A REMOTELY-OPERATED SYSTEM FOR ROUTINE PRODUCTION OF REGIOSELECTIVE NO-CARRIER-ADDED 6-[^{18}F]FLUORODOPA

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The importance of 6-[^{18}F]fluorodopa (6-FDOPA) in elucidating the central dopaminergic system has been documented and recognized. Recently, C. Lemaire et al. (1) reported a nucleophilic approach to produce regioselective, no-carrier-added 6-FDOPA. We examined in detail the chemistry and routine production of 6-FDOPA by this method. Here we describe the design of a remotely-operated system for the routine production and the results of our study.

Our remotely-operated system incorporates the following technical details. 1) Two separate syringe pumps were fixed onto a Pierce heating module so as to allow individual, vertical motion of two reacti-vials (10 ml) into the aluminum block which could be heated to 150 ^{o}C by a remote switch. 2) A third reacti-vial was easy-fitted to the mouth of an industrial heat gun which produces temperatures well over 300 ^{o}C and controlled by an external control switch. 3) Transfer of liquids was achieved remotely through two-port and three-port solenoids, under reduced pressure, by a membrane pump, rather than by compressed air or nitrogen. 4) A multi-port rotary valve was installed to deal with the complexity of the ^{18}F-fluoride displacement reaction step. 5) Loading of the sample into the HPLC loop was achieved by means of a syringe pump. 6) The whole assembly was installed in a hot cell with two sliding lead shields and a flexible mechanical arm, thus eliminating exposure to activity throughout the production.

Several production runs (n >10) were performed with the following results. The fluoride displacement was carried out with a radiochemical yield of 23% $\pm$ 3 EOB. The condensation step proceeded with a relative yield of 66%$\pm$6 (15%$\pm$2 EOB). However, the hydrolysis step gave racemic 6-FDOPA with only 20%$\pm$6 (3.0%$\pm$1 EOB) yield, contrary to the published results. The chiral separation yields 6-FDOPA with 35%$\pm$3 of the racemic activity. Thus, the total time required for the production of 6-FDOPA is 150 min $\pm$10 min and the overall yield is 1.0% $\pm$0.5 decay corrected. The Cu (II) ions in the final solution might pose some health concerns and hence have been sequestered as PTSM-Cu (II) complex . We are currently involved in improving the yield and the quality control measures.

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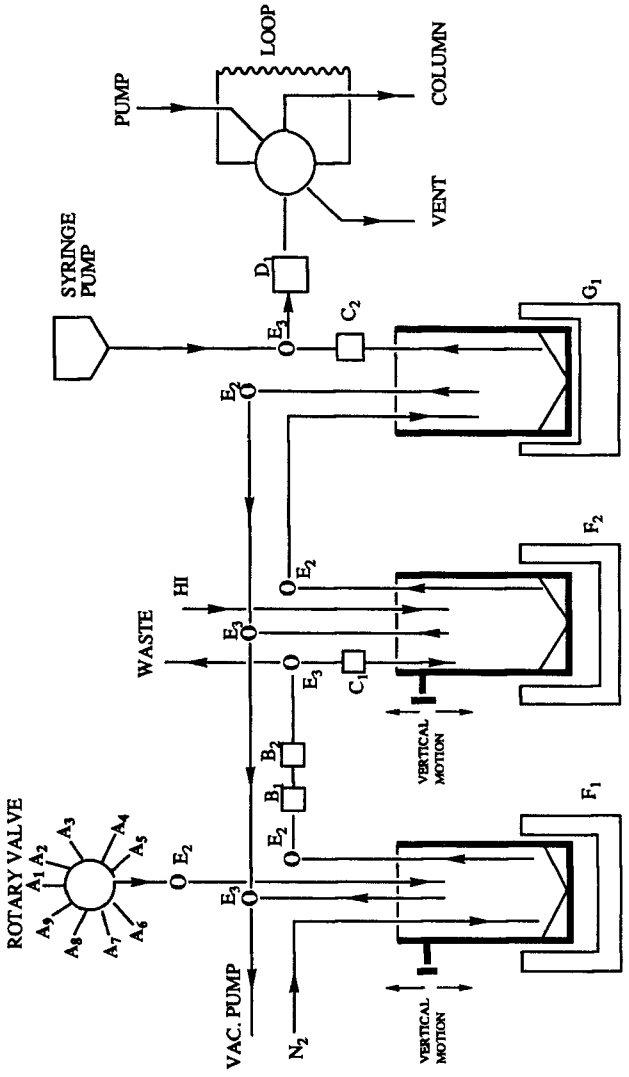

REMOTEL Y-OPERATED SYSTEM FOR ROUTINE PRODUCTION OF REGIOSELECTIVE NO-CARRIER ADDED 6-[^{18}F]FLUORODOPA

A_1-A_9 = DIFFERENT SOLVENTS/ MIXTURES, B_1-B_2 = C_{18} SEP PAKS, C_1-C_2 = CARTRIDGES (Na_2CO_3, GLASS WOOL), D_1 = MILLIPORE FILTER, E_2 = TWO-PORT SOLENOID, E_3 = THREE-PORT SOLENOID, F_1-F_2 = HEATING BLOCK, G_1 = HEAT GUN

REMOVAL OF MERCURY CONTAMINATION FROM 6-F-DOPA PREPARATIONS

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One of the highest yielding routes to ^{18}F-labelled 6-F-Dopa starts from a precursor containing a mercury trifluoroacetyl group in the 6- position(1,2). In spite of the fact, that two different methods (1,3) have been used to avoid mercury contamination of the final injectable solution, and even though the upper limit of 0.5 ppm given by the USP monograph (4) should be easily met by the above procedures, there still remains concern for potential users of this method (5).

In a series of test runs before the beginning of patient studies, we found low,(normally up to 100 ppb,measured by AAS) but variable amounts in spite of unchanged synthesis procedures. In a single case, the USP limit was exceeded in the final sample by about 40% due to a yet unknown reason.
Therefore, it was tried to add a further purification step to almost completely remove the contamination.

Chelite-S, a mercury-absorbing resin (SERVA) proved successful for this purpose. Tests with mercury-spiked solutions have shown, that 500 mg of chelite-S removes mercury to an undetectable level (below 1 ppb) from 10 ml of a 50 ppm mercury test solution. Such a high contamination level is extremely unlikely to occur as long as the synthesis procedure is followed correctly. Tests with 6- F-DOPA- samples showed also that mercury was removed to undetectable levels. Loss of product after chelite and sterile filtration was between 10 and 15 % of the collected activity after HPLC.

The procedure is as follows: 500 mg of Chelite-S (SERVA Nr. 41710) is placed in a small column and prewashed with 10 ml of water for injection, followed by an air bolus . The sample is passed slowly (ca. 8 ml/min) over the column, then the column is washed with additional 2 ml of water for injection. The sample is then made isotonic by addition of concentrated sodium chloride solution and drawn into a sterile vial via a sterile filter. After quality control (2)the sample is ready for injection.

By using this additional purification step, the tedious HPLC method for mercury determination (6) can eventually be omitted and replaced by occasional checks using the more sensitive and reliable AAS method.

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[^{11}C]COCAETHYLENE: SYNTHESIS AND ASSESSMENT OF ITS BIOAVAILABILITY RELATIVE TO COCAINE. J. S. Fowler, N. D. Volkow, R. R. MacGregor, J. Logan, S. L. Dewey, S. J. Gatley and A. P. Wolf, Brookhaven National Laboratory, Upton, NY, 11973

Recent epidemiological studies have shown that the combined use of cocaine and alcohol confers an eighteen-fold increase in the risk of sudden death relative to cocaine alone (1). Although the factors mediating the increased risk associated with the cocaine/alcohol combination are not fully understood, recent studies have presented evidence that cocaethylene (CE), the ethyl homologue of cocaine (COC) and a metabolite of COC formed in the presence of alcohol, may account for some of the behavioral and toxic effects of COC (2).

Although COC and CE are very similar in structure, differences in lipophilicity, plasma protein binding and the ability to serve as substrates for cholinesterases in plasma could result in a different uptake and residence time in target organs such as the brain. To assess the bioavailability of CE relative to COC, we have labeled CE with carbon-11, and compared its rate of uptake, distribution and clearance in baboon brain with that of [^{11}C]COC. We have also compared their plasma input functions, free fractions in plasma and their relative rates of metabolism.

[N-^{11}C-methyl]Cocaethylene was prepared by the C-11 methylation of norcocaethylene [prepared by the N-demethylation of cocaethylene (NIDA)] with [^{11}C]methyl iodide using conditions described for [^{11}C]cocaine (3) in a synthesis time of 40 minutes and a specific activity of 500 Ci/micromol (EOS).

Comparative PET studies of labeled COC and CE in baboon brain (n=5) over a 40 minute time period showed similar regional distribution and rapid rate of uptake of the two compounds with peak uptake at 2-5 minutes. The clearance of carbon-11 from all brain regions was generally slower for CE than COC as shown below.

	STRIATUM		THALAMUS		CEREBELLUM	
	COC	CE	COC	CE	COC	CE
Peak Uptake (%/cc)	0.05 ± 0.01	0.047 ± 0.01	0.049 ± 0.01	0.049 ± 0.01	0.047 ± 0.01	0.045 ± 0.01
Clearance $t_{1/2}$ (min)	15.2 ± 4.3	20.2 ± 4.9[a]	9.2 ± 3.0	13.4 ± 4.7[b]	5.2 ± 2.6	9.4 ± 3.6[c]

(a) $p < 0.05$
(b) $p < 0.01$
(c) $p < 0.005$ paired t-test (two tail)

The value of the integral of the arterial plasma input function for [^{11}C]CE was also higher than that of COC in 4 out of 5 baboons with the ratio of the integral for CE versus the integral for COC ranging from 1.0-1.2 over a 30 minute period. In vitro studies in which each compound was incubated with baboon plasma or purified horse butyrylcholinesterase showed that CE was metabolized at about one third the rate of COC. Plasma free fractions of COC and CE were 33.5 and 30 % respectively.

In summary, CE has a similar rate of uptake and distribution but shows a significantly slower clearance than COC. This is accounted for, in part, by the slower metabolism of CE relative to COC in plasma. Although the extent to which CE contributes to the enhanced toxicity when COC and alcohol are used in combination is not known, the results of these studies indicate that the increased bioavailability of CE, once formed, could lead to longer tissue exposures than cocaine thus enhancing its toxicity. Under conditions of continuous drug administration, CE would accumulate to a larger extent than COC leading more rapidly to toxic and lethal concentrations. Supported by NIDA and DOE/OHER.

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SCHEME 1. Synthesis of [N-^{11}C-Methyl]Cocaethylene

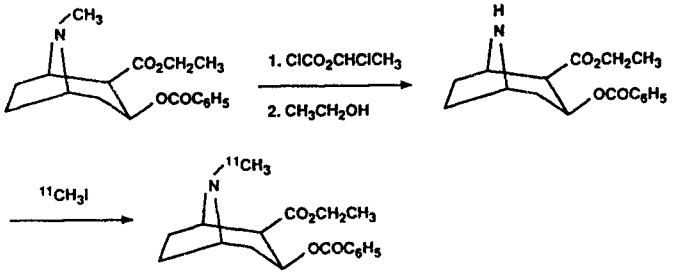
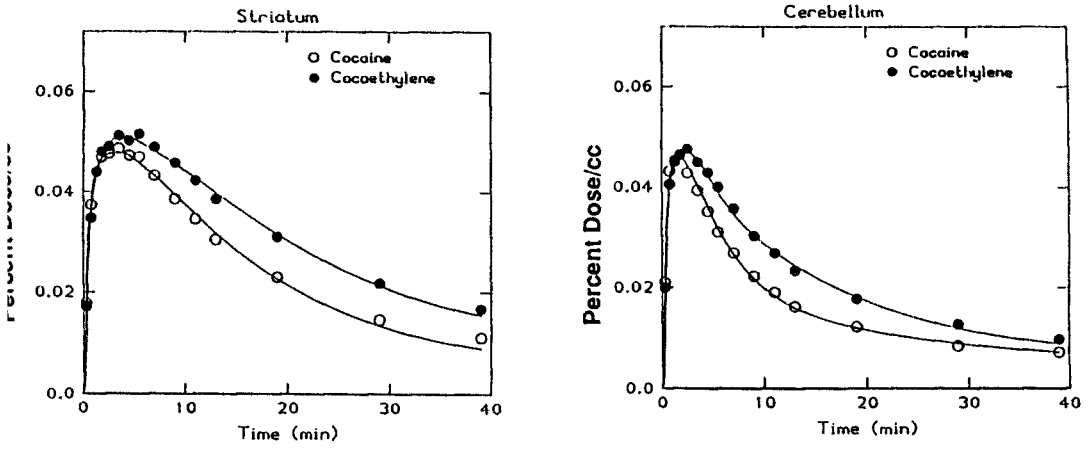


FIGURE 1. Comparative Uptake and Clearance of [^{11}C]Cocaine and [^{11}C]Cocaethylene in Baboon Brain



SYNTHESIS OF NO CARRIER ADDED [^{18}F] GBR 12936 *VIA* A WITTIG REACTION FOR USE IN A THE DOPAMINE REUPTAKE SITE STUDY

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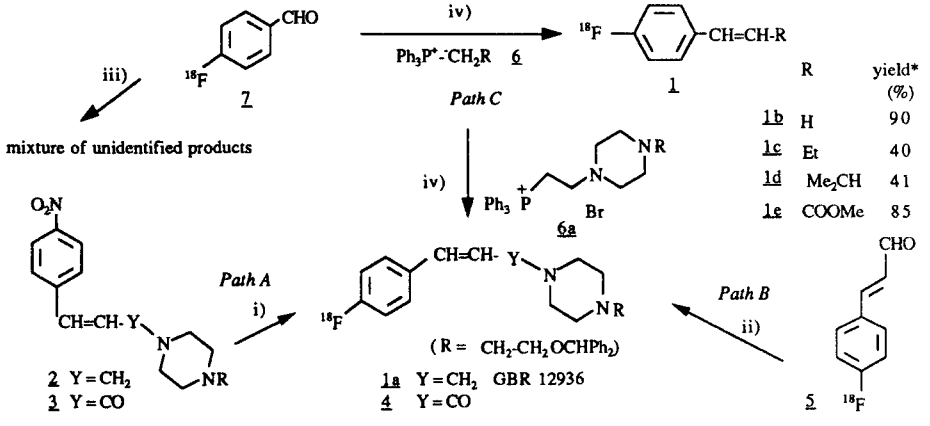
The dopaminergic system plays a prominent role in the etiology of certain psychiatric and neurological diseases (1). It has received much attention in positron emission tomography (PET)(2) and extensive research of a selective and potent inhibitor of presynaptic dopamine reuptake has been carried out during the last five years. Nomifensine has been labelled with carbon-11 (3), and a variety of N,N'-disubstituted piperazines have been synthesized and evaluated *in vitro* and in animal studies (4, 5). Some (6, 7) have also been labelled with fluorine-18 in order to obtain a suitable radiotracer for human use. The high affinity (IC_{50} : 1.2 nM) and selectivity [IC_{50} ratio dopamine/noradrenaline : 150) for the dopamine neurons of [1-[(diphenylmethoxy)ethyl]-4-[3-(4-[^{18}F]fluorophenyl)-2-propenyl) piperazine] [GBR 12936] (5) make this compound a suitable candidate for PET studies. With this aim, we have developed a synthesis of GBR 12936 1 labelled with fluorine-18 ($t_{1/2}$ 110 min).

