:cerebellar ratio was approximately constant at 5:1. The *in vivo* behavior of IMD is therefore quite different from that of epidepride and IBF, both of which display more constant striatal concentrations and increased ratios of striatum-to-cerebellum activities over similar time periods.

A possible explanation for the differences in the binding characteristics of these three structurally similar benzamides has been suggested for a series of analogous compounds (4). The steric configuration of the 2-position oxygen atom is apparently a critical parameter in determining the affinity of benzamides for the dopamine D-2 receptor. Thus, the 2,3-methylenedioxy ring system may constrain the 2-position oxygen atom in a relatively unfavorable conformation compared to those achieved with epidepride and IBF.

This work was supported by funding from the U.S. Department of Energy (DE-AC0376SF00098) and NIH (NS22899 and HL25840).

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"CLUSTERED LIPOPHILIC COMPENSATION" A NEW CONCEPT IN THE DEVELOPMENT OF LIPOPHILIC RADIO IODINATED AND BROMINATED RECEPTOR TRACERS.

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As in generally compounds showing in vivo receptor affinity are already lipophilic, introduction of a heavy radiohalogen can render the lipophilicity and resulting aspecific binding too high. Related to the development of 2-^{123}I-Ketanserin, a new radioligand for mapping serotonin S2 receptors with SPECT (1), we introduced Clustered Lipophilic Compensation (CLC) (2). This concept actually concerns compounds with a p-F-phenyl ketone group and is illustrated by means of Spiperone and Ketanserin analogues. The k' values of the compounds in RP-HPLC are used as their lipophilicity index.

Table 1 : n' and n respectively point to the phenyl ketone and the spiro-anilin group in SPIPERONE (k' at PH 4.9).

Analogue	k'	Analogue	k'
2'-H, 4-H, 4'-F	4.7	2'-H, 4-H, 4'-Br	10.0
*2'-Br, 4-H, 4'-F	8.0	2'-H, 4-H, 4'-I	13.0
*2'-I, 4-H, 4'-F	10.1	2'-H, 4-Br, 4'-F	13.6
*CLC		2'-H, 4-I, 4'-F	16.7

Substitution on the 2'-position (i.e. ortho phenyl ketone) yields the lowest increase of lipophilicity. CLC is obtained as in this position the halogen is clustered within the electronic cloud of the p-F-phenyl ketone system consisting of the aromatic resonance of the strong electronegative F (in the aromatic plane) and the polar C=O (perpendicular to the plane). 2'-^{123}I-Spiperone shows affinity comparable to Spiperone and is a suitable D2 receptor tracer for SPECT (3).

Table 2 : compares the Influence of Halogen Substitution on the Phenyl Ketone Group in SPIPERONE and KETANSERIN (k' at PH 4.9).

Position	k' Spiperone 2',4' analogues	k' Ketanserin 2, 4 analogues	k' Spip/k' Ket
o-H, p-F	4.7	2.8	1.7
o-Br, p-F	8.0	4.8	1.7
o-I, p-F	10.1	6.0	1.7
o-H, p-Br	10.0	6.0	1.7
o-H, p-I	13.0	7.8	1.7

In Ketanserin ortho substitution and the resulting CLC also limit the increase of lipophilicity with preservation of affinity. The constant k' ratio values show that substitution on the phenyl ketone group introduces a change in lipophilicity which is independant of the compound. This allows to predict the increase of lipophilicity for any molecule where substitution occurs on the phenyl ketone group.

The author thanks M. Gysemans, S. Soykenc and M. Thomas for the HPLC experiments.

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SYNTHESIS OF p-F-o-I-PHENYL KETONE COMPOUNDS, SUBSTRATES FOR RADIO BROMINATION, BY Cu(I) ASSISTED NUCLEOPHILIC EXCHANGE.

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We found that, on the analogy of the ^{123}I labelled compounds (1,2), 2-^{76}Br-Ketanserin and 2'-^{76}Br-Spiperone showed in vivo receptor affinity making these compounds candidates for PET tracers (3). This means that the 2-I-Ketanserin and 2'-I-Spiperone are required as substrates for radiobromination. As the ortho-position on p-F-butyrophenon (2'-Spiperone) and on (4-fluorophenyl)(4-piperidinyl) methanone (2-Ketanserin) is deactivated for electrophilic substitution, up to now rather cumbersome multistep syntheses, have to be used.
This paper proposes the Cu(I) assisted nucleophilic iododebromination as an alternative method for the synthesis of o-I-substituted p-F-phenyl ketone compounds.
The use of Cu(I) salts in organic synthesis is well described, although the mechanistics proposed are not evident. It has been shown that due to oxidation Cu(II) can be generated during the reaction, resulting in the formation of I_2 and side products. As our Cu(I) assisted radiohalogenation in reducing conditions (4) is successful, we decided to scale up this method for cold synthesis in the 10-100 mg range.
The exchange reaction was carried out by refluxing in 50% HOAc (reflux °t : 102 °C) a mixture of Br-compound ($2.5\ 10^{-2}$ M), KI ($2.5\ 10^{-2}$ M), $SnSO_4$ ($1.5\ 10^{-3}$ M), $CuSO_4$ ($2.5\ 10^{-4}$ M) and citric acid ($1.25\ 10^{-2}$ M) during 6 hours. The method was optimized using 2'-Br-acetophenon as a model molecule. Samples were taken at appropriate times and analyzed by RP-HPLC and UV detection (λ = 254 nm). As the amount of side products was negligible, the exchange yield η was determined as the ratio of the peak area corresponding to the iodinated compound to the sum of the areas of the iodinated and brominated compound, corrected for their respective molar extinction coefficient. A 65% exchange yield was obtained at the optimal substrate to Cu(I) ratio of 100 in presence of excess of $SnSO_4$ (Sn^{2+}/Cu^{2+} : 10) and citric acid. When plotting η or the reaction rate v as a function of the Cu(I) concentration a rectangular hyperbolic function is observed where at the maximal value η and v become independent of [Cu(I)], pointing to saturability of the substrate up from the optimal substrate/Cu(I) ratio mentioned.
Applying the described method directly to 2-Br-Ketanserin, yielded 52% of 2-I-Ketanserin. This method is actually applied for the preparation of 2'-I-spiperone.

This work was supported by a FGWO grant (3.0099.89).

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SYNTHESIS AND EVALUATION OF THREE IODINATED HALOPERIDOL DERIVATIVES AS POTENTIAL DOPAMINE RECEPTOR IMAGING AGENTS.

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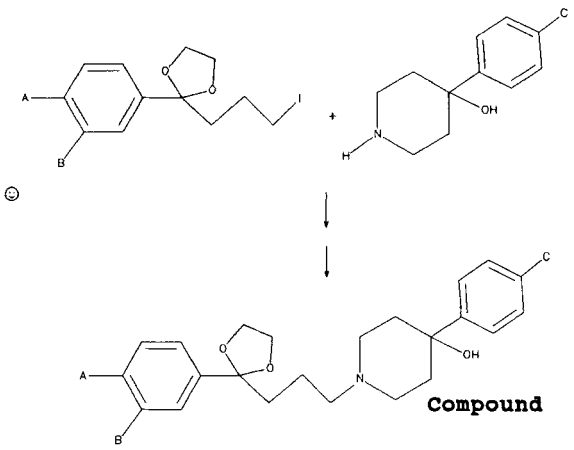

Compound	A	B	C	IC_{50}(nmol)
Haloperidol	F	H	Cl	1.5
1	I	H	Cl	156
2	F	I	Cl	26
3	F	H	I	1.0

The correlation between dopamine (D-2) receptor levels and neurological diseases such as Parkinson's disease, tardive dyskinesia and schizophrenia has been well documented.[1] An imaging agent which shows affinity and selectivity for D-2 receptors would be useful for the diagnosis of these diseases. Various neuroleptics have been labelled and evaluated for this purpose with varying success.[2] Haloperidol, a neuroleptic which shows D-2

receptor affinity and selectivity has been labelled primarily with positron emitting isotopes.[3] We have synthesized three iodinated analogues of Haloperidol, compounds 1, 2 and 3, to investigate the possibility of an ^{123}I labelled agent for SPECT imaging. To our knowledge, compounds 1 and 2 have not been previously synthesized but the synthesis and labelling and some biodistribution data for compound 3 has been reported.[4]

We will report on the our route to compounds 1, 2 and 3 employing ^{127}I. These compounds were obtained from the substitution of halogenated butyrophenones by halogenated arylpiperidols. The IC_{50}'s have been determined by *in vitro* receptor binding assay against [^{3}H]-spiperone. The correlation of this data with iodination position will be discussed. The relative binding affinities suggested we should further investigate compound 3. ^{131}I-3 was synthesised from ^{127}I-3, via the trimethyltin derivative. Its biodistribution in mice over a longer period of time than previously reported will be described.

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INFLUENCE OF THE SPECIFIC ACTIVITY ON THE BRAIN UPTAKE OF 2-RADIOIODO-KETANSERIN RELATED TO LOG P.

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2-^{123}I-Ketanserin was shown to be a promising tracer for mapping serotonin S2 receptor tracers for SPECT (1). During the first studies in human, it was observed that when 2-^{123}I-Ketanserin of high specific activity almost approaching the theoretical value of 40 10^8 MBq/mmol was intravenously injected the brain uptake decreased considerably. Optimal receptor visualization was obtained around 3.10^8 MBq/mmol corresponding to about 5.10^{-10} mol of 2-iodo-Ketanserin injected. 90 min. post injection >90% of the activity was bound to plasma proteins and was shown by HPLC to be unchanged ligand. In vitro binding experiments have shown that binding to human serum albumin is not saturable at concentrations corresponding to the in vivo application. This means that the radioactivity available for BBB passage should remain independent of the amount of cold product or specific activity injected
In vivo experiments on Male Wistar Rats had already shown that the brain uptake increased with the amount of 2-I-Ketanserin injected. Compared to the lineair increase of aspecific binding in cerebellum the uptake in the frontal cortex was high and saturable. This points to the fact that in rat the processes ruling the amount of free ligand in blood and the amount that crossed the BBB yield brain uptake linearly related to the amount injected.
Log P experiments using the shake flask method were carried out on 2-^{131}I-Ketanserin, to which appropriate amounts of cold compound were added to obtain a concentration range of 3.10^{-10} - 60.10^{-10} M. The partition between octanol and Tris/HCl buffer of PH 7.4 after 60 min. at 22 °C was estimated by measuring the activity in weighed aliquots of both phases. The next table shows the apparent log P as a function of the concentration of 2-I-Ketanserin.

10^{-10} mol	Log P_{app}
3.0	0.74
18.0	0.78
19.5	0.79
58.0	0.90

It is shown that the log P increases with the concentration in the involved concentration range.
This phenomenon can be correlated with the carrier effect for lipophilic transport in the BBB and could be an explanation of the observed brain uptake-specific activity relation.

This work was supported in part by the FGWO grant 39007.89.

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OPTIMIZED PREPARATION OF I123-IBZM AND I123-IBZP FOR CLINICAL SPECT STUDIES OF D-2 AND D-1 RECEPTORS.

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Since the initial reports on D-2 receptor binding of I125-S(-)-N-[(1-ethyl-2--pyrrolidinyl)methyl]-3-iodo-2-hydroxy-6-methoxy-benzamide (I125-IBZM) (1) and D-1 receptor binding of I125-R(+)-7-iodo-8-hydroxy-3-methyl-1-phenyl--2,3,4,5-tetrahydro-1H-3-benzazepine (I125-IBZP) (2) to rat striatum there has been great interest in patient SPECT studies with these ligands, I123-labeled. We aimed to develop reliable rapid high-yield methods for I123-IBZM and I123-IBZP radiopharmaceutical (RP).

Racemic N-ethyl-2-(aminomethyl)pyrrolidine was reacted with 6-methoxy-salicylic acid chloride (not isolated, prepared from 2,6-dimethoxy-benzoic acid) to yield about 50 % racemic BZM (Scheme 1) (modification of (1)) which was separated into R(+) and S(-)BZM of >99 % enantiomeric purity by chiral HPLC (Fig.1) for demonstration of stereoselectivity in vivo (3). A preparative HPLC system for I123-IBZM was developed: silica columns and polar eluents (combinations of MeCN, MeOH, EtOH/$(NH_4)_2HPO_4$, NH_4OH) did not provide good separations, but C18 columns with an eluent of 90% MeOH,10% 0.1M NH_4OH separated BZM (k'2.7) from IBZM (k'4.4) (Fig.2).

Electrophilic I123-labeling of BZM was initially performed with 0.35mM Chlor-aminT (CAT) in 0.01M HCl followed by neutralization, $CHCl_3$ extraction and HPLC purification (modification of (1)) giving isolated radiochemical yields (Y) of 69±6% (n=9) within 2 hrs. Further development resulted in using peracetic acid (HO_2Ac)as oxidant at 50° (avoiding ClBZM side-product), directly injecting the whole reaction mixture of only 150 µl (Tab.1) into the HPLC system and isolating I123-IBZM in 88±7% Y (n=17) (Fig.2). Evaporation of the HPLC-peak, recovery in EtOH, dilution with PBS and sterile filtration yield the RP: total prep. time 1 hr, radiochem. purity (RCP) >97 % (TLC:SG;i-propylether:MeOH: NH_4OH 100:10:1;Rf 0.5), spec. activity $\geq 10^3$ Ci/mmol. It was clinically applied for SPECT imaging and semiquant. estimation of D-2 receptors in patients (3).

I123-iodination of BZP was studied with a) HO_2Ac: Y was only ~40% and badly reproducible b) CAT: chlorination side-products (ClBZP) may be formed and must then be separated. C18 columns and 80% MeCN,20% 4mM PO_4 separated BZP (k'8.6), ClBZP (k'10.1) and IBZP (k'11.9) not ideal due to tailing of the large BZP substrate peak. Using silica columns and 50% MeCN, 50% 4mM$(NH_4)_2HPO_4$, IBZP (k'2.2) eluted prior to ClBZP (k'2.5) and BZP (k'4.0) but not enough resolved from ClBZP (Fig.3). c) Iodogen: with conventional coating technique Y was ~60%, but by reacting with 2 µg Iodogen in suspension followed by direct prep. HPLC of 100 µl reaction mixture an excellent Y of ~85% was obtained (Tab.3, Fig.3) without side-product as confirmed by ClBZP internal standard in HPLC. RP formulation was identical to IBZM, total prep. time ~1 hr, RCP ~97% by TLC (SG,MeOH;Rf 0.46), spec. activity $\geq 10^3$ Ci/mmol. This RP will soon be used for SPECT studies of D-1 receptors in patients.

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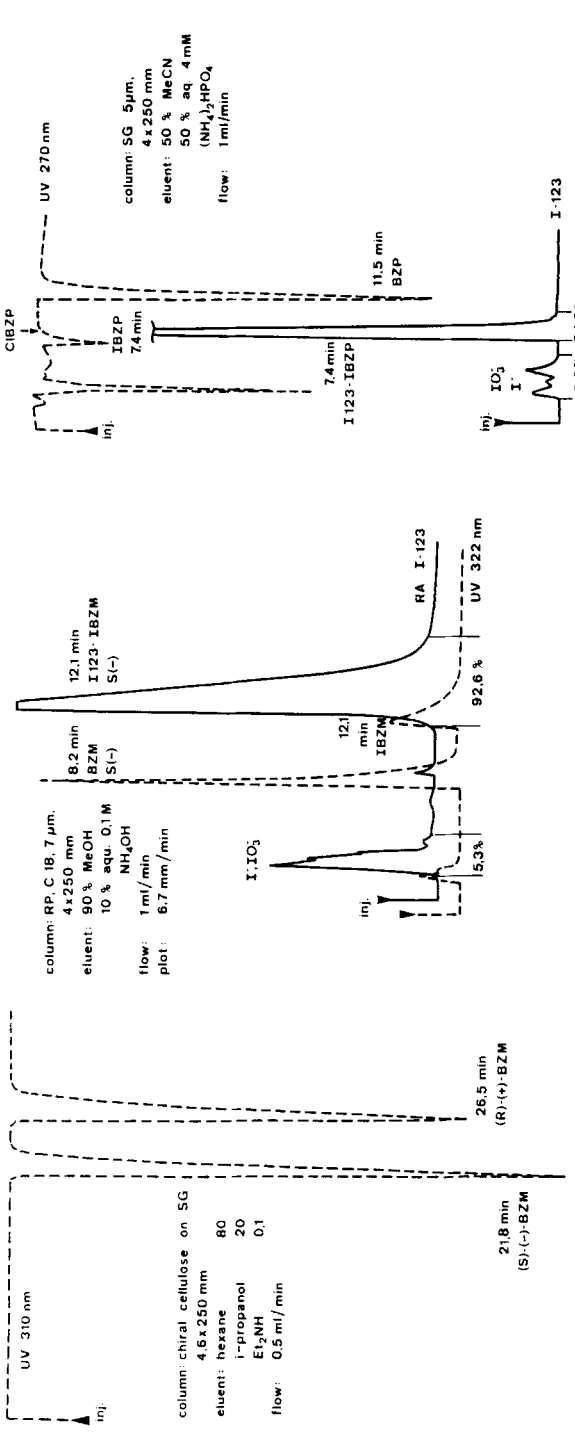
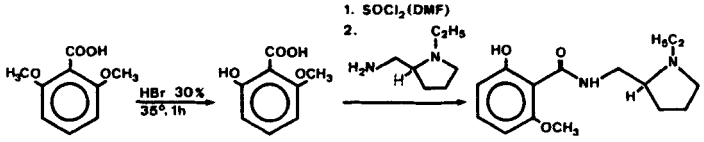


Fig.1 Prep. chiral HPLC of BZM-racemate. Conditions in Fig. 25-50 µg separated per run and used for labeling.

Fig.2 Prep. HPLC of I123-IBZM-S(-). Conditions in Fig. 10nmol NaI was added to make IBZM visible in UV.

Fig.3 Prep. HPLC of I123-IBZP-R(+). Conditions in Fig. 10nmol NaI was added to make IBZP visible in UV.

Scheme 1. **Synthesis of BZM**



2,6-dimethoxy-benzoic acid | 6-methoxy-salicylic acid | 1-ethyl-2-aminomethyl-pyrrolidin | N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-hydroxy-6-methoxy-benzamide BZM

Table 1.

I123 – Iodination of BZM with Peracetic Acid

reactant	add. volume	final concentration
30 µg BZM	30 µl 0.01 M HCl	∿1 mM
∿20 mCi I123 – NaI	∿60 µl 0.01 M NaOH	∿0.08 nmol I/mCi
	5 µl 0.1 M HCl	pH∿ 2
5.1 mg CH3COOH	5 µl 96%	0.77 M
3.3 mg H2O2	10 µl 30%	0.88 M
Vortex mix	110 µl Reaction mixture	50°C for 10 min
Neutralize	∿17 µl 5 M NaOH	
inject HPLC		

Table 2.

I123 – Iodination of BZP with Chloramine T

reactant	add. volume	final concentration
30 µg BZP	30 µl 0.01 M HCl	1.2 mM
∿20 mCi I123 – NaI	∿60 µl 0.01 M NaOH	∿0.08 nmol I/mCi
	5 µl 0.1M HCl	pH∿ 2
	5 µl CH3COOH	
6 µg Chloramin T	5 µl H2O	0.22 mM
Vortex mix	105 µl Reaction mixture	5 min at R.T.
inject HPLC		

Table 3.

I123 – Iodination of BZP with Iodogen

reactant	add. volume	final concentration
30 µg BZP	30 µl 0.1 M PO4 pH 7.5	1.2mM
∿20 mCi I123 – NaI	∿60 µl 0.01 M NaOH	∿0.08 nmol I/mCi
2 µg Iodogen	10 µl 0.1 M PO4 pH 7.5	0.046 mM
(suspension: 1mg Iodogen/0.1ml acetone + 4.9ml 0.1M PO4)		
stir	100 µl Reaction mixture	10 min at R.T.
inject HPLC through 0.45 µm microfilter		

CONVENIENT SYNTHESIS OF IODOETHYL RADIOTRACERS : APPLICATION TO SPIPERONE AND SPIROXATRINE DERIVATIVES.

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Neuroreceptors explorations are of great interest for research, diagnostic purpose and treatment evaluation of neurologic and psychiatric diseases. These explorations can be performed by positron emission tomography (PET)(1). However the number of patients investigated by this method is very limitated, because PET is expensive and difficult to use widely in nuclear medicine center. So to increase the number of investigations single photon emission computed tomography (SPECT) is more convenient than PET. For SPECT explorations it is essential to develop new ligands labelled with single photon emitting radionuclides as 123 -iodide.

For this aim to obtain no-carrier-added radioiodinated ligands we have developped a new method by carrying out nucleophilic substitution of tosylate derivatives by iodide. This procedure has been tested by synthesizing two new radioiodinated ligands derivated from spirodecanone series: spiperone (a D2 dopaminergic receptors ligand) and spiroxatrine (a 5HT1A receptors ligand). For this purpose the potassium salt of spiperone or spiroxatrine is opposed to ethylene glycol di-p-tosylate in equimolar ratio ; by this way precursors of radioiodinated ligands are obtained in one-pot synthesis (60% yield for 20 min at reflux of tetrahydrofuran). This method is described in the scheme 1.

To radiolabel the precursor of radioiodoethyl derivative of spiperone or spiroxatrine we used sodium radioiodide at reflux of acetone as shown in scheme 2.

Specificity of iodoalkylated ligands has been demonstrated by an *in vitro* binding method for D2 receptors with iodoethylspiperone and 5HT1A receptors with iodoethylspiroxatrine. Morever, cerebral biodistribution of [^{125}I] iodoethylspiperone in the rat showed a high uptake in striatum, rich in D2 receptors, with a maximal striatum/cerebellum ratio of 10 reached 4hr after injection (2).

In conclusion, we demonstrated that iodoalkylation on 3-N position in the spirodecanone part of spiperone and spiroxatrine is obtained with a good chemical yield. This 3-N alkylation does not prevent in vitro binding of these ligands on dopaminergic and serotoninergic receptors.

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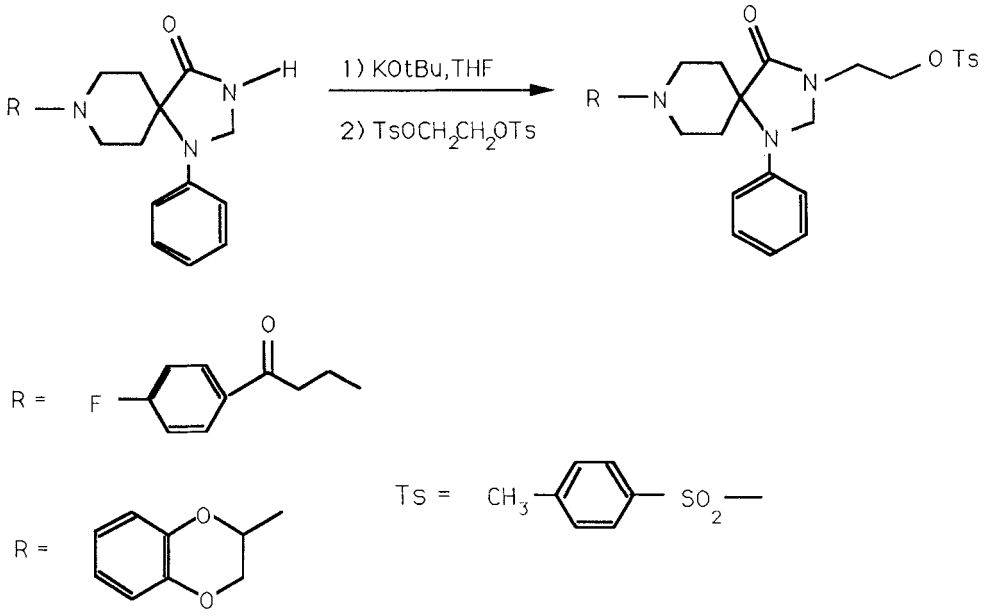
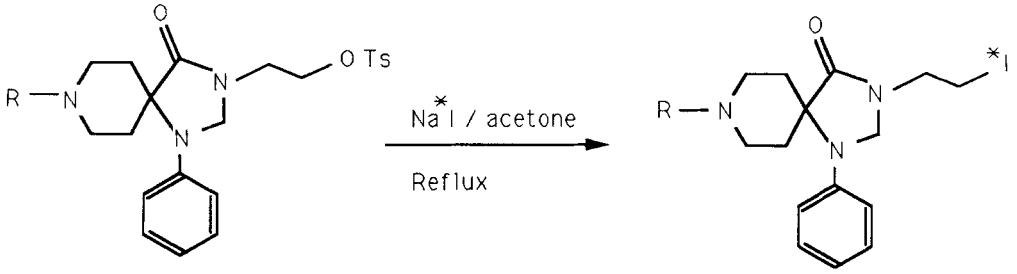


Scheme 1:
Synthesis of N-tosylethyl derivatives of spiperone and spiroxatrine



Scheme 2:
Radiolabelling of N-tosylethyl precursors to obtain N-radioiodoethyl derivatives of spiperone and spiroxatrine.

Fluorine-18 Fluoroalkyl Derivatives of Carazalol: Potential Ligands for the In Vivo Studies of the ß-Adrenergic Receptor.

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Carazalol (1: R= R'= H) is an antagonist for the ß-adrenergic receptor and has one of the highest association constants known for the receptor[1]. Therefore it has the potential of being a suitable ligand for in vivo studies if labeled with a positron emitting radionuclide. Because of the dynamics of the binding process, the radionuclide of choice would be fluorine-18; however, no fluorinated derivatives of carazalol are known. We have synthesized a series of alkyl carazalol derivatives (1: R= Me,Et; R'=H) and are investigating the effects of the substitutions on the association constants of the ligands. The synthetic pathway is based upon a Fischer indole synthesis using various alkylphenylhydrazones of cyclohexanedione as shown in the scheme. We have also prepared hydroxymethyl derivatives (2: R=5,6,7, or 8 $-CH_2OH$; R'=THP) which could be fluorinated following addition of a suitable leaving group. The syntheses of these compounds were straightforward but quite lengthy. Accordingly, we have made side-chain protected 6-hydroxyethylcarazolol (2: R= 6-CH_2CH_2OH, R'= H), the tosylate of which has been fluorinated with fluorine-18 fluoride in excellent yield. Acid hydrolysis then removes the acetate protecting groups to give the final product 3.
The in vitro association constant of 3 is being determined, and its suitability as a ligand for in vivo studies of the ß-adrenergic receptor is under investigation. So far, all of the compounds in this series are racemic, but they can be prepared enantiomericaly pure by substituting commercially available (2S)-(+) or (2R)-(-) glycidyl tosylate for epichlorohydrin. The availability of both enantiomers makes it possible to check many of the assumptions underlying the modeling used in the interpretation of the physiological data.

