AN APPROACH FOR CARBON-11 LABELLING OF CARDIAC RECEPTOR LIGANDS

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Several ^{11}C- and ^{18}F-labelled neurotransmittors, e.g. 6-[^{18}F]fluorometaraminol, [^{11}C]meta-hydroxyephedrine (MHED), 6-[^{18}F]fluorodopamine and 6-[^{18}F]fluoronorepinephrine, have been prepared for examination of cardiac sympathetic innervation and function with PET (1-4). However, it is desirable to avoid problems in cardiac PET studies caused by low specific radioactivity and the use of substituted analogues.

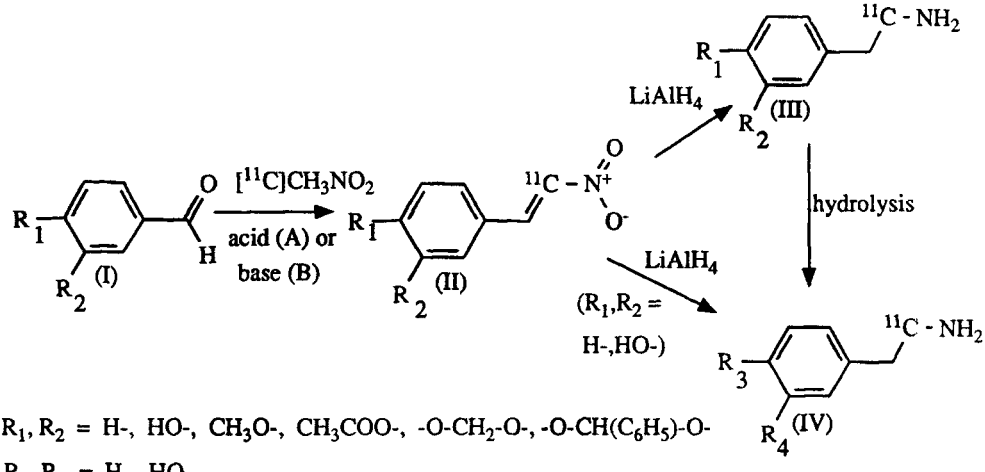
Recently, a method for the preparation of ^{11}C-labelled phenethylamine and amphetamine from [^{11}C]nitromethane and [^{11}C]nitroethane, respectively, was reported (5). The aim of this study was to develop general methods for the preparation of 3-hydroxy-, 4-hydroxy- and 3,4-dihydroxy-substituted phenethylamines. By this approach it would in principle be possible to obtain receptor ligands such as norepinephrine and metaraminol labelled with ^{11}C and with high specific radioactivity.

The first synthetic procedure, as outlined in Scheme 1, consists of three steps: (a) condensation of nca [^{11}C]nitromethane with a protected or un-protected hydroxy-substituted aldehyde, (b) reduction with $LiAlH_4$ and, if necessary, (c) hydrolysis of the protection group. The condensation reaction was investigated under both basic and acidic conditions (Table 1). After a Sep-Pak purification procedure and an almost quantitative reduction of both the nitro group and the alkene function, the hydrolysis of the protection groups was investigated using different reagents. The utility of this synthetic approach is examplified with the total synthesis of [^{11}C]dopamine, [^{11}C]tyramine and [^{11}C]meta-tyramine in 15-35 % yield from [^{11}C]CO_2.

In a second approach the one-pot reaction of aldehydes with nca [^{11}C]nitromethane to obtain β-nitroalcohols according to Scheme 2 (6) was investigated. In preliminary experiments using aldehydes Ic, Id and If a crude yield of 30-60 % (from [^{11}C]CH_3NO_2) of the corresponding nitroalcohols Vc, Vd and Vf has been obtained.

1. Mislankar S.G., Gildersleeve D.L., Wieland D.M., Massin C.C.,Mulholland G.K. and Tooronigan S.A. - J. Med. Chem. 31: 362 (1988).
2. Rosenspire K.C., Haka M.S., Van Dort M.E., Jewett D.M., Gildersleeve D.L., Schwaiger M. and Wieland D.M. - J. Nucl. Med. 8: 1328 (1990).
3. Ding Y-S., Fowler J.S., Gatley J., Dewey S.L., Wolf A.P. and Schlyer D.J. - J. Med. Chem. 34: 863 (1991).

4. Ding Y-S., Fowler J.S., Gatley J., Dewey S.L. and Wolf A.P. - J. Med. Chem. 34: 767 (1991).
5. Schoeps K-O., Stone-Elander S. and Halldin C. - J. Nucl. Med. 32: 994 (1991).
6. Fernandéz R., Gasch C., Gómez-Sánchez A. and Vílchez J.E. - Tetrahedron Letters 32: 3225 (1991).



R_1, R_2 = H-, HO-, CH_3O-, CH_3COO-, -O-CH_2-O-, -O-$CH(C_6H_5)$-O-

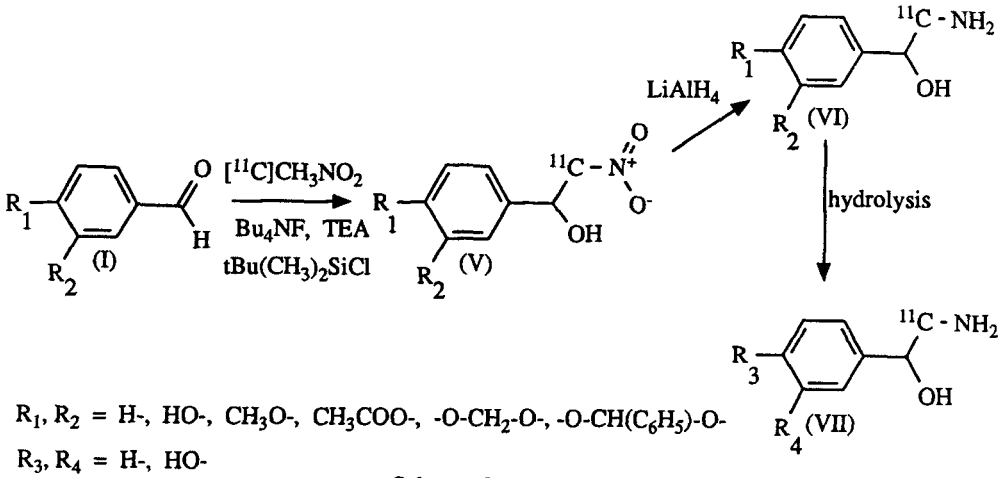
R_3, R_4 = H-, HO-

Scheme 1.

Table 1. Some $[^{11}C]CH_3NO_2$ - arylaldehyde condensations under acidic(A) or basic(B) conditions.

Aldehyde	R_1	R_2	method[1]	Yield[2]
Ia	H-	HO-	A	90
Ib	HO-	H-	A	95
Ic	H-	CH_3O-	B	88
Id	CH_3O-	H-	B	80
Ie	- O - CH_2 - O-[3]		B	50-95
If	- O - $CH(C_6H_5)$ - O-[4]		B	55
Ig	H-	CH_3COO-	B	23
Ih	CH_3COO-	H-	B	<5
Ii	CH_3COO-	CH_3COO-	B	<5

1: Method A: NH_4CH_3COO, CH_3COOH, 10 min 140 °C, Method B: NaOH, MeOH, 3 min 80 °C.
2: From $[^{11}C]CH_3NO_2$ (decay corrected). 3: 3,4-methylene dioxy. 4: 3,4-benzylidene dioxy.



R_1, R_2 = H-, HO-, CH_3O-, CH_3COO-, -O-CH_2-O-, -O-$CH(C_6H_5)$-O-

R_3, R_4 = H-, HO-

Scheme 2.

Paper G2a

[^{18}F]-Fluoroacetone for Radiopharmaceutical Synthesis: Preparation of ^{18}F-Carazolol

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[^{18}F]-Fluoroacetone is a potentially useful precursor for radiopharmaceutical synthesis. It could be used to incorporate ^{18}F through reductive alkylation of amines and through reactions at the carbonyl center. It can be used to prepare ^{18}F labeled beta-adrenergic receptor ligands in an analogous way to the ^{11}C labeled materials.[1,2,3,4]. Carazolol, labeled with ^{11}C, is currently under investigation in this laboratory with positive results.[4] It is restricted by its half-life in the time that is available for image collection and kinetic measurements. Availability of corresponding ^{18}F labeled analogs will allow longer data collection at higher count rates for accurate measurement of ligand kinetics. Also, the use of ^{18}F may allow production of ligands with higher specific activity than that of ^{11}C, through similar labeling chemistry.

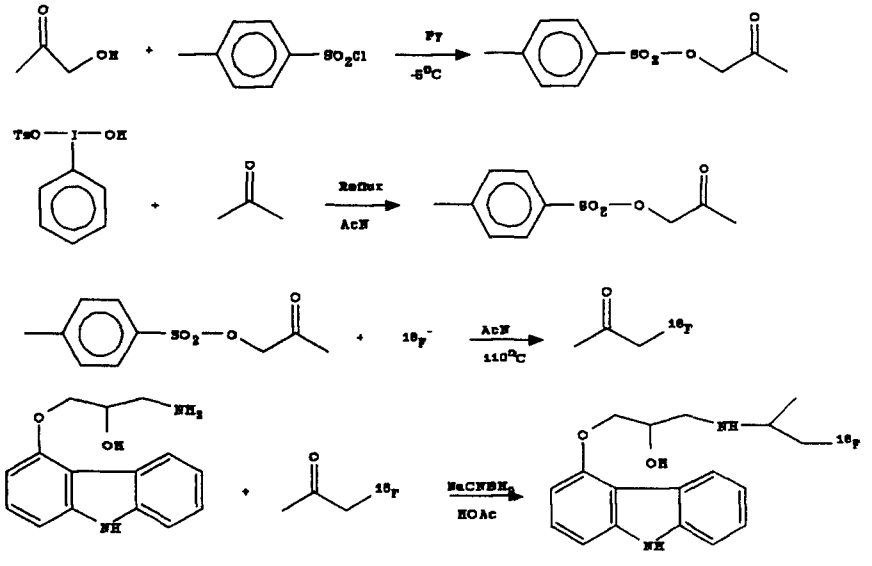
[^{18}F]-Fluoroacetone was prepared in two steps as shown in Scheme 1. The precursor used for fluorination, acetol tosylate, was synthesized in two ways. Direct tosylation of acetol (reaction 1) gave a yield of 15%. Alpha-tosylation of acetone using [hydroxy(tosyloxy)-iodo]benzene (reaction 2) proceeded with a yield of 60%[5]. Acetol tosylate was purified either by vacuum distillation (<1mmHg, bp.70°C) or by recrystallization from petroleum ether. (NMR (δ): acetol tosylate; 2.36 (s, 3H); 2.45 (s, 3H); 4.52 (s, 2H); 7.36 (d, 2H, J=8Hz); 7.8 (d, 2H, J=8Hz)).