Scheme 1 : synthetic routes to [^{18}F] GBR 12936



* the yields are decay corrected i) $^{18}F^-$ M^+ (M : K/K_{222}, Cs, NBu_4), DMSO or MeCN, 110 or 150°C, 25 min ; ii) piperazine (3mg), MeOH (100 µL), AcOH (8 µL) $NaBH_3CN$ (4 mg), 120°C, 10 min ; iii) 6 (10 mg), BuLi (15 µL of a 1.85 M solution in hexane), RT, 15 min ; iv) 6 (10 mg), propylene oxide (60 µL) o-dichlorobenzene (120 µL), 160°C, 10 min

Three synthetic approaches have been studied (Scheme 1). The shortest (path A), which involved a direct nucleophilic exchange of a nitro group on the aromatic ring of the precursor 2 or 3 gave less than 5% of the expected [^{18}F] fluoro compounds (respectively 1a or 4). This was as expected, due to the low reactivity towards nucleophilic substitution of an aromatic ring with no *para*

electron withdrawing group (8). The second route (path B), which used the reductive amination (yield : 50%) (9, 10) of 4-[^{18}F] fluorocinnamadehyde (7) was restricted by the difficulties encountered in preparing sufficient amounts of the starting [^{18}F] aldehyde 5. The last route (path C) consisted of a Wittig reaction of the now readily available [^{18}F] fluorobenzaldehyde (11) with the appropriate phosphonium salt 6a. The feasibility of the reaction was tested using different phosphonium salts (1b-e), because such a condensation was not known in fluorine-18 chemistry. The results are presented in scheme 1. They showed that, as in carbon-11 chemistry (12), the use of a strong base (BuLi) to generate the ylide resulted in side reactions that are not observed under the usual stoichiometric Wittig conditions. Using 1,2-epoxypropane as the base (13) it was possible to prepare [^{18}F] GBR 12936 1a in 25 % isolated yield from 4-[^{18}F]-fluorobenzaldehyde (decay corrected ; 95% radiochemically pure, overall synthesis time : 90 min). The structure and stereochemistry (Z/E ratio for 1a : 40/60) of the new compounds have been determined spectroscopically. All the labelled species have been identified by comparison of their Rf and retention times, both in TLC [CH_2Cl_2/MeOH (v/v : 95/5) Rf 1a : 0.3] and HPLC [μ Porasil, $CH_3CN/(NH_4)_2HPO_4$ 4mM (v/v : 80/20), 1 mLmin^{-1}, λ 254 nm , retention time 1a : 16 min] with those of standards.

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SYNTHESIS OF [^{18}F]-3-FLUOROMETHYL-N-[1-(2-BENZOTHIENYL)-CYCLOHEXYL]-PIPERIDINE, A POTENT RADIOLIGAND FOR THE DOPAMINE RE-UPTAKE COMPLEX.

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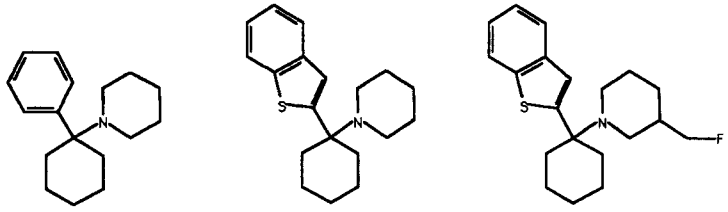
N-[1-(2-benzothienyl)-cyclohexyl]-piperidine (BTCP) was a new analog of phencyclidine (PCP) that had been developped recently for his specific binding to the dopamine re-uptake complex.

In order to visualize the receptor by positon emission tomography, cold and [^{18}F] analogs of BTCP were obtained by a similar synthesis that had been developped for the [^{18}F]-3-fluoromethyl-TCP. For the same reasons, the 3- position of the piperidine ring was choosen.



PCP BTCP 3-FLUOROMETHYL-BTCP

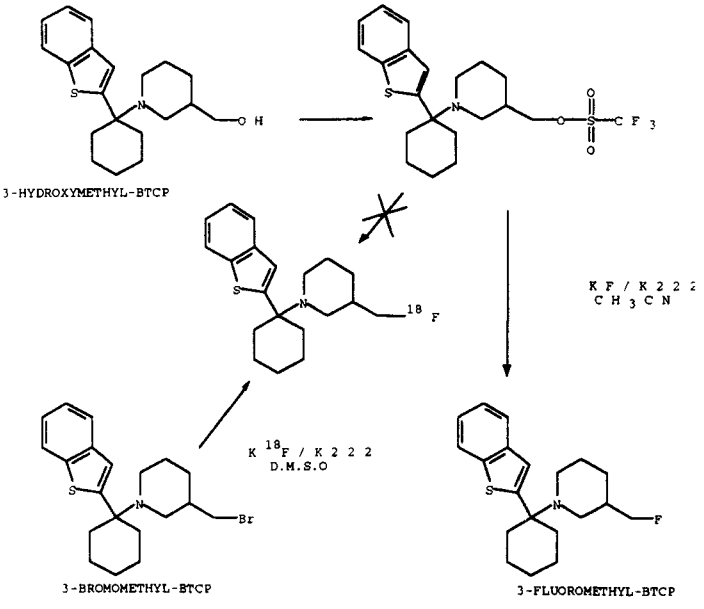
Cold 3-fluoro-BTCP was obtained in two steps via the triflate pathway with a 50% yield without isolation of intermediate product.

Radiosynthesis attempt was the nucleophilic substitution using [^{18}F$^-$] produced by a (p.n) reaction with 16 Mev protons on 50% [^{18}O] enriched water.
[^{18}F]-3-fluoromethyl-N-[1-(2-benzothienyl)-cyclohexyl]-piperidine ([^{18}F]-3-fluoromethyl-BTCP) was usefuly synthesized by direct exchange from 3-bromo-BTCP with K[^{18}F]/K 222 for 15 min in DMSO at 130 °C.

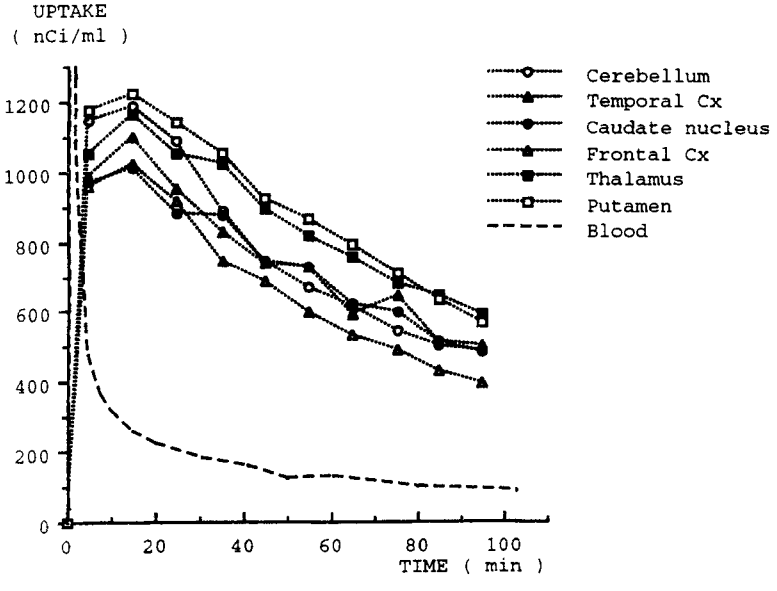
After elution on silica, then C-18 Sep-Pack cartridges, the crude material has a radiochemical purity of 85%, but was contaminated by the excess of unlabelled precursor and secondary products.Two HPLC purifications were performed on a C-18 µ-bondapack column with an eluant of 60% : acetonitrile, 20% : isopropanol, 20% : water, 0.05% : triethylamine.
10 mCi of [^{18}F]-3-fluoromethyl-BTCP was obtained (about 10% yield, decay uncorrected) with a specific radioactivity of 889mCi/µmol. Radiosynthesis procedure was found to be useful for routine use in *in vivo* studies.

In preliminary PET studies on baboon brain, [^{18}F]-3-fluoromethyl-BTCP also readily crossed the blood brain barrier. In general, the distribution of the radioligand was hetrogenous and similar to the known distribution of the dopamine re-uptake complex.

CHEMICAL AND RADIOCHEMICAL PATHWAY OF 3-FLUOROMETHYL-BTCP ANALOGS



[18F]-3-FLUOROMETHYL-BTCP UPTAKE ON BABOON BRAIN : REGIONAL DISTRIBUTION



Paper F6

SYNTHESIS OF [^{11}C]TETRABENAZINE AND A [^{11}C]METHOXY DERIVATIVE OF α-DIHYDROTETRABENAZINE FOR PET IMAGING OF MONOAMINERGIC NERVE TERMINALS

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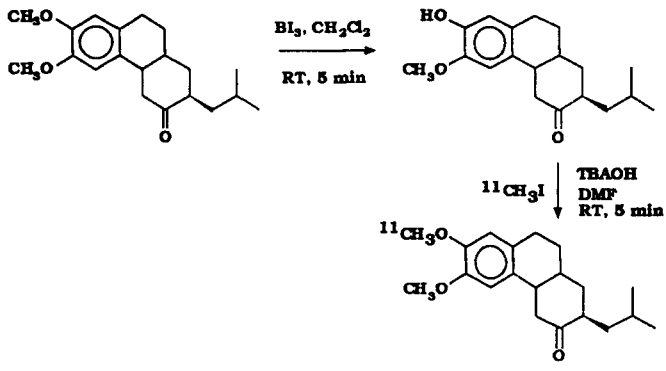
Tetrabenazine (TBZ), a potent specific inhibitor of the vesicular monoamine transporter, shows more CNS specificity, a shorter duration of monoamine depletion and a greater selectivity for depletion of brain catecholamines (dopamine > serotonin) as compared to reserpine (1). α-[^{3}H]Dihydrotetrabenazine ([^{3}H]TBZOH), obtained by simple reduction of TBZ, binds with high affinity to the vesicular monoamine carrier (K_d = 3 nM), and has been used as a radioligand for *in vitro* homogenate and autoradiographic studies of the monoamine vesicular transporter. [^{3}H]TBZOH shows high specific binding in the striatum of rodents (2). In 6-hydroxydopamine-lesioned rats, loss of [^{3}H]TBZOH sites correlated well with loss of tyrosine hydroxylase activity (3) and post-mortem studies of brains of patients with Parkinson's disease revealed lower binding of [^{3}H]TBZOH in the caudate nucleus and the putamen compared to controls (4).

Tetrabenazine and its derivatives are thus potential candidates for development of new PET radioligands to study losses of monoaminergic neurons in neurological diseases. The high binding affinities, moderate lipophilicities (TBZ, log P=2.68 (5)) and low toxicities of this class of drugs are particularly encouraging. We report here the synthesis of tetrabenazine in carbon-11 form and, since large substituents can be attached at the α-hydroxy function of TBZOH with retention of high binding affinities (5,6), the synthesis of the carbon-11 labeled O-methyl derivative of TBZOH.

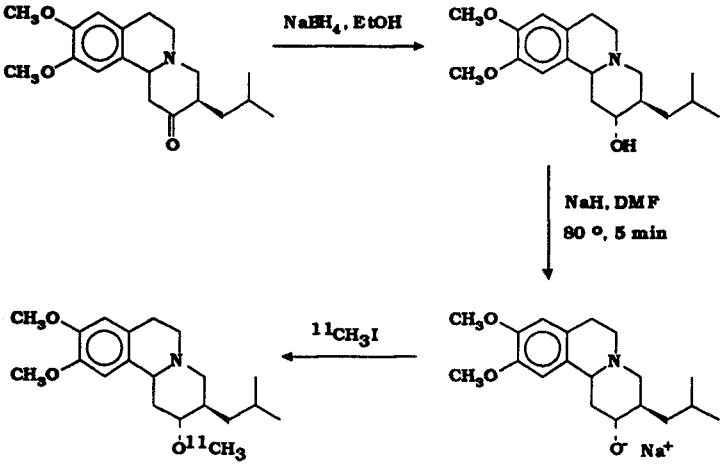
[^{11}C]Tetrabenazine was prepared by [^{11}C]methylation of the desmethyl-TBZ derivative with [^{11}C]methyl iodide (Scheme I). The demethylation of TBZ was carried out with BI_3 (1 equiv.) in CH_2Cl_2 under N_2 at room temperature for 5 min, more selectively and with a higher yield (10%) than with BBr_3, and the products were separated by silica gel chromatography ($CHCl_3$:MeOH gradient). The desmethyl-TBZ was further purified by HPLC on a semi-preparative silica gel column. [^{11}C]Methyl iodide was trapped in a reaction vessel containing the desmethyl-TBZ (1.0 mg), tetrabutylammonium hydroxide (MeOH solution, 0.5 equiv.) in dry DMF (200 μL) at -40 °C. After heating at 20 °C for 5 min, [^{11}C]TBZ was purified by HPLC using a semi-preparative reversed phase C_{18} column, with a high radiochemical yield (> 45%, decay corrected).