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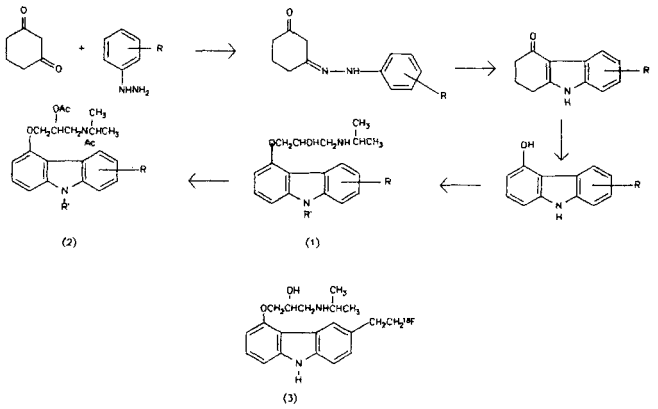

NCA SYNTHESIS OF AN N-ω-(^{18}F)-FLUOROETHYL ANALOG OF ALTANSERINE, A SEROTONIN S2 RECEPTOR LIGAND

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The preparation of fluorine-18 labeled altanserine for PET studies of serotonin S2 receptors involves the nucleophilic displacement of the nitro group in nitroaltanserine by NCA ^{18}F- (1). This nitro-precursor is not easy to prepare. Moreover, biological data in the literature indicate that N-alkyl [^{18}F]fluorospiperone derivatives have different properties compared to spiperone (2). Based upon these findings we decided to prepare the N-ω-[^{18}F]-fluoroethyl analog 4 of altanserine.

[^{18}F]-Fluoroethylaltanserine 4 was prepared via a two-step reaction (scheme 1). The first stage was the nucleophilic substitution of ^{18}F- on 1 followed by a rapid purification of 2 on a silica gel column and the second consisted of the alkylation of altanserine 3. The initial reaction was conducted at 90°C in acetonitrile using a mixture of potassium carbonate and Kryptofix 222 followed by passage of the [^{18}F]-fluorotosylethane 2 through a silica gel column (250 mm • 10 mm, silica gel 60, 230 - 400 mesh) eluted with pentane/ether (4 / 1) and evaporation to dryness of the solvent. 4 mg of altanserine 3 were alkylated using the same experimental conditions (10 min, 90°C, MeCN, K_2CO_3, K_{222}). Final purification of [^{18}F]-fluoroethylaltanserine was achieved by HPLC (Lichrosorb RP Select-B from Merck, 7μm, 250 mm • 25 mm) eluted with THF/MeOH/aqueous buffer pH 4 (43/17/40), at a flow rate of 7 ml/min. A radiochemical yield of 22 % decay corrected and a specific activity higher than 1Ci/ μmol were obtained in an overall preparation time < 90 min.

Compared to the results obtained with altanserine, the biological data in animal experiments showed that a few minutes after injection 0.5 % of the injected dose appeared in the total brain but this decreased very rapidly with time (figure 1). The frontal cortex/cerebellum ratio reached a maximum value of 2.3 and displayed a decrease with time indicating the very poor in vivo serotoninergic specificity of the radioligand (figure 2).

In conclusion, [^{18}F]-fluoroethylaltanserine does not appear to be a suitable radiopharmaceutical for PET imaging of serotoninergic receptors sites in humans.

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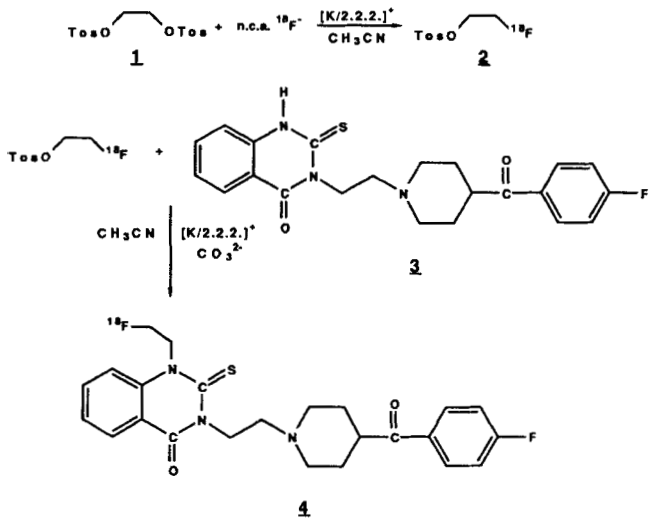
Acknowledgments :
We wish to thank Janssen Pharmaceutica (Belgium) for providing a sample of altanserine for these studies.

SCHEME 1



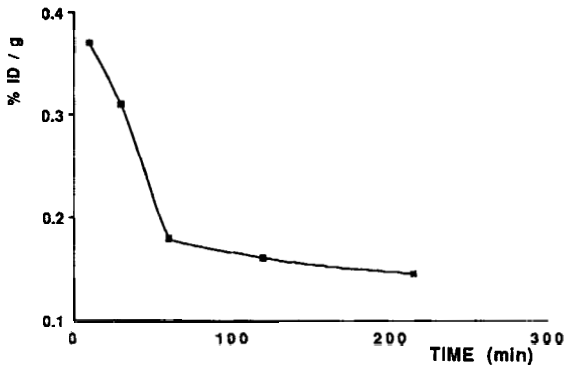


FIGURE 1 : CEREBRAL BRAIN UPTAKE OF [18F]-FLUOROETHYL-ALTANSERINE VERSUS TIME

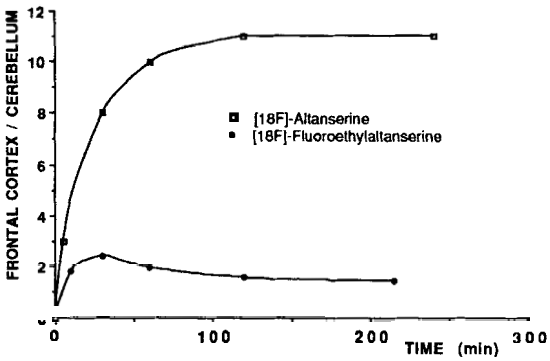


FIGURE 2 : TIME FRONTAL CORTEX/CEREBELLUM RATIO CURVES FOR [18F]-ALTANSERINE AND [18F]-FLUOROETHYLALTANSERINE

PREPARATION OF [^{123}I]-PRENALTEROL : A POTENTIAL RADIOTRACER FOR MYOCARDIAL β-ADRENERGIC RECEPTOR STUDIES BY SPECT.

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Prenalterol, 4-[2-hydroxy-3-[(1-methylethyl)-amino]propoxy]phenol, a β1-sympathomimetic agent with cardioselective properties, has been labeled with non carrier added ^{123}I. Thanks to its hydroxyl group, a regioselective labeling of Prenalterol, is obtain by electrophilic substitution, using Chloramine-T (CAT) as an oxydating agent.

Optimal labeling conditions have been determined using radio-TLC analysis of the reaction mixture. TLC separations were performed on RP18 plates (MERCK) using ethanol-0.01M acetate buffer (70-30) and on silicagel plates using $CHCl_3$-CH_3OH (85-15). Kinetic studies show that a high radiolabeling yield (80%) is obtained in 20 min using 1 µmol of Prenalterol and 0.3 µmol of CAT in 0.01M phosphate buffer (pH=7.4). The labeling process is stopped by addition of $Na_2S_2O_5$.

The reaction mixture is poured on a C18 cartrigde and polar by-products and excess of cold Prenalterol are eluted with 5 mL phosphate buffer-ethanol (90-10). [^{123}I]-Prenalterol is eluted with 5 mL ethanol and then purified by HPLC on a reverse phase analytical column (µ-Bondapak C18, 250x4, WATERS) using acetate buffer-ethanol (90-10) as the mobile phase. Monitoring UV absorption (288 nm) and radioactivity permits the radio-iodocompound collection. Retention volumes for cold or radioactive iodo-Prenalterol are identical (25 mL). The specific activity of the radioiodocompound measured on the HPLC radiochromatogram, is 20 Ci/µmol, its chemical and radiochemical purities, assessed by HPLC and radio-TLC, are higher than 95%. The whole procedure lenghts 2 hours and the total radiochemical yield is 70%.

Biodistribution of [^{123}I]-Prenalterol has been measured in rats after IV injection of 100 µCi of radioligand. In heart, a rapid and high uptake followed by a slow elimination process occur. 5 min post injection the heart radioactive concentration is 1% ID/g and the heart to whole blood ratio 6. In brain, 30 min post injection, the uptake is only 0.1% ID/g.

These preliminary results indicate that [^{123}I]-Prenalterol could be a useful tracer for in vivo SPECT studies of the myocardial adrenergic receptors.

AN IMPROVED RADIOSYNTHESIS OF THE D2 ANTAGONIST [^{123}I]-IODOLISURIDE.

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Lisuride , a semi-synthetic ergolene derivative, labeled with the β+ emitter ^{76}Br, has been successfully used for PET imaging of dopaminergic D2 receptors (1). For SPECT studies we have switched from radiobromine to radioiodine. Thanks to a NH group in position 2, Lisuride can be easily labeled by an electrophilic substitution. Iodiolisuride labeling using peracetic acid and Choramine T as oxydating agents have been described. Whatever the oxydant, labeling yields are lower than 50%. Here we report a high efficiency preparation of [123I]-iodolisuride using IODO-GEN (1,3,4,6-tetrachloro-3α,6α-diphenylglycouril).

IODO-GEN (1µmol) is set on the walls of a conical vial by evaporating a CH_2Cl_2 solution under a nitrogen stream. Lisuride (4µmol) in 200 µl of acetic acid (1M) and non-carrier-added ^{123}INa are successively added. Sequential radio-TLC analysis of the labeling mixture show that in 60 min at room temperature, the radiolabeling yield reaches 90% .

For [^{123}I]-iodolisuride purification, the solution is poured on a C18 cartridge and polar products are washed with 5 mL water. Then lipophilic products are eluted with 5 ml $CHCl_3$ and adsorbed on a silice cartridge. [^{123}I]-Iodolisuride is selectively eluted with 3 mL $CHCl_3$-CH_3OH (95-5), the cold precursor remaining on the column. The radio-iodoligand is purified by HPLC on a silicagel column (Lichrosorb-Si60, 250x10, MERCK) using chloroform-methanol-water-ethylamine (989-10-1-0.25) as the mobile phase. UV detection (254nm) and radioactivity measurement of the eluate allow to collect [^{123}I]-Iodolisuride. Using this procedure, iodolisuride can be easily separated from other halogeno derivatives (selectivity between iodo and bromo derivatives : 1.15), and its chemical purity is >95%. The specific activity measured on HPLC radiochromatogram ranges from 30 to 60 Ci/µmol. Radiopharmaceutical purity assessed by TLC using silicagel plates and $CHCl_3$-CH_3OH (90-10) is 99%.

[^{123}I]-Iodolisuride has been prepared in two pharmaceutical forms. A base form is obtained by taking off the radioligand in saline containing 4% of ethanol-propanediol (50-50). For the salt form preparation, the residue is taken off by 1M acetic acid; after sterilization through a 0.22µm PTFE membrane (MILLIPORE) the acidic solution is neutralized by 0.15M NaOH. The radiochemical stability of these preparations has been studied for 30h at 20°C by radio-TLC analysis. Whatever the preparation, the radiopharmaceutical purity remains always >96%.

We have elsewhere demonstrated that [^{123}I]-Iodolisuride possess physico-chemical (lipophilicity : log $P_{oct/water}$ = 2.3) and pharmacological properties (specificity, selectivity, affinity...) for in vivo SPECT imaging of D2 receptors (2). The use of IODO-GEN labeling, by increasing the preparation yield (>75%), should allow to minimize the cost of clinical D2 receptor pathology SPECT studies.

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The Determination of Unchanged [^{11}C]Diprenorphine, [^{11}C]L-Deprenyl and [^{11}C]Raclopride in Plasma by HPLC

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In PET studies a knowledge of the fraction of radioactivity in plasma representing unchanged ^{11}C-labelled ligand/tracer is essential for correction of the arterial plasma input curve.[1,2]

A number of different approaches (*e.g.* HPLC[1,3] and TLC[2]/OPTLC,[4]) to the determination of unchanged radio-ligand/tracer have been reported for ^{11}C-labelled carfentanil,[1] raclopride[2] and L-deprenyl[3] and for ^{76}Br-labelled bromolisuride.[4] We report here how we have applied the simple work-up and analytical procedure, originally reported for [^{11}C]carfentanil,[1] to establish the percentage of unchanged ^{11}C-labelled diprenorphine, L-deprenyl and raclopride in human and animal plasma as a function of time.

Cell free plasma, spiked with stable compound of interest, was passed through two activated C-18 SEP-PAKs arranged in series and these were then exhaustively washed with ammonium formate (0.1 M). Retained activity was eluted from the SEP-PAKs with methanol and the eluate adjusted to the composition of the HPLC mobile phase with appropriate volumes of ammonium formate. The resultant solution was analysed, with sensitive radioactivity detection, by HPLC (C_{18} "μ-Bondapak", 300 x 7.8 mm i.d.) at a flow rate of 3.0 ml min^{-1}. By simply adjustment of the composition of the mobile phase (methanol:ammonium formate), it was possible to elute all the injected activity within 12 min, and to achieve good resolution of unchanged ^{11}C-labelled compound from metabolites, for each of the three above-mentioned radioligands. A typical HPLC trace (Figure 1) and a time course study (Figure 2) are shown for [^{11}C]diprenorphine. The proportion of unchanged [^{11}C]diprenorphine in plasma declined from 90-65% at 5 min to approximately 40-35% at 30 min after injection. Unchanged [^{11}C]L-deprenyl varied from 80-25% between 5 and 25 min, in broad agreement with data reported using protein precipitation and subsequent HPLC.[3]

The described HPLC system can be applied to the analysis of a variety of ^{11}C-labelled compounds with only minor variations in the preliminary Sep-pak treatment and required HPLC eluent. The rate of loading of plasma onto the Sep-pak determines the extent of extraction of the labelled compound. For example, [^{11}C]raclopride requires slower loading than [^{11}C]diprenorphine and [^{11}C]L-deprenyl to obtain complete extraction.

In conclusion,the simple methodology reported by Frost *et al.*[1], with small modifications, shows general applicability to the preliminary analyses of unchanged ^{11}C-labelled compounds in plasma.

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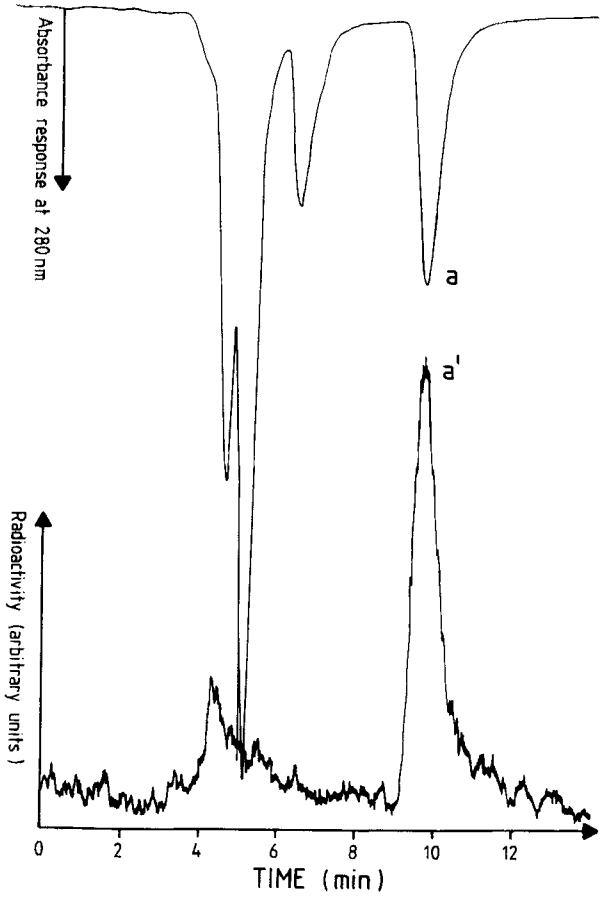

Fig. 1 HPLC trace of human plasma activity at 5 min post-injection of [^{11}C] diprenorphine (a'). Sample contains added cold diprenorphine (a).

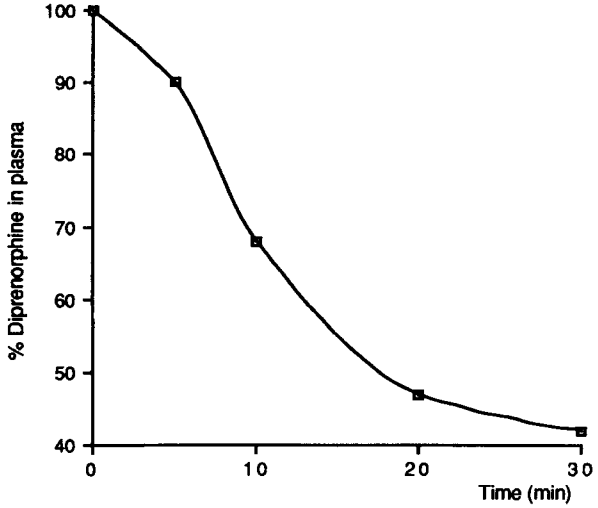

Fig 2. Time course of metabolism of [11-C]diprenorphine in human plasma

DEVELOPMENT OF (S)-N-[(1-ETHYL-2-PYRROLIDINYL)METHYL]-5-([F-18] FLUOROALKYL)-3-SUBSTITUTED-2-METHOXYBENZAMIDES AS POTENTIAL DOPAMINE D-2 RADIOTRACERS FOR PET.

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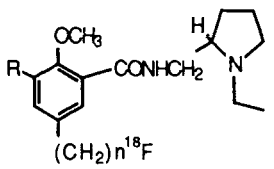
Dopamine D-2 receptors have been imaged *in vivo* by positron emission tomography (PET) in both primates and humans using a variety of positron-emitting radioligands (1,2). Substituted benzamides have been reported to show specific, high affinity and reversible binding for the dopamine D-2 receptors (3). Raclopride, which is a substituted benzamide has been labeled with carbon-11 and used for PET scans in primates and humans (4,5). Due to the short half-life of carbon-11 (20.4 min) development of a fluorine-18 (110 min) labeled benzamide derivative would provide a radiotracer which will be more tolerant to delays in synthesis time and also permit longer PET data acquisition times. Efforts to generate a fluorine-18 labeled derivative of raclopride have been pursued in several laboratories (6). Fluoroalkylation at the pyrrolidine nitrogen of raclopride lowers its affinity towards the D-2 receptor and therefore renders it incapable of being developed as a PET tracer. Also reported is the development of fluorinated derivatives of eticlopride which has a higher binding affinity than raclopride for the D-2 receptor. Although the fluoroethyl derivative of eticlopride has high affinity for the D-2 receptor *in vitro* , it showed poor *in vivo* selectivity in the rat brain (7).

We are involved in developing fluoroalkyl analogs of the related, clinically used antipsychotic sulpiride, as possible selective D-2 receptor antagonists. The aminosulfonyl moiety of sulpiride is replaced with a fluoroalkyl group in these analogs in order to avoid proximity of the fluorine atom to the pyrrolidine nitrogen. Other appropriate substituents in the C-3 position of the benzamide nucleus have also been incorporated. These fluorinated derivatives show high affinity (IC_{50} of 10^{-7} to 10^{-8} M) for the D-2 receptor binding sites labeled with ^{3}H-spiperone. Fluorine-18 analogs (Figure 1) of these compounds are therefore likely to be good candidates for development as radiotracers for PET.

Figure 1

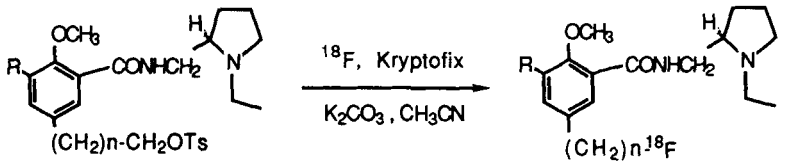


R= H, OCH_3

n= 2, 3

^{18}F-fluoride was solubilized with kryptofix and potassium carbonate in acetonitrile and taken in micro vials. Reaction of this ^{18}F-fluoride was carried out in small volumes of acetonitrile (typically 100-500 µL) with 3-5 mg of the respective tosylate precursor for 30 minutes at 85-90°C. The yields were in the range of 20-30%. Unreacted ^{18}F-fluoride was removed by water-methylene chloride extraction of the reaction mixture. The methylene chloride was dried and the residue was taken up in methanol and passed through a small column of neutral alumina. The eluate was purified by reverse phase HPLC. Radiolabeling was very clean and the respective ^{18}F-labeled benzamide derivatives were the only radiolabeled product. However, HPLC separation of the ^{18}F-

labeled product had to be carried out at least two times in order to remove traces of UV absorbing peaks. Chemical purity of the radiotracers were reduced due to traces of coeluting mass peaks. Specific activities of 0.8-1.2 Ci/μmole were computed using the extinction coefficient of the respective fluorine-19 compounds and assuming the coeluting peak to have a similar extinction coefficient.



Scheme-1 Direct fluoride-18 labeling of tosylate.

In conclusion, we report the development of new F-18 labeled fluorinated benzamide neuroleptics in moderate yields of 20-30% with specific activities in the range of 0.8-1.2 Ci/μmole. Preliminary PET studies in Cebus apella using (S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(3[F-18]fluoropropyl)-2,3-dimethoxybenzamide(^{18}F-FPMB) show localization of the radiotracer in the striata with the ratio of striata to cerebellum increasing from 1.4 at 16 minutes to 3.1 at 120 minutes, post injection (8). Further in-vivo characterization of ^{18}F-FPMB is in progress.

Acknowledgements

We wish to acknowledge support provided by the U. S. Department of Energy under contract No. DE-FGO2-86ER60438 and Brain Research Foundation of the University of Chicago.

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ROUTINE PRODUCTION AND IMPROVEMENT IN THE THE PURIFICATION OF 3-N-(2'-[^{18}F]FLUOROETHYL)SPIPERONE FOR CLINICAL USE

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The involvement of dopamine receptors in neurological diseases has precipitated an interest in *in vivo* imaging and quantification of cerebral dopaminergic receptor sites by positron emission tomography (PET). Two major appraoches have been previously reported (1-5) for the synthesis of 3-N-(2'-[^{18}F]fluoroethyl)spiperone (^{18}F-Et-Sp). The first one was a one-step reaction consisting of the direct nucleophilic substitution of $^{18}F^-$ on precursor 1 or 2 and the second one was a two-step reaction described in Scheme 1. Both routes relied on HPLC for final purification.

In order to allow the routine production of ^{18}F-Et-Sp (5) for clinical use, we have developed the two-step reaction procedure (4-5) and improved the final HPLC purification technique. The final purification was very important because the alkylation step required a significant amount (3-4 mg) of spiperone and led to a wide variety of by-products difficult to separate by a single chromatographic process. The most reliable results have been obtained by using two fast HPLC purifications instead of one long procedure. The first one consisted of a normal phase silica gel column (Lichrosorb Si-60, 7 µm, 250 mm x 10 mm from Merck) eluted with CH_2Cl_2 - 0.1% NH_4OH in CH_3OH (98 - 2) (solvent 1) at a flow rate of 5 ml/min. In these conditions there was a total baseline resolution between ^{18}F-Et-Sp and the large excess of unreacted spiperone sticking on the column. The ^{18}F-Et-Sp peak was collected, the solvent removed and the residue loaded onto a second HPLC column (Lichrosorb RP Select-B, 7 µm, 250 mm x 10 mm from Merck) eluted with CH_3OH - 0.1% triethylamine in water (70 - 30) (solvent 2) at a flow rate of 5 ml/min. After the second HPLC process, ^{18}F-Et-Sp was obtained in very high chemical and radiochemical purities (> 99%), the recovery yield of ^{18}F-Et-Sp from the HPLC system was > 95% and the specific activities were always > 2000 Ci/mmol. The entire purification procedure was realized in less than 30 min (retention times are listed in table 1). The conjunction of a normal phase followed by a reverse phase HPLC system appeared to be a powerful purification process which could certainly be used for other molecules.

Based on the two-step synthesis process and the purification procedure described above, we have developed a remote control system (Figure 1) for the routine production of ^{18}F-Et-Sp for clinical use. No-carrier-added aqueous [^{18}F]fluoride (in 1 ml of K_2CO_3 solution, 1 mg/ml) prepared by the $^{18}O(p,n)^{18}F$ reaction and isolated by anion exchange resin was added to an open pyrex vessel containing 6 mg of K_2CO_3 and 22 mg of Kryptofix (K/2.2.2). The water was removed under a nitrogen stream at 100°C, 1 ml of acetonitrile was added and the mixture was dried in the same conditions. A solution of 30 mg of 3 in 1 ml of acetonitrile was added to the dried K[^{18}F] and the mixture was kept under reflux for 5 min. The crude mixture was loaded onto a silica gel column prepared by hand (250 mm x 10 mm, silica gel 60, 230-400 mesh) and eluted with pentane - ether (4 - 1) at a flow rate of 8 ml/min. The peak corresponding to 4 was collected in the second reaction vessel kept at 85°C and containing a solution of spiperone (4 mg), K_2CO_3 (2 mg) and kryptofix (7 mg) in 1 ml of acetonitrile. A gentle stream of nitrogen was used to evaporate the solvents. The residue was dissolved in 1.5 ml of HPLC solvent 1 and loaded onto the first HPLC column (Lichrosorb Si-60). The peak corresponding to 5 was collected in the third vessel kept at 80°C and the solvents were removed using a stream of nitrogen. The residue was dissolved in 1.5 ml of HPLC solvent 2 and loaded onto the second HPLC column (Lichrosorb RP Select-B). The peak corresponding to 5 was collected and conditioned for injection. The overall yield was 10% (EOS) after 110 min.