[^{18}F]-fluoride was produced using the ^{18}O(p,n)^{18}F reaction with 17 MeV protons on [^{18}O]-water. After evaporation of water and drying of the fluoride by acetonitrile evaporation, acetol tosylate (2mg) was introduced in an acetonitrile solution. The fluorination reaction was performed at 110°C for 10 mins in a sealed vessel in the presence of potassium carbonate and kryptofix, in a procedure very similar to the common preparation of fluoroglucose. Labeled fluoroacetone is distilled from the labeling vessel into a second reaction vessel along with some acetonitrile using an Ar gas stream. It was radiochemically pure and identical to an authentic standard by HPLC (Alltech C-18 analytical column, 0.005 *N* sodium phosphate monobasic pH 3; fluoroacetone RT=5.2mins). The 2,4-dinitrophenylhydrazone of labeled fluoroacetone was also prepared and found to be identical by TLC and HPLC to a characterized authentic sample. (TLC: 10% EtOH/CH_2Cl_2, Rf=0.9; HPLC: Alltech C-18 analytical column, 60% EtOH/H_2O; RT=7.3mins). The synthesis requires a total of less than 30 mins and gives a radiochemical yield of 46% at 30mins EOB, giving a chemical yield of 55%.

The preliminary investigation of [^{18}F]-fluoroacetone application in [^{18}F]-carazolol synthesis (reaction 4) has given encouraging results. The ^{18}F-fluoroacetone from the first reaction vessel was distilled onto des-isopropylcarazolol ((1), 2mg), cyanoborohydride (1mg) and acetic acid(2ul) in methanol solvent. The reaction was performed at 110°C for 20mins. The TLC and HPLC of the radioactive product are identical to the product made from the authentic sample. (TLC: 0.5% conc. NH_4OH, 10% MeOH in $CHCl_3$; Rf=0.9; HPLC: 0.03% $MeNH_2$, 0.03% H_2O, 1.5% EtOH in $CHCl_3$; RT=6.2 min) The radiochemical yield of [^{18}F]-carazolol based on [^{18}F]-fluoroacetone is 66% measured at 30mins after the ^{18}F-fluoroacetone is introduced. The chemical yield is 80%.

References:

1. Berger G., Maziere M., Prenant C., et al. "Synthesis of Carbon-11 labeled Propanolol" *J. Radioanal. Chem.* **74,** 301 (1982).
2. Berger G., Prenant C., et al. "Synthesis of a beta-Blocker for Heart Visualization: [^{11}C]-Practolol" *Int. J. Appl. Radiat. Isot.* **34,** 1556 (1983).
3. Prenant C., Sastre J., et al. "Synthesis of Carbon-11 Labeled Pindolol" *J. Lab. Comps. Radiopham.* **24,** 227 (1987).
4. Berridge M. S., Terris A. "C-11 carazolol: A New Labeled Beta-adrenergic Ligand" *8th International Symposium on Radiopharmaceutical Chemistry,* 359 (1990).
5. Kosar G. F., et al. "One-Step alpha-Tosyloxylation of Ketones with [Hydroxy(tosyloxy)iodo] benzene" *J. Org. Chem.,* **47,** 2487 (1982).



Schema I

Paper G2b

Synthesis of Fluorine-18 Fluorocarazolol: A Ligand for the ß-Adrenergic Receptor.

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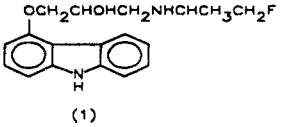
Imaging the ß-adrenergic receptor in the heart and lung presents a significant challenge on two counts. First, to image the receptor system ligands are needed whose association constants are significantly higher than those required in the brain and second there is no tissue within the field of view which can be used to estimate the non-specific binding of the ligand. In order to address these issues we have been seeking a derivative of pindolol or carazolol containing fluorine-18. The virtue of fluorine-18 is that the half life is long enough to permit observation of both the wash in and wash out curves, allowing estimation of non-specific binding. Pindolol and carazolol where chosen because of their binding characteristics but fluoroalkyl derivatives of the aromatic rings proved very difficult to make and caused a significant decline in the association constant of the ligand. We therefore investigated substitution on the nitrogen of the propanolamine side chain as a synthetic strategy for fluorine-18 labelling with considerably more success. One of the most promising compounds is fluoroisopropylcarazolol (fluorocarazolol) (1), with a K_d measured on the racemic compound of 113 pm^{-1}. This is slightly lower than that of carazolol itself in our system.

Fluorine-18 fluorocarazolol is prepared by co-distilling fluorine-18 fluoroacetone and acetonitrile, onto the amine (2) and acetic acid. The imine (3) forms and the yield can be checked by evaporation of unreacted fluoroacetone with the acetonitrile. The imine is reduced with sodium cyanoborohydride to give fluorine-18 fluorocarazolol. The product is separated from the starting amine on a silica Sep-Pak, using chloroform:methanol:triethylamine (95:5:0.05) as the eluating solvent. The specific activity of the product appears to be high and is currently being examined both by chromatographic means and by using the receptor binding assay.

The identity of the product has been confirmed by comparison with authentic material on TLC and three different HPLC systems (normal phase, reverse phase and strong cation ion exchange). The overall yields of purified product from fluoride are about 10%, i.e. 30 mCi's of fluoride give three mCi's of product, and the synthesis time is about an hour.



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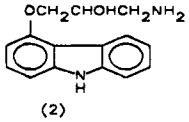

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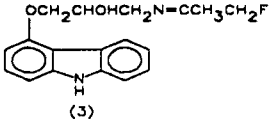
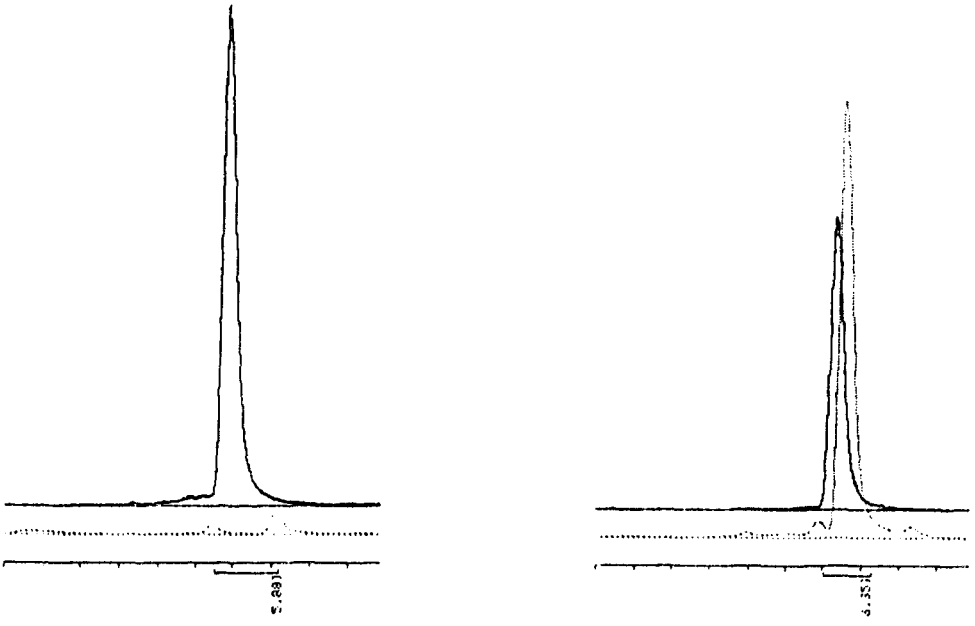


(3)

Summary of Fluorine-18 Fluorocarazolol Synthesis Runs

Starting Amount of Fluoroacetone (mCi's)	Amount of Amine Used(mg)	Reduction Yield
3.99	5	85%
11.65	3.3	50%
7.11	5	70%
12.4	5	79%
15.41	5	83%
15.94	5	73%



HPLC of Fluorine-18 fluorocarazolol. Silica column, Solvent Chloroform, methanol, triethylamine (95:5:0.05). 1 mL/min UV 285 nm.
a) Crude Reaction b) Co-injected with authentic

Synthesis of No Carrier Added Fluorine-18 Fluoroacetone, a Useful Synthetic Intermediate.

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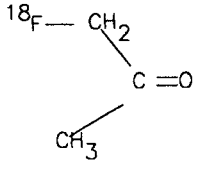
Labelled synthons as general intermediates for the synthesis of compounds containing short lived radionuclides have become widely used, with carbon-11 methyl iodide being the prime example. This intermediate has been used for the synthesis of N-methyl amines, methyl ethers and methyl esters. We had the need for a general synthesis of N-fluoropropyl amines for the synthesis of fluorine-18 labelled antagonists for the beta-receptor and so we developed the synthesis of fluorine-18 fluoroacetone (1) via nucleophilic displacement on acetone tosylate (2).

The tosylate (2) is prepared by refluxing the dimethyl ketal of bromoacetone with silver tosylate in acetonitrile. The methoxy groups are lost during the isolation and purification procedure and (2) is a stable crystalline solid with a melting point of 34-35°C. The fluorination reaction is then performed using fluorine-18 fluoride prepared by proton bombardment of oxygen-18 water and the fluoride is trapped by reaction with potassium carbonate/Kryptofix in the standard fashion and dried by azeotropic distillation with acetonitrile. The tosylate (2) is then added in an equivalent amount to the potassium carbonate present, the solution heated to 90°C for five minutes and then a slow stream of nitrogen is passed through the reaction mixture and the fluoroacetone distils with the acetonitrile where it is trapped at 0°C. The equipment used is simply two reaction vials connected by 1/16" Teflon tubing piercing septa. Typically 65-75% of the activity distils with the acetonitrile. The product is radiochemically pure and identical with authentic fluoroacetone on both GC and HPLC. The majority of the activity left behind in the reaction vessel is neither fluoroacetone nor fluoride, but its identity has not yet been established.

The specific activity of the fluoroacetone has not been measured directly as the solvent peaks serve to mask the mass peak in the GC and with HPLC the sensitivity to detection by UV is very low, but we believe it to be high. Measurements on the products made from the compound support the contention that the specific activity is high.

The compound can also be made using dimethylsulfoxide as the solvent. The fluoroacetone distils over at 90°C in a nitrogen stream in the absence of any solvent co-distilling. The yield is not as good under these circumstances, typically between 25-35% of the activity distils, but it does have the advantage that material or reactions that cannot tolerate acetonitrile can be used in subsequent steps.