Scheme I. Synthesis of [^{11}C]Tetrabenazine.

[^{11}C]TBZOMe was synthesized by O-[^{11}C]methylation of the alkoxide form of TBZOH (Scheme II). TBZOH was prepared by reduction of TBZ with $NaBH_4$ (3.5 equiv.) in dry EtOH at 0 °C for 90 min and the α-isomer was separated from the β-isomer by a silica gel chromatography. The sodium alkoxide of TBZOH was formed in the C-11 synthesis apparatus just prior to the O-[^{11}C]methylation, by heating (80 °C) the reaction vessel containing TBZOH (1.5 mg) and NaH (2 mg, 17.7 equiv.) in dry DMF (200 μL) under N_2 for 5 min. The vial was cooled to -31 °C, [^{11}C]CH_3I was bubbled into the reaction mixture, and the vial sealed and warmed to 0 °C for 5 min. Water (0.3 mL) was added at 0 °C to stop the reaction and decompose the excess NaH, and the solution was transferred onto a short reversed phase column filled with C_{18} Sep-pak packing that was pre-washed with MeOH (10 mL) and NH_4OH 0.2 N (20 mL). After blowing dry with N_2 for 1 min, the HPLC solvent was passed through this short column onto a silica gel semi-preparative HPLC column to give [^{11}C]TBZOMe in a high radiochemical yield (> 50%, decay corrected).

Isolated products were prepared for animal experiments by evaporating the HPLC solvent and dissolving the residue in a sterile solution of isotonic phosphate buffer (pH 6.0). Filtration provided an injectable formulation within 45 min from EOB. For both products, high specific activities (>2000 Ci/mmol), and high radiochemical and chemical purities (>95%) were observed by analytical HPLC. The identity of the [^{11}C]TBZ and [^{11}C]TBZOMe were confirmed by comparison of HPLC retention times and TLC R_f values, which were identical to those of authentic TBZ and authentic TBZOMe (prepared by O-methylation of the Na-alkoxide salt of TBZOH with 1 equiv. of CH_3I).



Scheme II. Synthesis of [^{11}C]TBZOMe.

Preliminary *in vivo* studies in mice with [^{11}C]TBZ showed good brain penetration, with high uptake in the striatum (rich in dopamine sites), and lesser uptake in hypothalamus, hippocampus and cortex (regions rich in serotonergic and/or noradrenergic sites). Rank order striatum> hypothalamus>hippocampus=cortex>cerebellum was the same as has been reported using *in vitro* studies with [^{3}H]TBZOH (2). Biodistribution studies in mice are in progress to evaluate the *in vivo* potential of [^{11}C]TBZOMe as an imaging agent for PET, and to determine the effect of methoxy substitution on the formation and distribution of labeled metabolites.

Acknowledgements. This work was supported by a postdoctoral fellowship from the Fonds de la Recherche en Santé du Québec (to J.N.D.) and USPHS grants NS 15655 and MH 47611.

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SYNTHESIS OF [F-18]-LABELLED α-FLUOROMETHYL-p-TYROSINE, A TYROSINE HYDROXYLASE-ACTIVATED DECARBOXYLASE SUICIDE INHIBITOR WITH POTENTIAL AS IMAGING AGENT FOR DOPAMINE NERVE TERMINALS.

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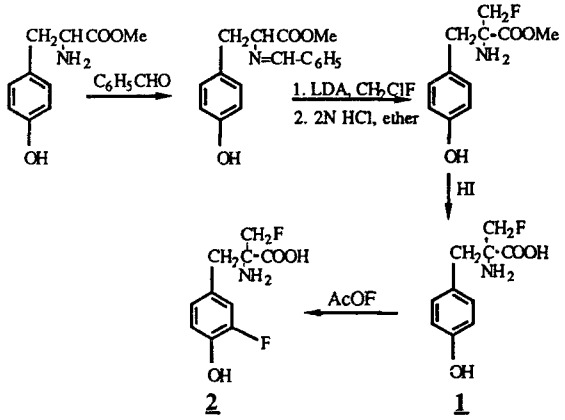
L-Dopa based-tracers currently in use or proposed for use in the imaging of central dopamine (DA) nerve terminals are 6-fluoro-L-Dopa (6-FD) (1), fluoro-m-tyrosine (FMT) (2) and fluoro-β-fluoromethylene-m-tyrosine (F-FMMT) (3). These compounds enter the dopamine metabolic pathway via the decarboxylation step which involve the enzyme L-aromatic amino acid decarboxylase (L-AAAD). This decarboxylation step, however, is fast and not rate determining in the biosynthesis of dopamine. The control for dopamine biosynthesis lies with the hydroxylation step prior to decarboxylation and involves the enzyme tyrosine hydroxylase (TH) (4). Since DA synthesis is tightly coupled to neuronal activity (5), a tracer technique to estimate TH activity may be useful in the assessment of DA neuronal activity in healthy as well as in individuals with movement disorders such as Parkinson's disease.

α-Fluoromethyl-p-Tyrosine (FMPT) was developed by Jung et al. (6) as a TH-activated irreversible inhibitor of L-AAAD aimed at limiting L-AAAD inhibition to catecholamine terminals sparing serotonin terminals which contain L-AAAD but not TH. FMPT has indeed been found to be selective for sites where active catecholamine synthesis occur (6) arising from the fact that the TH product, α-monofluoromethyl-L-dopa (MFMD), is one of the most potent L-AAAD inhibitors known (7). [F-18]-6-F-MFMD was recently prepared by Chirakal et al. and found to be useful in delineating L-AAAD-containing structures (8).

We have prepared [F-18]-labelled FMPT in order to explore the possible use of this compound in estimating TH activity. α-Fluoromethyl-p-Tyrosine (FMPT) was first synthesized via the scheme shown below.



Multinuclear (^{1}H, ^{13}C, ^{19}F) NMR spectroscopy was used to confirm the structure of the product, FMPT (1). Direct fluorination of FMPT with acetylhypofluorite (AcOF) resulted in a single product identified by multinuclear NMR analysis as 3-fluoro-FMPT (2).

Direct radiofluorination of DL-FMPT (10 mg) in 5ml 1:1 trifluoroacetic acid-glacial acetic acid using [F-18]-AcOF gave [F-18]-3-F-FMPT (2) with radiochemical yields of 19.3% ± 1.7% of HPLC purified product based on starting [F-18]-AcOF and decay corrected to the end of bombardment. Semipreparative radio-HPLC (reversed phase C-18 column, 250 x 10 mm, 10 µ, 0.02M NaOAc pH 3.5, 5 ml/min flowrate) was used to separate the product from the starting material (Fig. 1). The preparation time needed from the end of trapping [F-18]-AcOF to having the ready-to-inject HPLC-purified product was about 45 minutes.

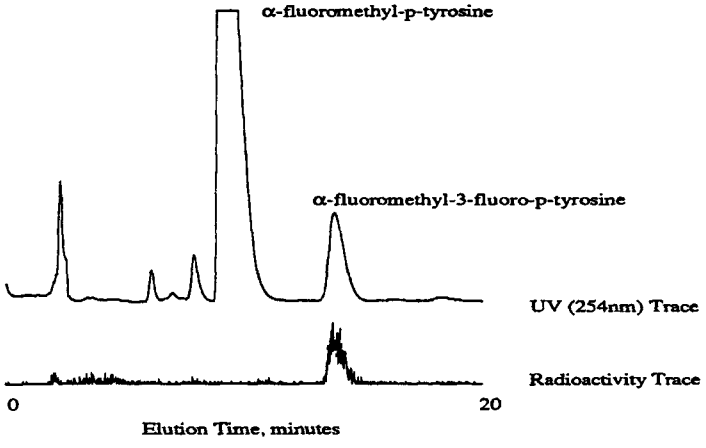


Figure 1. RadioHPLC of FMPT + [F-18]-AcOF reaction.

One potential problem with the use of [F-18]-3-F-FMPT as PET imaging agent is the slow rate of hydroxylation of the parent FMPT by TH compared with p-tyrosine. Biochemical studies are thus needed to validate [F-18]-3-F-FMPT as tracer for TH activity in the estimation of dopamine neuronal activity.

Support provided by NIH Grant NS 26621 is gratefully acknowledged.

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Structurally Modified Analogs of Clebopride and BRL 34778 as Potential ^{18}F-Labeled Ligands for PET Studies of the Dopamine D_2 Receptor.

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Clebopride and BRL 34778 are structurally related benzamide derivatives possessing a high affinity for the dopamine D_2 receptor.[1,2] The low affinity of each analog for dopamine D_1, serotonin 5-HT_2, and α-adrenergic receptors indicates that both clebopride and BRL 34778 are suitable lead compounds for PET radioligand development.[1,2] The presence of either an N-benzyl group (clebopride) or an N-4-fluorobenzyl group (BRL 34778) simplifies the radiosynthesis since a suitable ^{18}F-labeled analog can be prepared by alkylation of the N-desbenzyl precursor with [^{18}F]4-fluorobenzyl iodide ([^{18}F]FBI).[3] As part of an ongoing program in PET radiotracer development, a number of structurally modified analogs of clebopride and BRL 34778 were prepared and their affinity for the dopamine D_2 receptor was measured using [^{125}I]NCQ 298 as the radioligand.[4] The results (Table I) indicate that several of these analogs are suitable candidates for further evaluation as PET radiotracers.

Radiosynthesis of the ^{18}F-labeled analogs of fluoroclebopride, MBP, BBP, and MABN was achieved via N-alkylation of the corresponding desbenzyl precursor with [^{18}F]FBI. The radiolabeled products were obtained in overall yields of 5-15% and required a synthesis time of ~115 min. The specific activity of the final product ranged from 600-2200 mCi/μmol decay corrected to EOB.

In vivo studies with rats indicate that all of the ^{18}F-labeled analogs are capable of crossing the blood-brain barrier with brain uptake at 5 min displaying the following rank order: [^{18}F]MBP>[^{18}F]fluoroclebopride~[^{18}F]BBP>[^{18}F]MABN (Table II). A high accumulation in the striatum, a region with a high density of D_2 receptors, was observed for all four analogs; striatal uptake was blocked by co-injection of the radiotracers with 60 μg of spiperone. The striatum:cerebellum ratios displayed the following rank order: [^{18}F]MABN>[^{18}F]BBP>[^{18}F]MPB>[^{18}F]fluoroclebopride. [^{18}F]Fluoroclebopride displayed a relatively fast rate of washout from the striatum. These results suggest that [^{18}F]MABN, [^{18}F]BBP and [^{18}F]MBP are suitable candidates for further evaluation as potential ligands for studying dopamine D_2 receptors *in vivo* with PET.

This research was supported by USPHS Grants NS14867, MH43880, NS18591, and GM34781.

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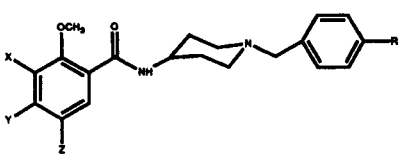
Table I. Structures and Binding Constants of the Benzamide Analogs.



Clebopride-based Analogs

BRL 34778-based Analogs

Analog	X	Y	Z	R	Ki (nM)
Clebopride	H	NH_2	Cl	H	1.13
Fluoroclebopride	H	NH_2	Cl	F	0.95
MBP	OCH_3	H	H	F	0.38
BBP	OCH_3	H	Br	F	0.07

Analog	X	Y	Z	Ki (nM)
BRL 34778	H	NH_2	Cl	0.017
MABN	OCH_3	H	H	0.028
BABN	OCH_3	H	Br	0.018

Table II. Brain Uptake of the ^{18}F-Labeled Benzamide Analogs in Rats.[a]

t	%ID Brain	%ID/g Brain	%ID/g Blood	Brain:Blood Ratio[b]
		[^{18}F]Fluoroclebopride		
5 min	1.01 ± 0.08	0.65 ± 0.05	0.115 ± 0.006	5.67 ± 0.15
30 min	0.48 ± 0.01	0.26 ± 0.03	0.057 ± 0.008	4.47 ± 0.41
1 h	0.47 ± 0.09	0.16 ± 0.04	0.031 ± 0.007	5.10 ± 0.09
2 h	0.19 ± 0.02	0.11 ± 0.01	0.023 ± 0.002	5.18 ± 0.17
3 h	0.09 ± 0.01	0.06 ± 0.01	0.011 ± 0.001	5.04 ± 0.22
		[^{18}F]MBP		
5 min	1.28 ± 0.15	0.81 ± 0.11	0.155 ± 0.010	5.32 ± 0.45
30 min	0.61 ± 0.02	0.39 ± 0.01	0.066 ± 0.010	6.34 ± 0.97
1 h	0.57 ± 0.05	0.36 ± 0.04	0.043 ± 0.009	9.12 ± 1.27
2 h	0.50 ± 0.02	0.33 ± 0.02	0.029 ± 0.002	11.26 ± 0.49
3 h	0.38 ± 0.02	0.25 ± 0.02	0.017 ± 0.002	14.91 ± 1.05
4 h	0.17 ± 0.01	0.11 ± 0.01	0.010 ± 0.001	11.44 ± 1.09
5 h	0.19 ± 0.01	0.12 ± 0.01	0.012 ± 0.001	10.04 ± 1.31
		[^{18}F]BBP		
5 min	1.12 ± 0.10	0.58 ± 0.06	0.088 ± 0.017	7.56 ± 0.85
30 min	0.55 ± 0.05	0.32 ± 0.04	0.036 ± 0.013	7.15 ± 0.80
1 h	0.36 ± 0.01	0.20 ± 0.01	0.025 ± 0.002	7.40 ± 0.29
2 h	0.27 ± 0.02	0.14 ± 0.01	0.016 ± 0.001	8.72 ± 0.48
3 h	0.20 ± 0.01	0.10 ± 0.01	0.013 ± 0.003	10.21 ± 0.80
4 h	0.21 ± 0.01	0.12 ± 0.01	0.012 ± 0.001	10.00 ± 0.64
5 h	0.20 ± 0.06	0.12 ± 0.03	0.012 ± 0.004	10.29 ± 0.55
		[^{18}F]MABN		
5 min	0.64 ± 0.05	0.39 ± 0.04	0.156 ± 0.018	2.63 ± 0.43
30 min	0.35 ± 0.04	0.20 ± 0.02	0.089 ± 0.017	2.63 ± 0.73
1 h	0.31 ± 0.03	0.23 ± 0.03	0.044 ± 0.003	4.55 ± 0.45
2 h	0.44 ± 0.07	0.27 ± 0.03	0.024 ± 0.001	14.77 ± 2.50
3 h	0.37 ± 0.04	0.23 ± 0.03	0.019 ± 0.002	19.37 ± 2.77
4 h	0.36 ± 0.05	0.22 ± 0.03	0.013 ± 0.003	20.25 ± 2.88
5 h	0.23 ± 0.03	0.14 ± 0.02	0.005 ± 0.002	23.50 ± 3.67

[a]Mean ± SE (n = 4-8); [b](%ID/g brain)/(%ID/g blood).