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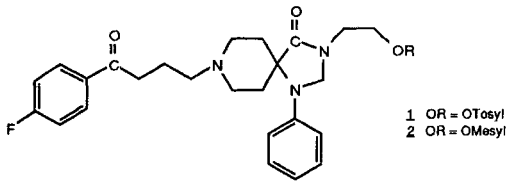

SCHEME 1

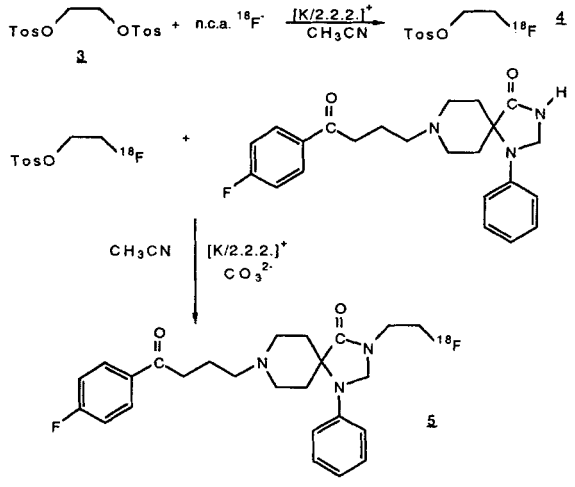

TABLE 1

HPLC retention times of ^{18}F-Et-Sp, ^{18}F-Et-O-alkylated spiperone (O-alkyl.) and spiperone (Sp) in min.

	Lichrosorb Si-60
^{18}F-Et-Sp	11.3
O-alkyl.	6.2
Sp	> 30
	Lichrosorb RP Select-B
^{18}F-Et-Sp	12

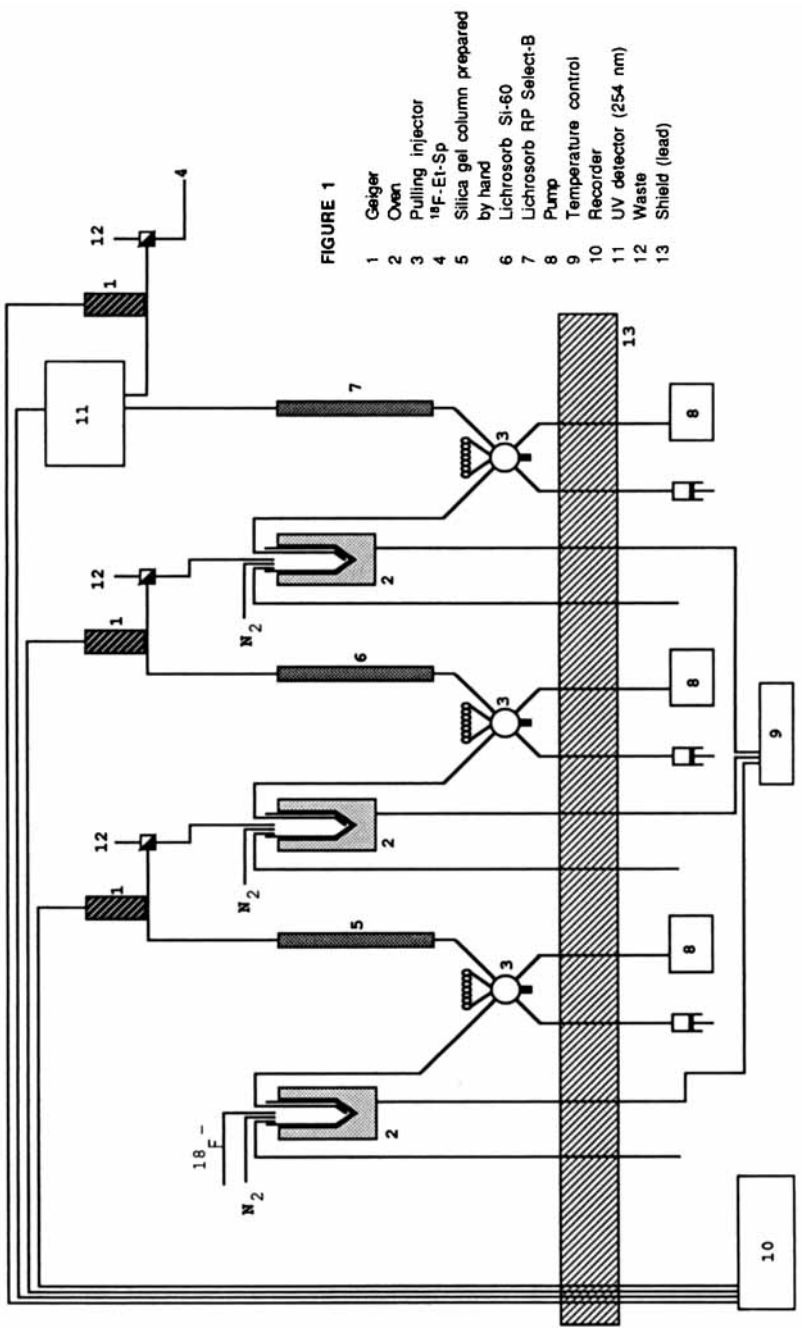
FIGURE 1
1 Geiger
2 Oven
3 Pulling injector
4 18F-Et-Sp
5 Silica gel column prepared by hand
6 Lichrosorb Si-60
7 Lichrosorb RP Select-B
8 Pump
9 Temperature control
10 Recorder
11 UV detector (254 nm)
12 Waste
13 Shield (lead)
18 F-
N2
N2
N2

Synthesis of Fluorine-18 Fluoroalkyl Pindolol Derivatives: Ligands for the ß-Adrenergic Receptor.

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[I-125]Iodocyanopindolol, an antagonist for the ß-adrenergic receptor, has been shown to accumulate in vivo in areas rich in ß-adrenergic receptors, presumably through saturable receptor mediated binding[1]. In order to perform PET studies of the ß-adrenergic receptor in the heart and lung we have prepared fluoroalkyl analogs of iodocyanopindolol and are evaluating these compounds for this purpose.
The synthesis of suitably 2,3-substituted 4-hydroxyindoles, as required for fluoroalkyl pindolol derivatives, represented a considerable synthetic challenge and finally required a synthetic pathway starting with 2-substituted 1,3-cyclohexane dione derivatives. Using this sequence we have prepared the two derivatives 1 and 2 containing good leaving groups and these can be fluorinated with fluorine-18 fluoride. Acid hydrolysis removes the protecting acetates to give the fluorine-18 ligands 3 and 4. The mono substituted compounds 2 and 4, the synthetic pathway for which is shown in scheme 1, are considerably easier to prepare than the di-substituted derivatives 1 and 3 which are prepared in an essentially similar manner but starting with 3-bromo-4-oxopentyl acetate. The sequence has the advantage that using the commercially available enantiomerically pure glycidyl tosylates both enantiomers of the compounds can be prepared. With both enantiomers available many of the assumptions intrinsic in any modeling can be tested. The in vitro association constants of the racemic compounds are being determined and their suitability for the imaging of the ß-adrenergic receptor in vivo is being assessed.

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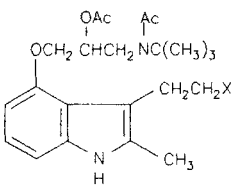
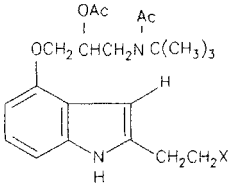
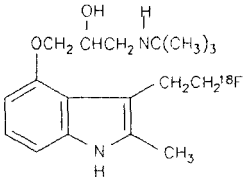


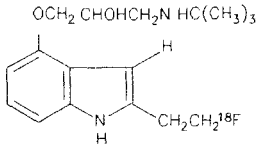
(1)



(2)



(3)



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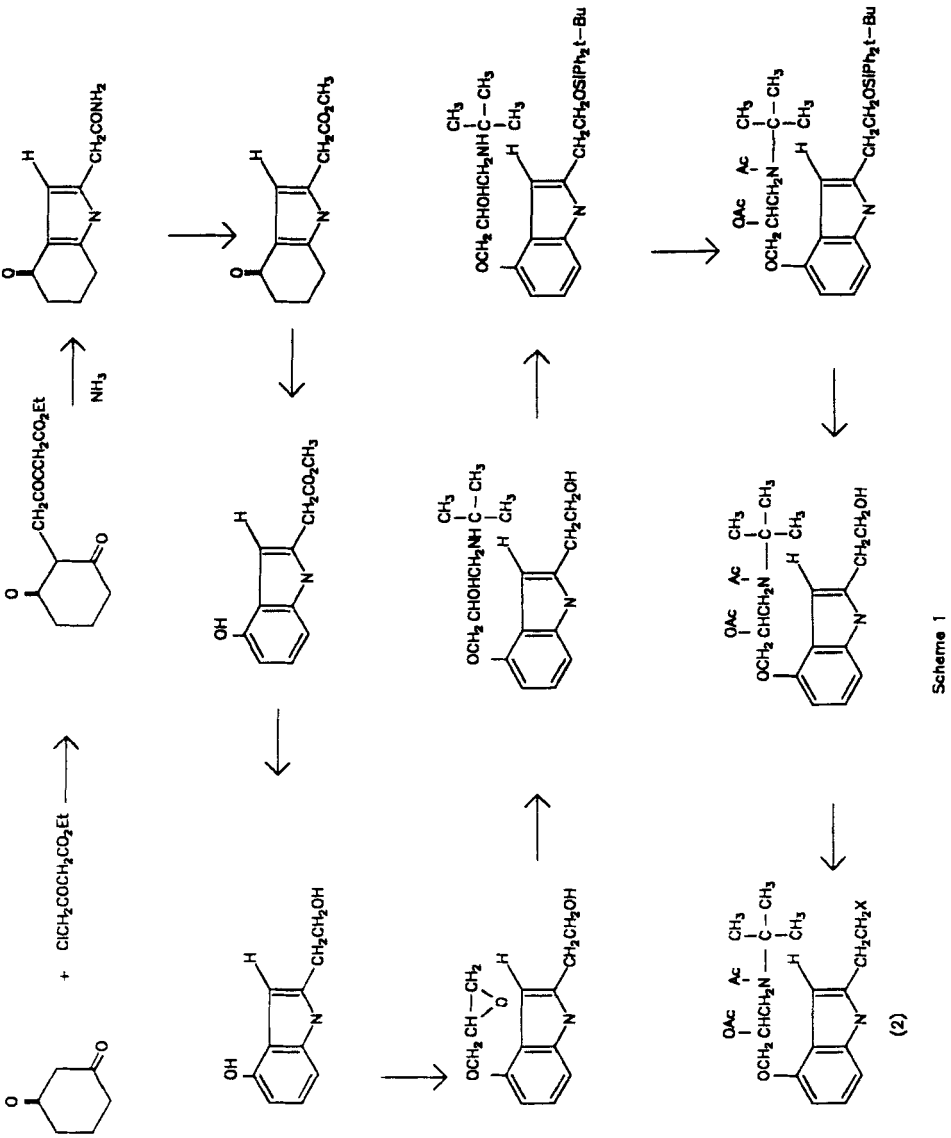

Scheme 1

SYNTHESIS OF (±)-I-125-IODOBENZOVESAMICOL -- A CHOLINERGIC NEURON MARKER.

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A number of mapping agents for the adrenergic neuron were developed in our laboratory during the 1980's. We are now focusing efforts on developing similar markers for the cholinergic neuron. Vesamicol, a potent inhibitor of vesicular acetylcholine storage, has been adopted as a basis for the design of a presynaptic cholinergic nerve marker. SAR studies and kinetic data suggest that vesamicol binds stereospecifically to the outside of the vesicle membrane at a locus distinct from the active site of the acetylcholine transporter (1). Parsons and coworkers recently reported an elegant study of the acetylcholine blocking activity of 84 analogs of vesamicol (2). One of these analogs, benzovesamicol (BVM), is equipotent with vesamicol and displays remarkable bulk tolerance in the 5-position. Racemic 5-amino-BVM was synthesized and converted to the (±)-5-iodo analog via the diazonium salt. Solid-state radioiodide exchange labeling in the presence of ammonium sulfate gave (±)-[I-125]-5-IBVM in 80% radiochemical yield following silica Sep-Pak purification. Radiochemical purity was 99%; specific activity was >140 Ci/mmol. Preliminary in vivo screening results are presented in Table 1. The 4 hr brain distribution is consistent with the known pattern of cholinergic innervation. Pretreatment of mice with cold (±)-vesamicol lowered striatal and cortical accumulation 52-53%.

Since the binding of vesamicol is highly stereospecific, the enantiomers of (±)-IBVM were resolved by preparative TLC of their diastereomeric (S)-(-)-a-methoxy-a-(trifluoromethyl)phenyl acetates [(S)-(-)-MTPA] as depicted in Figure 1. Diastereomeric purities are >98% as determined by ^{1}H-NMR (360 MH$_2$). Studies of the enantioselectivity of [I-125]-5-IBVM are in progress.

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Figure 1 **Resolution of (±)-5-Iodobenzovesamicol**

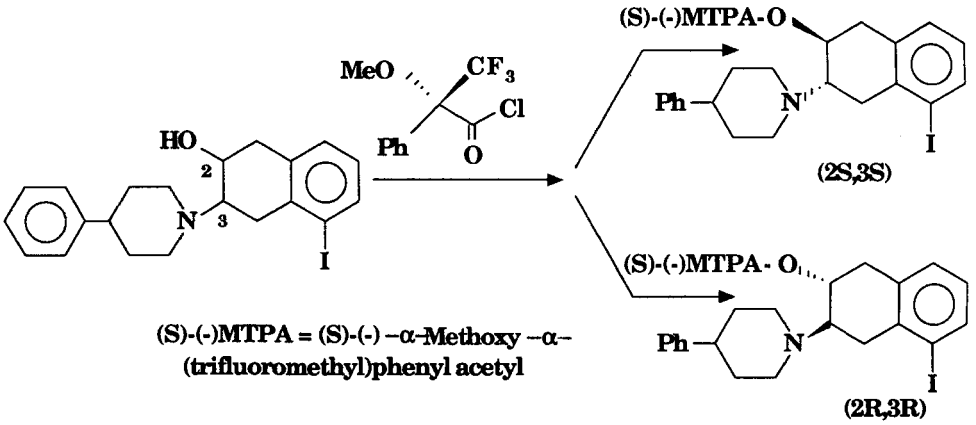

TABLE 1

BRAIN DISTRIBUTION OF 5-[^{125}I]IODOBENZOVESAMICOL IN MICE*

	CONCENTRATION (% Dose/g)				TISSUE RATIOS	
GROUP	STRIATUM	CORTEX	CEREBELLUM	BLOOD	STRIATUM CEREBELLUM	CORTEX CEREBELLUM
Control	4.12±.65	1.46±.25	0.26±.06	1.01±.70	15.8	5.6
Blocked‡	1.97±.35	0.69±.13	0.24±.10	0.009±.002	8.2	2.9

*N=5 per group. Mice (CD-1) weighed 19-21 g. Concentrations are normalized to a 20g mouse.

‡Blocking dose (0.53 mg/kg, ip) of (±)-vesamicol was given 10 min prior to i.v. tracer injection; all animals were killed 4 h after tracer injection. Controls received an equal volume of vehicle.

‡In vivo autoradiography in rat 90 min after i.v. injection of 5-[^{125}I]IBVM confirms the selective accumulation of tracer in the striatum.

SYNTHESES OF FLUORINE-18 LABELED INHIBITORS OF GABA (γ-AMINOBUTYRIC ACID) REUPTAKE

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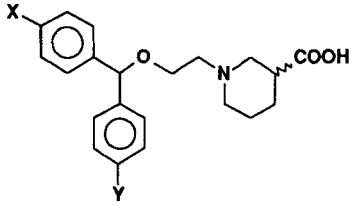
GABA (γ-aminobutyric acid) is a major inhibitory neurotransmitter. GABA is involved in the control of neuronal activity in the central nervous system, and dysfunctions of the GABA system have been implicated in a number of neurological disorders, particularly epilepsy and other seizure disorders. To study the GABA system in vivo using PET, previous investigators have prepared a variety of radiolabeled ligands known to bind to the GABA/benzodiazepine receptor complex. In order to examine the presynaptic physiology of the GABA system, and perhaps assess the degeneration of GABAergic neurons in disease, we have now prepared fluorine-18 radiotracers with demonstrated affinity for the presynaptic reuptake system for GABA. The compounds, diarylmethoxyalkyl derivatives of the amino acid nipecotic acid (1,2,3), are sufficiently non-polar to penetrate the blood-brain barrier, and are potent in vivo inhibitors of GABA reuptake.

We have prepared fluorine-18 labeled derivatives bearing one (I) or two (II) fluoroaryl rings, as well as a bis(trifluoromethyl) derivative (III, CI-966 (3)). All three radiotracers were prepared by the condensation of the appropriate fluorine-18 labeled benzhydryl chloride with N-(2-hydroxy)nipecotic acid methyl ester, followed by acid hydrolysis to the free amino acid. The preparation of 4-[^{18}F]fluorobenzhydryl chloride and 4-[^{18}F]fluoro-4'-fluorobenzhydryl chlorides has been previously reported (4,5). The bis(trifluoromethyl) derivative was prepared as follows: NCA [^{18}F]fluoride ion was reacted with 4-(bromodifluoromethyl)-4'-(trifluoromethyl)benzophenone (prepared by bromination of the corresponding difluoromethyl compound) to yield the fluorine-18 labeled bis(trifluoromethyl)benzophenone (40-60%yield), which was then reduced ($LiAlH_4$) and chlorinated ($SOCl_2$) to yield the necessary 4-([^{18}F]trifluoromethyl)-4'-(trifluoromethyl)-benzhydryl chloride. The final products (I,II, and III) were all obtained in NCA form in high specific activity (>2000 Ci/mmol). All intermediates and final products were characterized by HPLC and/or TLC and compared to authentic standards (1). The final amino acids were obtained in racemic forms (stereoisomers at the carboxylic acd position for all three compounds; isomers also at the benzylic position for compound I). As more potent GABA inhibitory activity resides in the R(-) isomer of nipecotic acid (1,3), resolution of the final products may give improved radiotracers for in vivo studies.



1 $X = H, Y = {}^{18}F$
2 $X = F, Y = {}^{18}F$
3 $X = CF_3, Y = CF_2{}^{18}F$

These radiotracers form a new approach to the in vivo study of the GABA system. Preliminary in vivo study of regional brain distribution in mice shows low but adequate brain penetration, and a distribution not inconsistent with the distribution of GABA-ergic nerve terminals.

Acknowledgements. This work supported in part by National Institutes of Health Grant NS15655 and Department of Energy Grant DE-AC02-76EV02031.

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RADIOSYNTHESIS OF [*O-METHYL*-^{11}C]VIQUALINE THROUGH AN *N*-TRITYL PROTECTED INTERMEDIATE FOR EVALUATION AS A PET RADIOLIGAND FOR 5-HT RE-UPTAKE SITES

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

The isoquinoline derivative, viqualine, is a potent inhibitor of 5-HT re-uptake.[1,2] Our calculation suggests viqualine also has modest lipophilicity. Thus viqualine might be expected to show a high ratio of specific to non-specific binding *in vivo*. We therefore considered that the labelling of viqualine with carbon-11 might provide a radioligand for PET studies of 5-HT re-uptake sites. The structure of viqualine (Scheme I) suggested the possibility to introduce carbon-11 at the *O*-methyl group using nca [^{11}C]iodomethane as labelling agent.

The direct methylation of *O-desmethyl*viqualine (I) hydrochloride (4 mg) with nca [^{11}C]iodomethane in DMSO (500 μL) with sodium hydroxide (5 M, 10 μL) as base for 5 min at 80 °C yields a mixture of radioactive products. ^{13}C-NMR spectroscopy on the products obtained by running the reaction with ^{13}C-enriched (90 atom %) iodomethane in place of [^{11}C]iodomethane indicated that the main product is from methylation at the piperidino-nitrogen. No conditions could be found to provide viqualine as major product from the direct reaction of *O-desmethyl*viqualine (I) with either [^{13}C]iodomethane or [^{11}C]iodomethane. Hence, to provide for the possibility of higher radiochemical yield and ease of separation in the radiosynthesis of [*O-methyl*-^{11}C]viqualine (III), we sought to protect the piperidino-nitrogen of *desmethyl*viqualine (I) during methylation.

The trityl group can be used to protect phenolic hydroxyl groups during the [^{11}C]methylation of aliphatic hydroxyl groups,[3] since it exhibits excellent stability to strong base and yet is easily removeable by acid. We were therefore interested to assess its suitability for the protection of the piperidino-nitrogen in *O-desmethyl*viqualine (I).

*O-Desmethyl*viqualine (I) hydrochloride was treated with trityl chloride and triethylamine in dichloromethane at 18 °C for 4 h and the products separated by silica gel chromatography (AcOEt/pentane, 3:7 by vol). The second major product to elute was characterised by ^{1}H- and ^{13}C-NMR spectroscopy and mass spectrometry as the desired *piperidinyl-N*-trityl derivatine (II). Treament of the *N*-trityl derivative (II) (3.5 mg) with nca [^{11}C]iodomethane in DMSO (400 μL) containing sodium hydroxide solution (5 M, 10 μL) at 110 °C for 6 min, followed by brief (3 min) treatment with hydrochloric acid (1 M; 100 μL) gives [*O-methyl*-^{11}C]viqualine (III) as major radioactive product (*ca* 27 %). Work up of the preparation on a C18 Sep-Pak followed by HPLC on a silica gel column (30 cm X 0.7 cm i.d.) eluted at 2.5 mL/min with ethanol/dichloromethane (1/3 v/v) provides radiochemically and chemically pure [*O-methyl*-^{11}C]viqualine (III) as assessed by TLC, HPLC and mass spectrometry. The product (III) is formulated for *i.v.* injection by removal of HPLC solvent, dissolution of the residue in normal saline for injection (0.9% v/v; 9.8 mL) plus ethanol (0.2 mL) and finally millipore filtration. By this method useful activities of nca [*O-methyl*-^{11}C]viqualine (III) have been obtained for biological evaluation. Early results indicate that [*O-methyl*-^{11}C]viqualine (III) has low uptake into rat brain, but progressively accumulates in certain regions, including thalamus, olfactory lobes, cingulate cortex and caudate putamen.

In conclusion the chemistry described here, as well as providing carbon-11 labelled viqualine for biological assessment as a potential PET radioligand, demonstrates that the trityl group, because of its great stability to base and rapid removal by acid, is excellent for *N*-protection during the [^{11}C]alkylation of phenols.

Acknowledgement. The authors are grateful to Drs C. Gueremy and A. Noble (Rhone Poulenc) for useful discussions and the provision of *O-desmethyl*viqualine and viqualine.

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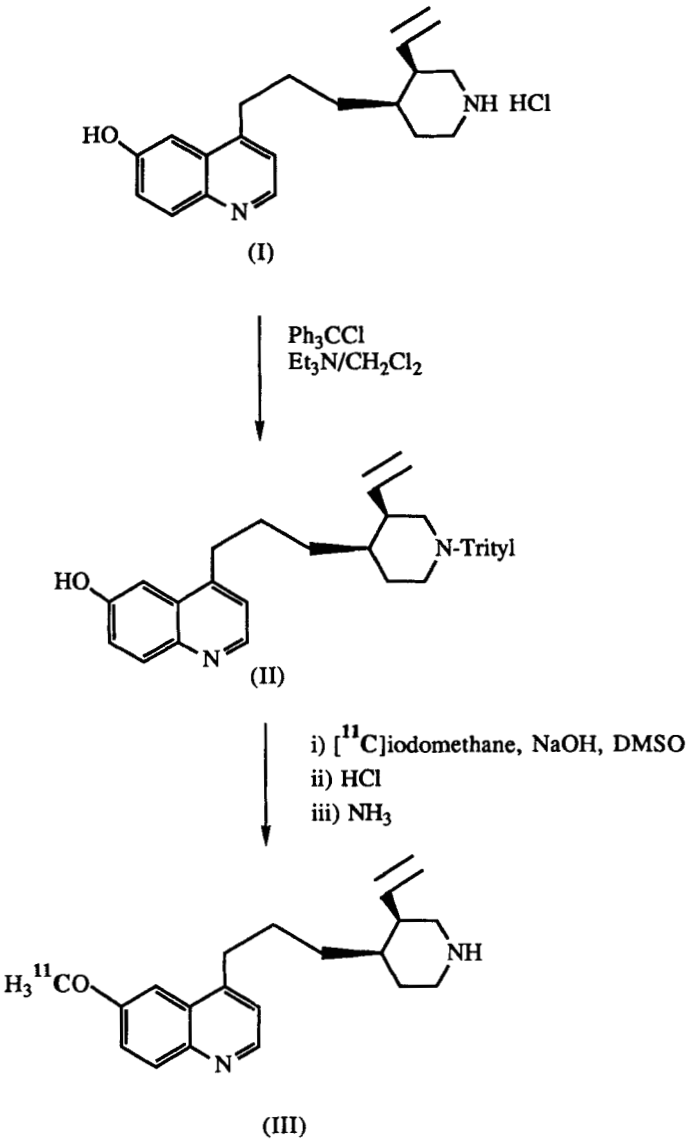

Scheme 1. The preparation of [*O-methyl*-^{11}C]viqualine (III)

CITALOPRAM: LABELLING WITH ^{11}C AND EVALUATION IN RAT AS A POTENTIAL PET RADIOLIGAND FOR 5-HT RE-UPTAKE SITES

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Much *in vitro* and *in vivo* evidence supports the involvement of a dysfunction in serotonergic neurotransmision in clinical depression. Some hypotheses specifically implicate abnormal levels of either pre-synaptic 5-HT re-uptake sites or post synaptic $5HT_2$ receptors. Clearly, suitably radiolabelled markers of the serotonergic system might allow the aetiology of depression to be investigated using PET. The recently marketed antidepressant, citalopram, is one of the most potent and selective of the known inhibitors of 5-HT re-uptake.[1] Its lipophilicity ($Log_{10}P$) calculates to be modest (1.3) suggesting that it might give relatively low non-specific binding *in vivo*. For these reasons we chose to evaluate the potential of carbon-11 labelled citalopram as a radioliogand for PET studies of 5-HT re-uptake sites.