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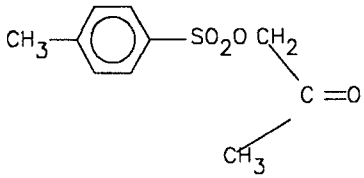


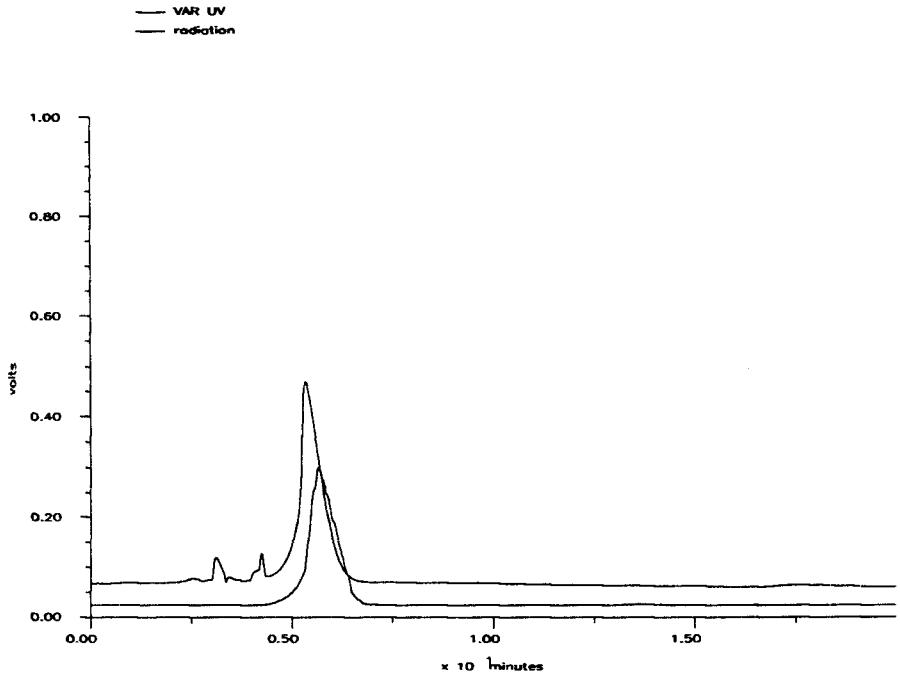
(2)

Supplementary Data.

Summary of Yields from Fluoroacetone Syntheses

Fraction of (Spread) Fluoride Run Used[a]	Tosylate (mg)[b]	Number of Runs	Conditions	Av.Yield
0.3 to 0.5	3.4-4.3	8	Heat 5 min, Distill	67% (49-75)
1.00	7.6	4	Heat 1-10 min Distill	58% (31-72)
0.5	3.7	2	Distill Immed.	34%,53%
0.5	3.4 + 3.4	1	Heat, Distill Add more Tosyl, Distill	30% 50%
1.00	7.6 + 3	1	Heat, Distill Add more Tosyl Distill	27% 41%
1.00	4 + 3	1	Distill Immed. Add more Tosyl, Distill	38%
0.5	3.5 + 3.6	2	Distill Immed. Add more Tosyl Distill	28,50% 42,60%
0.5	3.0 + 2.0	1	Heat, Add more Tosylate, Distill	40%

a. The full run of fluoride contains 4.6 mg of potassium carbonate, 33 micromoles. b 7.6 mg of the tosylate is 33 micromoles.



HPLC of Fluorine-18 fluoroacetone. Column: Phenyl Fatty Acid Column.
Solvent: 10% Acetonitrile in 1M Ammonium Acetate pH 3.2
UV Detector 230 nanometers
5 microlitre of Fluoroacetone co-injected.

Paper G3

SYNTHESIS OF A PET RADIOTRACER FOR STUDYING SEROTONIN UPTAKE SITES: [^{11}C]McN-5652-Z-68.

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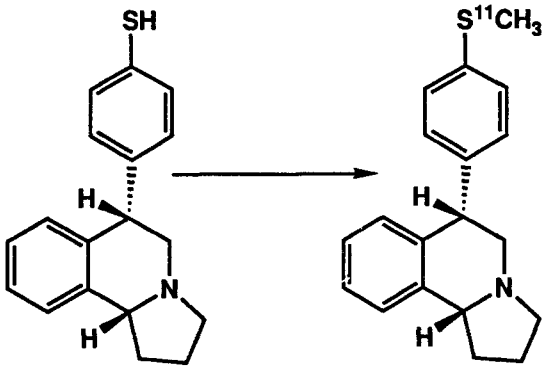
The development of an appropriate radioligand that would selectively label the presynaptic element of central serotonergic (5-HT) neurons may make possible positron emission tomographic (PET) imaging of these neurons in the living human brain in both health and disease.

Although a number of 5-HT uptake site blockers have been labeled with carbon-11, none of these ligands has been found suitable for imaging the 5-HT transporter in the human brain. High levels of non-specific binding compared to specific uptake site binding *in vivo* have been reported. [^{11}C]Imipramine (1), [^{11}C]chloripramine (2) and [^{11}C]cyanoimipramine (3) have been studied, as well as [^{11}C]citalopram, [^{11}C]fluoxetine (4 - 6), [^{11}C]sertraline (7) and [^{11}C]methyl-paroxetine (8).

Recently, McN-5652-Z-68 has been described as a highly potent inhibitor of serotonin uptake (9). The ^{11}C-S-methylation of the nor-methyl precursor, prepared by demethylation of the parent compound using sodium thiomethoxide, was performed in dimethylformamide at 80° C for 1 minute (see figure). 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 16 minutes following the end-of-bombardment (E.O.B.) with an overall radiochemical yield of 5%

(not corrected for decay). The average specific activity determined at end-of-synthesis was 3655 mCi/μmole; this corresponds to approximately 6323 mCi/μmole E.O.B.

Preliminary *in vivo* results in mice indicate that after intravenous injection [^{11}C]McN-5652-Z-68 binds selectively and potently to central serotonin uptake sites, whereas binding to nonspecific sites is relatively low.

Acknowledgements: The authors would like to thank Dr. Bruce E. Maryanoff of McNeil Pharmaceutical for providing samples of McN-5652-Z-68. This work was supported in part by U.S.P.H.S. grant numbers NS-15080, CA-32845, and DA-06309.

1. Maziere M, Berger G, Comar D: ^{11}C-Clomipramine: Synthesis and analysis. J Radioanal Chem 45: 453-457, 1978.
2. Berger G, Maziere M, Knipper R, Prenant C, Comar D: Automated synthesis of carbon-11 labeled radiopharmaceuticals: Imipramine, chlorpromazine, nicotine, and methionine. Int J Appl Radiat Isot 30: 393-400, 1979.
3. Hashimoto K, Inoue O, Suzuki K, Yamasaki T, Kojima M: Synthesis and evaluation of [^{11}C]cyanoimipramine. Nucl Med Biol 14: 587-592, 1987.
4. Kilbourn MR, Haka MS, Mulholland GK, Jewett DM, Kuhl DE: Synthesis of radiolabeled inhibitors of presynaptic monoamine uptake systems: Fluorine-18 GBR-13119 DA, carbon-11 nisoxetine NE and carbon-11 fluoxetine 5-HT. J Labelled Compd Radiopharm 26: 412-414, 1989.
5. Scheffel U, Dannals RF, Suehiro M, Wilson AA, Ravert HT, Stathis M, Wagner HN Jr: Evaluation of ^{11}C-citalopram and ^{11}C-fluoxetine as *in vivo* ligands for the serotonin uptake site. J Nucl Med 31: 883-884, 1990.
6. Hume SP, Pascali C, Pike, VW, Turton DR, Ashier RG, Myers R, Bateman DM, Cremer JE, Manjil LG, Dolan R: Citalopram: Labelling with carbon-11 and evaluation in rat as a potential radioligand for *in vivo* PET studies of 5-HT re-uptake sites. Nucl Med Biol 18: 339-351, 1991.
7. Lasne MC, Pike VW, Turton DR: The radiosynthesis of N-methyl-carbon-11-sertraline. Appl Radiat Isot 40: 147-152, 1989.
8. Dannals RF, Ravert HT, Wilson AA, Scheffel U, Frost JJ, Wagner HN Jr: Synthesis of a radiotracer for studying serotonin uptake sites: Carbon-11 labeled N-methylparoxetine. J Label Compds Radiopharm 26: 205-206, 1989.
9. Shank RP, Vaught JL, Pelley KA, Setler PE, McComsey DF, Maryanoff BE: McN-5652: A highly potent inhibitor of serotonin uptake. J Pharm Exp Ther 247: 1032-1038, 1988.

Paper G4

SYNTHESIS OF ^{18}F-LABELED FLUOXETINE : A SELECTIVE SEROTONIN UPTAKE INHIBITOR.

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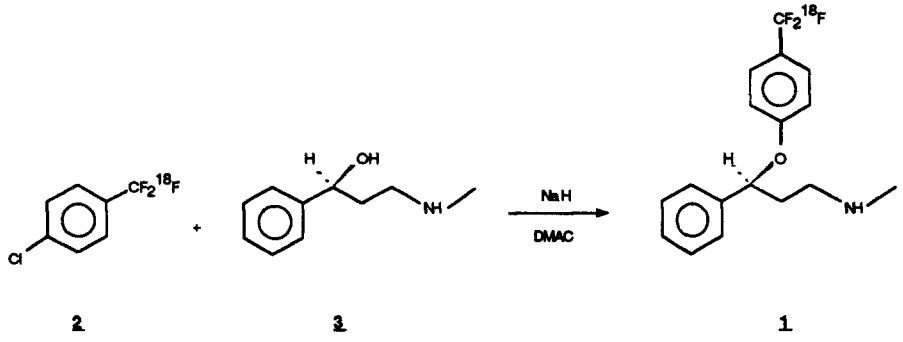
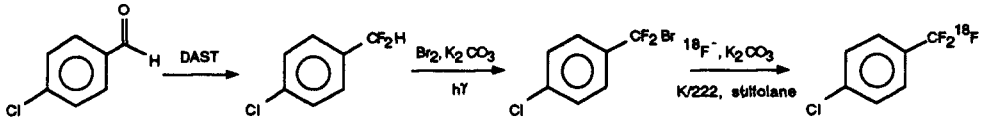
Fluoxetine, N-methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine is an antidepressant with potential applications in treatement of other symptoms (1). The mode of action of fluoxetine has been proposed as a selective inhibition of serotonin uptake in presynaptic neurons. Synthesis of [^{11}C]fluoxetine has been reported by Kilbourn and co-workers (2) involving the methylation of the nor-methyl amine with [^{11}C]methyl iodide or [^{11}C]formaldehyde. However, N-demethylation of fluoxetine occurs during metabolism. Synthesis of fluoxetine with ^{11}C in any other position than the methyl is presently difficult. For these reasons the fluorine-18 labeled fluoxetine is desired for PET studies of reuptake sites. In the present work, synthesis of (S)-(+)-[^{18}F]fluoxetine **1** (Figure 1) is divided into two major parts, the preparation of the labeled 4-chlorobenzotrifluoride **2** followed by condensation with the (S)-(-)-3-(methylamino)-1-phenyl-1-propanol **3** (3).