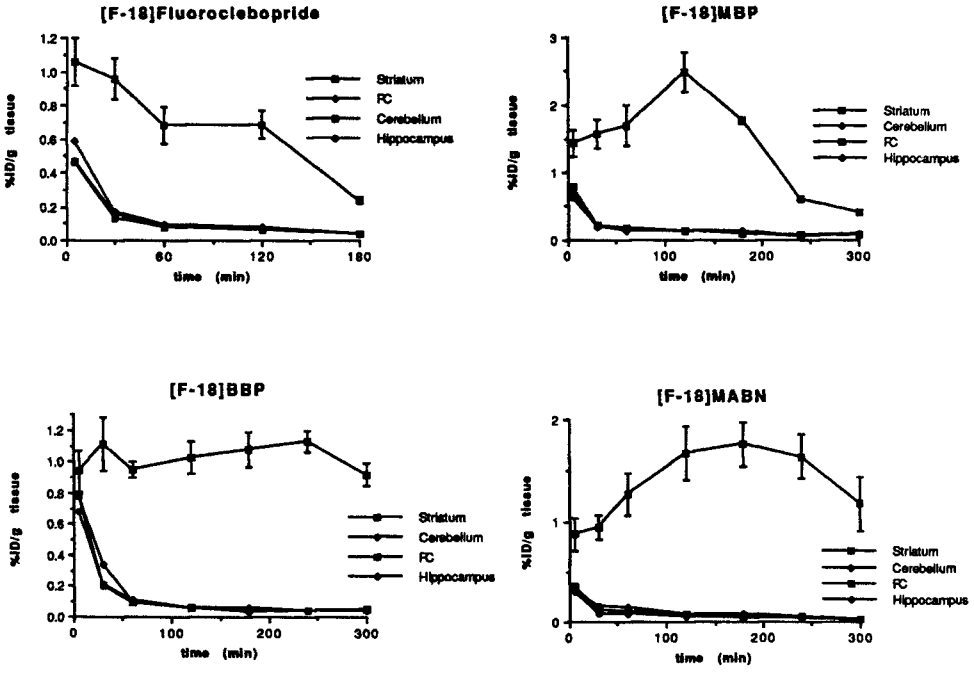


Figure 1. Tissue-time activity curves for the ^{18}F-labeled benzamide analogs.

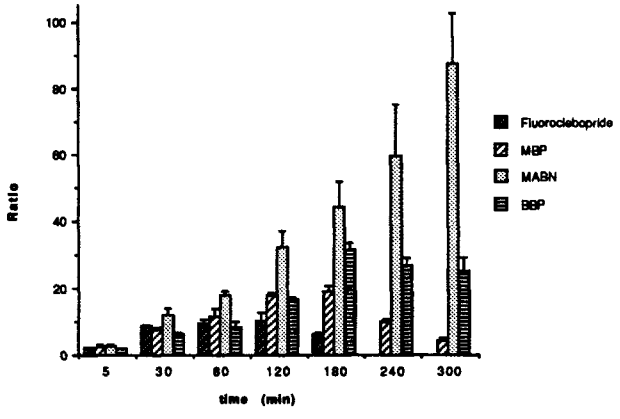


Figure 2. Striatum:cerebellum ratios for the ^{18}F-labeled benzamide analogs.

PREPARATION OF TWO POTENT AND SELECTIVE DOPAMINE D-2 RECEPTOR AGONISTS: (R)-[propyl-^{11}C]-2-OH-NPA AND (R)-[methyl-^{11}C]-2-OCH_3-NPA.

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(R)-Apomorphine (APO) and (R)-N-n-propylnorapomorphine (NPA) are considered to be centrally active dopamine receptor agonists with IC_{50}-values of 66.7 and 4.8 nM, respectively (1). Recently, the more potent and selective dopamine D-2 agonists (R)-2-OH-NPA and (R)-2-OCH_3-NPA (IC_{50} = 0.32 and 1.0 nM) have been developed (2). (R)-2-OH-NPA and (R)-2-OCH_3-NPA were labelled with ^{11}C and the potential of these dopamine D-2 agonists for PET was examined.

(R)-[Propyl-^{11}C]-2-OH-NPA was labelled by N-alkylation of the free base of the secondary amine with [^{11}C]propyl iodide (Scheme 1). Reaction in acetonitrile/DMF at 140°C for 12 min with subsequent reversed-phase semi-preparative HPLC purification resulted in 20-25% radiochemical yield (from EOB and decay-corrected) with a total synthesis time of 45 min and a radiochemical purity >99%. The specific radioactivity obtained at EOS was about 1000-2000 Ci/mmol. (R)-[Methyl-^{11}C]-2-OCH_3-NPA was labelled by O-alkylation of the trihydroxy-precursor with [^{11}C]methyl iodide (Scheme 2). Reaction in DMSO at 80°C for 5 min with subsequent reversed-phase semi-preparative HPLC purification resulted in 25-30% radiochemical yield (from EOB and decay-corrected) with a total synthesis time of 35-40 min and a radiochemical purity >99%. The specific radioactivity obtained at EOS was about 1000 Ci/mmol. To further examine the labelled product, derivatization of the catechol to acetate was performed and compared with a reference material.

Preliminary *in vitro* autoradiographic studies on human post mortem brain sections showed binding in striatum with (R)-[propyl-^{11}C]-2-OH-NPA. It was possible to partially block the binding with raclopride or dopamine. In PET-studies (R)-[Propyl-^{11}C]-2-OH-NPA and (R)-[methyl-^{11}C]-2-OCH_3-NPA were injected into Cynomolgus monkeys. Plasma HPLC metabolite studies showed a lower metabolism for (R)-[methyl-^{11}C]-2-OCH_3-NPA compared to (R)-[propyl-^{11}C]-2-OH-NPA (55% and 86%, respectively, at 15 min post injection). (R)-[propyl-^{11}C]-2-OH-NPA did not pass the blood-brain-barrier to any significant degree, most likely depending on its hydrophilic character. (R)-[Methyl-^{11}C]-2-OCH_3-NPA passed the blood-brain-barrier readily and showed a marked uptake of radioactivity in the monkey striatum. The striatum/cerebellum ratio was about 2. The accumulation of radioactivity in the striatum indicates that (R)-[methyl-^{11}C]-2-OCH_3-NPA binds predominantly to dopamine receptors. Further displacement studies must be performed to qualitatively examine the specificity of (R)-[methyl-^{11}C]-2-OCH_3-NPA binding.

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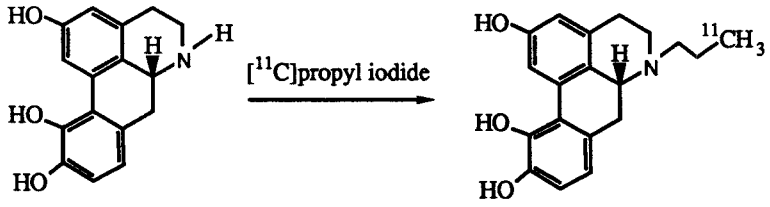
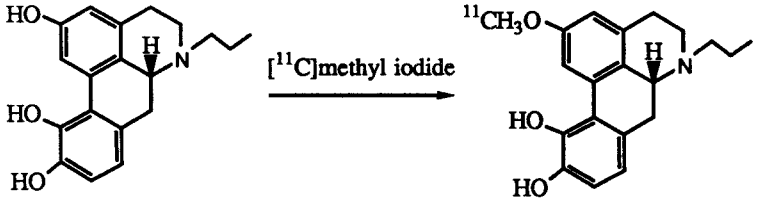


(R)-[propyl-^{11}C]-2-OH-NPA

Scheme 1.



(R)-[methyl-^{11}C]-2-OCH$_3$-NPA

Scheme 2.

Paper F10

The radiolabelling of [18F]-fluoralkylnorapomorphines via sodiumiodide induced nucleophilic substitution reactions in microwave ovens.

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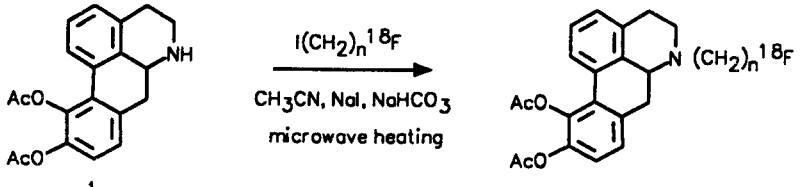
It has been shown that the electromagnetic field generated in microwave ovens also generates alterations in chemical reactivity of substrates[1,2]. The applicability of this technique is determined by several parameters like sample geometry, microwave intensity, polarity of the medium and ionic strength of the sample solution. Microwave heating provides a powerful technique for accelerating rates in the production of carbon-11 and fluorine-18 labelled radiopharmaceuticals[3]. It is known that introduction of an electrolyte into the reaction medium, strongly depends on the solubility of this electrolyte, dramatically increases the energy absorption of the medium during microwave heating[4]. The possibility of adjustment of microwave energy absorption by increasing the ionic strength of the solution, might be a very useful tool in radiolabelling reactions with very low reaction kinetics. We found that labelling of norapomorphine 1 (a potential D_2 receptor agonist), via fluoralkylation of substrates under thermal heating conditions (oil bath, refluxing) was not successful. However, using microwave heating, we were able to label the norapomorphine 1 as depicted in the scheme.

Reaction scheme;



1

2a, n=2

2b, n=3

So, after intermittant runs of 6 x 1.5 minutes resp. 5 x 2 minutes (depending on the substrate used; power level 600 Watt) we acquired derivatives 2a and 2b in 1.2% and 2.3% yield respectively (total synthesis time 50 minutes). Concerning compound 2b, this yield could even be improved for by a factor of 10 after adding 4 mg of NaI to the reaction medium. At this moment, work is in progress to optimize experimental parameters in this type of alkylation experiments.

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Table 1.

Salt	mg salt	Time P=5 atm	Time P=10 atm
NaI	0 mg	4.40 min	12.20 min
NaI	1 mg	1.45 min	7.15 min
NaI	10 mg	0.24 min	0.33 min
NaI	50 mg	0.07 min	0.18
NaBr *	50 mg	1.17	2.30
NaCl *	145 mg	3.30	10.20

* low soluable.

A CEM MDS-81D microwave oven was used, equipped with an electronic module designed to monitor and control pressure inside a vessel during microwave heating. As solvent 1 ml acetonitrile was used.

Table 2:

product	NaI (mg)	reaction time (min)	radiochemical yield
2a	0	6 x 1.5	1.2%
	2	6 x 1.5	2.4%
	3	6 x 1.5	4.5%
2b	0	5 x 2	2.3%
	2	5 x 2	10.5%
	4	5 x 2	28.8%

A MIELE De Luxe M686 was used for the radiolabelling of norapomorphines.

Paper F11

Synthesis of [^{18}F]-*o*-Fluoromethyl MPTP

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Small amounts of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produce in man and other primates a clinical and neurochemical state similar to Parkinson's disease[(5,2)]. Much effort has been devoted to the exploration of MPTP-induced Parkinsonism as a model for studying the natural disease. The study of the behavior of MPTP in animal brains using PET may provide new information concerning the factors which underlie the action of this toxin. For this purpose, we have chosen to label an analog of MPTP, *o*-fluoromethyl MPTP (**6**), with fluorine-18.

Labeled MPTP for this purpose must retain the neurotoxicity of the parent compound. Our previous work showed that the *o*-fluoro and the *o*-trifluoromethyl derivatives of MPTP had similar neurotoxicity to the parent compound, while other *o*-substituted derivatives had decreased toxicity. This indicated that the ortho position could be labeled without diminishing the toxic action of the compound. The ^{18}F could be placed by nucleophilic aromatic substitution[(1,3)], through use of a piperidyl triazene[(7)], or by substitution on a functionalized *o*-alkyl MPTP. The lack of suitable ring activation in this compound and the presence of a tertiary amine indicated that straightforward nucleophilic substitution would not be a fruitful approach. We report the preparation of [^{18}F]-*o*-fluoro MPTP (**5**) in poor yield via the corresponding piperidyl triazene and the preparation of [^{18}F]-*o*-fluoromethyl MPTP (**6**) in useful yield by nucleophilic substitution on the corresponding chlorinated and brominated materials.