In vivo autoradiography showed a differential regional localisation of [*N-methyl*-^{3}H]citalopram in rat brain which correlated with the localisation of specific binding sites defined *in vitro*. A comparison of the biodistribution over a 2 h period after *i.v.* injection of [*N-methyl*-^{3}H]citalopram in (1) control rats (2) rats pre-dosed with either citalopram or paroxetine and (3) rats chemically-lesioned with *p*-chloroamphetamine gave an estimate of specific binding relative to total binding *in vivo*. The ratio of binding in certain regions (*e.g.* cingulate) to binding in a reference tissue (*e.g.* cerebellum) at 30-120 min post injection was *ca* 1.4. In view of these results we set out to label citalopram with carbon-11 for PET studies.

Nca [^{11}C]iodomethane was prepared conventionally from cyclotron-produced [^{11}C]carbon dioxide by reduction to [^{11}C]methanol with lithium aluminium hydride in THF and treatment with hydroiodic acid. Reaction of [^{11}C]iodomethane with *N-desmethyl*citalopram hydrochloride (5 mg, 14 μmol) in ethanol (400 μL) containing 2,2,6,6-tetramethylpiperidine (3 μL) for 5 min at 95 °C gives [*N-methyl*-^{11}C]citalopram in up to 60% radiochemical yield, decay-corrected (Scheme 1). HPLC of the crude product on a silica gel column (30 X 0.7 cm i.d.) eluted with chloroform/methanol (9/1 by volume) at 3 mL/min provides radiochemically and chemically pure [*N-methyl*-^{11}C]citalopram, as assessed by TLC, HPLC and mass spectrometry. The radioactive product is formulated for *i.v.* injection by evaporation of solvent, dissolution in normal saline for injection (9.8 mL; 0.9% v/v) plus ethanol (0.2 mL) and finally millipore filtration. From the carbon-11 generated in an irradiation of nitrogen (200 psi) with a 30 μA beam of 19 MeV protons for 30 min the preparation yields *ca* 2 GBq of product with a specific activity of *ca* 15 GBq/μmol at 40 min from EOB.

In biological experiments [*N-methyl*-^{11}C]citalopram behaves as [*N-methyl*-^{3}H]citalopram. Both compounds are racemates. However only one of the enantiomers has high affinity for the re-uptake site. The *N-desmethyl* analogue of the biologically active enantiomer has recently become accessible. We have applied the above radiosynthesis to this enantiomer and have found that the product gives enhanced specific signal in the rat following *in vivo* administration, the ratio of uptake in regions of interest relative to cerebellum approaching 2 at 90 min after *i.v.* injection of [*N-methyl*-^{11}C]citalopram.

Acknowledgement. The authors are grateful to Drs J. Hyttel and K. Bogeso of H. Lundbeck and Co A/S for kindly providing [*N-methyl*-^{3}H]citalopram, citalopram and related compounds.

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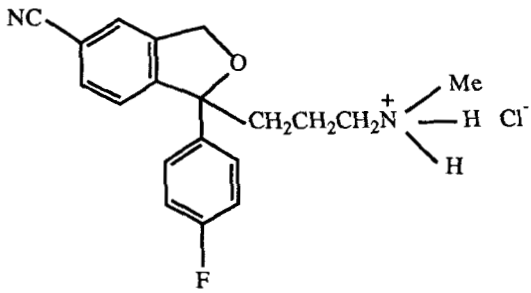
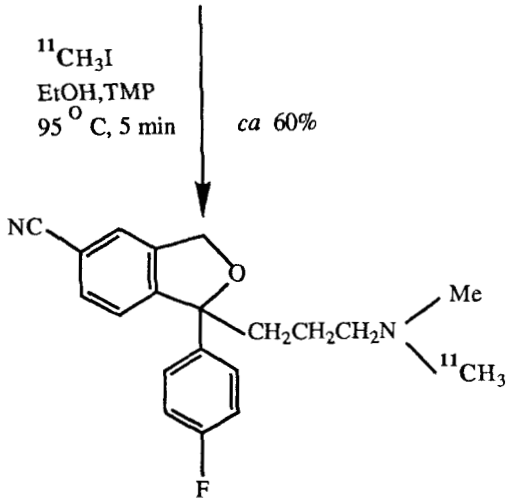


N-Desmethyl-citalopram hydrochloride (5 mg)



[*N-methyl*-^{11}C]CITALOPRAM

Scheme 1. Radiosynthesis of [*N-methyl*-^{11}C]citalopram

HALOGENATED COCAINE ANALOGS FOR PET AND SPECT STUDIES.

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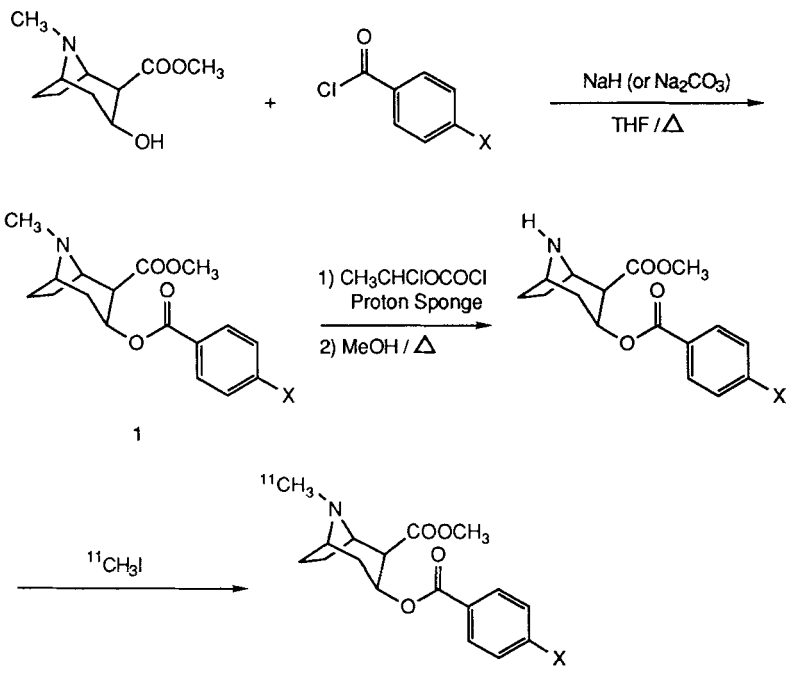
As part of our program in substance abuse [*N*-^{11}C-methyl]cocaine has been prepared and studied in baboons and in humans.[1-5] While the behavior of labeled cocaine itself is of intrinsic interest in defining the neurochemical factors responsible for cocaine's euphorogenic and reinforcing properties, labeled cocaine is also of interest as a marker for the dopamine transporter complex and may be useful in tracking the progress of neurodegenerative diseases like Parkinson's disease.[6]

We report here the synthesis of fluorine and iodine phenyl substituted derivatives of cocaine in order to examine the sensitivity of the parent molecule to structural modification and to explore the possibility of labeling this molecule with the longer lived nuclides fluorine-18 and iodine-123 for PET and SPECT studies. These halogenated cocaine derivatives were labeled with carbon-11 to compare their regional distribution and kinetics to those of the parent molecule. *p*-Fluorococaine and *p*-iodococaine were prepared by reaction of ecgonine methyl ester with the appropriate halogenated benzoyl chloride. *N*-Demethylation followed by methylation with [^{11}C]H_3I produced the labeled fluoro and iodo cocaines (**2** and **3**) (**Scheme 1**).[7] Following intravenous injection in baboons,

Scheme 1

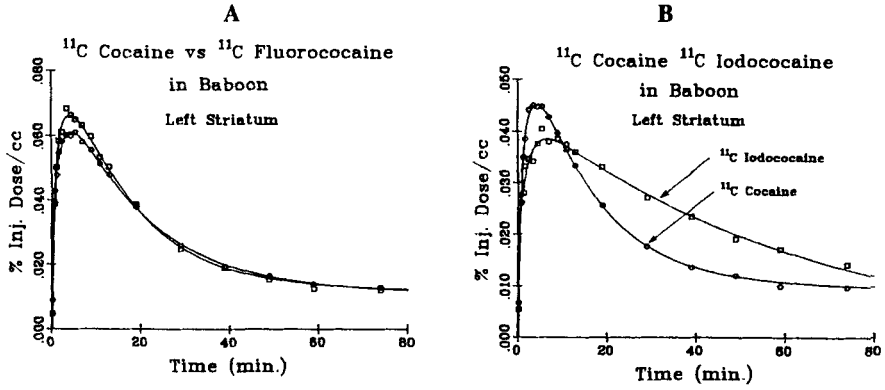


1

2, X = F
3, X = I

[*N*-^{11}C-methyl] *p*-fluorococaine showed almost identical regional distribution and kinetics to cocaine itself with the highest uptake occuring in the corpus striatum, bilaterally, while the carbon-11 labeled *p*-iodo derivative showed a different regional distribution and a different rate of uptake and clearance (**Figure A-B**). Radioactivity was distributed throughout all cortical regions including frontal, parietal, temporal and occipital cortices, as

Figure A and B



well as in subcortical structures including the corpus striatum (bilaterally) and thalamus. Analysis of arterial plasma showed that [^{11}C]iodococaine was also more rapidly metabolized than either [^{11}C]cocaine or [^{11}C]fluorococaine (**Table 1**).

Table 1

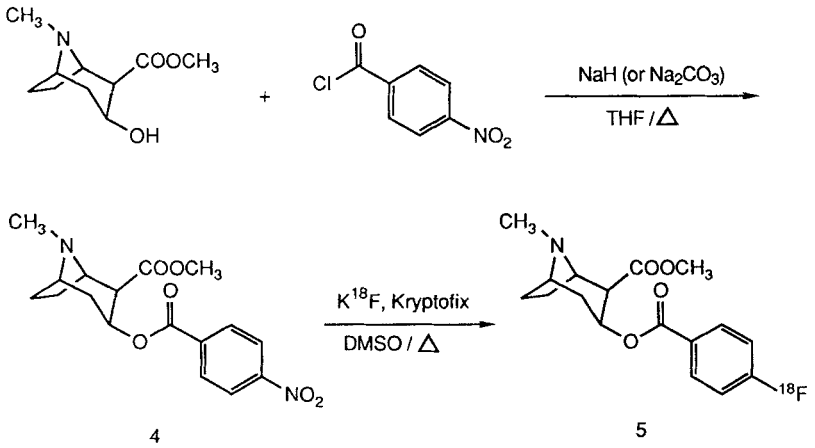
Baboon Plasma Metabolites

	Unchanged (%) Time (minutes)			
Compounds	1 min.	5 min.	10 min.	30min
^{11}C-Cocaine	90	62	45	15
^{11}C-*p*-Fluorococaine	97	84	56	39
^{11}C-*p*-Iodococaine	89	42	26	6

The fluorine-18 labeled cocaine molecule **5** may prove useful in studying the dopamine transporter complex and has the advantage of a convenient half life and potential distribution from a regional center, it can be prepared from *p*-nitrococaine (**4**) using the nucleophilic aromatic substitution reaction (**Scheme 2**).

This study exemplifies the ability of many small molecules to tolerate fluorine substitution on an aromatic ring with minimal perturbation of the biological properties of the parent molecule in contrast to iodine substitution where the regional binding and kinetics bear little resemblance to those of the parent molecule. Thus the assumption that

Scheme 2



4 5

SPECT studies using PET analogs are valid must be considered on an individual basis recognizing the changes that can occur in pharmacokinetics and sites of action.

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CHEMICAL AND ENZYMATIC APPROACHES FOR ^{11}C-LABELLED OCTOPAMINE SYNTHESIS USING HYDROGEN [^{11}C]CYANIDE

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Octopamine, the β-hydroxy derivative of tyramine, has been the objects of growing interest as biogenic trace amine. Studies using [^{3}H]-p- and m-octopamine have shown that they are both taken up in noradrenergic nerve terminals, accumulated in storage vesicles, and released together with noradrenaline on stimulation (1). [^{11}C]-p- and m-Octopamine have been synthesized from [^{11}C]HCN in a two-step sequence. Chemical and enzymatic approaches were used for the production of the [^{11}C]cyanohydrin intermediates as the key step. The enantiomeric composition of the labelled products obtained through the enzymatic process was assayed by HPLC.

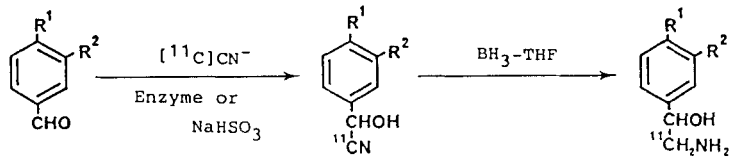
Chemical Approach. p-Hydroxy- and m-hydroxybenzaldehyde (**I** and **II**) were converted to their [^{11}C]cyanohydrins (**III**) via the addition of $NaHSO_3$ and NCA [^{11}C]-NaCN (2). The cyanohydrins were subsequently reduced with BH_3-YHF complex to the desired [^{11}C]aminoalcohols (**IV** and **V**), after purification by reversed phase HPLC, in radiochemical yields of 1.2% and 2.3% respectively at EOS. The entire procedure required about 40 min.

Enzymatic Approach. This approach is an extension of our previously reported method for the synthesis of [^{11}C]phenylethanolamine (3). The enzyme-catalyzed cyanohydrin reactions were carried out by adding the aldehyde (**I** and **II**) and enzyme [1-2 unit, hydroxymandelonitrile lyase toward (**I**), mandelonitrile lyase toward (**II**)] to a solution of 0.05 M 50% methanolic acetate buffer (pH 5.4) containing NCA [^{11}C]HCN. The [^{11}C]cyanohydrins (**III**), obtained after evaporation of the extracts from the enzyme reaction mixtures, were subsequently reduced with BH_3-THF complex to the [^{11}C]octopamines, which were purified by reversed phase HPLC. The enzyme contaminants were occasionally detected in the final preparations. Several methods were taken to avoid contamination of the enzyme in the final products. These included ion-exchange chromatography, gel permeation chromatography, Sep-Pak columns, immobilized enzyme column by absorption on ECTEOLA-, AVICEL- or DEAE-cellulose. These methods, however, were not always favorable in respect of reproducibility and simplicity. Of the commercially available ultrafiltration units we tested, the use of the Ultrasart 20 ultrafiltration unit under pressure proved to be most suitable. Thus addition of this procedure prior to the extraction step of the enzyme reaction mixtures eliminated the enzyme contamination in the final preparation. Radiochemically pure [^{11}C]-p- and m-octopamine (**IV** and **V**) were obtained in average radiochemical yields of 0.7-0.9% at EOS, and with a specific activity of 20-50 Ci/mmol. The total preparation time was around 50-60 min, counted from the start of the enzymatic reaction. There was no observable formation of the [^{11}C]cyanohydrin of m-hydroxybenzaldehyde (**II**) when the Sorghum enzyme was used in place of the almond enzyme, and the almond enzyme did not accept p-hydroxybenzaldehyde (**I**) as the substrate.

Direct separation of enantiomers for racemic octopamines was achieved by HPLC using analytical Crownpak CR(+) column without derivatization: The R-enantiomers were eluted prior to the S-enantiomers. This technique was used for the determination of the enantiomeric excess of the labelled products. The result is shown in Table 1. The absolute configuration for the resolved enantiomers was determined by HPLC analysis after derivatization with (R)-(+)-MTPA of the enantiomerically enriched material. The configuration of the each diastereomeric MTPA derivatives isolated was determined by ^{1}H-NMR analysis. It is evident that

the addition process of cyanide to aldehyde by enzymes from two different plants principally takes place on the opposite enantiotopic sides of the aldehyde molecule.

Several attempts involving the use of p- and m-acetoxybenzaldehyde as the substrate in the enzymatic step were also made to improve the labelling efficiency of the procedure without significant success.



(I) R^1=OH, R^2=H
(II) R^1=H, R^2=OH
(III)
(IV) R^1=OH, R^2=H
(V) R^1=H, R^2=OH

Table 1. Enantiomeric Purity of [^{11}C]Octopamines

Enzyme*	Octopamine	Enantiomeric excess(%)
HM	(S)-[^{11}C]-p-octopamine	92
M	(R)-[^{11}C]-m-octopamine	42

*HM: Hydroxymandelonitrile lyase(EC 4.1.2.11) from Sorghum seedings
M: Mandelonitrile lyase(EC 4.1.2.10) from almonds

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ENZYMATIC SYNTHESES OF SOME ^{11}C-LABELLED ANALOGUES OF L-TYROSINE AND L-TRYPTOPHAN

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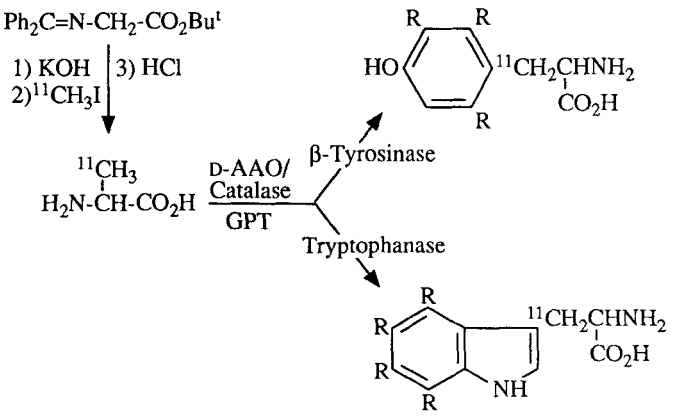
In the elucidation of biochemical processes by use of positron emission tomography (PET), the multi-tracer approach can be valuable. In previous work, we have been studying the dopaminergic and serotonergic neurosystems by use of ^{11}C-labelled L-DOPA and 5-hydroxy-L-tryptophan, respectively.[1] We have now developed the syntheses of several analogues of tyrosine and tryptophan, labelled with ^{11}C in the β-position, which are of interest for use in similar applications.

The syntheses were carried out by a combination of conventional organic synthetic methods and enzymatic transformations.[2,3] DL-[3-^{11}C]Alanine was prepared by alkylation of a glycine derivative, *N*-(diphenylmethylene)glycine *tert*-butyl ester, with [^{11}C]methyl iodide followed by hydrolysis with hydrochloric acid. The racemic ^{11}C-alanine was then used in combination with the appropriate substrate in the enzymatic reactions to form the labelled amino acids, Scheme 1. The multi-enzymatic syntheses were performed in a one-pot procedure, using D-amino acid oxidase (D-AAO)/catalase, glutamic-pyruvic transaminase (GPT), and β-tyrosinase or tryptophanase. The different analogues produced are presented in table 1.



Scheme 1.

Table 1. The β-^{11}C-labelled amino acids synthesized from DL-[3-^{11}C]alanine.

Substrate	Amino acid synthesized	Radiochemical yield (%) a	b
2-Fluorophenol	3-Fluoro-L-tyrosine	54	30
3- " -	2- " -	56	-
4-Fluorocatechol	6-Fluoro-L-DOPA	40	20
4-Methylindole	4-Methyl-L-tryptophan	15	-
5- " -	5- " -	56	30
6- " -	6- " -	53	-
7- " -	7- " -	26	-
5-Fluoroindole	5-Fluoro-L-tryptophan	43	-
6- " -	6- " -	48	-

a) Crude product, based on [3-^{11}C]alanine.
b) Purified product, based on [^{11}C]O_2.

The decay corrected radiochemical yields of the unisolated amino acids ranged from 15 to 56 %, based on [3-^{11}C]alanine. Purification of the amino acids can be carried out by reversed phase HPLC, and has been performed for 3-fluoro-L-tyrosine, 6-fluoro-L-DOPA, and 5-methyl-L-tryptophan. The total synthesis time, including purification, was 50 min, counted from the start of the [^{11}C]methyl iodide synthesis. The enantiomeric purities were assessed for 3-fluorotyrosine and 5-methyltryptophan to be over 99 % L.

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TWO SYNTHETIC ROUTES FOR THE ASYMMETRIC SYNTHESIS OF [β-^{11}C]AMINO ACIDS WITH HIGH ENANTIOMERIC PURITIES.

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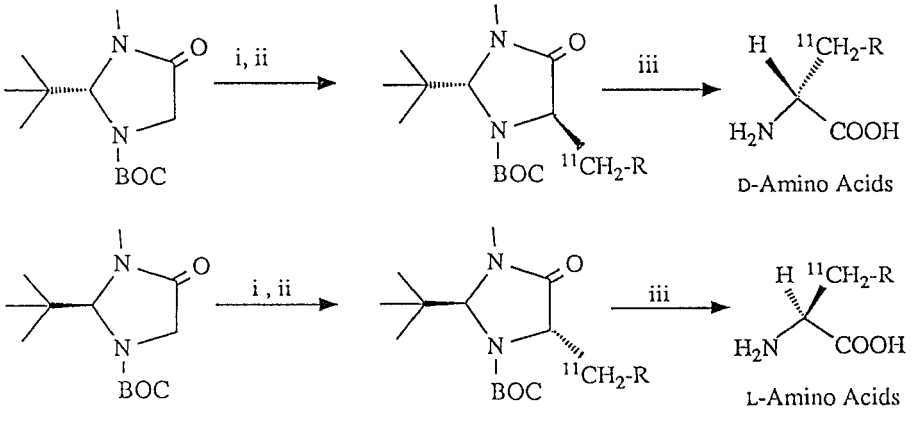
Many compounds of interest in PET are chiral and it is therefore desirable to synthesize them in any of their stereochemical forms. The most convenient way to achieve this is to establish the proper stereochemistry before the labelling-synthesis is carried out, as in the synthesis of D- and L-[methyl-^{11}C]methionine.[1] When this can't be performed, the use of asymmetric synthesis, either by conventional chemical reactions[2] or by enzymatic catalysis,[3] is a suitable method for obtaining the compounds with the desired stereochemistry. Another approach is the resolution of racemic mixtures, which has a consequential drawback that half of the radioactivity is lost in the resolution procedure.[4]

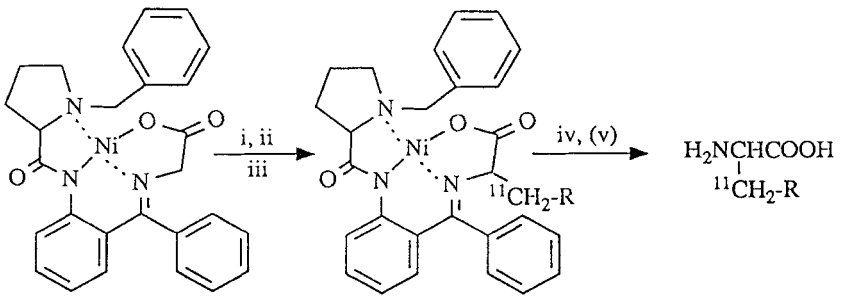
Routes for the asymmetric synthesis in [^{11}C]-labelling of amino acids have been of interest because it presents the possibility to prepare either enantiomer in high radiochemical yields. Though enzymes have been shown to be useful, a disadvantages is that some enzymes are not easily available and that it could be difficult to produce the labelled amino acids in both enantiomeric forms or to synthesize some non-natural amino acids.

The asymmetric synthesis of some [β-^{11}C]-amino acids by highly stereoselective alkylations of imidazolidinone glycine derivatives[5] with ^{11}C-labelled alkyl iodides is presented (Scheme 1). [β-^{11}C]Labelled alanine and phenylalanine enriched in the L or D-form were obtained, after acid hydrolysis, in 20 and 75% radiochemical yields (decay corrected) within 25 and 50 min respectively. The radiochemical purities were higher than 98 % and the enantiomeric purities 98% enantiomeric excess (e.e.).



Scheme 1. i, BuLi/TMP in THF, -78 °C ; ii, R-$^{11}CH_2$-I ; iii, 9M HCl, 200 °C

A chiral glycine Ni-complex[6] was employed in the asymmetric synthesis of L-[β-^{11}C]-amino acids (Scheme 2). Alkylation reactions with ^{11}C-labelled alkyl iodides under mild conditions followed by acid hydrolysis gave the amino acids in 12-60% radiochemical yields and with higher than 98% radiochemical purity. The results are summarized in table 1.



Scheme 2. i, NaOH(s)/Acetone; ii, R$^{11}CH_2$I; iii, MeOH; iv, HCl/H_2O; v, HI
R= (a) H; (b) Ph; (c) Ph-OMe; (d) Ph-OH.

Table 1. Asymmetric induction, radiochemical yields and synthesis times for the syntheses of the [β-^{11}C]-labelled amino acids.

[β-^{11}C]-Labelled Amino Acid	Asymmetric Induction[a] % e.e.	Radiochemical Yield[b] %	Synthesis Time[c] min
Alanine	80	60	30
Alanine[d]	>99[d]	40	50
Phenylalanine	90	30	40
O-Metyltyrosine	90	15	45
Tyrosine	90	12	55

[a] L.c. analysis after derivatisation with N-(5-fluoro-2,4-dinitrophenyl)-L-alanineamide.
[b] Decay corrected and based on the amount of [^{11}C]CO_2 released from the molecular sieves.
[c] From the start of releasing [^{11}C]CO_2 to the purified product.
[d] After preparative HPLC of the diastereoisomers.

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(b) Antoni G. and Långström B., *Acta Chem. Scand. B*, **41**, 511 (1987).

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SYNTHESIS OF [^{11}C]N-METHYL TETRAHYDROAMINOACRIDINE, A POTENT ACETYLCHOLINE ESTERASE INHIBITOR.