The synthesis of the ^{18}F-labeled precursor **2** is outlined in Figure 2. Reaction of 4-chlorobenzaldehyde with diethylaminosulfur trifluoride (DAST) give 4-chloro-α,α-difluorotoluene in 70% yield. α-bromo-4-chloro-α,α-difluorotoluene is then prepared from bromination of the difluoromethyl group according to the reported method (72% yield)(4).The ^{18}F-labeled trifluoromethyl group is obtained by nucleophilic aliphatic substitution of bromide with nca [^{18}F]fluoride. Various conditions are explored for this exchange reaction. Optimal reaction conditions are found to be 160°C for 18 min, using 27 µmol of Kryptofix 2.2.2, 18 µmol of K_2CO_3 and 4 µmol of α-bromo-4-chloro-α,α-difluorotoluene in 0.4 ml of tetramethylene sulfone (sulfolane). The desired fluoro compound **2** is isolated by distillation of the reaction mixture in reacti-vial containing sodium alkoxide **3** in 0.5 ml of dimethylacetamide (DMAC). The condensation of **2** with the amino alcohol **3** occurs when this solution is heated at 120°C for 45 min. The mixture is then transferred onto a C-18 Sep-Pak cartridge with water and the final product **1** is eluted with CH_2Cl_2. Purification by HPLC on a silica gel column with CH_2Cl_2/MeOH/NH_4OH (100/10/1 v/v) as elution solvent, gives (S)-(+)-[^{18}F]fluoxetine with a radiochemical yield of 6-12% (corrected for decay). The overall synthesis time is nearly 150 min. The identity of the product is confirmed by comparison of its HPLC retention time (9 min) with unlabeled fluoxetine. Unfortunately, specific activities of **2** and **1** are low (50 mCi/ µmol). This result can be explained by the superconjugativity of CF_2Br-compound followed by a side reaction (Figure 3).The transformation of α-bromo-4-chloro-α,α-difluorotoluene to 4-chlorobenzotrifluoride occurs at high temperature (reaction performed without [^{18}F]fluoride, under the same experimental conditions as for the synthesis of the ^{18}F-labeled precursor **2**). Further work to clarify the influence of temperature and improve the specific activity is in progress.



2 3 1

Figure 1. Synthesis of [^{18}F]-Fluoxetine 1



2

Figure 2. Synthesis of [^{18}F]-4-chlorobenzotrifluoride 2

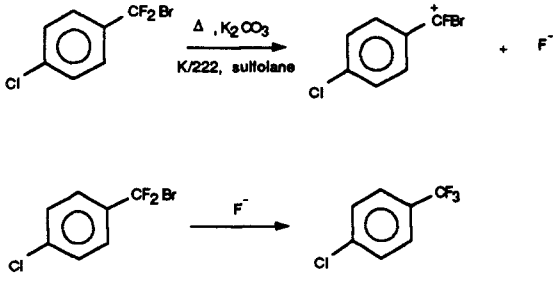


Figure 3. Transformation of α -bromo-4-chloro- α,α -difluorotoluene at high temperature

(1) Murphy D.L., Mueller E.A., Garrick N.A. and Aulakh C.S. J. Clin. Psychiatry, 47, 9 (1986).
(2) Kilbourn M.R., Haka M.S., Mulholland G.K., Jewett D.M. and Kuhl D.E. J. Label. Compd. Radiopharm., 26, 412 (1989).
(3) Robertson D.W., Krushinski J.H., Fuller R.W. and Leander J.D. J. Med. Chem., 31, 1412 (1988).
(4) Haas A., Spitzer M. and Lieb M.; Chem. Ber., 121, 1329 (1988).

Paper G5

CHOLINERGIC NERVE MARKERS: BENZOVESAMICOL STRUCTURAL STUDIES. Y-W. Jung, M.E. Van Dort, D.L. Gildersleeve, D.E. Kuhl, D.M. Wieland. University of Michigan, Ann Arbor, MI. 48109-0552.

We have reported that (-)-5-IBVM is a highly specific marker for central cholinergic neurons(1). We have expanded this initial study to address the following goals: 1) to develop a rapid, chiral synthesis of 5-[^{123}I]IBVM for clinical studies; 2) to evaluate structural variants of IBVM; 3) to determine if hydroxyl- or aminobenzovesamicols can be regiospecifically radioiodinated.

Based on extensive *in vivo* testing in rodents, (-)- 5-[^{123}I]IBVM was chosen for clinical SPECT studies. Our initial synthesis of (-)-5-[^{125}I]IBVM entailed a final purification by chiral HPLC. However, this route is not feasible for the routine clinical synthesis of (-)-5-[^{123}I]IBVM in view of the high cost of iodine-123. A chiral synthesis using iododestannylation has been developed as shown in Figure 1. Initially Chloramine-T was used as the oxidant and excellent radiochemical yields (>90 %) were obtained. However, to exclude the possibility of generating 5-chlorobenzovesamicol as an impurity in the final product, peracetic acid was adopted as the oxidant. Following HPLC purification, (-)-[^{123}I]IBVM was obtained with a specific activity >20,000 Ci/mmol. A 10 mCi dose to an adult human will contain <5 pg/kg of carrier; an important consideration in view of the potent neuromuscular blocking activity of this drug.

Eight radioiodinated derivatives of benzovesamicol have been synthesized as shown below. Placement of iodine in position 5 or 6 gives derivatives with excellent *in vivo* neuronal selectivity (ie. compounds 1 and 2); benzovesamicols with iodine substitution in the 8 or 4' position show no selectivity (ie. compounds 3-7). The 7 and 2' positions are under study. Negative results with the *m*-iodobenzamide analog 8 are likely due to rapid *in vivo* hydrolysis of the amide linkage.

The compounds 5-hydroxy and 5-aminobenzovesamicol were synthesized and attempts were made to regiospecifically introduce radioiodine in the 6 position. Electrophilic [^{127}I]iodination favored formation of the *para* isomer(position 8) under all reaction conditions tested. Tracer level iodination with iodine-125 under basic conditions, as shown in Figure 2, gave the highest proportion (50%) of 6-iodo product, due possibly to complexation of the iodinating species with the phenoxide anion.

In view of the low regioselectivity of the radiosynthesis of compound 2, the chiral iododestannylation route and (-)-5-[^{123}I]IBVM remain the synthetic method and the radioiodinated benzovesamicol of choice for clinical SPECT studies of cholinergic nerve integrity.

1. Jung, Y-W., Van Dort, M.E., Gildersleeve, D.L., Wieland, D.M., **J. Med. Chem.** 33: 2065-2068 (1990).

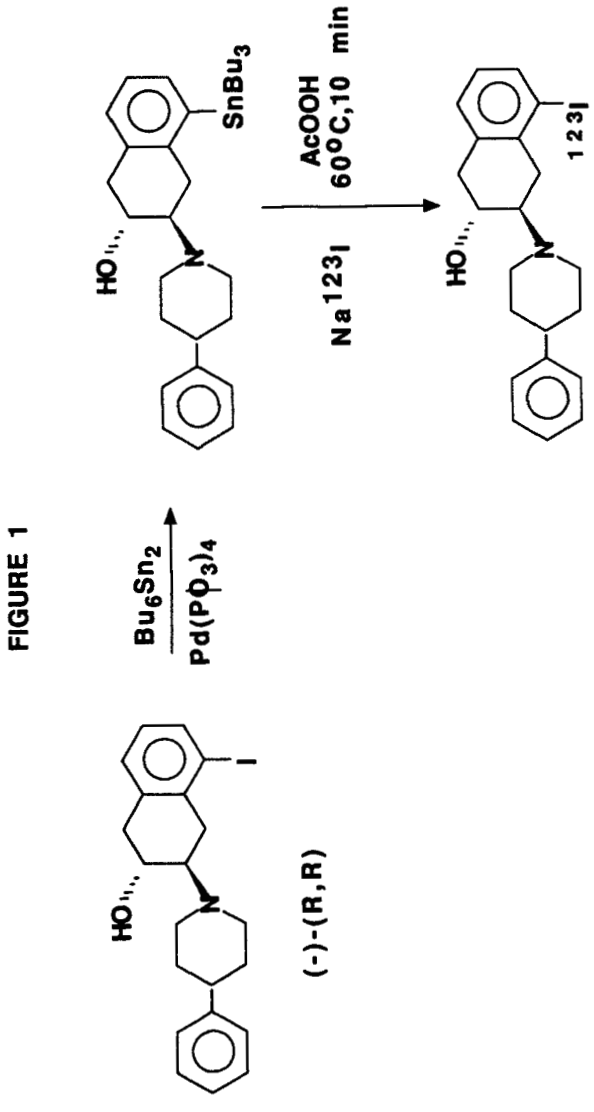

HO
I
(-)-(R,R)
Bu_6Sn_2
$Pd(P\phi_3)_4$
HO
$SnBu_3$
AcOOH
60°C,10 min
$Na^{123}I$
HO
^{123}I

FIGURE 1

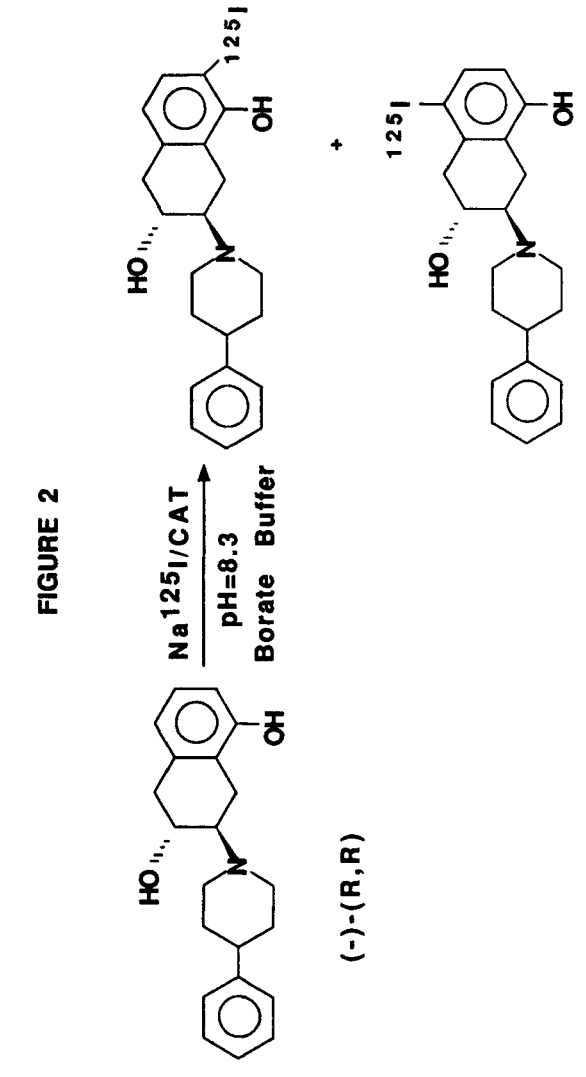
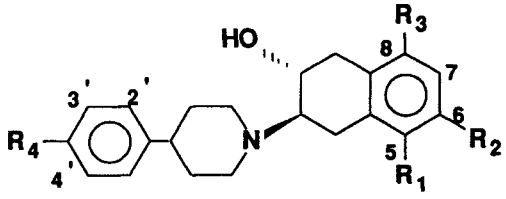