[F-18]-1-methyl-4-[*o*-fluorophenyl]-1,2,3,6-tetrahydropyridine (5) was produced via triazene decomposition (**1**) in methanesulfonic acid[(3)] at up to 185°C for up to 30 min. The chemical yield based on ^{18}F was only 0.4 ± 0.34%, maximum 2%, giving a radiochemical yield (45 min) of 0.3%. This approach was attempted because the product was known to be neurotoxic[(8)] and because a yield of a few percent would be sufficient for a limited number of experiments in small animals. Although a low yield was expected, it was insufficient for use in PET. The simultaneous preparation of [^{18}F]-p-fluorobenzonitrile[(3)] was used as a reference reaction and gave yields in agreement with previous results.

[^{18}F]-1-methyl-4-[*o*-(fluoromethyl)-phenyl]-1,2,3,6-tetrahydropyridine (5) (Fluoromethyl MPTP) was produced by benzyl halide substitution on *o*-(chloromethyl)-MPTP hydrochloride (**2**) (1mg), or the corresponding brominated compound (**3**), with ^{18}F-fluoride in dry acetonitrile. Before reaction the acetonitrile solution of the precursor was passed over 20 mg granular potassium carbonate. After reaction, the solution was passed over a small potassium carbonate column, and purified by HPLC (1:1 2mM aq. HNa_2PO_4 in THF, 2 mL/min. TLC: 1%NH_4OH/MeOH, Rf= 0.6).

Due to the reactivity of benzyl fluorides, loss of label by hydrolysis or halide exchange was tested in water and isotonic saline. Hydrolysis to free fluoride ion occurs with first order kinetics and a half-time of approximately one hour. Unlabeled *o*-fluoromethyl MPTP was tested for toxicity in rats. It was shown to be approximately twice as toxic as MPTP itself, while the *o*-chloromethyl and *o*-hydroxymethyl ana-

logs were much less toxic. This indicates that the hydrolysis of the fluoride is not sufficiently severe to interfere in the processes which will be measured. Hydrolysed label is expected to accumulate in bone and to show low brain uptake[6,4]. The effects of tracer degradation must be determined during the initial *in-vivo* trials.

The brominated (3) and chlorinated (2) precursors both gave reasonable product yields, 30% and 60% respectively. Yields from the brominated compound were not optimized. The reaction yield did not have a strong dependence on the concentration of precursor (1-10mg/0.5mL). Reaction reached completion within 2 minutes. At the conclusion of the reaction unreacted fluoride and Cl-methyl-MPTP were observed, but further time did not increase the yield. Temperatures below 95°C reduced yield to 10-48%, and a small improvement with further temperature increase gave maximum yield (60%) at the maximum practical temperature of 125°C. Pretreatment with potassium carbonate removed trace acids to release the free base, did not cause measurable hydrolysis, gave reproducible high yields, and simplified the purification by reducing unlabeled products in the mixture.

Original attempts to remove unreacted fluoride on alumina resulted in hydrolysis of the product on evaporation of the treated solution, releasing [^{18}F]-fluoride. Passage of the product over potassium carbonate before evaporation avoids this hydrolysis, and unreacted fluoride is removed in the HPLC purification.

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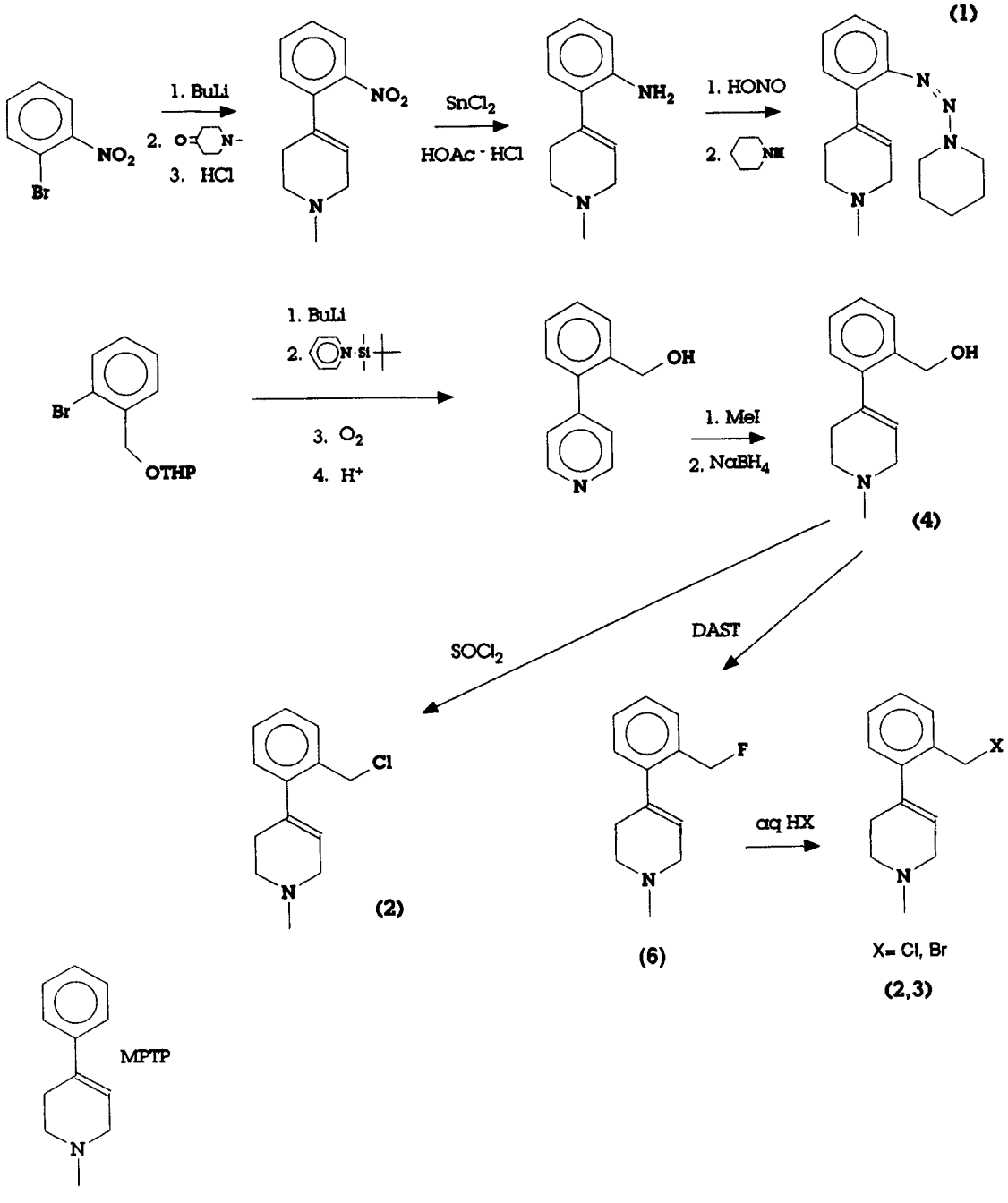
(1)
1. BuLi
2.
3. HCl
NO_2
Br
$SnCl_2$
HOAc · HCl
NH_2
1. HONO
2.
N
N
N
OTHP
1. BuLi
2.
3. O_2
4. H^+
OH
1. MeI
2. $NaBH_4$
(4)
$SOCl_2$
DAST
Cl
(2)
F
(6)
aq HX
X
X= Cl, Br
(2,3)
MPTP

RADIOFLUORINATED ANALOGUES OF BENPERIDOL FOR PET STUDY OF DOPAMINERGIC D-2 RECEPTOR BINDING IN VIVO.

S.M. Moerlein[1], J.S. Perlmutter[1,2], W.R. Banks[1] and D. Parkinson[3].
[1]Edward Mallinckrodt Institute of Radiology, [2]Department of Neurology and Neurological Surgery, and [3]Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110

Although positron-emitting analogues of spiperone have been used in several PET centers for evaluation of dopaminergic D-2 receptor-binding *in vivo*, this radioligand is relatively nonspecific, with high affinity for binding *in vivo* to serotonergic S-2 receptors as well (1-3).

With the goal of identifying a fluorine-18 labeled tracer with improved selectivity for binding to D-2 receptors, we have synthesized novel radiofluorinated analogues of benperidol for evaluation as PET tracers. Benperidol is a selectively potent D-2 ligand, and analogues of this butyrophenone have been labeled with ^{11}C (4), ^{18}F (5) and ^{75}Br (6) for use with positron emission tomography. N-methyl benperidol (NMB, 1) shows particular promise as a PET radiopharmaceutical due to its specific, reversible binding to dopamine receptors *in vivo* (4).

Synthetic pathways to [^{18}F]N-methyl benperidol (NMB, 1) and N-[^{18}F]fluoroethyl-benperidol (FEB, 2) were developed. These D-2 derivatives were expected to show differing behavior *in vivo*, since the radiolabel is attached to an aromatic site in NMB and an aliphatic position in FEB.

Standard NMB and FEB were synthesized via N-alkylation of benperidol. *In vitro* binding assays using primate cerebral tissues indicate that these ligands have high affinity for binding to D-2 receptors (K_i = 3.6 nM for NMB and 5.2 nM for FEB) and relatively poor affinity for S-2 receptors (K_i = 89 nM for NMB and 31 nM for FEB).

[^{18}F]FEB was produced at the no-carrier-added (nca) level via a two-step, one-pot synthetic sequence (Scheme 1). Radiochemical yields of 25-30% and specific activities >1000 Ci/mmol were accomplished within an overall preparation time (including HPLC purification) of 90 min.

Nca [^{18}F]NMB was prepared using a three-step procedure (Scheme 2) similar to that used for other butyrophenones (7,8). The tracer was produced in 5-10% radiochemical yield with a specific activity >1000 Ci/mmol within an overall preparation time of 110 min.

The results of imaging experiments with these promising D-2 receptor-binding PET tracers will be presented.

This work was supported in part by NIH grants 1R29NS26788 and HI-13851, the McDonnell Center for the Study of Higher Brain Function, and the Greater St. Louis Chapter of the American Parkinson's Disease Foundation.

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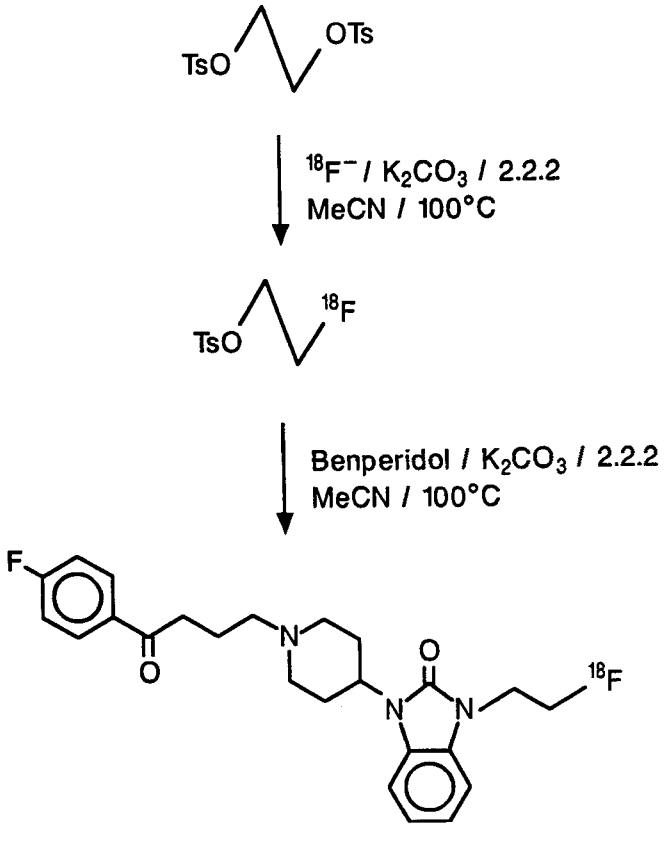
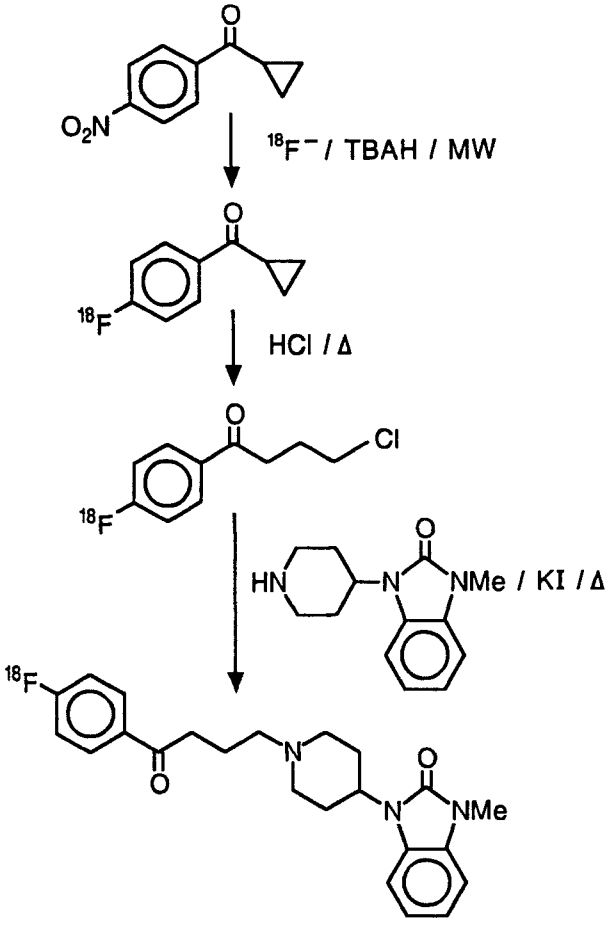
SCHEME 1



[F-18]FEB (2)

SCHEME 2



[F-18]NMB (1)

Paper F13

$[^{18}F]F_2$ From ^{18}O Gas Target for Direct Electrophilic Fluorination: Synthesis of 6-$[^{18}F]$Fluorodopamine.