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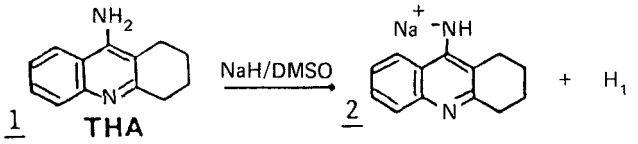
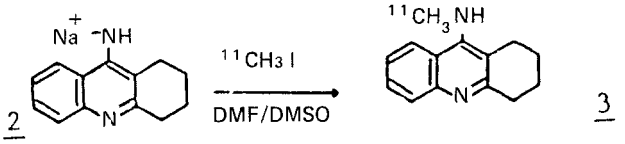
Tetrahydroaminoacridine (THA) is a potent central acting acetylcholine esterase (AChE) inhibitor which might be used as therapeutic agent in the treatment of Alzheimer's disease (AZD) (1). In order to study the AChE activity in the brain by PET, we selected N-Methyl THA, a potent AChE inhibitor (2), as a potential radioligand. In this paper we report the synthesis and labelling of N-methyl THA with [^{11}C]methyl iodide.
The process took place in four stages.
In a first place the sodium amide of THA 2 was synthesized just before the begining of the radioactive synthesis : to a solution of THA 1 was added sodium hydride in dry dimethylsulfoxide. After heating at 100°C for 2 min, a fraction of this solution containing the compound 2 was transfered in dry dimethylformamide.



1 THA 2

[^{11}C]methyl iodide was synthesized by standard procedures from $^{11}CO_2$ (3). Methylation occured on sodium amide of THA 2. The [^{11}C]methyl iodide was distilled in the vial containing the compound 2 in DMSO/DMF. At the end of the $I^{11}CH_3$ trapping, the solution was heated at 100° for 10 min.



2 3

$^{11}CH_3$THA

After heating, the reaction mixture containing 3 was purified by HPLC and the appropriate fraction collected. Following evaporation and sterile filtration, the [^{11}C]N-Methyl THA was ready for biomedical application.
The same procedure was applied to the synthesis of N-Methyl THA and "cold" methyl iodide was used as methylating agent. The N-Methyl THA was characterized by mass spectrometry and $^1_1$H NMR.
A 30 mn irradiation of nitrogen by a 20 MeV proton beam (30 µA intensity), under 8 bars pressure, yielded approximately 3.7 GBq (100 mCi) of injectable $^{11}CH_3$-THA with a specific radioactivity of about 35 GBq/µmol (950 mCi/µmol) at the end of synthesis (total time of synthesis : 45 min from EOB).
Studies are now in progress in our laboratory to further characterize the pharmacology of [^{11}C]N-Methyl THA binding in baboon brain, in view of its application to AZD patients.

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SYNTHESIS OF [^{11}C]CITALOPRAM AND BRAIN DISTRIBUTION STUDIES IN RATS.

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The central serotoninergic system has been implicated in several neuropsychiatric disorders (1,2), and is extensively investigated in affective disorders and in suicide victims (3). One particular index of serotoninergic function, the imipramine binding sites, has been a focus of particular attention. High affinity imipramine binding sites are found in the brain, and are located primarily on serotoninergic terminals. A decrease in imipramine binding sites in platelets has been reported to be a biological marker for major depression (4).

Citalopram [1-(3-dimethylamino)propyl-1-(p-fluoro-phenyl)-5-phthalancarbonitrile] is a selective serotonin uptake inhibitior, and this prototype drug possesses high affinity (Ki = 0.7 nM) for serotonin uptake sites (5) and is used in the treatment of depression. The study of serotonin uptake sites in the living human brain by Positron Emission Tomography (P.E.T.) with [^{11}C]citalopram (^{11}C-half life 20.4 min) may be valuable in investigating the anatomic locus and the therapeutic role of depression and prevention of suicide. For this purpose, we have synthesized [^{11}C]citalopram by alkylation approach. The procedure involves the reaction of [^{11}C]iodomethane with desmethylcitalopram in acetone in the presence of sodium hydroxide base at 65°C for 8-10 min, and followed by purification by column, which contained, in series silica gel and basic alumina produces pure [^{11}C]citalopram with a specific activity of 150-434 Ci/mmol (at EOS). The radiochemical yields were 18% to 66% (at EOB), with a radiochemical purity range 92% to 99%.

In vivo biodistribution of [^{11}C]citalopram in Sprague-Dawley rats brain clearly differentiates regions of high (frontal cortex, substantia niagra and hypothalamus) and low (cerebellum) uptake corresponding to the known distribution of serotonin uptake sites in rats and primates. These results demonstrate that this ligand is suitable for study of serotonin uptake sites by P.E.T., and may be useful as a biochemical diagnostic imaging tool in various psychiatric disorders. Further studies with this ligand are in progress.

This work was supported by the Department of Radiology, Duke University Medical Center.

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Supporting Data

Chemistry and Results:

[^{11}C]Citalopram was prepared by reaction of [^{11}C]iodomethane with desmethylcitalopram as described in abstract. The radiochemical yields and purity data for labeled citalopram prepared on six (N = 6) separate occasions using the method described are given in Table 1. The radiochemical yields obtained in acetone were 18% to 66% based on the [^{11}C]iodomethane, with a radiochemical purity range 92% to 99%. The time required for the unoptimized manual synthesis and purification is 42-45 minutes from the end of bombardment. The final product, [^{11}C]citalopram, was characterized on the basis of its chromatographic behavior (HPLC, TLC) by comparing it with an authentic sample of [^{12}C] citalopram (H. Lundbeck & Co. A/S, Copenhagen, Denmark].

Table 1. Physicochemical data of [^{11}C] citalopram prepared by reaction of [^{11}C]CH_3I and desmethylcitalopram.

Entry	Substrate[b] (μmole)	Base[c] (μmole)	Reaction time(min)	Radiochemical Yield[a](%)	Radiochemical Purity(%)
1	8.64	120	8	66	95
2	3.17	60	10	43	92
3	1.73	40	8	34	95
4	1.73	56	10	20	94
5	1.73	60	8	37	93
6	1.44	48	8	18	99

[a]Radiochemical yields are corrected to EOB and based on [^{11}C] CH_3I.
[b]Norcitalopram. HCl is used as starting material.
[c]4N-NaOH is used as base.

On HPLC (Adsorbosphere, 10 μm silica gel column, size 4.6 X 250 mm), citalopram and desmethylcitalopram had retention times of 2.56 min and 6.57 min, respectively, using $CH_3CO_2C_2H_5$:CH_3OH:28% NH_4OH (4:0.9:0.1;V/V) as the mobile phase with a flow rate of 2 ml/min. Separated components were monitored by U.V. absorption at 280 nM. However, radioHPLC of the labeled reaction products shows two peaks at 1.72 min and 2.62 min, respectively. On the basis of HPLC and TLC analysis of cold citalopram, the second peak at 2.62 min was assigned [^{11}C]citalopram. The minor product with the short retention time (1.72 min) has not yet been identified. The R_f values for cold citalopram and desmethylcitalopram were 0.48 and 0.21, respectively [silica gel, E. Merck plates, $CHCl_3$:CH_3OH:28% NH_4OH (5:0.9:0.1; V/V]. The R_f value of radiolabeled citalopram was the same as with authentic compound. The radiochemical purity of [^{11}C] labeled citalopram was determined by HPLC (Perkin Elmer LC-4 model) using the procedure described by Dannal et al.

1985 (6). The specific activity of [^{11}C]citalopram was found to be 150-434 Ci/mmole [at the end of synthesis].

Biodistribution of [^{11}C]citalopram in rat brain

Sprague-Dawley rats (260-700g) were anesthetized with ketamine (15 mg/kg, I.P.) and sodium pentobarbital (35 mg/kg, I.P.) and the skin covering the femoral vein incised and retracted. The [^{11}C] citalopram (doses 220—336 μCi) was injected into the femoral vein, the skin was closed with wound clips and the animals were kept warm. After 30 minutes, animals were decapitated by guillotine and brains were rapidly removed and dissected on ice into eight sections (frontal cortex, nucleus accumbens, anterior caudate, septum, hypothalamus, amygdala, substantia nigra and cerebellum). Each section was placed in a thin-walled plastic vial, weighed and counted in NaI well counter and Nuclear Data Inc. 65 multichannel analyzer. The distribution of [^{11}C] citalopram in these tissues was counted and is shown, expressed as ratio, (counts/mg of tissue)/(injected dose μCi/wt of animal)X 10^3 in table 2 and table 3.

Table 2 Brain regional distribution of [^{11}C] citalopram in rats (n=7)

Name of tissue	Ratio (In control rats) Mean ± SD
Frontal Cortex	93.05±25.22
Nucleus Accumbens	90.02±30.62
Anterior Caudate	81.07±24.34
Septum	90.48±28.57
Hypothalamus	94.72±24.11
Amygdala	82.45±24.24
Substantia Nigra	102.07±26.10
Cerebellum	76.76±22.41

Table 3. Brain regional distribution of [^{11}C]citalopram in rats (n=4) at 30 min after treatment with cold citalopram.

Names of tissue	Ratio (in control rats) mean ± SD	Ratio (in cold citalopram treated rats) mean ± SD
Frontal Cortex	111.47±18.44	80.21±15.69
Anterior caudate	96.85±13.75	81.00±5.85
Septum	105.80±6.87	91.50±13.20
Hypothalamus	107.78±8.58	94.76±9.89
Amygdala	102.18±3.89	78.62±13.31
Substantia Nigra	115.75±6.00	99.50±15.25
Cerebellum	66.42±6.87	52.16±6.86

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SYNTHESIS OF IODOVINYLMISONIDAZOLE FOR IMAGING HYPOXIA

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Misonidazole and its derivatives are metabolically trapped by viable hypoxic tissues. This property allows the imaging of hypoxic tissue in tumors and in ischemic heart and brain by PET using F-18 labeled fluoromisonidazole.(1) Our desire to develop a misonidazole derivative for SPECT has led to the synthesis of iodovinylmisonidazole (IVM, (E)-5-(2-nitroimidazolyl)-4-hydroxy-1-iodopent-1-ene), which has been labeled with I-131. The preparative scale synthesis of IVM has been accomplished in a 10% overall yield, from (E)-1,2-bis(tributylstannyl)ethylene (1). The lithium anion of 1, prepared by reaction with n-BuLi in THF at -78°C under an argon atmosphere, was treated with one equiv. of $BF_3{\cdot}Et_2O$. The resulting solution was then treated with glycidyl tosylate to afford (E)-1-tributylstannyl-5-tosyl-4-hydroxypent-1-ene (2) in 50% yield after silica column chromatography (1:4 EtOAc/hexanes). The use of the Lewis acid, $BF_3{\cdot}Et_2O$, was critical to the success of the reaction. This reagent has been previously reported to successfully promote the nucleophilic addition of simply substituted organolithiums to epoxides and oxetanes.(2) Treatment of 2 with powered NaOH in monoglyme at RT for 1 h gave (E)-4,5-epoxy-1-tributylstannylpent-1-ene (3, 76% yield) after silica chromatography (1:9 EtOAc/hexanes). The labeling precursor, (E)-5-(2-nitroimidazolyl)-4-hydroxy-1-tributylstannylpent-1-ene (4) was synthesized in 50% yield by the nucleophilic ring opening of the epoxide 3 with 2-nitroimidazole in analogous fashion to the synthesis of F-MISO. Treatment of the labeling precursor with sodium iodide using hydrogen peroxide-acetic acid (2:1) in sodium acetate buffer (pH 4.5) gave IVM radiochemically pure (25-35% radiochemical yield, specific activity approx. 400 Ci/mmol) in 2 h after reverse phase HPLC (Whatman M9/25 ODS-3, 1:1 $EtOH/H_2O$). Preliminary biological evaluations show that the uptake of IVM in hypoxic cells is equivalent to that of FMISO.

This research was supported by USPHS grants, HL38736 and CA34570.

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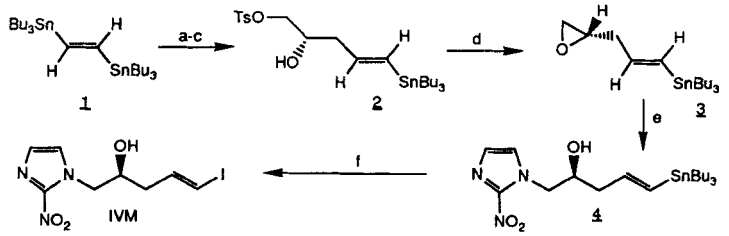

a)BuLi (1 eq); b)$BF_3{\cdot}Et_2O$; c)2(S)-(+)-glycidyl tosylate; d)NaOH(s); e) 2-nitroimidazole, KOH, DMF/CH_3CN; f)NaOAc buffer, pH 4.5, NaI, $H_2O_2/HOAc$

SYNTHESIS OF ORGANOBORANE POLYMERS FOR USE IN THE PREPARATION OF NITROGEN-13 LABELED AMINES.

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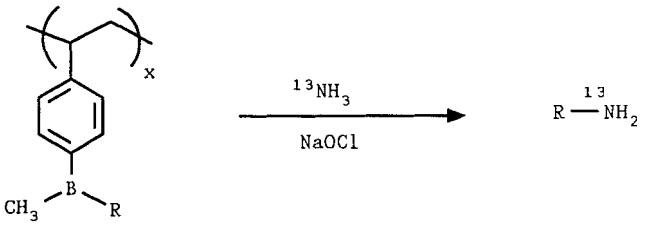
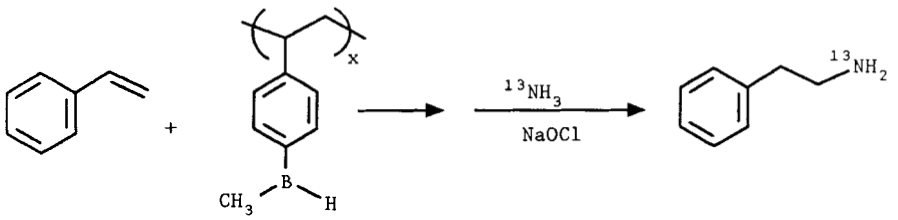
Organic amines labeled with short-lived, positron-emitting isotopes have proven valuable in nuclear medicine research (1,2). However, nitrogen-13 has been used only sparingly to label physiologically active amines due to its short half-life. Organoboranes can now be utilized to introduce a variety of positron emitting nuclides into functionally substituted materials via the direct reaction of trialkylborane with the appropriate, cyclotron-produced intermediates (3). The reaction has been used to successfully prepare nitrogen-13 labeled alkylamines (4).

We wish to report the synthesis of polymeric organoborane derivatives which can be used to prepare nitrogen-13 labeled amines. The polymers are polystyrene-based methylboranes which take advantage of the fact that methyl and phenyl groups do not readily participate in the amination reaction (5,6).



The polymeric reagents afford efficient utilization of the alkyl portion of the borane moiety; an important consideration in instances in which the alkene is expensive and/or not readily available. In addition, the reaction is regioselective, affording only the 1-aminoalkanes. Significantly, the polymer is solid and insoluble in standard reaction media which makes it an ideal candidate for automated radiopharmaceutical generators.



Research funded by DOE (DE-FG05-86ER60434) and NIH (GM-39081).

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[^{18}F]-FLUCONAZOLE: SYNTHESIS, BIODISTRIBUTION IN RATS, AND IMAGING OF RABBITS.

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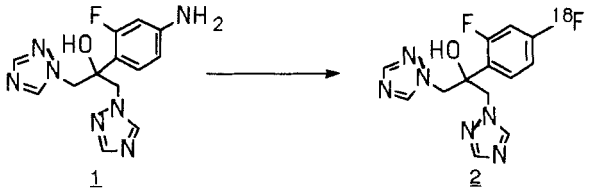
Flucanozole [FL] is a new antifungal agent effective in the treatment of systemic yeast infection. To study the in vivo pharmacokinetics of FL the drug was labeled with 18F by a modified Schiemann reaction on the amino precursor 1 (Scheme 1).



1 2

Scheme 1

Water (2 mmol), 50% HBF4 (0.2 mmol), 1 (0.033 mmol),and aqueous $NaNO_2$ (0.033 mmol) were stirred at 0°C for 15 min. A solution of ^{18}F in $H_2{}^{18}O$ (1.1 ml) was added to the diazonium salt of 1, the solution was stirred for 15 min. and evaporated under argon at 50°C. The residue was decomposed at130°C for 30 min. and purified on NH_2 SEP PAK. The time required for synthesis and purification was 2.5 h (from EOB) and the yield was 0.33% to 2.66% (decay corrected EOB). The radiochemical purity of 2 was ascertained by TLC (>95%, SG/Al in CH_2Cl_2:MeOH, 9:1, R_f of 2 0.42). The synthesis of 1 is shown in Schme 2.

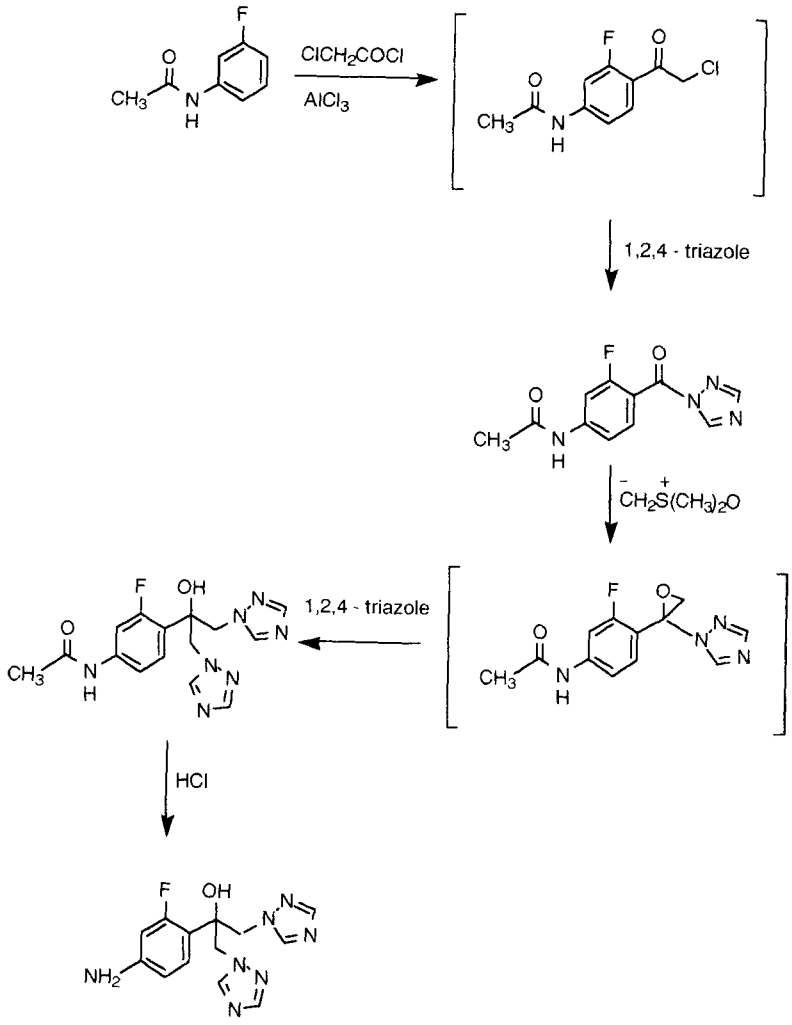
The biodistribution of F-18[FL] was determined in rats at 5,30,60, and 120 min (% dose/gm).

Organ	5min	30min	60min	120min
blood	0.44	0.51	0.33	0.22
liver	2.57	2.58	2.87	3.40
brain	0.16	0.23	0.29	0.14
kidney	0.65	0.57	0.41	0.51
bone	0.39	0.43	0.43	0.43
muscle	0.26	0.33	0.34	0.31

When pharmacolgical doses are administered, these concentrations are in excess of that required to inhibit growth of invading yeast.

Whole body PET images of rabbits injected with F-18[FL] revealed the highest uptake in the liver and kidneys, and demonstrable accumulation in the other organs as well.

This approach will allow us to delineate the in vivo distribution of FL in a variety of disease states.



Scheme 2

FLUORINE-18 LABELED TETRAHYDROCANNABINOL: SYNTHESIS AND PET STUDIES IN A BABOON.

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Cannabinoids, the active components of marijuana are known to be psychotic (1-3). The most active components of this class of compound are delta-9-tetrahydrocannabinol (Δ^9-THC) and its delta-8 isomer. While Δ^8-THC and Δ^9-THC have similar psychotic activity, Δ^8-THC is more stable than its Δ^9 analog. Recently, several cannabinoids are found to have high binding affinity to the brain (4,5). However, little is known about the mechanisms of their actions (6). In order to study its pharmacokinetic in animals, we have synthesized fluorine-18 labeled 5'-fluoro-Δ^8-THC and studied its distribution in mice and in a baboon brain.

Fluorine-18 labeled 5'-fluoro-Δ^8-tetrahydrocannabinol (5'-[^{18}F]fluoro-Δ^8-THC;2) was synthesized by the nucleophilic fluorination of the triflate (1) with K[^{18}F]/Kryptofix followed by deprotection and purification with HPLC (Beckman Ultrasphere semi-preparative silica gel column, 10x250mm, hexane:EtOAc, 95:5, 3ml/min) in 8% yield in a sysnthesis time of 90 minutes from EOB (Scheme 1). The uptake of 5'-[^{18}F]fluoro-Δ^8-THC in mouse tissues was high at 5 minutes, but radioactivity then declined rapidly in all tissues studied (Table 1). Pretreatment of mice with Δ^8-THC had no effect on the distribution of 5'-[^{18}F]fluoro-Δ^8-THC in different regions of the brain. Following I.V. administration, 5'-[^{18}F]fluoro-Δ^8-THC uptake in the baboon brain was similar in the striatum, thalamus and cerebellum, and the clearance from these regions was relatively rapid. Radioactivity in the base of the skull corresponding perhaps to the sella turcica increased with time, indicating *in vivo* defluorination may have occurred (Fig. 1). 5'-[^{18}F]fluoro-Δ^8-THC was also metabolized in baboon plasma rapidly. These results suggest that the uptake of 5'-[^{18}F]fluoro-Δ^8-THC in mouse and in baboon brain is probably non-specific. The synthesis of other cannabinoids and the study of their specific binding sites in the brain is in progress.

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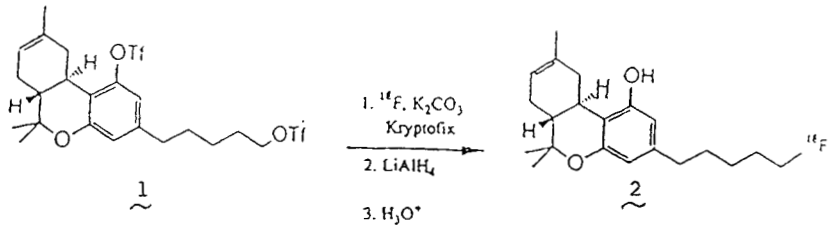
TABLE 1. Tissue Distribution of 5'-[^{18}F]Fluoro-Δ^8-Tetrahydrocannabinol in Mice

Mean ± S.E.M. (n = 4)
Time After Injection

Organ	5 Minutes		30 Minutes		60 Minutes	
	%/Organ	%/Gram	%/Organ	%/Gram	%/Organ	%/Gram
Brain	1.07 ± 0.14	2.23 ± 0.34	0.52 ± 0.05	1.07 ± 0.09	0.24 ± 0.01	0.52 ± 0.02
Blood	----	2.65 ± 0.21	----	2.27 ± 0.69	----	1.38 ± 0.12
Heart	0.88 ± 0.17	6.45 ± 1.10	0.19 ± 0.03	1.53 ± 0.15	0.10 ± 0.01	0.88 ± 0.06
Lungs	0.81 ± 0.12	4.82 ± 0.74	0.39 ± 0.17	1.72 ± 0.23	0.19 ± 0.05	1.15 ± 0.15
Liver	25.31 ± 2.73	20.07 ± 2.06	13.49 ± 7.90	9.08 ± 5.20	9.72 ± 0.60	7.44 ± 0.33
Spleen	0.41 ± 0.07	2.85 ± 0.43	0.16 ± 0.04	1.07 ± 0.09	0.10 ± 0.01	0.67 ± 0.09
Kidneys	3.17 ± 0.46	7.33 ± 0.85	0.75 ± 0.16	1.86 ± 0.26	0.41 ± 0.02	1.04 ± 0.08
Small Intes.	6.84 ± 2.74	3.42 ± 1.04	30.89 ± 13.56	16.08 ± 5.02	42.66 ± 20.19	22.92 ± 10.14
Femur	----	1.38 ± 0.31	----	1.50 ± 0.19	----	1.65 ± 0.26
Muscle	----	2.57 ± 0.30	----	1.06 ± 0.23	----	0.68 ± 0.19



Scheme 1 - Synthesis of 5′-[^{18}F]Fluoro-Δ^8-THC

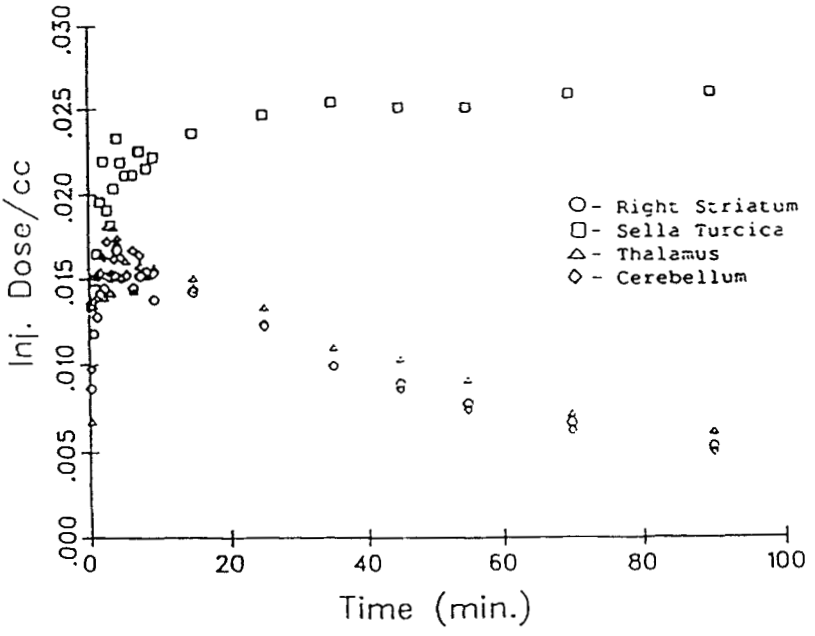


Fig. 1
Time Courses for 5′-[^{18}F]Fluoro-Δ^8-THC
Distribution to Baboon Brain

SYNTHESIS OF CARBON-11 LABELED ANALOGS OF VESAMICOL, A POTENT INHIBITOR OF VESICULAR UPTAKE OF ACETYLCHOLINE

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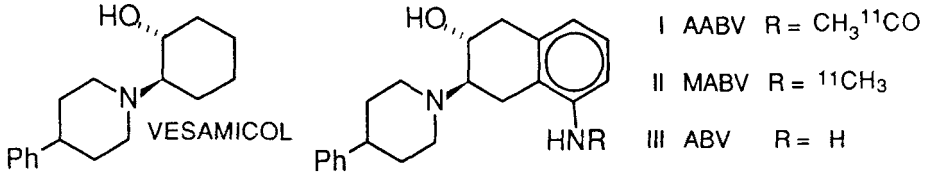
Vesamicol (AH5183) is a potent, noncompetitive inhibitor of the vesicular uptake of acetylcholine which binds to sites on cholinergic nerve terminal storage vesicles. It is of interest in PET and SPECT as a potential presynaptic marker for cholinergic neurons in vivo. Although vesamicol itself does not appear to be easily amenable to labeling with a poitron-emitting radionuclide, a closely related series of benzovesamicols have been recently identified which show great in vivo potency (1) and which would be suitable for radiolabeling with carbon-11 or fluorine-18. We describe here the synthesis of two carbon-11 labeled benzovesamicols (I,II) as potential PET tracers for the cholinergic system.