FIGURE 2
Na125I/CAT
pH=8.3
Borate Buffer
HO
OH
125I
(-)-(R,R)



Cmpd #	R_1	R_2	R_3	R_4
5-IBVM (1)	I			
2	OH	I		
3			I	
4	NH_2		I	
5	OH		I	
6				I
7	NH_2			I
8	HNCO-*m*-I-C_6H_4			

Paper G6

The Vesamicol Receptor (VR): Radioligand Selectivity and the Detection of Central Cholinergic Hypofunction. S.M.N. Efange,* D.C. Mash,+ M. Basile,+ J. Pablo,+ H. F. Kung,#J. Billings,# R. H. Michelson* & J. R. Thomas*.
*University of Minnesota, Minneapolis, MN 55455. #University of Pennsylvania,Philadelphia, PA 19014. +University of Miami School of Medicine, Miami, FL 33136.

Cortical cholinergic hypofunction associated with Alzheimer's disease (AD) is attributable to the loss of cholinergic long axon projections (and their corresponding perikarya) originating from the nucleus basalis of Meynert. Consequently, radiotracers which bind selectively to unique presynaptic cholinergic sites may prove to be useful in the detection of the cholinergic lesion in AD. In developing such radiotracers, we have synthesized (-)-2-hydroxy-3-(4-[^{125}I]iodophenyl)-1-(4-phenylpiperidinyl)propane (3 , 4-HIPP), a conformationally mobile analog of vesamicol, the prototypical vesamicol receptor (VR) ligand (1).

Compound 3 was synthesized, by iododestannylation of 2, from the corresponding bromo analog 1, a potent inhibitor of [^{3}H]vesamicol binding to cholinergic synaptic vesicles (2). Radioiodination was achieved in 73 % radiochemical yield with specific activities ranging between 100 and 200 Ci/mmol.

In biodistribution studies, 2% of the injected dose of 4-HIPP was detected in the rat brain at 5 min post-injection. This level of radioactivity remained stable for up to 3 hr, the duration of the study. Co-administration of 3 with vesamicol (1.1 umol/kg) resulted in a 70% reduction in the brain levels of radioactivity, suggesting that both vesamicol and 3 compete for the same binding site *in vivo*. Finally, preliminary metabolite analysis of rat brain extracts indicates that at 3 hr post-injection, 4-HIPP accounts for 75 - 80% of the radioactivity. In SPECT imaging studies on the monkey, an estimated 3% of the injected dose of (-)-4-[^{123}I]HIPP was found localized within the brain at 2 min post-injection. The half-life of 3 in the monkey brain was 9 hr.

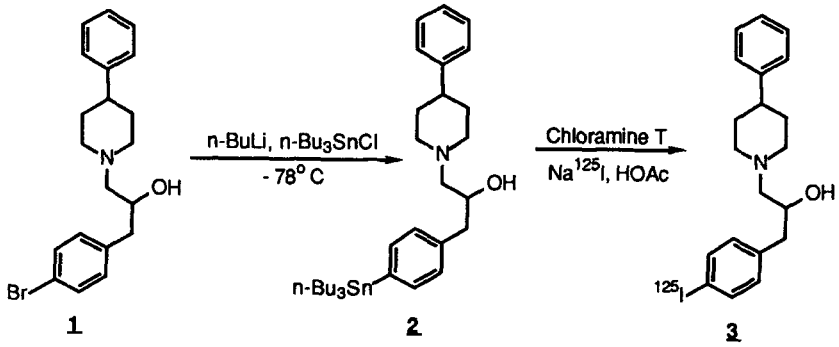
Characterization of (-)-4-[^{125}I]HIPP binding in human cortical membranes revealed the presence of two binding sites: a high-affinity site (Kd = 3.5 nM, B_{max} = 42.5 pmol/g) and a low-affinity site (Kd = 31.1 nM, B_{max} = 102.1 pmol/g). The low-affinity site is haloperidol sensitive, suggesting that 4-HIPP binds to both the VR and the sigma binding site. Characterization of 4-HIPP binding in membranes obtained from the basal forebrain of subjects with no history of neurological disorder reveals a pattern similar to that observed in the cortex. However, in corresponding basal forebrain samples obtained from age-matched Alzheimer's subjects, a significant reduction in 4-HIPP binding was observed. Scatchard analysis reveals that this reduction is attributable to the loss of the vesamicol receptor (or cholinergic) component of 4-HIPP binding. Such a reduction is not only consistent with the neurochemistry of AD, but it also suggests that the VR may be a useful target for the design of radiotracers for mapping cholinergic innervation *in vivo*. However, the reduced selectivity of 4-HIPP for the VR and the relative abundance of the competing sigma receptor greatly decrease the sensitivity of this radiotracer to changes in vesamicol receptor levels. We therefore conclude that more selective VR ligands will be needed to further explore the feasibility of mapping cholinergic pathways in the living human brain.***Supported by the American Health Assistance Foundation***

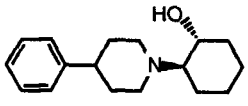
REFERENCES

1. Bahr, B. A. & Parsons, S. M. *Proc. Natl. Acad. Sci. U. S. A.* 83: 2267-2270 (1986).
2. Efange, S. M. N., Michelson,. R. H., Dutta, A. K. & Parsons, S. M., *J. Med. Chem.*, 34: 2638-2643 (1991).

Supporting data for:

The Vesamicol Receptor (VR): Radioligand Selectivity and the Detection of Central Cholinergic Hypofunction. S.M.N. Efange,* D.C. Mash,+ M. Basile,+ J. Pablo,+ H. F. Kung,#J. Billings,# R. H. Michelson* & J. R. Thomas*.
*University of Minnesota, Minneapolis, MN 55455. #University of Pennsylvania,Philadelphia, PA 19014. +University of Miami School of Medicine, Miami, FL 33136.





VESAMICOL (AH5183)

[^{18}F]FLUORO-*tert*-BUTYL BICYCLOORTHOBENZOATE (FTBOB). A POTENTIAL PET TRACER FOR THE GABA$_A$ CHLORIDE CHANNEL

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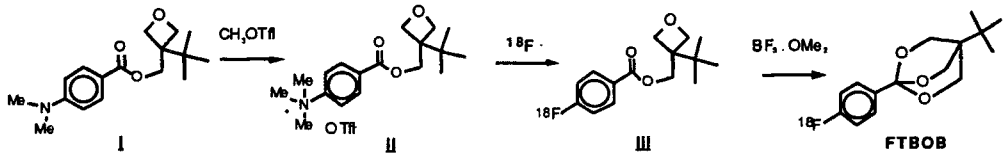
The key event in the function of central GABAergic synapses following binding of GABA to its receptor is the opening of the chloride channel and increase of chloride influx . This response is modulated by a variety of pharmacological agents. Benzodiazepine agonists, barbiturates and ethyl alcohol augment the actions of GABA, while a class of bicyclic molecules, referred to as cage convulsants or chloride channel blockers, have been found to potently and noncompetatively block GABA stimulated chloride conduction. The binding sites for cage convulsants, GABA and benzodiazepines have distinct loci within the same GABA receptor/ Cl^- channel complex. Rich interactions exist within this system which can markedly increase or decrease the binding of channel blockers. Radiolabeled channel blockers have been proposed as "biochemical tools to understand *in vivo* dynamic functional changes of the inhibitory GABAergic synapses elicited by physiological, pharmacological and pathological conditions" (1). A suitable positron labeled chloride channel blocker might be of value in PET studies of epilepsy, anxiety disorders, alcoholism, or drug efficacy.

A large series of cage convulsants have been studied (2). Our initial target, FTBOB, has a receptor IC_{50} of 42 nM vs [^{35}S]TBPS and an LD_{50} of 0.77 mg/kg in mice (2). [^{18}F]FTBOB was prepared by the scheme below. Transesterification of 3-tert-butyl oxetane-3-methanol (2) with ethyl 4-dimethylaminobenzoate under basic conditions yielded the oxetane ester I in 60% yield after chromatography and crystallization (mp 135-138°). Reaction of I with methyl triflate at room temperature gave the aryl trimethyl ammonium triflate precursor II (mp 215-220°) in excellent yield. [^{18}F]Fluorination of II under standard (3) or "dry" (4) conditions afforded [^{18}F]III in >60% yield, (coincident with authentic III on RP-HPLC C-18, 1:1:1 CH_3CN: MeOH: 12mM pH 6.7 $KHPO_4^-$. After extractive removal of excess II and drying with $MgSO_4$, III was converted to [^{18}F]FTBOB in unoptimized yield of 25% by a slight modification of reported methods (2) ($BF_3 \cdot OMe_2$, -78°- +50°, 30 min, neutralization w/ Et_3N, and normal phase HPLC to remove the 20% of unchanged III and and cold polar materials). It is anticipated this general route can be used to prepare ^{18}F- and ^{11}C-analogs from this interesting class with a spectrum of binding affinities.



Studies to examine the biodistribution and fate of [^{18}F]FTBOB and compare it with that of [^{11}C]flumazenil are presently underway.

Work supported by DOE DE-FG2-87ER60561 and NIH NS 15655 .

(1.) Concas et al., in *GABA and Benzodiazepine Receptor Subtypes.* ed. Biggio and Costa; Raven Press NY. 1990, p 89. (2.) Casida et al., Molecular Pharm. 28:246, 1985. (3.) Haka et al., J Lab Compd 27:823, 1989. (4.) Mulholland et al., J Nucl Med. 32:1010(S), 1991.