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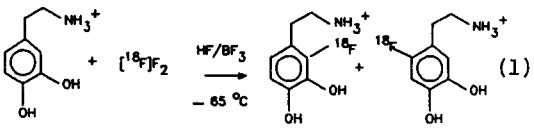
The aim of this study is two fold: (a) to demonstrate the use of $[^{18}F]F_2$, produced by the double shoot method from ^{18}O gas target[1], for routine preparation of ^{18}F-radiopharmaceuticals and (b) to use PET to measure the regional distribution of 6-$[^{18}F]$FDA and the rate of decline of ^{18}F in the hearts of normal volunteers.

Fluorine-18 $[F_2]$ is produced from the $^{18}O(p,n)^{18}F$ reaction using Siemens RDS 112 10 MeV cyclotron (30μA for 60 min). The labelled 2- and 6-FDA are produced by bubbling $[^{18}F]F_2$ (20 - 30 μmol) in neon (0.3% F_2 in neon) through a solution of dopamine hydrochloride (105 μmol) in anhydrous HF containing BF_3 at -65 °C (Equation 1).



(1)

HPLC analysis of the reaction mixture (Fig.1. A) showed that the radiochemical yields of 2- and 6-$[^{18}F]$FDA (identified using high resolution mass spectrometry and ^{19}F NMR[2]) are 13 and 24%, respectively, with respect to $[^{18}F]F_2$. From 100 mCi of $[^{18}F]F_2$, 8 - 9 mCi of 6-$[^{18}F]$FDA is produced after a synthesis time of 120 min. The radiochemical purity is 98 ± 1% (Fig. 1. B) and the specific activity of 6-$[^{18}F]$FDA is 17 - 20 mCi/mg (2.9 - 3.4 Ci/mmol).

PET scans of two normal volunteers were obtained after they were injected with 6-$[^{18}F]$FDA. These studies show intense uptake of ^{18}F in the left and right ventricles. In separate experiments on the same volunteers activities of the sympathetic system by vigorous exercise caused a marked increase in washout of ^{18}F from the heart reflecting increased turnover of FDA.

In summary, our results show that 6-$[^{18}F]$FDA is a good tracer to study cardiac neuronal function with PET. The ^{18}F yield at saturation from $[^{18}O]O_2$ thick target is 150 mCi/μA for 10 MeV protons.[3] We expect to produce higher specific activity $[^{18}F]F_2$ after longer (2 hours) irradiation of the target with higher beam current (40 μA). Therefore the direct fluorination method has the potential to produce $[^{18}F]$6-FDA with specific activity greater than 20 Ci/mmol which will correspond to less than 50 μg of 6-$[^{19}F]$FDA (the pharmacological dose of DA is 2 - 10 μg/Kg/min.) for an injection of 5 mCi of 6-$[^{18}F]$FDA.

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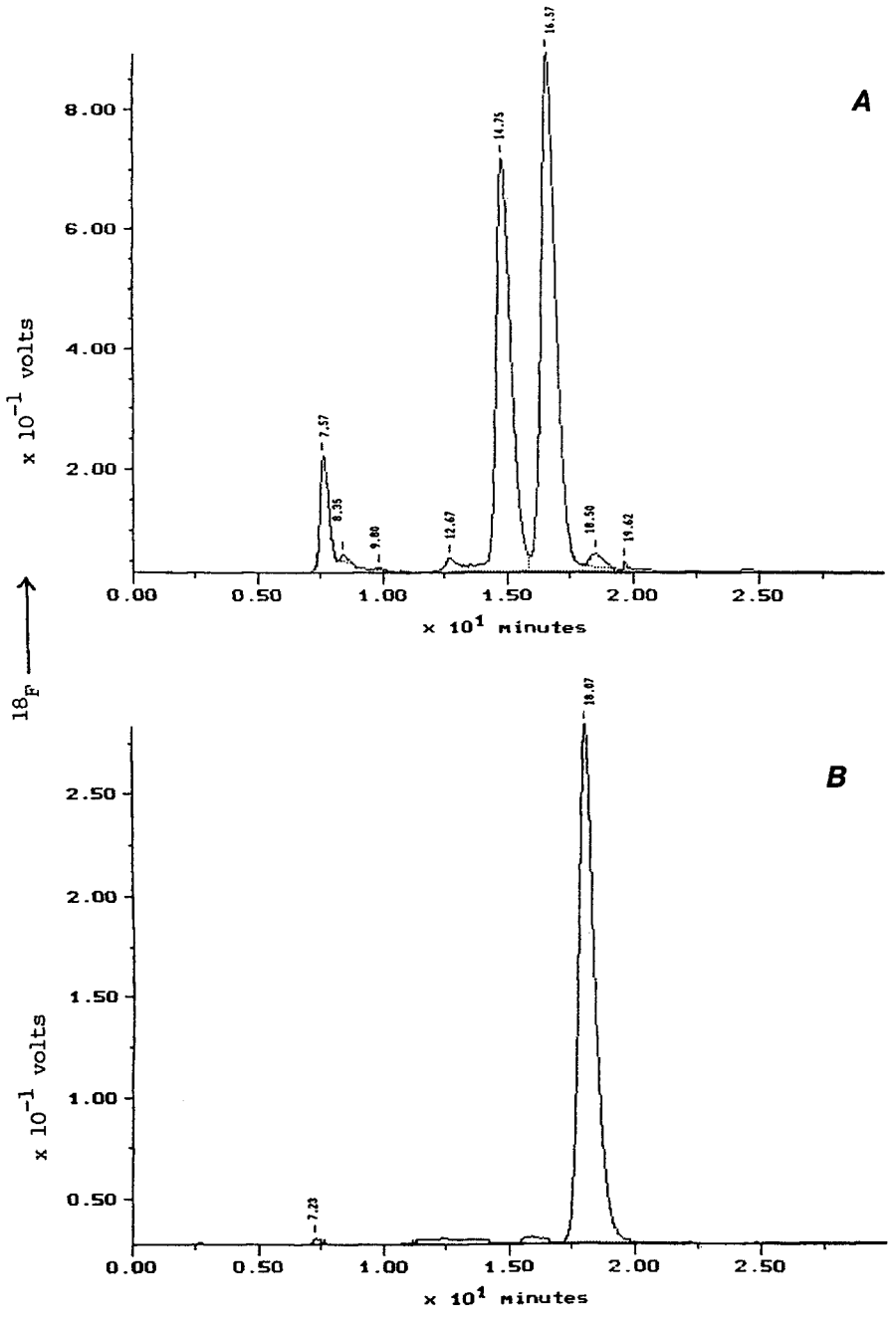

Fig.1. (A) Radiochromatogram of the reaction mixture obtained after the direct fluorination of DA.HCl in HF/BF_3 at -65 °C. (B) Radiochromatogram of [^{18}F]6-FDA. (Column: Waters μBondaPak C_{18} 0.78 cm x 30 cm. Mobile phase: 0.15% TFA in water containing 4.5% CH_3CN. Flow rate: 2.5 mL/min.)

RADIOLABELED ANALOGS OF COCAINE AS TRACERS FOR STUDYING THE DOPAMINE TRANSPORTER USING PET.

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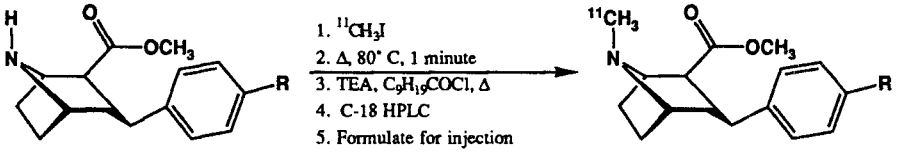
Abnormalities in dopaminergic neurotransmission are believed to be involved in a variety of neurologic and neuropsychiatric disorders (1). Specifically, changes in the dopamine transporter have been observed in Parkinson's disease (2,3), Huntington's disease (4), and schizophrenia (3,5), as well as in normal aging (6). Recently, a series of cocaine analogs have been reported that are more potent than cocaine in the inhibition of [^{3}H]dopamine uptake (7). One of these analogs has been radioiodinated and shown to be useful for studying the transporter with single photon emission tomography (8,9). The ready availability of [^{11}C]methyl iodide and the presence of an N-methyl group on the tropane moiety make these analogs attractive candidates as radiotracers for studying the dopamine transporter using PET (see figure).



where R= H, Cl, or I

The ^{11}C-N-methylations of the nor-methyl precursors were performed in dimethylformamide at 80° C for 1 minute. The unreacted secondary amine precursors were derivatized to form long chain amides by the addition of decanoyl chloride and triethylamine. Reversed phase high performance liquid chromatography was used for semipreparative purification and determination of the specific activity of the final products. The syntheses were completed in an average of 23 minutes following the end-of-bombardment (E.O.B.) with an overall radiochemical yield of 9% (not corrected for decay). The average specific activity determined at end-of-

synthesis was 2475 mCi/μmole; this corresponds to approximately 5500 mCi/μmole E.O.B.

Acknowledgements: This work was supported in part by U.S.P.H.S. grant numbers NS-15080, CA-32845, DA-06309, and DA-05477.

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Paper F15

SOME THINGS TO CONSIDER WHEN PREPARING 6-[^{18}F]FDOPA BY RADIOFLUORODEMERCURATION

H. Richard Adams, Michael A. Channing and William C. Eckelman
National Institutes of Health Bethesda, MD 20892

Currently there are two approaches to the preparation of 6-[^{18}F]FDOPA by electrophilic substitution. One is the direct electrophilic substitution of H; there are several methods of this type described in the literature, but the method most commonly used is described by Adam et al[1]. The other is the substitution of Hg resulting in a regiospecific reaction; this reaction is described by Luxen et al[2] and Adam et al[3].

In the preparation of 6-[^{18}F]FDOPA by the substitution of H by [^{18}F]acetyl hypofluorite there are two major problems: first, the reaction is not regiospecific; and second, the resulting usable 6-[18F]FDOPA yield is low. To overcome these disadvantages, we decided to investigate the synthesis of [^{18}F]6FDOPA described by Luxen et al[2].

Upon completion of the synthesis of 6-[18F]FDOPA, a higher than permitted level of Hg was found in the product (0.5 µg / mL as stipulated by the US Pharmacopeia, 1991), so, we decided to study the factors that could lead to a high Hg content. The first factor we looked at was the silica/thiosulfate column. As can be seen in Figure 1, the amount of Hg is dependent on both the amount of silica and the amount of thiosulfate. If the ratio of silica to thiosulfate is chosen correctly the amount of Hg removed is in the same range as that found using the modified silica described by Adam et al [3]. It does not matter if the silica and thiosulfate are mixed, and we have shown that both thiosulfate and the silica are needed to remove the Hg.

The Hg content of the final product is also affected by the composition of the HPLC solvent. We found that the EDTA had to be removed from the solvent described by Luxen et al[2]. If this was not done the level of Hg in the final product was above usable limits. Because the remaining Hg is removed by the HPLC, the column must be rinsed and the eluatent checked for Hg. We found that at least 500 mL of solvent must be used to remove the remaining Hg.

It can be seen in Figure 2 that the radiofluorodemercuration method increases the usable 6-[18F]FDOPA yield by about 6 times. The average yield by this method is 52.5 ± 8.3 % , as compared to 8.6 ± 3.1% from the H substition method (N=14). We have also found that the HPLC column and solvent have an effect on the yield. Using the HPLC system described by Adam et al[3], we get a 33.7 % yield, but if we add 0.01 % ascorbic acid to the solvent we increase the yield to 58.1 %. The above yields are all based on the fluorinated starting material in the hydrolysis flask (RBF).

In conclusion, we found that a great deal of care must be taken in order to obtain a product both free of Hg and in high yield. We have shown that the ratio of silica to thiosulfate is critical to keep the level of Hg in the acceptable range and that the HPLC solvent has an effect on both the amount of 6-[18F]FDOPA recovered from the column and the ability to keep the level of Hg low.