[^{11}C]Acetamidobenzovesamicol (AABV, I). The N-acetyl derivative of amino-benzovesamicol was prepared by reaction of aminobenzovesamicol (ABV,III, 2 mg) with [^{11}C]acetyl chloride (2) (300 µL of 10:1 THF: diisopropylethylamine, 2 min, 25°C) and purified by C-18 HPLC : $R_{t\ AABV}$ = 8.9 min, $R_{t\ ABV}$ = 12.8 min. Its chromatographic properties were identical to those of authentic AABV, as was the biological activity (1) of the same product isolated from a 10 µmol scale "cold" reaction. The final product (spec activity >130 Ci/mmol) was prepared in < 40 min synthesis time in an unoptimized yield of 4-9% (corrected, based on [^{11}C]CO_2).

[^{11}C]Methylaminobenzovesamicol (MABV, II). The N-methyl compound was prepared by alkylation of ABV with NCA [^{11}C]CH_3I (2.9 mg of ABV as camphorsulfonate salt, 250 µl DMF, 100-120 °C, 15 min). The product was isolated by HPLC (C_{18}, 3:1 CH_3CN : 0.1 M NH_4OAc, 1 ml/min: $R_{t\ ABV}$ = 3.3 min, $R_{t\ MABV}$ = 4.7 min, R_t N,N-dimethylamino-benzovesamicol = 8.0 min). The product co-chromatographed (HPLC as above: TLC, silica gel, 1:1 hexane:ethylacetate) with an authentic sample of MABV prepared by an different synthetic route. Overall yields from [^{11}C]CH_3I were 10-26% (corrected), with a specific activity of > 500 Ci/mmol.

[^{11}C]AABV and [^{11}C]MABV represent new approaches to the in vivo study of the cholinergic system via PET.

This work was supported by National Institutes of Health grants NS 24896, NS 22908 and NS 15655.

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MALONIC ESTER SYNTHESIS OF VARIOUS α-(^{11,14}C-METHYL)-CARBOXYLIC ACIDS AND RELATED COMPOUNDS

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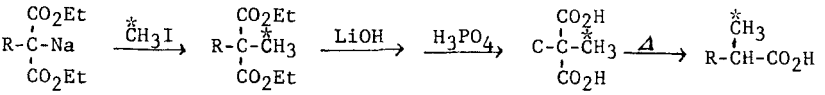
We continue the study of the malonic ester synthesis mainly of α-(^{11,14}C-methyl)-carboxylic acids and their behavior in mouse (1). Preparation of (2-^{11,14}C-ethyl)-ketones is also studied. This synthesis consists of three steps: methylation, hydrolysis and decarboxylation. Various conditions in them were examined to find out suitable procedure for ^{11}C labeling.



A Teflon mini-vial (5 ml content) set in a metal overcoat vessel in intimate contact was used for the synthesis, in which some pressurization is needed. The substrate was methylated with $^{*}CH_3I$ in a few varieties of solvents at various temperatures. An alkali hydroxide was added to the resultant solution, which was heated for de-esterification. The vial was then opened and the solvent evaporated. The residue, after the addition of H_3PO_4, was heated to give the final product.

The methylation was found to be completed for most substrates in 0.1 mol dm^{-3} tetrahydrofuran solution at 50 °C in 5 to 10 min. The yields are shown in Table 1. The yield decreased with the decrease of substrate concentration. For example, 0.01 and 0.001 mol dm^{-3} diethyl malonate substrate gave methylation yields of 84 % and 0.5 %, respectively. Higher temperatures were preferable for the methylation of diketones, such as acetylacetone (93 % yield at 80 °C in diethyleneglycol monomethylether) and benzoylacetone (34 % yield at 80 °C in THF). Suitable conditions in the hydrolysis were shown to be warming with LiOH to 120 °C for 5 to 10 min. Due to higher solubilities in organic solvents, LiOH is preferred to NaOH or KOH. Higher temperatures and/or prolonged heatings were required for the hydrolysis of phenyl-ethyl-malonate and cyclohexyl-methyl-malonate. Although the decarboxylation in an ordinary scale synthesis begins at about 160 °C almost independently of the substituent, heating to 180 °C in a closed vessel was necessary for the rapid labeling. The final product is accompanied with its unmethylated (non-radioactive) homologue and remaining intermediates, i.e., the dicarboxylic acid and its monoester. The intermediates can be eliminated by liquid chromatography, but rapid removal of the unmethylated homologue is difficult, especially in long chain carboxylic acids. It usually takes 40 to 60 min for the conversion of $^{*}CH_3I$ into the product ready for injection, with chemical yield of 70 to 80 %.

The product, usually racemic and with the unmethylated homologue, was injected into mouse, and the organ distribution was measured with the respiratory excretion of the radio-carbon. Various different features were observed in in vivo behavior of the α-(^{11,14}C-methyl)-carboxylic acids according to the chain length and substituent. Similar measurement was also undertaken for some related compounds labeled at the carboxyl position. Time course of the radio-carbon concentration in the brain is shown in Fig. 1. We are now in the stage of discussing the metabolism of these compounds to find out good utilization of the products of the malonic ester synthesis.

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Table 1. Reaction Yield

Substrate	Yield (%)		
	Methylation	Hydrolysis	Decarboxylation
$H\text{-}CH(CO_2C_2H_5)_2$	100	99	80
$CH_3\text{-}CH(CO_2C_2H_5)_2$	100	99	97
$C_2H_5\text{-}CH(CO_2C_2H_5)_2$	100	99	100
$C_4H_9\text{-}CH(CO_2C_2H_5)_2$	100	99	97
$C_8H_{17}\text{-}CH(CO_2C_2H_5)_2$	100	98	97
$C_{14}H_{29}\text{-}CH(CO_2C_2H_5)_2$	99	99	100
$(CH_3)_2CH\text{-}CH(CO_2C_2H_5)_2$	95		
$C_{13}H_{27}CH(CH_3)\text{-}CH(CO_2C_2H_5)_2$	100	95	96
$CH_3O\text{-}CH(CO_2C_2H_5)_2$	92		
$cyclo\text{-}C_6H_{11}\text{-}CH(CO_2C_2H_5)_2$	100	33	97
$C_6H_5\text{-}CH(CO_2C_2H_5)_2$	85	32	98
$CH_3COCH_2CO_2C_2H_5$	100		

Reaction condition: methylation — 0.1 mmol substrate, in THF(1 ml), 50°C, 10 min; hydrolysis — (after addition of 1 mmol LiOH in 1 ml MeOH) 120°C, 10 min; decarboxylation — (after solvent evaporation and H_3PO_4 addition) 180°C, 10 min.

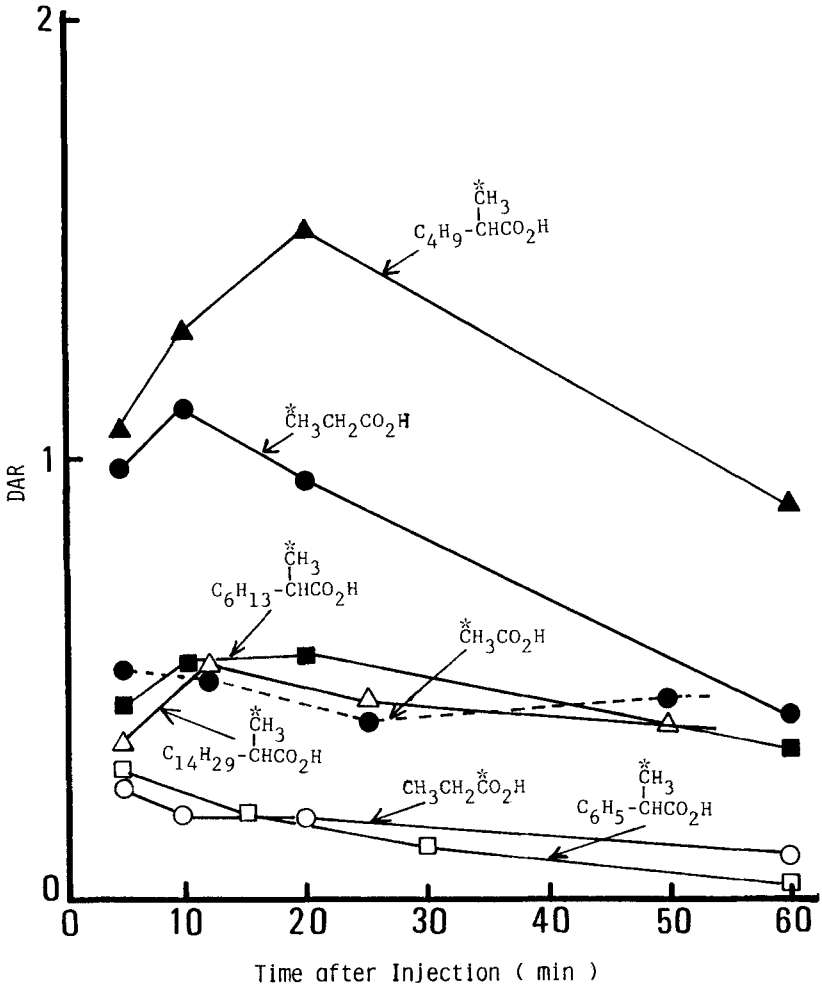

Fig. 1. Radio-carbon Concentration in Mouse Brain.

Biochemical Evaluation of Metyrapone Derivatives - Inhibition Kinetics and Affinity Studies

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Enzyme inhibition was studied with C-14-DOC as the substrate and bovine adrenocortical mitochondria as a source of 11ß-hydroxylase activity. Initial rate measurements of the formation of C-14-corticosterone were used to determine the kinetic parameters of the enzyme reaction. 4'-bromo-metyrapone was synthesized (1) and K_i-values determined for metyrapone and the 4'-bromo-derivative (2).

To study the effect of substitution on the inhibition kinetics of metyrapone, various derivatives were synthesized. Syntheses included the replacement of ring A by a phenyl-ring and derivatization at the ortho-position as well as modifications at C_2 with elimination of the geminal methyl-groups (Tab.1). 4'-bromo-metyrapone was reexamined and served as an internal standard of the biological assay system. Metyrapol and metyrapone-N-oxide were also included. The results of the inhibition studies obtained with these derivatives are presented in Fig.1. Metyrapone and metyrapol showed identical results (X_3 and X_{13}). Replacement of 3-pyridyl-ring A by a phenyl-ring had a positive effect (X_4). Substitution in ortho-position with Br, F, OCH_3 or OH (X_9 - X_{12}) showed a considerable increase in inhibitory potency, as observed previously with m-iodo-phenylmetyrapone (3). Modifications on ring B had a negative effect on enzyme inhibition as indicated by 4'-bromo-metyrapone (X_8) and the mono-N-oxide (X_{14}), the demethylated compound is practically ineffective (X_1).

High affinity mitochondrial binding sites for metyrapone were demonstrated using H-3-metyrapol as the tracer and metyrapol-concentrations between 1×10^{-9} to 1×10^{-5} M for binding. Different amounts of mitochondria were used, namely 0.25, 0.5 and 1 mg/ml. Scatchard analysis of experimental data is presented in Fig.2. The number of H-3-metyrapol-sites ranged from 0.5 to 0.7 nmol/mg protein, K_D-values between $2.0\text{-}3.9 \times 10^{-8}$ mol/L. This is in good agreement with values published by Satre (4).

Enzyme inhibition- and binding studies provide sensitive parameters for the characterization of metyrapone derivatives as suitable ligands for labelling.

This work was supported by the Fonds zur Förderung der wissenschaftlichen Forschung, Vienna 1090, Project No. P 5174

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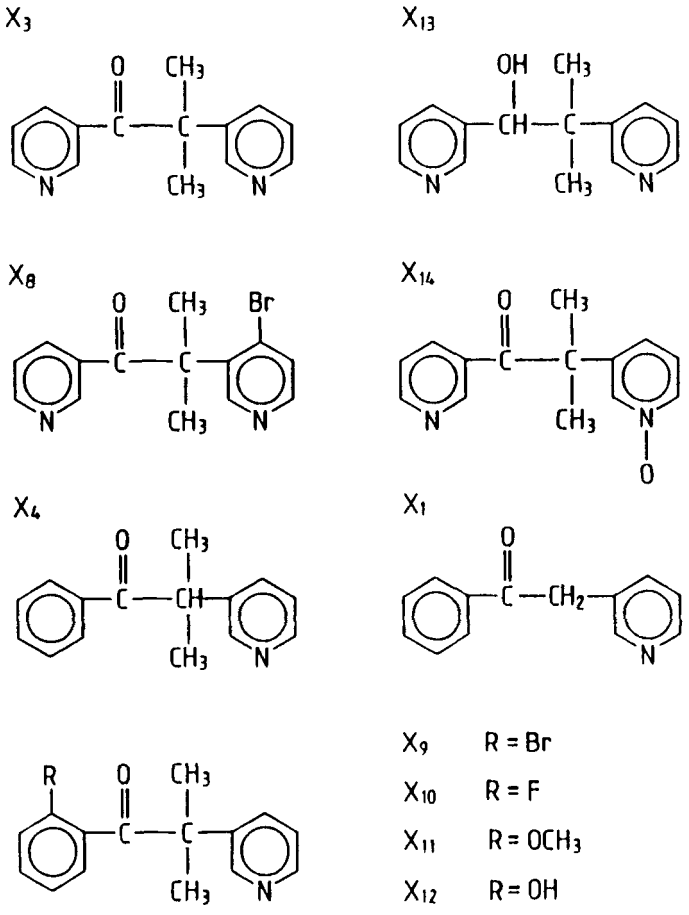

Tab.1 Derivatization of metyrapone: X_3 = metyrapone, X_{13} = metyrapol, X_8 = 4'-bromo-metyrapone, X_{14} = metyrapone-mono-N-oxide, X_4 = phenyl-metyrapone, X_1 = 2-nor-phenyl-metyrapone, X_9 = 2'-bromo-phenyl-metyrapone, X_{10} = 2'-fluoro-phenyl-metyrapone, X_{11} = 2'-methoxy-phenyl-metyrapone, X_{12} = 2'-hydroxy-phenyl-metyrapone

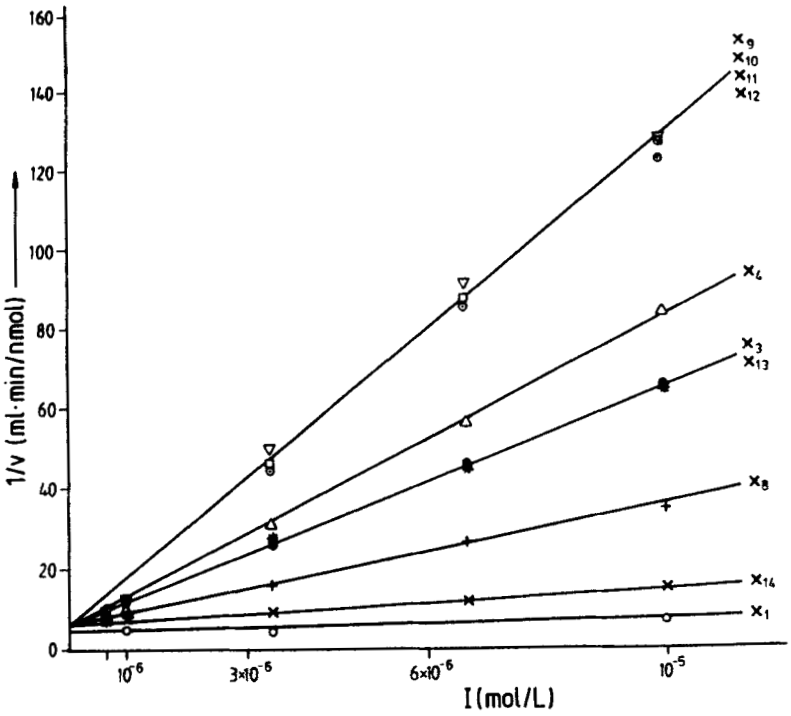

Fig.1 Comparison of the inhibitory effect of metyrapone-derivatives on the 11ß-hydroxylation of deoxycorticosterone (C-14-DOC)

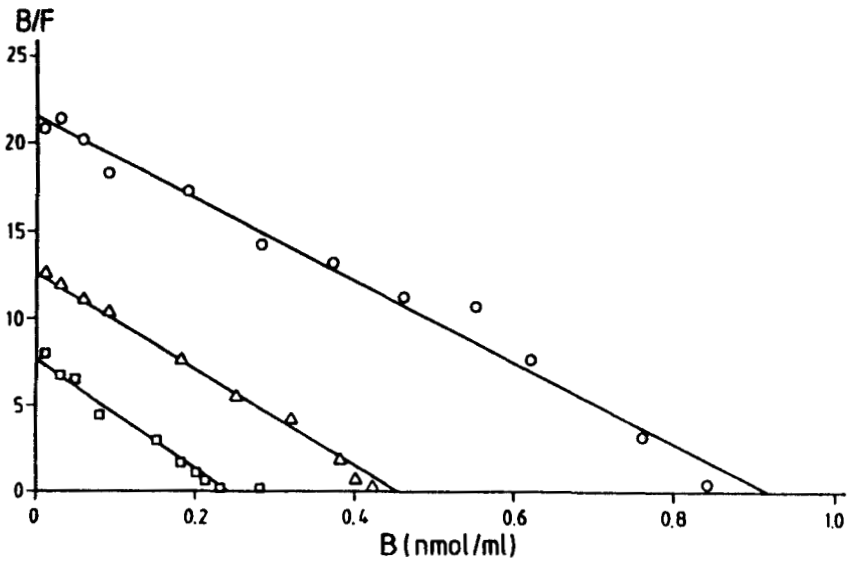

Fig.2 Scatchard analysis on the equilibrium binding of H-3-metyrapol. Amount of protein: ⊡ 0.25 mg, △ 0.5 mg, ⊙ 1 mg

TOTAL SYNTHESIS OF [2-^{11}C] THYMIDINE FROM [^{11}C]UREA : A TRACER OF CHOICE FOR MEASUREMENT OF CELLULAR PROLIFERATION USING PET.

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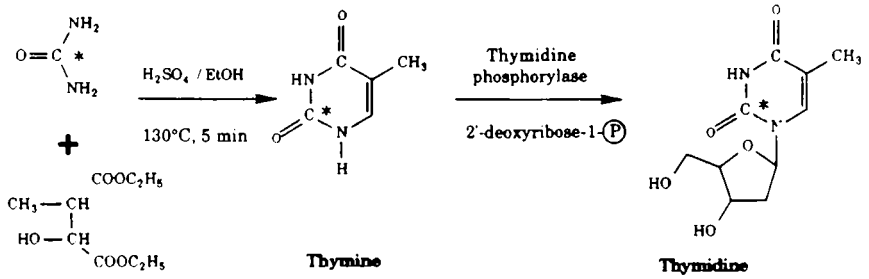
Since thymidine has been used extensively in studies of cellular proliferation, [methyl-^{11}C] thymidine was expected to be an obvious candidate for the in vivo determination of the mitotic tissue activity by PET. Nevertheless, it has been observed that the [methyl-^{11}C] labeled degradative products mask the progressive incorporation of the tracer into DNA. In contrast, using thymidine labeled in 2C-position, the labeled degradative products were rapidely eliminated in the form of labeled CO_2 so that radioactivity remaining in the cell was mainly carried by the DNA synthetic pathway (1).

The [2-^{11}C] thymidine synthesis was based on the [2-^{14}C] thymidine synthetic procedures (2, 3) and we suggested a three-step strategy starting with the production of [^{11}C]urea.



[^{11}C] Urea was prepared following the Emran procedure (4); the crude reaction mixture was cleared out of MnO_2 by filtration and the solvent was evaporated to dryness. To the anhydrous residue, were added a 20µl solution of 0.3µmol of cold urea and 50µmol of diethyl-β-methylmalate in absolute ethanol, and 100µl of fuming sulfuric acid (15% SO_3). The reaction mixture was heated at 130°C for 5.0 minutes. The pH of the crude [^{11}C]thymine solution was adjusted to 7.0 using 5.0N NaOH and stabilized with Tris-buffer.

The enzymatic transfer of deoxyribose onto thymine was performed according to the method described by Filip (5). The ionic strength of the above solution was reduced using a retardation resin (AG 11A8, Bio-Rad) and the eluate was collected in a vial containing 50µl of a 0.15M aqueous solution of 2'-deoxyribose-1-phosphate and 25 U.I. of thymidine phosphorylase. The reaction mixture was incubated at 40°C for 8.0 minutes and [2-^{11}C] thymidine was isolated by semi-preparative HPLC (RP-18; H_2O-methanol, 9-1; 6.0mL/min) with a 30-35% decay corrected yield. The specific activity was found in the range of 115 ± 46 Ci/mmol. (Synthesis time from $^{11}CO_2$: 60 minutes)

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IODOAZOMYCIN ACYCLONUCLEOSIDE: A POTENTIAL SPECT IMAGING AGENT WITH HIGH TUMOR UPTAKE.

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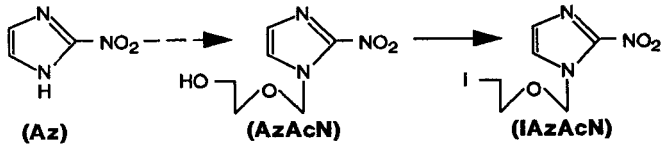
Synthesis of acyclonucleosides as analogues of naturally occurring ribonucleosides has been the subject of major investigation since the advent of the chemotherapeutic agent acyclovir (Zovirax), an acyclic analogue of guanosine.[1] The antibiotic azomycin (2-nitroimidazole, Az) and the corresponding ribonucleoside[2] preferentially concentrate in hypoxic tumor cells and show cytotoxicity to these cells.[3,4] Because of the potent chemotherapeutic activity exhibited by certain acyclonucleosides in contrast to their inactive ribonucleoside natural congeners, we have synthesized the 2-nitro-1-[(2-hydroxyethoxy)methyl]imidazole (azomycin acyclonucleoside, AzAcN). The AzAcN was radiolabeled with ^{125}I, and its biodistribution in a tumor model was evaluated.

Azomycin was silylated and condensed with the alkylating agent, 2-benzoyloxyethoxymethylene chloride, in dimethylformamide in the presence of triethylamine. Silica gel column chromatography provided the blocked intermediate which after debenzoylation with methanolic ammonia at ambient temperature yielded (72%) azomycin acyclonucleoside (AzAcN) after crystallization from chloroform-petroleum ether: mp 65-66°C; MS: m/z 188 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 7.4 (s, 1H, H-5), 7.15 (s, 1H, H-4), 5.8 (s, 2H, H-1'), 3.7 (s, 4H, H-3' & H-4'). Iodination of AzAcN with methyltriphenoxyphosphonium iodide gave 4'-deoxy-4'-iodoazomycin acyclonucleoside (IAzAcN) characterized by ^1H NMR and elemental analysis (C, H, N, & I). Exchange radiolabeling gave [^{125}I]-IAzAcN in 80% radiochemical yield. The [I-125]-IAzAcN (0.6 μCi ID) was evaluated in athymic nude mice bearing LS174T human colon cancer xenografts (tumor weight range, 0.24-0.39 gm, n=5; 1, 4, and 24 h). The [^{125}I]-nucleoside showed high tumor uptake (3.5% ID/gm at 24 h); relatively low uptake (less than 1% ID/gm) in the stomach, small intestine, kidneys, bone, muscle, and liver; and showed no uptake in the bone marrow. The [^{125}I]-nucleoside also showed activity in the blood, lungs, and spleen (3-4% ID/gm, 24 h) due perhaps to *in vivo* deiodination or metabolism. Attempts to stabilize iodine on AzAcN and further studies with this agent are being pursued.



ORNL research supported by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc., and at UM research is supported by National Cancer Institute Grant CA44173.