SYNTHESIS OF [^{18}F]-FLUOROBEPRIDIL, A POSITRON LABELED CALCIUM ANTAGONIST

Jialing L. Gong*, Carmen S. Dence and Michael J. Welch
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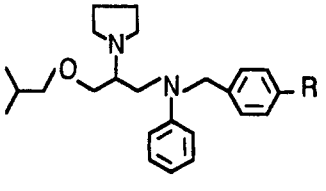
Bepridil, {1-[2-(N-benzylanilino)-1-isobutoxy methyl} pyrrolidine (Fig. 1a) is an intracellular calcium antagonist. Among other important pharmacological actions, bepridil may prove useful as an antiarrhythmic as well as an antianginal agent (1). These modes of action derive from the ability of bepridil to combine with calmodulin, an intracellular calcium receptor (2,3). To evaluate the possibility of using [^{18}F]-fluorobepridil in probing calmodulin content in vivo by PET, we first synthesized the non-radioactive 4-fluorobepridil (Fig. 1b). 4-Fluorobepridil was synthesized by debenzylation of bepridil with 10% Pd/C and 4.4% HCOOH in methanol at room temperature, to give the free base (2-methylpropoxy)-methyl-n-phenyl-1-pyrrolidine in 91% yield. The free base was alkylated with either 4-fluorobenzaldehyde or 4-fluorobenzylbromide. This last reagent gave the best yields about 44% of 4-fluorobepridil. The desired product was identified by NMR and MS.

Figure 1



(a) R = H Bepridil

(b) R = F p-F-Bepridil

The biological activity of the fluoro-analog was tested by radio-receptor assay (RRA) technique using [^{3}H]-bepridil and calmodulin. The results were as follows:

Bepridil IC_{50} = 3.47 ± 0.70 μM (n=3)

F-Bepridil IC_{50} = 4.09 ± 0.79 μM (n=3)

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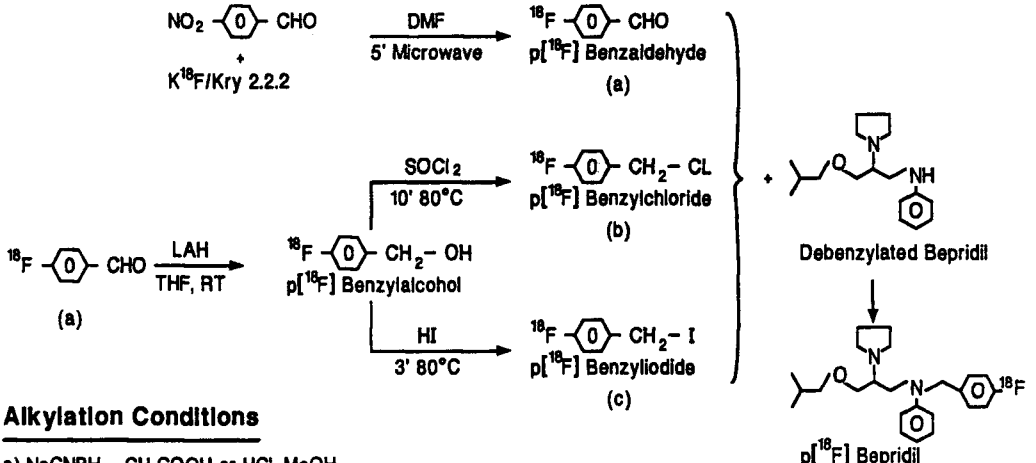
Encouraged by their close IC_{50} values, [^{18}F]-fluorobepridil was synthesized. The synthetic pathways and overall radiochemical yields are illustrated in Fig. 2. As seen in this figure, the best overall yield was obtained with the use of p-[^{18}F]-benzyliodide (4) as the N-alkylating agent. Three sets of rats experiments with [^{18}F]-fluorobepridil obtained from either (b) or (c) route, have been done. The results are being evaluated for future work.

This work was supported by NIH grant HL 13851.

References:

1. Schwartz A., Matlib M.A., Balwierczak J. and Lathrop D. Am. J. Cardiol. 55, 3C (1985).
2. Brostrom C.O. and Wolff D.J., Biochem. Pharm. 30, 1395 (1981).
3. Means A.R. and Dedman J.R., Nature 285, 73 (1980).
4. Mach R.H., Scripko J.G., Ehrenkaufer R.L. and Morton T.E., J. Lab Compd. Radioph. 30, 154 (1991).

Figure 2
Pathways for the Synthesis of p[^{18}F] Bepridil



Alkylation Conditions

a) $NaCNBH_3$, CH_3COOH or HCl, MeOH
35' 110°C, overall yield <1%

b) CH_3CN/H_2O (7/3), KI, $NaHCO_3$ 30' 80°C
overall yield 15%

c) DMF, 20' 70°C, overall yield 35%

SYNTHESIS AND EVALUATION OF FLUORINE-18 LABELED BENZODIAZEPINE ANTAGONISTS AS PET TRACERS.

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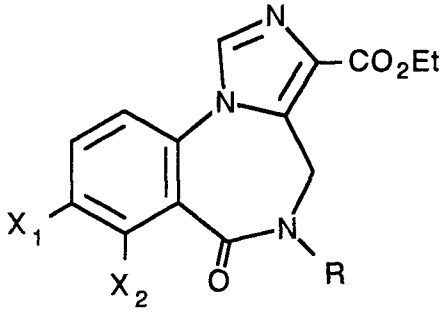
[1]Edward Mallinckrodt Institute of Radiology, [2]Department of Neurology and Neurosurgery, and [3]Department of Cell Biology and Physiology, Washington University, St. Louis MO 63110.

The involvement of central benzodiazepine (BZP) receptors in drug action and in disease pathophysiology has generated interest in the application of labeled benzodiazepine antagonists as radiopharmaceuticals. The use of carbon-11 labeled flumazenil (1) as a tracer for study of BZP receptor binding *in vivo* was originally established in 1984 (1). [^{11}C]Flumazenil has subsequently found widespread application with human subjects and PET for drug evaluation (2-4) and diagnosis of disease (5,6). More recently, reports have appeared concerning the use of the iodine-123 labeled BZP antagonist iomazenil (2) with SPECT (7,8).

Because fluorine-18 has a relatively long half-life of 110 min which is more convenient for radiopharmaceutical production and distribution, and makes possible longer imaging intervals than the 20 min half-lived carbon-11, we have prepared radiofluorinated analogues of flumazenil (FEF, 3) and iomazenil (FEI, 4) for evaluation as potential PET radiotracers. Aside from the advantage of longer half-life, the metabolism *in vivo* of these radiohalogenated tracers was anticipated to differ from corresponding carbon-11 labeled ligands.



	X_1	X_2	R
Flumazenil (1)	F	H	CH_3
Iomazenil (2)	H	I	CH_3
FEF (3)	F	H	CH_2CH_2F
FEI (4)	H	I	CH_2CH_2F
nor-FEF (5)	F	H	H
nor-FEI (6)	H	I	H

The standard ligands FEF and FEI were prepared from the corresponding nor compounds 5 and 6 via alkylation with 1-fluoro-2-tosyl ethane. The ligands were purified using flash chromatography and isolated in 70-75% yield. The affinity of these ligands for binding to primate BZP receptors was ascertained by displacement of [^{3}H]flumazenil from binding sites on cortical tissues from nonhuman primates. K_i = 11.6 $\pm$ 1.0 nM and 1.6 $\pm$ 0.1 nM for 3 and 4, respectively.

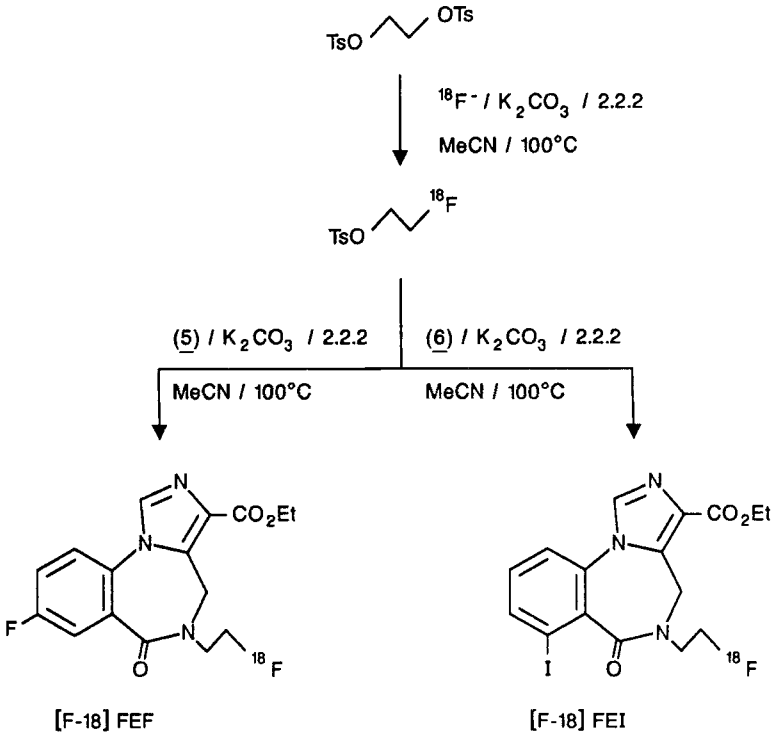
Fluorine-18 labeled 3 and 4 were prepared in high specific activity via a two-step, one-pot reaction method (see Reaction Scheme). [^{18}F]fluoride was resolubilized into acetonitrile and used to label 1,2-bis tosyl ethane as previously described (9). The resulting [^{18}F]fluoroethyltosylate was subsequently used to alkylate 5 or 6 to yield the target compounds [^{18}F]FEF and [^{18}F]FEI. Final radiopharmaceutical products were isolated using preparative normal-phase HPLC in 25-30% radiochemical yield and a specific activity > 1000 Ci/mmol within an overall preparation time of 90 min.

Preliminary applications of these tracers in mapping central BZP receptor areas with PET are promising, with selective localization within BZP receptor-rich brain tissues *in vivo*. Results of imaging experiments will be presented.

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

1. Hantraye P, Kaijima M, Prenant C, et al. *Neurosci Lett* 48: 115 (1984).
2. Samson Y, Hantraye P, Baron JC, et al. *Eur J Pharmacol* 110: 247 1985).
3. Pappata S, Samson Y, Chavoix C, et al. *J Cereb Blood Flow Metabol* 8: 304 (1988).
4. Persson A, Pauli S, Halldin C, et al. *Hum Psychopharmacol* 4: 21 (1989).
5. Samson Y and Bernuau J. *N Engl J Med* 316: 414 (1987).
6. Savic I, Persson A, Roland P, et al. *Lancet* 863 (1988).
7. Innis RB, Al-Tikriti MS, Zoghbi SS, et al. *J Nucl Med* 32: 1754 (1991).
8. Schubiger PA, Hasler PH, Beer-Wohlfahrt H, et al. *Nucl Med Commun* 12: 569 (1991).
9. Block D, Coenen HH, Laufer P, Stöcklin G. *J Lab Comp Radiopharm* 23: 1042 (1986).