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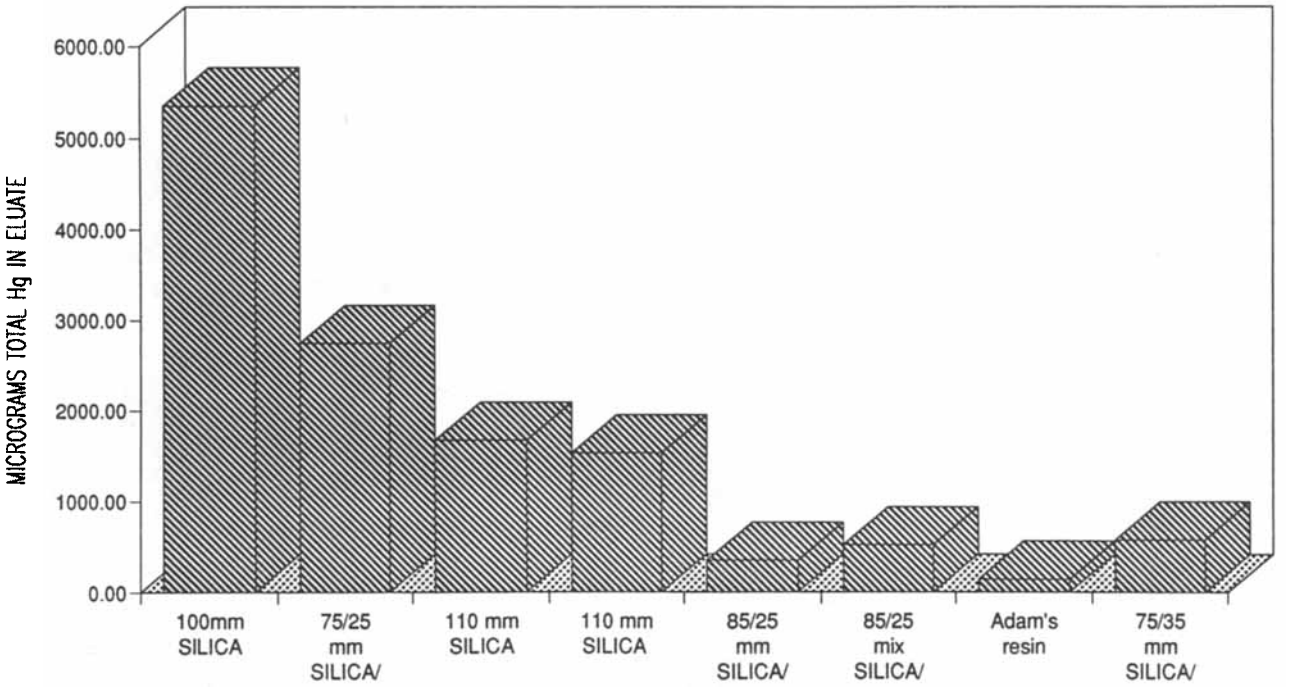
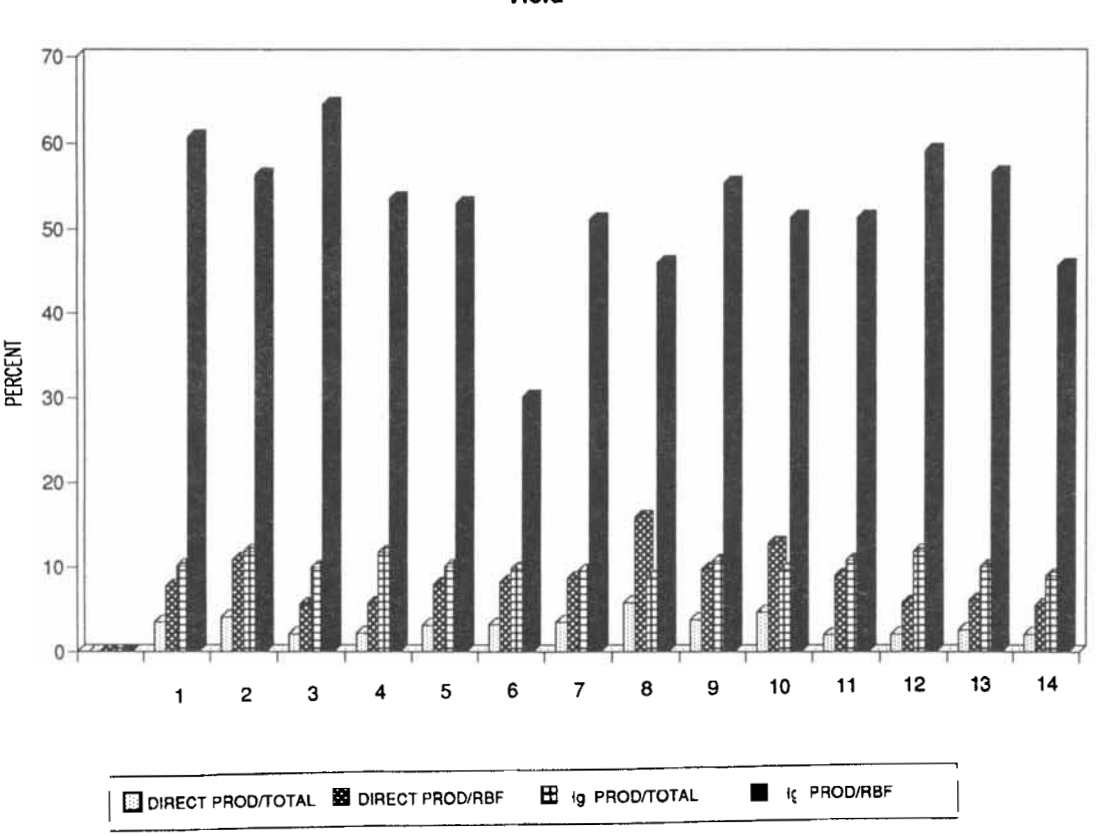
Hg After Si/S2O3 Column Treatment



Page 2 Figure 1



Page 3 Figure 2

PREPARATION OF TWO HIGHLY POTENT AND SELECTIVE DOPAMINE D-2 RECEPTOR ANTAGONISTS FOR PET: [^{18}F]NCQ 616 AND [^{11}C]FLB 457.

C. Halldin, T. Högberg, H. Hall, P. Karlsson, C.-E. Hagberg, P. Ström and L. Farde. Department of Psychiatry and Psychology, Karolinska Institute, Stockholm, Astra Arcus, Södertälje and Astra Draco, Lund, Sweden.

Several substituted benzamides with high affinity and selectivity for central dopamine D-2 receptors have been labelled with ^{11}C or ^{18}F for the examination of receptor binding in man (1-2). NCQ 616 ((S)-5-bromo-N-((1-ethyl-2-pyrrolidinyl)methyl)-2-fluoroethoxy-3-methoxybenzamide) and FLB 457 ((S)-5-bromo-N-((1-ethyl-2-pyrrolidinyl)-methyl)-2,3-dimethoxy-benzamide) are two new selective dopamine D-2 receptor antagonists (3). The compounds inhibit the binding of [^{3}H]raclopride and [^{3}H]spiperone in picomolar concentrations and are two of the most potent dopamine D-2 antagonists so far developed.

[^{18}F]NCQ 616 was prepared by O-alkylation of FLB 604 (2-phenol precursor) with [^{18}F]-2-fluoroethyl bromide (produced from (K/2.2.2.)$^{+}$^{18}F^{-} and 1,2-dibromoethane and isolated by a sequential Sep-Pak and distillation procedure) in DMSO with sodium hydroxide as a base. [^{11}C]FLB 457 was prepared by O-alkylation of FLB 604 with [^{11}C]methyl iodide in DMSO with sodium hydroxide as a base. The purification of both products was performed by semi-preparative reversed-phase HPLC with total radiochemical yields (EOB and decay-corrected) of 5-8% and 20%, total synthesis times of 90 and 35-40 min, respectively, and with radiochemical purities >99% (Scheme 1).

Both [^{18}F]NCQ 616 and [^{11}C]FLB 457 were used for *in vitro* autoradiography on human post mortem brain sections. Both compounds bound specifically to dopamine D-2 receptors in human striatum. Virtually all binding was inhibited by raclopride indicating a very low proportion non-specific binding. In PET-studies on Cynomolgus monkeys [^{18}F]NCQ 616 showed a high uptake of radioactivity in the striatum and a possible equilibrium already after 30-40 minutes with a striatum/cerebellum ratio of about 4. After i.v. injection of [^{11}C]FLB 457 there was a continous accumulation of radioactivity in the striatum. The striatum/cerebellum ratio was about 13 after 65 minutes. This ratio is to our knowledge the highest seen with any dopamine D-2 receptor ligand that have been examined in PET. In a displacement experiment, radioactivity in the striatum was almost completely displaced after injection of 5 mg unlabelled raclopride, thus demonstrating specificity and reversibility of [^{11}C]FLB 457 binding to dopamine D-2 receptors. Both [^{18}F]NCQ 616 and [^{11}C]FLB 457 should be useful PET ligands for the examination of dopamine D-2 receptors in man.

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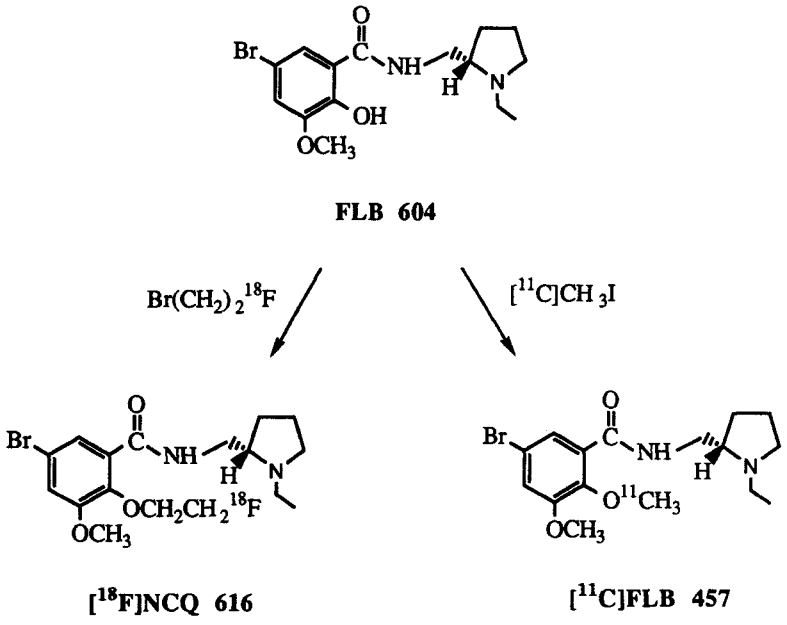
Br
O
C
NH
H
N
OH
OCH3
FLB 604
Br(CH2)2 18F
[11C]CH3I
OCH2CH2 18F
O11CH3
[18F]NCQ 616
[11C]FLB 457

Scheme 1.

Paper F17

A RAPID AND EFFICIENT HPLC-METHOD FOR DETERMINATION OF LIGAND METABOLISM DURING PET-STUDIES - EXAMPLIFIED WITH [^{11}C]RACLOPRIDE.

C.-G. Swahn, C. Halldin, Jan Lundström, Eva Erixson and Lars Farde.
Department of Psychiatry and Psychology, Karolinska Institute, Stockholm and Astra Arcus, Södertälje, Sweden.

In experiments with PET and radiolabelled ligands it is important to determine the occurrence of ligand metabolites in plasma. A time-curve for the fraction of radioactivity in plasma representing unchanged ligand is necessary for calculation of the metabolite corrected arterial plasma input curve (1). Identification of the main metabolites in plasma is fundamental if the metabolites are radiolabelled, and pass the blood-brain-barrier and may bind to the receptors.

Different methods have been used for the determination of unchanged [^{11}C]raclopride in human plasma during PET studies: thin layer chromatography (TLC), ion exchange chromatography (IEC) and high performance liquid chromatography (HPLC) (2-3). The TLC procedure is experimentally simple but time-consuming and allows only a few samples to be processed during a PET-experiment. The ion exchange procedure allows more samples to be analyzed but is dependent on acidic or basic properties of the compounds and can not resolve all metabolites. The Kontron gradient HPLC and 450 Multitasking PC system is rapid, efficient and reliable. It gives a better resolution between radioligand and metabolites, the chemical identity of metabolites can be more easily determined by simultaneous addition of standards and 2 different detectors (UV and radioactivity) give simultaneous information of mass and radioactivity.

The plasma handling procedure developed is rapid (total time 4-5 min) with a recovery of >95% (4). The plasma sample containing standards together with acetonitrile (1 ml) was injected on to the HPLC column. The peaks of UV and radioactivity were integrated by the PC and the chemical identity was simultaneously verified by the standards. Experiments showed that [^{11}C]raclopride was metabolized comparatively slowly and unchanged compound represented 70-80% after 42 minutes. The total time for separation of unchanged tracer and the two metabolites was 7.5 min (Figure 1) and >98% of the radioactivity in plasma was recovered from the column. Radioactive fractions could also be collected and counted in a well-counter.

Several pathways can be suggested for the metabolism of raclopride. The most likely is an oxidation to a lactam and conjugation with sulphate or glucuronic acid at the phenolic hydroxyl group. In the present study the lactam and the sulphate of raclopride were used as standards to identify the main metabolite as the lactam.

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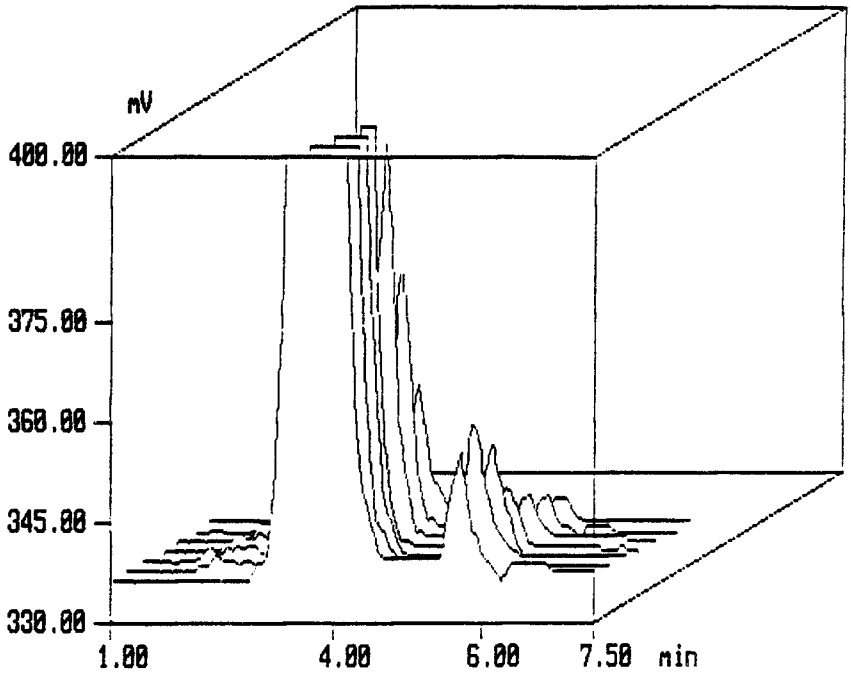

Figure 1. HPLC chromatograms of blood samples from a healthy volunteer after i.v. injection of [^{11}C]raclopride. From front to rear, the curves represent the consecutive samples, obtained after 4, 8, 12, 18, 24, 30, and 42 minutes after administration. During the course of the experiment the main metabolite, lactam, (right peak) and the second metabolite (left peak) appears in the blood and the concentration of [^{11}C]raclopride (middle peak) decreases.