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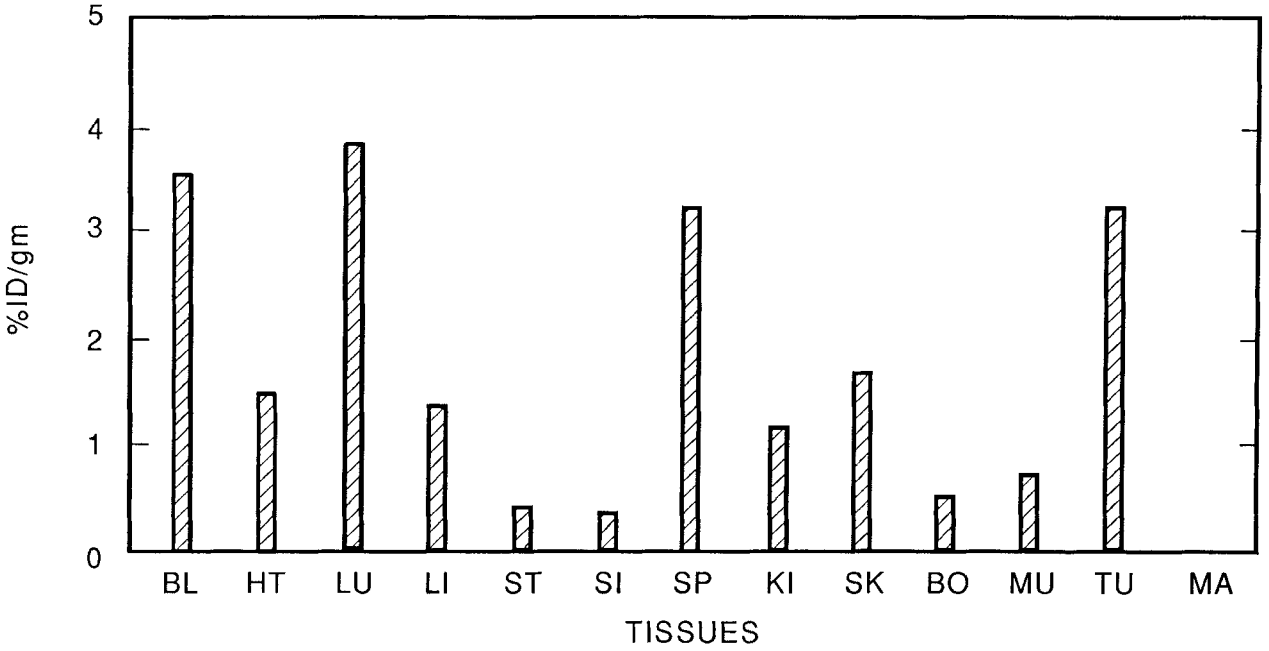
ORNL-DWG 90Z-5629

TISSUE[a] DISTRIBUTION AT 24h FOLLOWING i.p. ADMINISTRATION OF [I-125]-IODOAZOMYCIN ACYCLONUCLEOSIDE[b] IN NUDE MICE[c] BEARING LS174T HUMAN COLON CANCER XENOGRAFTS



[a] TISSUES: BL=BLOOD, HT=HEART, LU=LUNGS, LI=LIVER, ST=STOMACH, SI= SMALL INTESTINE, SP=SPLEEN, KI=KIDNEYS, SK=SKIN, BO=BONE, MU=MUSCLE, TU=TUMOR, MA=BONE MARROW

[b] INJECTED DOSE (ID)=0.6 μCi

[c] TUMOR WEIGHT RANGE=0.24-0.39 gm, n=5

THE USE OF CULTURED LIVER CELLS FROM RATS AND HUMANS TO STUDY THE METABOLISM OF THE PET IMAGING AGENT (F-18)-FLUOROESTRADIOL

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Evaluation of new radiopharmaceuticals for human use is routinely carried out first in rats or other animal models. However it is difficult to use animal data *in vivo* to predict the response in humans because major differences in metabolism exist among species (see 1), and there are significant sex-dependent differences in the metabolism of some compounds (2). Since the liver is generally the major site of metabolism, an *in vitro* procedure for testing liver cell metabolism in animal species and humans may provide useful information. Primary cultures of hepatocytes prepared from different species including humans have been used to study the metabolism of C-14 labeled amphetamine (3). The purpose of the present work was to determine the feasibility of using such cultures to determine the metabolic fate of PET radiopharmaceuticals prepared at high specific radioactivities with isotopes having short half-lives. 16 alpha-(F-18)fluoro-17 beta-estradiol (FES), a PET imaging agent for estrogen receptors (4), was chosen for this study.

Liver cells were prepared in primary culture by a collagenase perfusion technique (5), and incubated in supplemented Waymouth medium at a concentration of 1 million per ml with 250 uCi/ml of no-carrier added FES (about 1 Ci/umole). Both the incubation medium and an ethanol extract of the cells were analyzed by TLC using silica gel plates developed in benzene containing 10% ethanol. When freshly prepared cells from the liver of a male Fisher rat was used more than 40% of the FES was taken up by the cells at zero time. FES itself was not detected in the medium or the cell extract after 30 minutes of incubation but more polar F-18 labeled compounds were observed. The results were very similar when cryopreserved cells (5) from a male rat liver were used instead of the freshly prepared cells. In contrast, FES metabolism was much slower when a similar culture of cells freshly prepared from the liver of a 61 year old man was incubated with FES using the same conditions and cell concentration. 40-60% of the FES remained after 30 minutes of incubation in this case, with the rest of the radioactivity present in polar metabolites. After incubation of the human liver cells with FES for 70 minutes at least 7 radioactive metabolites were detected in the medium by HPLC analysis using a C18 column with a mobile phase of 25% acetonitrile in 7.5 mM KH2PO4. These results suggest it is feasible to use primary cultures to study the metabolism of FES (and presumably other radiopharmaceuticals) with liver cells from different species and sexes.
(This work was supported by Department of Energy Grant DEFG0284ER60218.)

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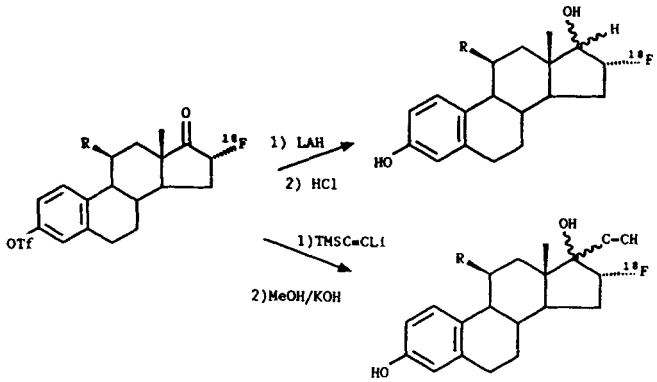
FLUORINE-18 LABELED ESTROGENS: SYNTHESIS AND BIOLOGICAL EVALUATION OF 11β AND 17α SUBSTITUTED ESTRADIOLS. H.F. VanBrocklin, M.J. Welch, J.W. Brodack, C.J. Mathias, M.G. Pomper*, K.E. Carlson*, J.A. Katzenellenbogen*, Mallinkrodt Institute of Radiology, Washington University Medical School, St. Louis, MO 63110, and *Department of Chemistry, University of Illinois, Urbana, IL 61801.

Substituted estrogens labeled with fluorine-18 have shown high selectivity for estrogen receptor rich target sites.[1,2,3] Human breast tumor imaging and *in vivo* receptor concentration quantification has been accomplished using 16α-[^{18}F]fluoroestradiol, 1.[4] Thus, in order to enhance the *in vivo* characteristics of 1, we have investigated 16α- and 16β-[^{18}F]fluorine labeled estradiols with substituents in the 11β (ethyl and methoxy) and 17α (ethynyl) positions. These positions are known to alter both the estrogen receptor binding and rate of metabolism of the radiopharmaceutical.[5]

The 16α-fluoro agents were produced by the fluoride displacement of estrone-16β-trifluoromethanesulfonates (triflates)[1] followed by either reduction with lithium aluminum hydride (LAH) or nucleophilic addition of lithiumtrimethylsilyl- acetylide (LiTMSacetylide) at the 17 carbonyl. Subsequent hydrolysis and deprotection yielded the labeled estradiol analogs, (scheme 1). Both reaction pathways produced the 17α- and 17β- hydroxy isomers, separable by HPLC, whose ratio (α/β) was dependent on the 11β substituent. Displacement of the 16α-triflates afforded the 16β-fluoro compounds. Reaction of these intermediates with either LAH or LiTMSacetylide gave only the desired 17β-hydroxy product.



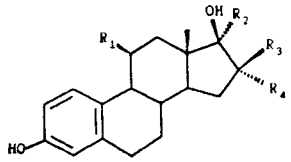
SCHEME 1

The uptake data from 25 day old female rats, 1 hour postinjection, and the relative binding affinities (RBA) for compounds 1-8 are presented in Table 1. The metabolic consumption of the labeled estradiols was followed in the female rat blood, muscle and uterus up to 3 hours postinjection. Ethanol extracts from the homogenized tissues were assayed by silica gel chromatography. A typical metabolic consumption time curve is shown in figure 1.

The uptake selectivity (uterus/blood or uterus/muscle) while quite high in the 16α-fluoro-ethynyl series, varies over a wide range in 1-8 and does not correlate well with either the

Table 1

1 hr uterus uptake, tissue ratios and relative binding affinities



	1	2	3	4	5	6	7	8
R_1	H	CH_2CH_3	OCH_3	H	CH_2CH_3	OCH_3	H	H
R_2	H	H	H	C≡CH	C≡CH	C≡CH	H	C≡CH
R_3	^{18}F	^{18}F	^{18}F	^{18}F	^{18}F	^{18}F	H	H
R_4	H	H	H	H	H	H	^{18}F	^{18}F
Uterus[a]	8.00	13.50	6.76	12.15	9.93	12.92	5.27	7.46
Ut/Bld	128	54	70.5	154	169	145	9.2	16
Ut/Musc	40	17	38	30	35	41	17.4	24
RBA[b]	60	891	28	61	--	--	30	60

a: %ID/gram; b: Estradiol = 100

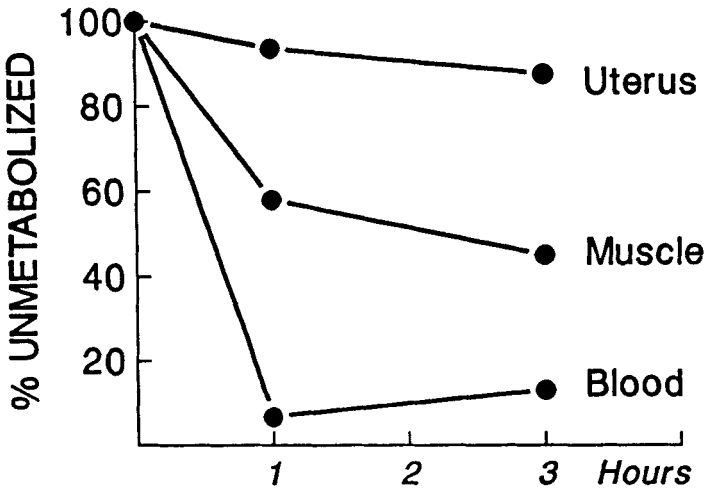


Figure 1. Plot of percent unmetabolized 17α-ethynyl-16α-fluoroestra-3,17β-diol extracted from the female rat blood, muscle and uterus versus time.

RBA or their respective lipophilicities. The specific uptake by the uterus is high and relatively equal for all agents despite the differences in the RBA's for these compounds.

These results suggest that the uptake of these analogs into receptor rich target tissues is limited by tissue blood flow. *In vivo* titration studies of 1 with estradiol and tamoxifen have revealed that the flow limited conditions exist. These new estrogen ligands exhibit an array of desirable characteristics for optimal imaging of estrogen receptor rich targets, however, further biological evaluation will have to be undertaken to determine which of these may be "best" clinical agent.

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SYNTHESIS OF A NEW SERIES OF 17α-IODOVINYLESTRADIOLS VIA BORANES

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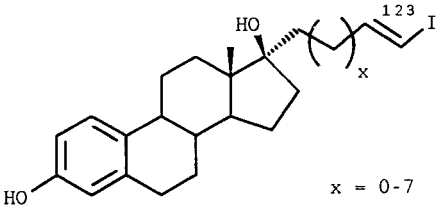
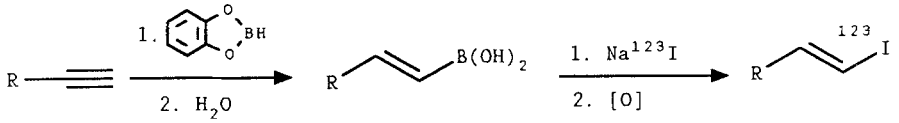
Radiohalogenated estrogens have long been recognized as potential probes for the detection of estrogen dependent cancers (1,2). However radioiodine-labeled pharmaceuticals are prone to in-vivo de-iodination and researchers have developed a number of approaches to stabilize iodinated molecules. A particularly interesting approach involves the preparation of iodovinyl analogs which have proven to be more stable, in-vivo, than the corresponding alkyl derivatives (3-5).

We wish to report the syntheses of a new series of 17α-iodovinylestradiols in which the vinyl iodide is systematically removed from the 17-position of the steroid nucleus trough the addition of methylene groups.



Radioiodination reactions were performed via reaction of the corresponding vinylboronic acids with iodine-123 labeled sodium iodide in the presence of a mild oxidizing agent (6,7). The boronic acids were synthesized from the corresponding alkynyl estradiol derivatives which were prepared using classical coupling reactions.



The work was funded by DOE (DE-FG-86ER60434) and the Squibb Institute for Medical Research

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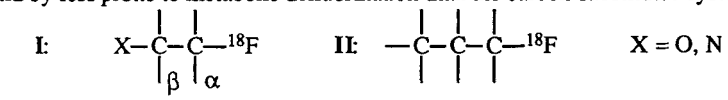
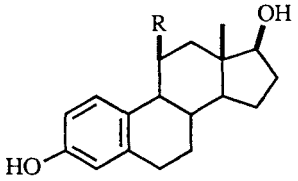
THE β-HETEROATOM EFFECT IN METABOLIC DEFLUORINATION: THE INTERACTION OF RESONANCE AND INDUCTIVE EFFECTS MAY BE A FUNDAMENTAL DETERMINANT IN THE METABOLIC LABILITY OF FLUORINE-SUBSTITUTED COMPOUNDS. A. N. French,[a] E. Napolitano,[a] H.F. VanBrocklin,[b] J.W. Brodack,[b] R.N. Hanson,[c] M.J. Welch,[b] J.A. Katzenellenbogen[a], [a]Department of Chemistry, University of Illinois, Urbana, IL 61801, [b]Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110, [c]Department of Medicinal Chemistry, Northeastern University, Boston, MA 02115.

The utility of fluorine-18 labeled radiopharmaceuticals as in vivo diagnostic imaging agents depends upon the metabolic stability of the carbon-fluorine bond, as its scission will release labeled fluoride ion, which will accumulate in bone and will degrade the selective localization of the radiotracer. Using an estimate of total bone activity as an index of metabolic defluorination, we have noted in several tissue distribution studies in rats, involving a series of F-18 labeled compounds of diverse structure, that those that are metabolically stable have a heteroatom substituent situated β to the fluorine atom (I: estimate of total bone activity <10% ID), whereas those that are metabolically labile (II: 25-90% ID/total bone) lack this heteroatom substituent. These results are consistent with an interesting interplay between inductive and resonance effects.

It is known that the metabolism of halogenated alkanes generally involves a preferred hydroxylation at the halogen-bearing carbon, followed by an elimination of halide ion (1); thus, the metabolic lability of the carbon-fluorine bond will be determined by the reactivity of the (F)C–H bond towards hydroxylation. The activating effect of fluorine towards hydroxylation at this site results from electron donation by a resonance mechanism (that stabilizes an incipient radical or carbenium ion intermediate in the metabolic hydroxylation process) which exceeds electron removal by an inductive mechanism (that destabilizes such intermediates). The β-heteroatom substituent, however, provides an additional metabolically deactivating inductive effect, as it is too distant to donate electron density by resonance. Thus, the β-heteroatom substituted-system (I) should by less prone to metabolic defluorination than the carbon-substituted system (II).



In a controlled investigation of this phenomenon, we compared the bone activity in immature female rats 3 h after injection of two closely related estrogens bearing F-18 labeled 11β-substituents, as a fluoroethyl (**III**) or a fluoroethoxy (**IV**) group. As anticipated, the fluoroethyl system (**III**) showed 28% ID/total bone, whereas the fluoroethoxy system (**IV**) showed only 1.3% ID/total bone.



R = ^{18}F-CH_2-CH_2- **III**
R = ^{18}F-CH_2-CH_2-O- **IV**

While other structural and stereochemical features will undoubtedly affect the acccess of fluorine-substituted systems to the enzymes involved in metabolic defluorination, the β-heteroatom stabilization effect may be a primary electronic determinant of metabolic lability, and it should be considered in the design of fluorine-18 labeled radiopharmaceuticals.

Supported by grants from the National Institutes of Health (CA25836, CA41339 and HL13851), Department of Energy (DE FG02 84ER60218 AND 86ER60401) and the American Health Assistance Foundation.

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Table 1. Biodistribution data for 11β-(2-[^{18}F]Fluoroethyl)estradiol (**III**)[a]

	1 h	1 h blocked[b]	3 h
% ID/organ:			
blood	0.8391 ± 0.0933	1.11 ± 0.1405	0.5315 ± 0.1514
liver	6.2525 ± 1.0289	7.6872 ± 1.9505	3.6573 ± 0.7931
spleen	0.0693 ± 0.0075	0.0386 ± 0.0071	0.0398 ± 0.0091
kidney	0.596 ± 0.0749	0.3628 ± 0.0453	0.2898 ± 0.068
muscle	3.3923 ± 0.4842	0.9497 ± 0.1443	1.4722 ± 0.3428
fat	4.6868 ± 0.6245	1.0565 ± 0.0967	2.4509 ± 0.5873
bone	24.5043 ± 4.71	23.2371 ± 5.0316	27.6794 ± 8.3096
uterus	0.5236 ± 0.1742	0.0618 ± 0.016	0.3439 ± 0.1332
ovaries	0.1324 ± 0.0288	0.0278 ± 0.0027	0.084 ± 0.0253
% ID/g:			
blood	0.2171 ± 0.0213	0.3086 ± 0.0335	0.1452 ± 0.0325
liver	2.1772 ± 0.3152	2.9467 ± 0.5568	1.4461 ± 0.1963
spleen	0.2838 ± 0.0282	0.1764 ± 0.0223	0.1893 ± 0.0292
kidney	1.7481 ± 0.2108	1.0689 ± 0.1228	0.9761 ± 0.1937
muscle	0.441 ± 0.0733	0.1326 ± 0.0215	0.2019 ± 0.0321
fat	0.6169 ± 0.0866	0.1496 ± 0.0174	0.3423 ± 0.0638
bone	4.0586 ± 0.627	4.1198 ± 0.638	4.8362 ± 1.1422
uterus	9.2446 ± 3.4371	1.3015 ± 0.2767	7.2475 ± 2.7399
ovaries	3.184 ± 0.6593	0.816 ± 0.126	2.3819 ± 0.7911
Ratios			
uterus/blood	42.97 ± 16.13	4.23 ± 0.838	49.06 ± 16.58
ovaries/blood	14.72 ± 3.13	2.67 ± 0.458	15.99 ± 2.80
uterus/muscle	21.43 ± 8.06	9.85 ± 0.458	35.11 ± 12.14
ovaries/muscle	7.36 ± 1.16	6.24 ± 1.06	11.48 ± 2.57

[a]Administered i.v. in 10% ethanol-saline (n=5, ±SD). [b]36 μg estradiol co-administered.

Table 2. Biodistribution for 11β-(2-[^{18}F]Fluoroethoxy)estradiol (**IV**)[a]

	1 h	1 h blocked	3h
% ID/organ:			
blood	0.8335 ± 0.0789	0.832 ± 0.153	0.8656 ± 0.0664
liver	4.4947 ± 1.0997	4.9744 ± 1.3793	2.6868 ± 0.4722
spleen	0.0356 ± 0.0051	0.0608 ± 0.0758	0.0344 ± 0.0025
kidney	0.1711 ± 0.0341	0.1118 ± 0.0504	0.0765 ± 0.0052
muscle	1.5527 ± 0.1896	1.1215 ± 0.1977	1.0276 ± 0.1013
fat	1.6683 ± 0.3901	1.404 ± 0.2508	0.7727 ± 0.2439
bone	1.3065 ± 0.4115	1.0186 ± 0.1372	2.2259 ± 0.3668
uterus	0.1212 ± 0.0284	0.0111 ± 0.0035	0.0508 ± 0.0077
ovaries	0.0319 ± 0.0103	0.0069 ± 0.0011	0.0134 ± 0.0019
% ID/g:			
blood	0.2198 ± 0.0251	0.2321 ± 0.0362	0.2244 ± 0.013
liver	1.5788 ± 0.2238	1.9812 ± 0.4968	0.9041 ± 0.1993
spleen	0.1748 ± 0.0178	0.2355 ± 0.178	0.1368 ± 0.0147
kidney	0.5237 ± 0.1232	0.3869 ± 0.1242	0.2417 ± 0.0287
muscle	0.2048 ± 0.0314	0.1567 ± 0.025	0.1336 ± 0.0152
fat	0.2223 ± 0.0475	0.2008 ± 0.0477	0.1015 ± 0.0295
bone	0.2241 ± 0.0834	0.1826 ± 0.0191	0.3713 ± 0.0618
uterus	3.9353 ± 1.0003	0.3066 ± 0.041	1.5503 ± 0.6057
ovaries	0.9293 ± 0.363	0.2147 ± 0.0355	0.3749 ± 0.0976
Ratios			
uterus/blood	18.2762 ± 5.7950	1.3342 ± 0.1772	7.0387 ± 3.2399
uterus/muscle	19.0618 ± 2.6466	1.9734 ± 0.2164	12.0173 ± 6.2409
ovaries/blood	4.4451 ± 2.4498	0.9266 ± 0.0867	1.6600 ± 0.3832
ovaries/muscle	4.5688 ± 1.8273	1.3713 ± 0.0941	2.7917 ± 0.6173

[a]Administered i.v. in 10% ethanol-saline (n=5, ±SD). [b]36 μg estradiol co-administered.

ANDROGEN RECEPTOR-BASED IMAGING AGENTS FOR THE PROSTATE: SYNTHESIS AND TISSUE DISTRIBUTION STUDIES WITH TRITIUM AND FLUORINE-18 LABELED ANDROGENS. A. Liu,[a] K. E. Carlson,[a], J. A. Katzenellenbogen,[a] H. F. VanBrocklin,[b] C. J. Mathias,[b] and M. J. Welch,[b] [a]Department of Chemistry, University of Illinois, 1209 W. California St., Urbana, IL 61801, and.[b]Division of Radiation Sciences Research, Mallinckrodt Institute of Radiology, Washington University School of Medicine, 510 S. Kingshighway, St. Louis, MO 63110

The growth and development of the prostate depends on androgens, and prostatic tissue and most prostate tumors contain receptors for androgens. These receptors offer a means for the selective localization of a suitable radiolabeled androgen that could act as an imaging agent for prostatic tumors. If such imaging agents could provide definitive determination of the extent of tumor invasion and metastasis, which is difficult to achieve with diagnostic methods in current use, it would be very helpful in guiding the selection of alternative therapies for the management of prostatic cancers.

In order to develop suitable androgen-receptor based imaging agents, we first investigated the uptake of five androgens, available commercially in high specific activity tritium-labeled form, in the prostate of one-day castrated rats (250 g) (1). Testosterone (**T**; Relative Binding Affinity (RBA) = 5.9), 5α-dihydrotestosterone (**DHT**; RBA = 60), 19-nortestosterone (**nor-T**; RBA = 31), mibolerone (**Mib**; RBA = 120) and methyltrienolone (**R1881**; RBA = 100) all showed selective uptake by the ventral prostate that was 61-90% displaceable by co-injection of an excess of unlabeled steroid. The greatest uptake was with **R1881** (0.69% ID/g at 1 h), and **Mib** (0.56% ID/g); the other three showed lower uptake (ca. 0.4% ID/g). The target tissue activity remained high for all compounds up to 4 h after injection, and at 2-4 h the prostate to blood ratio for **Mib** and **R1881** exceeded 10 and 20, respectively. The uptake efficiency and selectivity of these five androgens appear to be related to their affinity for the androgen receptor and their resistance to metabolism. **Mib** and **R1881** have substantial affinity for other steroid receptors, but competition studies with triamcinolone acetonide and **DHT** show that prostate uptake depends upon binding to the androgen receptor. The prostate uptake of **Mib** and **R1881** in intact animals was significantly lower than in castrated animals, but treatment of the intact animals with diethylstilbestrol restored uptake nearly to the level seen in castrated animals.

Based on these uptake studies, we have synthesized 20-fluoromibolerone (2). The synthesis begins with 7α-methyltestosterone and proceeds to the 17β,20-spiro cyclic sulfate, which undergoes fluoride substitution at C-20 in good yield. This compound has high affinity for the androgen receptor (RBA = 56), and it was prepared in fluorine-18 labeled form in reasonable yield and in high effective specific activity. Tissue distributions in diethylstilbestrol-treated (1 mg s.c. at -24 and -3 h) rats (175 g) (2) indicated selective uptake and prolonged retention by the target tissue: The % ID/g prostate and the prostate/muscle ratio were 0.9 and 4, respectively, at 0.5 h, and 0.58 and 12, respectively, at 4 h. Co-administration of a high dose (36 μg) of testosterone reduced prostate uptake to the level of non-target tissues.

Thus, high affinity androgens – particularly derivatives of mibolerone (**Mib**) or methyltrienolone (**R1881**) – appear to be promising candidates for receptor-based imaging of androgen target tissues and tumors, even when patients are already receiving hormonal therapy.

This work was supported by DOE grants DE FG02 86ER60401 and 84ER60218.

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2. A. Liu, J. A. Katzenellenbogen, H. VanBrocklin, C. J. Mathias, and M. J. Welch, *J. Nucl. Med.*, submitted.