REACTION SCHEME



Paper G10

Strategies for labelling radiopharmaceuticals with ^{123}I

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Two different brain receptor binding compounds were labelled with ^{123}I for a later use as SPET imaging agents, namely ^{123}I-Ro 16-0154 (Iomazenil, Fig. 1) (1) and ^{123}I-SCH 23982 (2), shown in Fig. 2. The two compounds differ markedly in their chemical reactivity.

In the case of Ro 16-0154 a labelling should be achieved in position 7, ortho to the oxygen bearing carbon 6. Starting with the dehalogenated compound an electrophilic attack would lead to an iodination at carbon 8 and 10 because of the deactivating influence of the oxygen at position 6 and the activating influence of the bridging nitrogen. Another position expected is at carbon 1 in the imidazol ring. Starting with Ro 19-3797, the 7-bromo compound, iodination is possible by a nucleophilic attack at carbon 7. A nucleophilic substitution with the well established method in acidic aquous medium assisted by Cu(I) (3) is in that case not useful because of the hydrolytic sensitivity of the ethyl-3-carboxylate moiety, which would complicate the purification and diminish the yield. The search for a suitable solvent led us to the use of glacial acetic acid which by protonation activates further carbon 7 with respect to a nucleophilic substitution. Cu(I) assistance is therefore no longer necessary to perform the halogen exchange (Fig 3).

The compound SCH 23982 (Fig. 2) on the other hand is an aromatic compound activated by the 8-hydroxy group for electrophilic substitution with iodine. The labelling with ^{123}I was therefore investigated in detail using different oxidants as H_2O_2, chloramine-T and Iodogen (2). Different labelled positions (ortho to the hydroxy group) are expected and, in consequence, labelled byproducts were found during HPLC analysis. This complication is avoided using the second labelling pathway (Fig. 4), the nucleophilic substitution starting with SKF 83566, the 7-bromo derivative. Due to the 8-hydroxy group the 7 position is deactivated with respect to a nucleophilic attack and therefore a labelling using the glacial acetic acid approach as for Ro 16-0154 fails. Whereas with the Cu(I) assisted halogen exchange the introduction of ^{123}I specifically to the 7 position is successful, avoiding labelled by-products.

Materials and methods

Ro 16-0154: [^{123}I]NaI (up to 11.1 GBq) in 0.1 N NaOH was evaporated to dryness by means of of a gentle stream of N_2 at 90 °C. Afterwards 1.5 mg of the Ro 19-3797 dissolved in 100 µl glacial acetic acid was added and the reaction mixture heated for 1 h at 155 °C. After cooling, this mixture was dissolved in 5 ml water and purified by HPLC. The HPLC conditions were as follows: RP-18 column (Knauer Lichrosorb 10 µm, 8x250 mm), EtOH/H_2O 20/80, 2 ml/min. The peak fraction was collected and diluted 1:1 with 10% glucose, the solution was passed through a sterile filter and was diluted to 37 MBq/ml with 5% glucose to give a radiopharmaceutical ready for use containing less than 10% EtOH.

SCH 23982 (electrophilic): [^{123}I]NaI (9.25-18.5 GBq) was evaporated to dryness as described above. 500 µg of R(+) SCH 23390 des-chloro HCl were dissoved in 1 ml 0.36 M H_3PO_4 and added to a vial with the radioactivity. This mixture was transferred to a vial containing 500 µg of Iodogen and stirred for 20 min at room temperature. The reaction mixture was purified by HPLC using an RP-18 column (Knauer Lichrosorb 10 µm, 4x250 mm) and EtOH/0.36 M H_3PO_4 at 1.5 ml/min. The product peak was collected, neutralized 1:1 with 0.45 NaOH, passed through a sterile Anotop 0.22 µm filter and diluted to 37 MBq/ml with isotonic phosphate buffer to give the ready radiopharmaceutical.

SCH 23982 (nucleophilic): The exchange labelling was performed in 2 ml 0.36 M H_3PO_4, in which 1.5 mg R(+) SKF 83566, 5 mg ascorbic acid, 50 µl 1% $CuSO_4x5H_2O$ and [^{123}I]NaI (up to 925 MBq) were dissolved and heated for 1 h at 150 °C. The work up was the same as above.

Conclusion

The different labelling techniques used are examples of adapting the chemical conditions to the different reactivities of similar (aromatic) compounds, keeping the final labelling conditions so simple as possible so as to allow routine labelling to be performed by untrained staff. To our knowledge it is the first time that radiopharmaceuticals are purified by HPLC with a solvent mixture that is easily transformed in to an injectable solution by a simple dilution step avoiding the cumbersome evaporation in a hot cell rotavapor. Evaporation is time consuming and the partial decomposition of the product was observed.

Whereas the ^{123}I-Ro 16-0154 was successfully introduced into clinical investigation, the ^{123}I-SCH 23982 did not result in reproducable SPET images and is therefore not clinically used.

Literature

1) H.-F. Beer, P. Bläuenstein, P.H. Hasler, B. Delaloye, G. Riccabona, I. Bangerl, W. Hunkeler, E.P. Bonetti, L. Pieri, J.G. Richards, P.A. Schubiger, In Vitro and in vivo evaluation of iodine-123-Ro 16-0154: a new imaging agent for SPECT investigations of benzodiazepine receptors. J. Nucl. Med. 31: 1007-1014 (1990)

2) H.-F. Beer, Sha Lin, I. Novak-Hofer, P. Bläuenstein, P.A. Schubiger, Large scale preparation strategy for labelling of ^{123}I-SCH 23982, a dopamine D1 receptor binding agent. Internat. J. Appl. Rad. Isot., Part A, accepted

3) J. Mertens, M. Gysemans, Cu(I) assisted nucleophilic exchange radiohalogenation. Application and mechanistic approach. in: A. Emran (Editor) New trends in radiopharmaceutical synthesis, quality assurance and regulatory control, Plenum Press, New York, 1991, p. 53-65

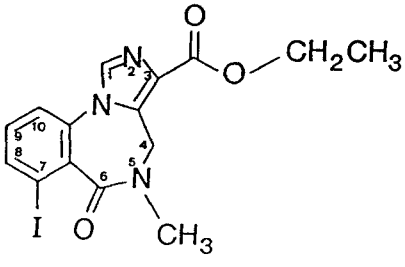


Fig. 1
Ro 16-0154: ethyl-5,6,-dihydro-7-iodo-5-methyl-6-oxo-4H-imidazo[1,5a][1,4] benzodiazepine-3-carboxylate

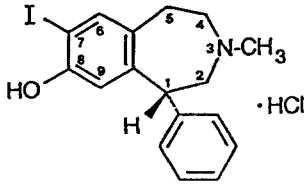


Fig. 2
SCH 23 982: R(+)-7-iodo-8-hydroxy-3-methyl 1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride

Fig. 3

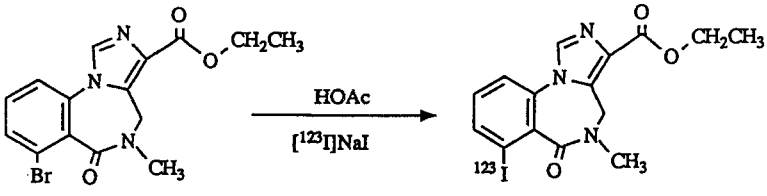
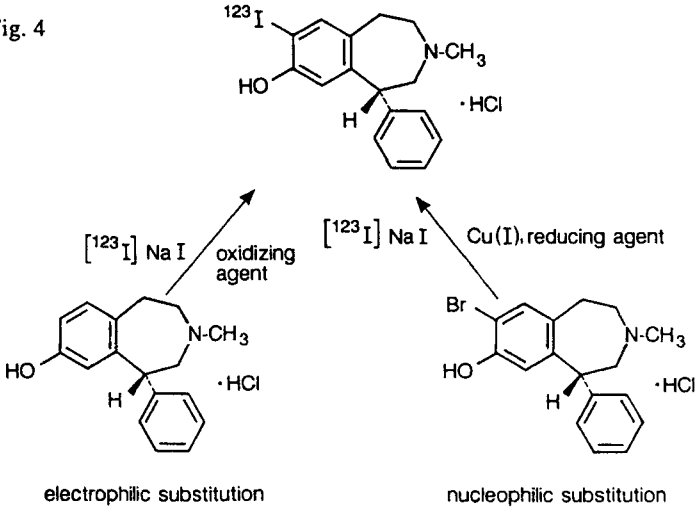

HOAc
[123I]NaI
Br
123I
CH2CH3
CH3

Fig. 4


123I
HO
N-CH3
H
·HCl
[123I] Na I
oxidizing agent
[123I] Na I
Cu(I), reducing agent
Br
electrophilic substitution
nucleophilic substitution

Radiosynthesis of 1-Methyl-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1H-indazole-3-carboxamide: A PET tracer for $5HT_3$ receptors.

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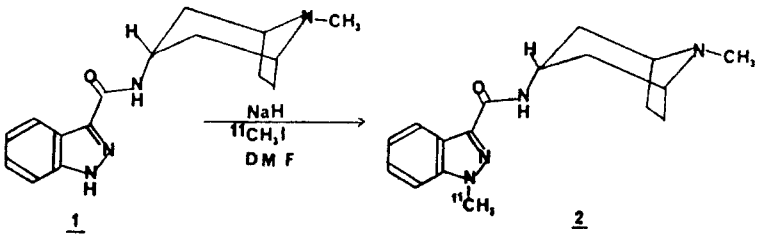
There is much interest in studying the indazole class of serotonin (5HT) antagonists with $5HT_3$ receptors at the biochemical level. The biochemical evaluations of $5HT_3$ receptors with PET requires a radioligand with high specificity. A highly selective and potent $5HT_3$ receptor 1-Methyl-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1H indazole-3-carboxamide was evaluated recently by Robertson and his collaborator using Tritium labeled compound (1). Selective $5HT_3$ receptors are being studied in human for the treatment of Schizophrenia, anxiety, substance abuse and other central nervous system (CNS) disorders (2). In vivo demonstration of receptor binding using PET will give a closer look at the receptor site to provide us with very useful information to understand the receptor at the biochemical level and will finally lead to its quantification. To achieve this one has to device a simple and short synthetic procedure with high specific activity. In order to label this compound with short lived radioisotope without altering the chemical structure of the molecule, we looked into the possibility of labeling this compound by N-methylation using [^{11}C]Iodomethane.