Paper F18

SYNTHESIS OF CARBON-11 LABELED (1R-2-EXO-3-EXO)-2-CARBOMETHOXY-8-METHYL-8-AZABICYCLO[3.2.1]OCTYL-3-N-(3'-NITROPHENYL)CARBAMATE AS A POTENTIAL PET DOPAMINE RECEPTOR IMAGING AGENT.

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Cocaine is a powerful addictive drug which is abused by a considerable number of people worldwide. It has been reported that cocaine's pharmocologic behavior is the result of cocaine inhibition of the reuptake of dopamine into the mesocorticolimbic dopaminergic nerve terminals which leads to prolonged simulated dopamine action at the dopamine receptor.

Cocaine and several analogs have been radiolabeled with carbon-11 (1) and fluorine-18 (2) and iodine-123 (3) as potential radiotracers for imaging and quantifying cocaine receptor sites in the brain using positron emission (PET) and single photon emission tomography (SPECT) techniques. Recently a meta- nitro- 3-carbamoylecognine methyl ester analog (4) of cocaine was found to be twice as potent in inhibiting [^{3}H]cocaine binding and more potent in inhibiting [^{3}H]dopamine binding to rat striatal tissue than (-)-cocaine itself. These findings prompted us to synthesize carbon-11 labeled (1R-2-exo-3-exo)-2-carbomethoxy-8-methyl-8-azabicyclo[3.2.1]octyl-3-N-(3'-nitrophenyl)carbamate (3) for evaluation as a potential PET cocaine receptor imaging agent.

Compound 3 (Figure 1) was synthesized by treatment of ecgonine methyl ester (1) with 3-nitrophenyl isocyanate (2) in refluxing toluene. The nor substrate (1R-2-exo-3-exo)-2-carbomethoxy-8-azabicyclo[3.2.1]octyl-3-N-(3'-nitrophenyl)carbamate (7) was prepared from cocaine (4) by a five step sequence of reactions shown in Figure 1. Treatment of 7 with $^{11}CH_3I$ in CH_3CN gave carbon-11 3. Evaluation of C-11 labeled 3 in laboratory animals will be presented.

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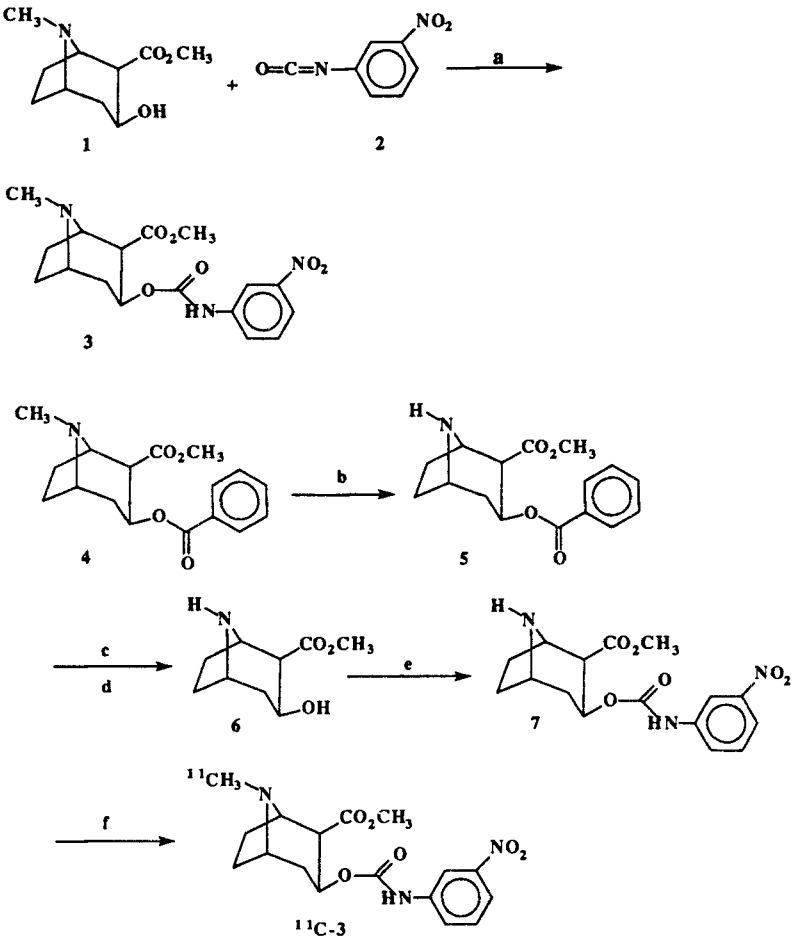

Reagents: a: toluene, Δ; b: 2,2,2-trichloroethylchloroformate, Zn;
c: 10% HCl; d: CH_2N_2; e: 3-nitrophenyl isocyanate, toluene, Δ;
f: $^{11}CH_3I$, CH_3CN, Δ

Paper F19

SYNTHESIS AND C-11 LABELING OF SOME 3 β-PHENYLTROPANE-2-CARBOXYLIC ESTERS (WIN) ANALOGS FOR PET.

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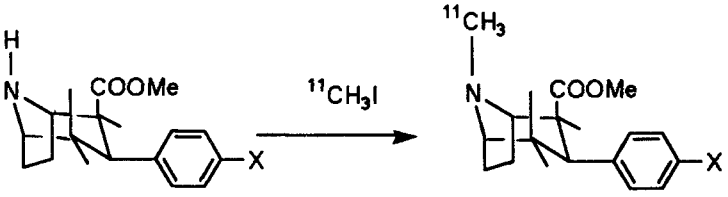
*MASSACHUSETTS GENERAL HOSPITAL, BOSTON MA 02114, **ORGANIX, WOBURN, MASSACHUSETTS 01801, ***NEW ENGLAND REGIONAL PRIMATE RESEARCH CENTER, SOUTHBOROUGH, MASSACHUSETTS 01772.

A series of 3β -aryltropane-2β-carboxylic ester analogs (1) and their N-substituted derivatives (2) displayed high binding affinity to the cocaine recognition sites. Here we report on the synthesis and radiolabeling of some N-[C-11]methyl-2β-carbomethoxy-3β-aryl tropanes (I).



X=H, F, Cl, CH_2Cl

(I)

A series of these tropane analogs were prepared according to the chemical route outlined in schemel, as originally described by Clarke et. al.(1) and others(3). Slight modifications of the Grignard reaction conditions and workup improved the yield of the 2βcarbomethoxy isomer. The N-demethylated precursors were obtained by milder demethylation conditions using α-chloroethyl chloroformate.
We labeled this series by reacting 30-50ug of the N-demethylated (free base) precursor with [C-11]CH_3I in acetonitrile at 90^0C for 5 min. A short evaporation process (1 min.) of the solvent and unreacted C-11 precursor, provide acceptable yields of a high specific activity product. $^{11}CH_3I$ was produced by an automated system form $^{11}CO_2$ as reported earlier(4).
The potential utility of this series for imaging presynaptic dopamine terminals will be reported.

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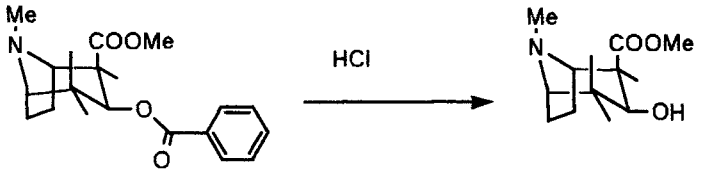
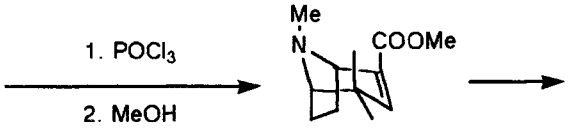
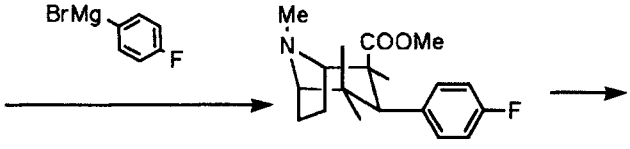
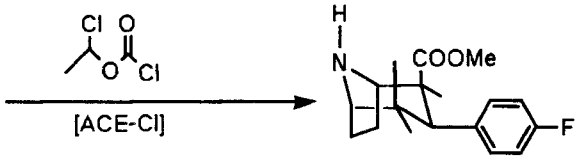


(-)-cocaine



anhydroecgonine





Paper F20

PREPARATION OF [76Br]-BROMOTROPAPRIDE, A NEW DOPAMINE D2 RECEPTOR LIGAND FOR PET STUDIES.

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For the in vivo mapping of dopamine D2 receptors, the radioligands used are dopamine antagonists belonging to the butyrophenone, the ergolene and the benzamide families. In a systematic study of radiobrominated ligands for imaging the dopaminergic neurotransmission system, we have already developped [76Br]-labelled derivatives of compounds belonging to the butyrophenone (bromospiperone) and to the ergolene (bromolisuride) families (1,2). To investigate the pharmacological and/or clinical interests of radiobromo derivatives from the third neuroleptic family, we have labelled with [76Br], Tropapride, a nortroprane substituted benzamide antagonist. Tropapride and its halogenated derivatives, possess high affinity and selectivity for D2 receptors. Iodotropapride has been labelled with iodine 123 for SPECT studies (3). For PET imaging, [76Br]-Bromotropapride has been prepared by nucleophilic substitution of the iodinated analog.
The exchange between [76Br] (non carrier added [76Br]NH4 and the cold iodine of the precursor (2 μmol) is performed at 180°C in a sealed vial in presence of Cu+ and an excess of reducing agents (gentisic acid, ascorbic acid, citric acid), according a method described by MERTENS (4). In these conditions the labelling yield reaches 60% in 45 to 60 min. For purification, the reaction mixture is poured on a C18 cartridge and polar products are washed with water. Halogeno derivatives of tropapride are eluted with methanol and finally [76Br]-Bromotropapride is separated from the iodo precursor by HPLC on a μ-Bondapak C18 column (300*7.8 mm) with a mixture of water, methanol, acetonitrile, acetic acid and ethylamine (60/20/20/1/1) as mobile phase. The recording of UV absorption (254 nm) and radioactivity measurements permits the recovery of [76Br]-Bromotropapride which is eluted before the iodo precursor (selectivity = 1.23). For radiopharmaceutical preparation, the HPLC solution is poured on a C18 cartridge which is washed with saline and the radiobromocompound is eluted with 1 mL ethanol. 10 mL saline are added and the solution is sterilizated through a .2 μm PTFE membrane. In these operating conditions the radiopharmaceutical preparation yield of [76Br]-Bromotropapride is 50%. The specific activity is higher than 50 MBq/nmol and the radiochemical purity is higher than 98%. The lipophilicity has been measured by the shake-flask method. The logarithm of the partition coefficient of the radioligand between octanol and a phosphate buffer (pH=7.4) is 2.65.
In vitro saturation experiments have been performed on rat striatal membranes with increasing doses of [76Br]-Bromotropapride. The analysis of saturation data using a non linear least square regression program revealed a single population of binding sites (Kd = .1 nmol/L, Bmax = 280 fmol/mg of protein). The ex vivo distribution of the radioligand have been studied by autoradiography. The highest radioactivity concentration corresponds to the caudate nucleus; 4 h after injection the striatum to cerebellum concentration ratio is around 40 which demonstrate the in vivo specificity of [76Br]-bromotropapride for D2 receptors. A four hour PET study was performed in a baboon which was injected with 75 MBq of [76Br]-Bromotropapride. The radioactivity concentrations in cortical and cerebellar regions decrease throughout the experiment while the radioactivity uptake in the striatum increases rapidly and reaches a plateau 1 h after injection. Four hours after injection, the radioactivity concentration in putamen and caudate are 20 fold higher than in cerebellum. Unchanged radioligand and radiolabelled metabolites have been measured in plasma by radio TLC analysis. In vivo, [76Br]-bromotropapride is rapidly metabolized: 2h after injection, unchanged radioligand represents only 15% of the plasmatic radioactivity.
All these studies clearly demonstrate that [76Br]-Bromotropapride is suitable for the in vivo studies of central D2 dopaminergic receptors. Therefore, this new ligand will be compared to others radio bromo ligands an will be used in PET studies of the pathophysiology of the dopaminergic neurotransmission.

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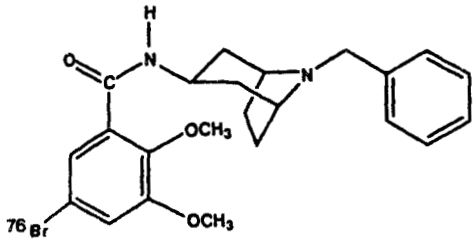
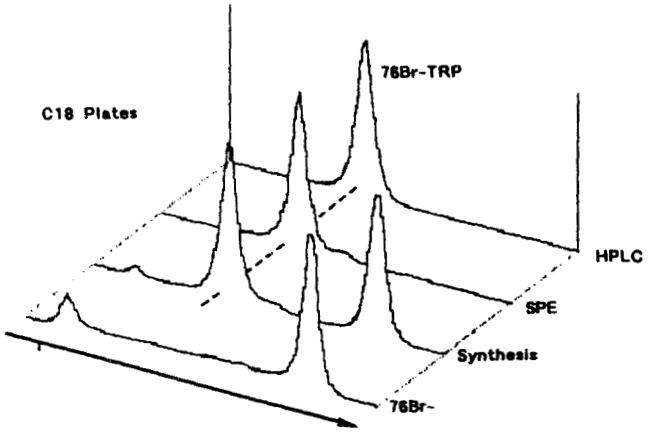


RADIO TLC ANALYSIS



76Br-BROMOTROPAPRIDE
BABOON