Scheme 1

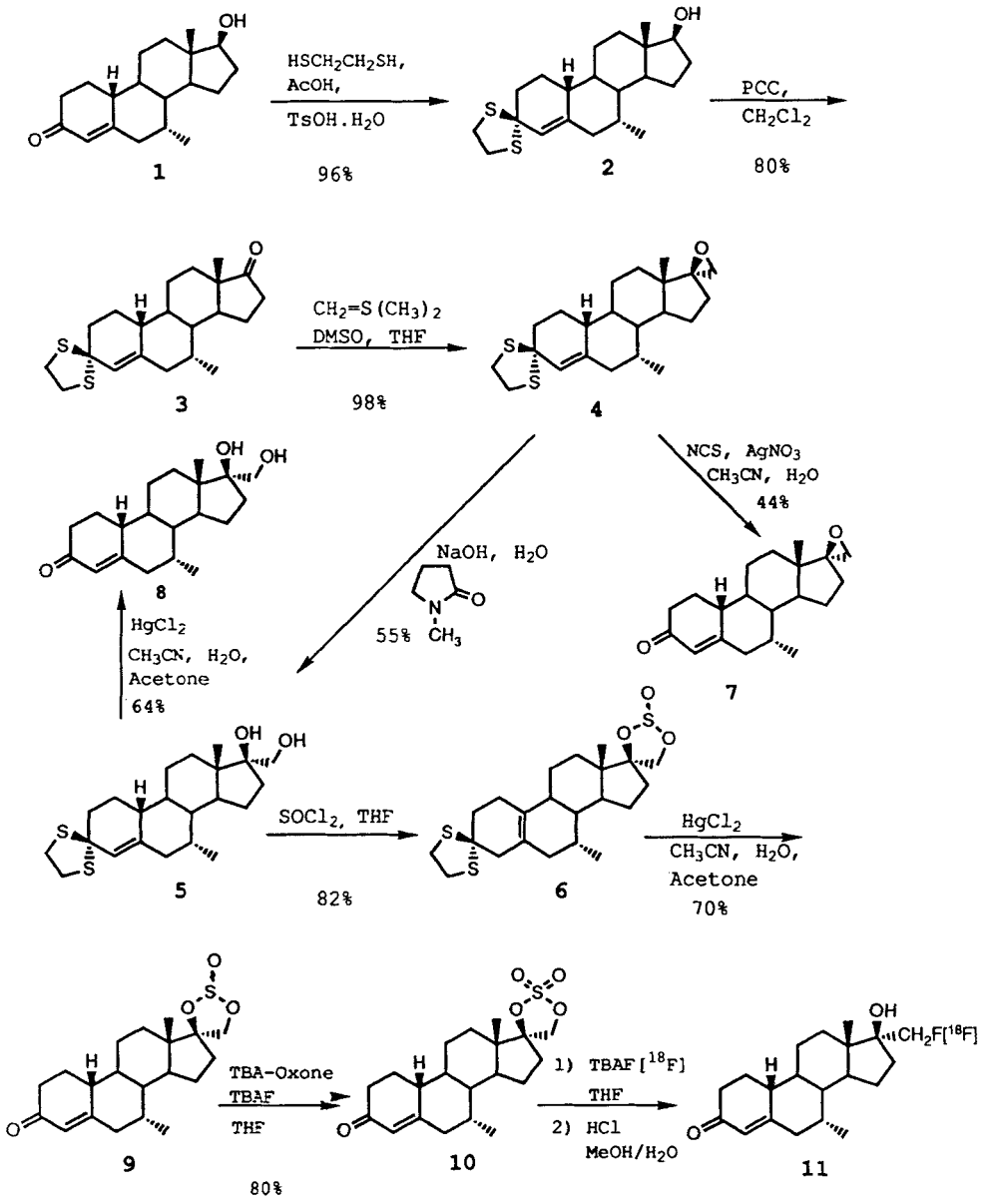
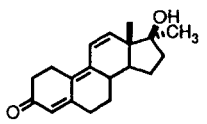
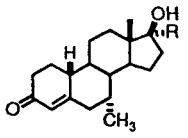
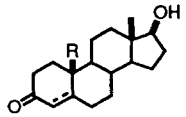
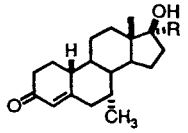

HSCH2CH2SH, AcOH, TsOH.H2O
96%
PCC, CH2Cl2
80%
CH2=S(CH3)2 DMSO, THF
98%
NCS, AgNO3 CH3CN, H2O
44%
NaOH, H2O
55%
HgCl2 CH3CN, H2O, Acetone
64%
SOCl2, THF
82%
HgCl2 CH3CN, H2O, Acetone
70%
TBA-Oxone TBAF THF
80%
1) TBAF[18F] THF
2) HCl MeOH/H2O
CH2F[18F]

Table 1. Binding Affinity for Rat Prostatic Androgen Receptor[a]

Compound	Relative Binding Affinity (RBA)	Compound	Relative Binding Affinity (RBA)
 R1881 Methyltrienolone	100		
		R = H (1)	420
		R = CH_3 (Mibolerone)	120
		R = CH_2OH (8)	1.4
		R = CH_2F (11 F-Mib)	56
			
R = CH_3 Testosterone (Δ)	5.9		
R = CH_3 5α-Dihydrotestosterone	60	R = $-CH_2-$ (7)	3.1
R = H 19-nor-testosterone (Δ)	31	R = $-S(O)-O-CH_2-$ (9, mixture A)[b]	3.2
		R = $-S(O)-O-CH_2-$ (9, mixture B)[b]	2.6

a The binding affinity was determined in our Lab by a competitive binding assay using [^{3}H]R1881 as a tracer.

b Mixture A: the ratio of epimer a and b is ca.70 to 30; mixture B: the ratio is ca.30 to 70.

Table 2. Tissue Radioactivity Distribution of 20-[^{18}F]Fluoro-mibolerone[a]

organ	0.5h	1 h	2 h	2h(unl)[b]	2h(blocked)[c]	4 h
% ID/g ±SEM						
blood	0.234±0.003	0.159±0.014	0.091±0.006	0.064±0.002	0.081±0.011	0.050±0.001
bone	0.189±0.011	0.197±0.010	0.251±0.014	0.184±0.017*	0.220±0.007	0.226±0.006
muscle	0.232±0.006	0.177±0.008	0.092±0.005	0.069±0.003	0.067±0.011	0.050±0.009
spleen	0.237±0.011	0.170±0.013	0.092±0.006	0.064±0.002	0.070±0.011	0.045±0.001
lung	0.417±0.011	0.300±0.012	0.165±0.011	0.107±0.004	0.078±0.011	0.080±0.006
liver	2.362±0.075	1.839±0.095	0.805±0.032	0.514±0.036	0.739±0.069	0.402±0.029
fat	0.573±0.049	0.264±0.012	0.132±0.020	0.112±0.014	0.137±0.020	0.067±0.003
kidney	0.880±0.038	0.753±0.035	0.423±0.018	0.266±0.012	0.361±0.078	0.174±0.008
prostate (v)[d]	0.886±0.037	0.969±0.140	0.601±0.056	0.384±0.073	0.150±0.034*	0.611±0.034
prostate (d)	0.923±0.028	1.188±0.304	0.775±0.135	0.393±0.068	0.111±0.009	0.545±0.057*
Ratio ± SEM						
prostate/ (v)	3.825±0.189	5.532±0.935	6.541±0.543	5.615±1.113	2.773±0.389*	13.25±2.22
muscle (d)	3.985±0.138	6.913±1.919	8.563±1.775	5.709±0.924	1.761±0.306	10.82±3.14
prostate/ (v)	3.790±0.105	6.385±1.302	6.707±0.673	6.128±1.388	2.159±0.298*	12.41±0.92
blood (d)	3.953±0.103	8.021±2.502	8.818±1.953	6.200±1.204	1.428±0.184	10.95±1.46*

a In the study, mature Sprague-Dawley rats were injected with 100 μCi dose of 20-[^{18}F]fluoro-mibolerone in 10% ethanol-saline. Average animal weight at time of experiment was 173±16g. All rats were treated with 1mg of DES in 0.2mL sunflower oil per rat 24h and 3h prior to injection with activity except "c" then untreated. n=4, except *, then n=3. SEM is standard error of mean.

b Animals are untreated (see a).

c Blocked: in order to block receptor-mediated uptake, 36μg of testosterone was added to each injected dose

d "v" = ventral, "d" = dorsal.

STRUCTURE-ACTIVITY RELATIONSHIPS FOR 17α-E/Z VINYL AND 11-SUBSTITUTED-17α-E/Z VINYL ESTRADIOLS

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Because of the ongoing interest in imaging estrogen responsive tumors, a number of 17α-X-vinyl estradiol derivatives have been prepared in the radioactive form and evaluated in animal models. However, there has been no attempt to evaluate these derivatives in a systematic fashion and develop a coherent structive-activity relationship (SAR) for them. Our expertise in preparing the requisite stannylvinyl intermediates has permitted us easy access to a variety of estradiol derivatives and thus undertake such a study. This presentation will describe the results of this work.

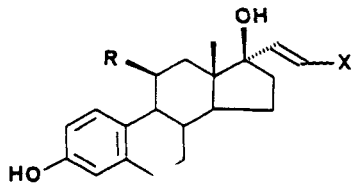
The intermediate 11-unsubstituted-17α-ethynyl estradiols were prepared by synthesis from estradiol methyl ether or obtained from Roussel-Uclef (Romainville, France). Stereoselective conversion to the E- or Z-tributylstannylvinyl estradiol-3-acetates was accomplished using a method developed in our laboratory. The destannylation with the appropriate electophile followed by hydrolysis gave the desired 17α- E- or Z- X-vinyl estradiol in an 85-95% yield. The products were characterized and then evaluated for their relative binding affinity (RBA) in a competitive receptor binding assay. The results are shown in Table 1.

For the 11-unsubstituted compounds, all of the x-vinyl derivatives demonstrated reasonably high affinity compared to estradiol and to ethynyl estradiol. Significant differences were noted between the Z- and E- isomers, however. The Z-isomers demonstrated higher affinity than the corresponding E-isomers, an effect that was exaggerated when the incubation was performed at 25°C. Also affinities for the Z-isomers increased in the series I > Br > Cl whereas the Cl-product had the highest affinity in the E series.

The introduction of a substituent at the 11-position has a variable effect. A polar OCH_3 reduces the RBA for both E- and Z- isomers compared to the 11-unsubstituted analog. Conversely, the lipophilic 11-vinyl group, while having no effect at 0°C, markedly enhances the RBA determined at 25°C. Again, the Z- isomers have higher RBA values than the E- isomers.

The results suggest that the introduction of a radiohalogen into the Z-vinyl estradiols results in the more favorable product. The corresponding E-vinyl estrogens have potential if also accompanied by a lipophilic 11β-substituent.

This work was supported in part by NIH Grant CA 41399.



R	X	E	Z
H	Cl	102(89)	126(199)
H	Br	78(56)	195(661)
H	I	77(62)	202(776)
H	C_6H_5	14(7)	49(26)
H	SC_6H_5	25(31)	117(107)
H	SeC_6H_5	32(25)	31(25)
H	$SnBu_3$	1.3	-
α-OCH_3	$SnBu_3$	0.02	-
β-OCH_3	$SnBu_3$	0.3	-
α-OCH_3	I	12.5(1.6)	-
β-OCH_3	I	34(65)	-
C_2H_5	I	43(891)	-
C_3H_7	I	74(393)	-
-OCH_3	Cl	29(65)	22(195)
-OCH_3	I	34(65)	48(513)
-OCH_3	SeC_6H_5	-	56(32)
C_2H_3	Cl	107(724)	101(794)
C_2H_3	I	78(447)	98(1202)
C_2H_3	SeC_6H_5	59(347)	30(195)

RBA = 0%(25%)

7α-METHYL AND 11β-ETHOXY DERIVATIVES OF [^{125}I]16α-IODOESTRADIOL: EFFECT ON ESTROGEN RECEPTOR-MEDIATED TARGET TISSUE UPTAKE.

Hasrat Ali, Jacques Rousseau, Johan E. van Lier.
MRC Group in the Radiation Sciences, Faculty of Medicine, University of Sherbrooke, Québec, Canada J1H 5N4.

A radioiodinated estrogen analog with high binding affinity for estrogen receptors could have an important role in the management of human breast cancer through the use of the ^{123}I-analog and SPECT imaging. Many estrogen analogs have been studied for this purpose and among them 16α-iodoestradiol stands out as a simple derivative which closely resembles the parent molecule, estradiol, and which exhibits high affinity for the estrogen receptor (1). However, rapid in vivo metabolism masks receptor mediated localization of this radiopharmaceutical and attempts to slow down its degradation via further substitution at the 11β-position have shown promise (2). To further explore this approach we prepared the 7α-methyl and 11β-ethoxy derivatives of [^{125}I]7β-estradiol. Receptor-mediated uterus uptake and in vivo stability was studied in immature female rats.

The synthesis of the radiolabeled 7α-methyl and 11β-ethoxy estradiols [^{125}I]7a,b is similar to the original procedure of Hochberg for the preparation of the non-substituted 16α-iodoestradiol (7c) (1). The corresponding estrone derivatives (1a,b) were converted to the 17-enol 3-acetates (2a,b) which were subsequently brominated with Br_2 in acetic acid to yield exclusively the 16α-bromo isomers of the 7α-methyl and 11β-ethoxy derivatives of 16-bromoestrone 3-acetate (3a,b). Epimerization with LiBr in 2-butanone gave the 16β-bromo isomers (5a,b) which upon reduction with $NaBH_4$ in EtOH gave the corresponding 16β-bromo-17β-estradiol derivatives 6a,b. The [^{125}I]7α-methyl-16α-iodoestradiol ([^{125}I]7a) and the [^{125}I]11β-ethoxy-16α-iodoestradiol ([^{125}I]7b) were obtained by halogen exchange on the 16β-bromo derivatives 6a,b with [^{125}I]NaI in 2-butanone for 2 h. The radiolabeled products were purified by C-18 reverse phase HPLC in MeOH/H_2O to give [^{125}I]7a and [^{125}I]7b in 60-75% radiochemical yield.

Tissue distribution of the 7α-methyl and 11β-ethoxy estradiols [^{125}I]7a and [^{125}I]7b in immature female Fischer rats showed a high uterus uptake with peak activities at 2 h postinjection of 6.35 % I.D./g and 13.08 % I.D./g respectively. These values are substantially higher than published data on the nonsubstituted [^{125}I]17α-iodoestradiol (2.8 % I.D./g, 1h, lit.[2]) whereas the 11β-ethoxy derivative [^{125}I]7b gives uptake values comparable to those reported for the 11β-methoxy analog (14.4 % I.D./g, 1 h, lit.[2]). Co-injected nonradioactive estradiol (60 μg/rat) sharply decreased (93-94 %) uterus uptake of both radiopharmaceuticals confirming an estrogen receptor-mediated uptake mechanism.

This work was supported by the Medical Research Council of Canada.

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Table I. Tissue distribution of ^{125}I-labeled 7a in immature female Fischer rats.

	%I.D./g (%CV)[a]						
	Time in hours.						
Tissue n	0.08 3	0.5 3	1 5	1+Est.[b] 3	2 4	5 4	24 3
Blood	0.99 (25%)	0.45 (2%)	0.38 (19%)	0.33 (9%)	0.32 (21%)	0.28 (14%)	0.03 (18%)
Plasma	1.55 (25%)	0.68 (2%)	0.58 (19%)	0.51 (11%)	0.49 (24%)	0.41 (12%)	0.04 (19%)
Lungs	2.41 (16%)	0.82 (13%)	0.54 (7%)	0.43 (12%)	0.32 (18%)	0.19 (15%)	0.03 (13%)
Liver	4.52 (27%)	1.94 (10%)	1.26 (8%)	1.09 (9%)	1.09 (10%)	0.72 (19%)	0.13 (7%)
Spleen	1.10 (47%)	0.57 (4%)	0.43 (6%)	0.43 (20%)	0.33 (12%)	0.22 (29%)	0.08 (27%)
Kidneys	4.40 (25%)	1.30 (6%)	0.83 (3%)	0.51 (9%)	0.50 (24%)	0.25 (22%)	0.03 (19%)
Uterus	1.95 (34%)	4.32 (25%)	5.25 (28%)	0.34 (11%)	6.35 (26%)	3.86 (35%)	1.64 (13%)
Muscle	1.43 (21%)	0.54 (1%)	0.32 (11%)	0.27 (5%)	0.15 (32%)	0.06 (33%)	0.01 (44%)
Thyroid	59.31 (22%)	40.33 (31%)	45.15 (24%)	43.50 (15%)	66.19 (40%)	230.54 (35%)	334.84 (20%)
Fat	1.27 (36%)	2.20 (49%)	1.79 (28%)	1.64 (8%)	1.12 (25%)	0.38 (41%)	0.03 (11%)

[a]Average %I.D./g for n rats. Each rat was injected i.v. with 3 μCi of carrier free ^{125}I-labeled 7a The incertitude is given in brackets as the %CV, e.g. the percent coefficient of variation corrected for small sample size effect.

[b]The animals were coinjected with 60μg of unlabeled estradiol.

Table I. Tissue distribution of ^{125}I-labeled **7b** in immature female Fischer rats.

	%I.D./g (%CV)[a]						
	Time in hours.						
Tissue	0.5	1	1+Est[b]	2	5	24	48
n	3	5	2	4	4	4	4
Blood	1.29 (11%)	1.01 (17%)	1.09 (9%)	0.89 (16%)	0.70 (13%)	0.17 (18%)	0.06 (10%)
Uterus	7.85 (17%)	11.67 (18%)	0.72 (9%)	13.08 (18%)	7.76 (19%)	1.64 (35%)	0.20 (26%)
Thyroid	105.65 (19%)	110.96 (32%)	156.51 (34%)	218.87 (21%)	703.10 (10%)	773.37 (45%)	587.49 (35%)
Muscle	1.05 (22%)	0.69 (21%)	0.72 (20%)	0.52 (35%)	0.35 (29%)	0.13 (52%)	0.03 (41%)
Fat	2.47 (23%)	1.51 (24%)	1.63 (20%)	0.94 (32%)	0.52 (14%)	0.15 (24%)	0.09 (14%)
Brain	0.68 (16%)	0.39 (21%)	0.42 (25%)	0.25 (33%)	0.14 (22%)	0.04 (17%)	0.01 (14%)
Kidneys	2.26 (10%)	1.83 (25%)	1.61 (6%)	1.78 (28%)	0.91 (17%)	0.24 (21%)	0.10 (16%)
Spleen	1.44 ( )	1.49 (19%)	1.60 (23%)	1.76 (19%)	1.28 (21%)	0.70 (23%)	0.53 (25%)
Lungs	1.72 (13%)	1.30 (22%)	1.18 (14%)	1.01 (19%)	0.76 (8%)	0.23 (11%)	0.13 (22%)
Liver	5.91 (30%)	3.48 (26%)	4.50 (19%)	3.24 (18%)	2.31 (9%)	0.96 (11%)	0.48 (10%)

[a]Average %I.D./g for n rats. Each rat was injected i.v. with 7 μCi of carrier free ^{125}I-labeled **7b** The incertitude is given in brackets as the %CV, e.g. the percent coefficient of variation corrected for small sample size effect.

[b]The animals were coinjected with 60μg of unlabeled estradiol.

Z-11ß-CHLOROMETHYL-17α-IODOVINYL ESTRADIOL, A VERY HIGH AFFINITY ESTROGEN FOR RADIOIMAGING AND TARGETTED RADIOTHERAPY OF ESTROGEN RECEPTOR (ER) POSITIVE TUMORS.

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In order to prepare high specific estrogen bearing I-123 for imaging and therapy of estrogen receptor (ER) containing tumoral tissues, we have substituted the steroidal skeleton with chemical groups confering :
- high affinity and low dissociation for ER
- low non-specific binding (e.g. to plasma steroid-binding proteins).

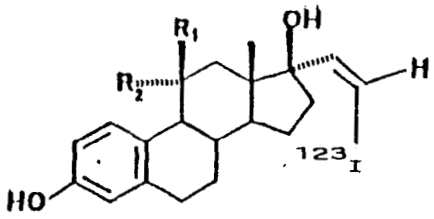
11ß-chloromethyl and 17α-iodovinyl substitutions can fulfill these conditions. The challenge was to prepare a steroidal molecule bearing two different halogens which one radiolabelled. An original chemical synthetic route starting from Δ^1-adrenosterone after 13 steps produced E or Z-11ß-chloromethyl-17α-iodovinyl estradiol. Binding studies on human MCF-7 and calf uterus ER showed very high affinity of these two isomers E and Z with either cold or I-125 labelled, respectively by indirect competitive assay with tritiated estradiol and direct binding (Scatchard plot).

The Z isomer has an affinity for the estrogen receptor 10 times higher than estradiol and an affinity for the plasma sex steroid binding protein 400 times lower than estradiol. In biodistribution experiments in mice and rats, high target versus non target tissues ratios were obtained with this molecule. The Z isomer was labelled with I-123 and excellent radioimaging of human tumor grafted onto nude mice were obtained. As evaluated by the ROI method, the specific tumor uptake was reduced to 5% and 6.5%, if excess cold estradiol and cold Z isomer respectively were coinjected with I-123 labelled Z isomer.

AUGER electrons emitting radionuclides like I-123 release most of their energy within a few nanometers of the parent isotope. If the decay occurs in the vicinity of the DNA, it will induce double strand breaks and less than 100 disintegrations are sufficient to kill a cancer cell. however, if it occurs at the cell surface or within the cytoplasm it has a negligible effect on cell survival. One way to bring these radioisotopes near the DNA is to attach them to steroids with high affinity to the nuclear associated estradiol receptor (ER).

In vitro cytotoxicity of the I-123 Z isomer was assessed using a clonogenic assay and a modified MTT assay. The Z isomer was 1000 times more toxic for MCF-7 human breast carcinoma cells than for human bone marrow cells. Our data suggest the estradiol receptor could be a suitable site for radioimaging and for targetted radiotherapy.

This work is supported by an European EUREKA grant (EU/181/18).



$R_1 = CH_2Cl$ $R_2 = H$

Z-11β-CHLOROMETHYL-17α-IODOVINYL-ESTRADIOL (Z-IV)

SYNTHESIS OF "NO-CARRIER ADDED " CARBON-11 FOTEMUSTINE.

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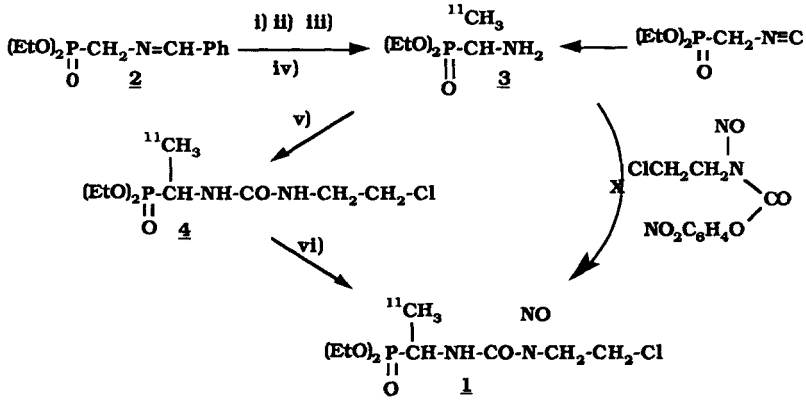
Nitrosoureas are commonly used for chemotherapeutic treatment of some tumours (1) and the search for new drugs less toxic and more selective is always under investigation. Efficacy of fotemustine **1**, a nitrosourea type compound [N-(2-chloroethyl)-N-nitroso-N' 1-(1-diethyl phosphonate) 2-[^{11}C] ethyl urea] in the treatment of malignant gliomas (2) prompted us to label it with^{11}C so that its pharmacokinetics and metabolic behaviour in human brain tumors could be studied *in vivo* using positron emission tomography (PET). The labelling at the ethylene carbon atom is of limited use because of the rapid formation *in vivo* of the chloroethylcarbonium ion (3). BCNU [1,3-bis (2-chloroethyl)nitrosourea], the most commonly used drug in the treatment of gliomas, has been labelled with nitrogen-13 (4) and carbon-11 (5) for studies of metabolic degradation of nitrosoureas. More recently, the less toxic [^{11}C] SarCNU, has also been prepared (6) . Both of them have been labelled on the ureido carbon using [^{11}C] phosgene. Due to the good stability of a carbon-carbon bond in comparison with a nitrogen-carbon bond and due to the possibility of preparing [^{11}C] iodomethane in high yields and high specific radioactivity, we have chosen to label the methyl group located on the carbon α to the phosphonate group.



Scheme 1 : Preparation of [^{11}C] fotemustine : i) BuLi, -78°C, THF ii) [^{11}C] CH_3I, 0°C, 4 min iii) NH_2OH, HCl, pH 5.6; 5 min, 140°C iv) K_2CO_3 v) $ClCH_2CH_2NCO$, CH_2Cl_2, 55°C, 5 min vi) $NaNO_2$, HCOOH, 0°C, 3 min.

The synthesis and the different approaches we have described for [^{11}C] fotemustine are outlined in scheme 1. The methods are based on the synthesis of [^{14}C] fotemustine developed in the I.R.I S group (7) and in the litterature (1). After [^{11}C] methylation (at 0°C, 4 min) of the carbanion derived from the imine **2** (obtained by addition at -78°C of 13.5 µL of a 2.08 M solution of butyllithium to 35.3 µ mol. of **2**) then hydrolysis (5 min) with hydroxylamine hydrochloride (300 µL, 0.5M) the crude product is passed through a small column filled with potassium carbonate.The THF is evaporated and the free amine **3** is obtained in 75-85% yield. Dichloromethane is then added and chloroethylisocyanate (50 µL) is allowed to react at 50°C for 5 min. The nitrosation is carried out in

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the same pot by adding to the cooled mixture (0°C) formic acid (500 µL) then sodium nitrite (100 µL, 9.7 M). After 5 min at room temperature, and neutralisation of the solution with potassium carbonate, the [^{11}C] fotemustine is purified by passing through two Sep-paks (C18 then silica). By this purification technique avoiding HPLC, [^{11}C] fotemustine has been obtained with a radiochemical purity greater than 96%. For example, starting from 592 MBq [^{11}C] iodomethane,162 Mbq of [^{11}C] fotemustine ready for injection have been prepared in 27% yield from [$^{11}CO_2$] decay corrected at EOB (4%, yield at the end of synthesis, 69 min counted from EOB). The complete results are summarized in table 1. All the new [^{11}C] compounds co-elute both in HPLC and TLC with their respective standards.

Table 1.

Compounds	Synthesis time (a)	Radiochemical yield (b)	Rf (c)	RT(d)(min)
3	35	80	0.45	(e)3.9
4	50	75	0.56	(f)9.3
1	65	30	0.70	(g)4.1

(a) in min from EOB (b) decay corrected at EOB from [$^{11}CH_3I$]; mean value for **3** and **4** (c) TLC : silica plate, eluents : dichloromethane/methanol (90/10, v/v) (d) HPLC : µ-Porasil (l x id : 300 x 7.8 mm), λ: 254 nm, flow rate : 2 mL.min^{-1} for **4** and **1**, 3 mL.min^{-1} for **3**.

Both the reliability of the different steps of this synthesis and the easy purification make this preparation of [^{11}C] fotemustine valuable for futher PET studies. Work is now in progress to reduce the reaction times and to adapt it to routine synthesis.

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