1 2

The precursor compound 1 (13.3 mg) was dissolved in anhydrous DMF (1 ml) in a reacti-vial(3 ml). The solution was stirred with Sodium hydride (80% dispersion in mineral oil, 1.5 mg) under nitrogen for 15 minutes. [^{11}C]Iodomethahe was prepared and passed over P_2O_5 and then bubbled through the reaction mixture at room temperature for 10 minutes. The reaction mixture was stirred for an additional 10 minutes at room temperature. The solvent was removed by rotary evaporation under vacuum. The crude product was extracted with ethanol (1 ml x 2) and filtered through a milipore filter (0.22 micron). Preparative HPLC separation of the product using C-18 column, eluted with acetonitrile/water/triethylamine (250:250:1) yielded the labeled compound in 98% purity. 10 ul of the purified product was reinjected into an analytical HPLC C-18 column (Perkin Elmer/ HS-5 C18, Ser No. 2997) eluted with acetonitrile/water/triethylamine (250:250:1) at 1.5 ml/minute. Chemical purity was established by comparing the chromatograms of the [^{11}C] product with the chromatographic mobility of an authentic sample of 2 by UV spectroscopy (302 nm). The radiochemical yield without correcting for the decay, was 28%. Total synthesis time

was 60-65 minutes including [^{11}C]Iodomethane preparation and preparative HPLC.

Acknowledgements: The authors would like to thank Dr. Robertson, Lilly Research Laboratories for supplying the precursor and the authentic compound for the above experiment.

References:

1. Robertson, D.W., Bloomquist, W., Cohen, M.L., Reid, L.R., Schenck, K., Wong, D.T., J. Med. Chem., 33, 3176-3181 (1990).
2. Fozard, J.R., The peripheral actions of 5-Hydroxytryptamine, Fozard, J.R., Ed., Oxford Medical Publications, Oxford, p.354 (1989).

Paper G12

SYNTHESIS OF N^1-3-[^{18}F]FLUOROPROPYL-N^4-2-([3,4-DICHLOROPHENYL]ETHYL)PIPERAZINE, A HIGH AFFINITY LIGAND FOR SIGMA SITES.

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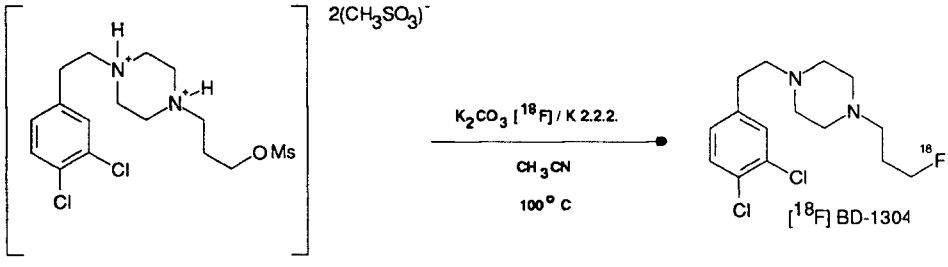
N^1-3-Fluoropropyl-N^4-2-([3,4-dichlorophenyl]ethyl)piperazine [BD 1304] is a high affinity and specific ligand for the sigma receptor. The biology and function of the sigma receptor was the subject of a recent review (1). The sigma receptor has been implicated in the physiological aspects of motor disorders such as tardive dyskinesia and dystonia. Previous studies of sigma ligands have appeared in the PET radiopharmaceutical literature (2,3).

BD 1304 displayed a K_i of 4.2 nM versus [^{3}H]R-(+)-3-(3-hydroxyphenyl)-N-propyl piperidine ([^{3}H]R-(+) 3-PPP) in the assay for sigma receptor affinity (4). In assays for affinity to phencyclidine, kappa opiate, D_2 dopamine, and cholinergic receptors, BD 1304 displayed K_i values greater than 10 μM. Because of these highly desirable *in vitro* properties, we prepared fluorine-18 labeled BD 1304.

The chemical precursor for labeling by fluoride displacement is the primary methanesulfonate. We conducted the fluoride displacement on both the free base and the bis-methanesulfonate salt using either tetrabutylammonium hydroxide or K_2CO_3/Kryptofix-222 in acetonitrile. The results were similar regardless of the substrate. We optimized the reaction utilizing the bis-mesylate salt since it is the stable form of the precursor. To our knowledge, this is the first example of an [^{18}F]fluoride displacement reaction using an amine salt as the substrate.



Approximately 1.1 mg (1.9 μmol) of bis-methanesulfonate salt was added as the solid to a solution of [^{18}F]fluoride, 5.5 μmol K-2.2.2, and 5.5 μmol K_2CO_3 in acetonitrile (0.2 mL). This mixture was heated at 100° C for five minutes. The crude product was filtered through a 1 mL BONDELUT-SI column and subsequently purified by HPLC. The preparative HPLC utilized a Beckman-ODS column (5μ, 9.4 x 250 mm) with an eluant of 50% acetonitrile and 50% buffer (5 mM Et_3N, 5 mM NaH_2PO_4, pH 7.8).

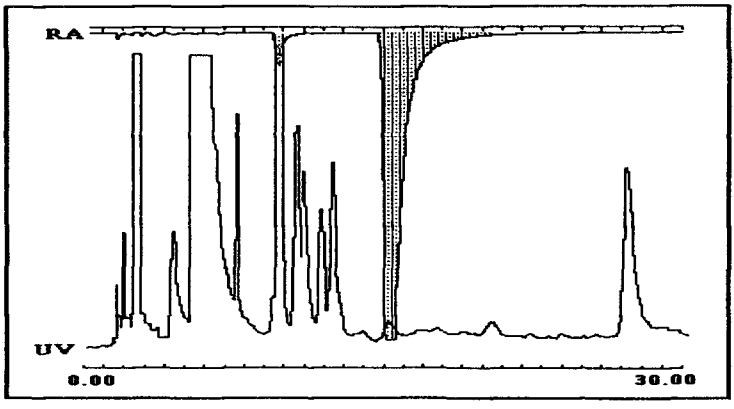
The product was isolated from the HPLC column in 48.5 +/- 8.9 % (n=10) radiochemical yield (corrected to start of synthesis) with a synthesis time of approximately 60 min. Additional time will be required to isolate the product from the HPLC eluant and formulate for injection. The HPLC purification provided a product with high radiochemical (>99%) and chemical purity. The specific activity for the product, determined in two separate preparations, was 4.0 and 3.7 Ci/μmol (EOB). Thus, starting from 5.8 mCi of [^{18}F]fluoride, 1.8 mCi (45%) of product was collected from the HPLC column within 60 minutes.

(1). Walker J.M., Bowen W.D., Walker F.O., Matsumoto R.R., DeCosta B., Rice K.C. - Pharmacological Reviews, 42 (4): 355, (1990).
(2). Wilson A.A., Dannals R.F., Ravert H.T., Wagner H.N.,Jr. - J. Labelled Compd. Radiopharm. 30: 260, (1991).
(3). Wong D.F., Gibson R., Burns H.D., Dannals R.F., Shaya E., London E.D., Brenner N., Wilson A.A., Ravert H.T., Chen C., Minkin E., Wagner H.N.,Jr. - J. Nucl. Med. 32 (5 Supplement): 1071, (1991).
(4.) Bowen W.D., unpublished results.

SYNTHESIS OF N^1-3-[^{18}F]FLUOROPROPYL-N^4-2-([3,4-DICHLOROPHENYL] ETHYL)PIPERAZINE, A HIGH AFFINITY LIGAND FOR SIGMA SITES. ***KIESEWETTER and DeCOSTA***

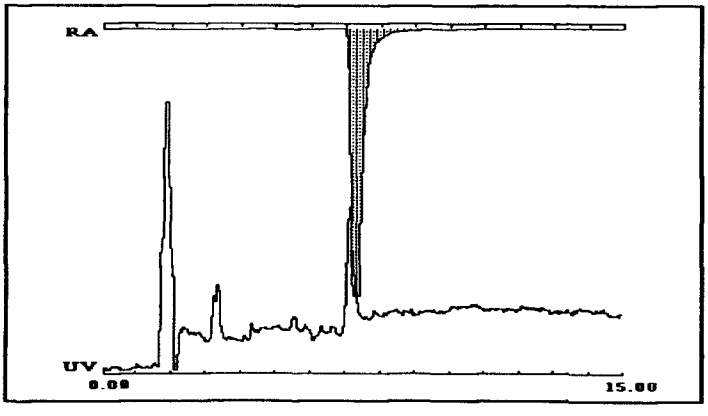
Semipreparative HPLC

Beckman ODS (9.4 x 250 mm)
50 % ACN / 50 % Buffer
1 mL / min



Analytical HPLC

Beckman ODS (4.6 x 250 mm)
80 % ACN / 20 % Buffer
1 mL / min



Labelling of N-[^{11}C]methyl-N-diphenylbutenyl-GABA, a GABA uptake inhibitor.

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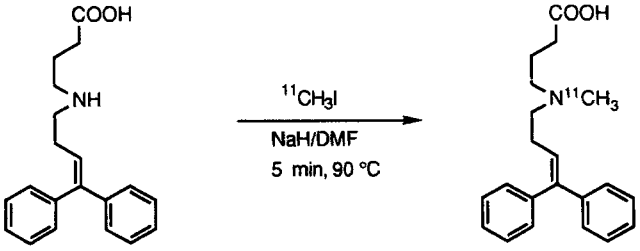
CERMEP, 59 Bd Pinel, 69003 Lyon, France & *PharmaBiotec, Royal Danish School of Pharmacy, 2 Universitetsparken, 2100 Copenhagen, Danmark.

PET exploration of the GABA system has so far been so far limited to the use of labelled benzodiazepines, such as RO15-1788. The compact heterocyclic nature of the specific GABA agonists muscimol and THIP, capable of penetrating the blood-brain barrier, makes their labelling with positron emitting isotopes extremely difficult.

We wish to report here the radiosynthesis of a GABA uptake inhibitor, N-methyl-N-diphenylbutenyl-GABA; this class of ligand could be helpful for the exploration of the GABA system using PET techniques.

GABA itself has been labelled with carbon 11 [Antoni & Langstrom 1989], but this neurotransmitter does not cross the blood-brain barrier. Introduction of a bulky diphenylbutenyl substituent on the amino group of GABA-related amino acids interacting with the uptake system leads to a serie of very potent and lipophilic GABA uptake inhibitors [Falch & Krogsgaard-Larsen 1991]

N-methyl-N-diphenylbutenyl-GABA is labelled via a classical methylation reaction, using [^{11}C]CH_3I on the desmethyl precursor dissolved in DMF/NaH, for 5 minutes at 90°C:



The reaction can be monitored by radioTLC, using acetonitrile/water/triethylamine 7/2/1, Rf: N-Me-N-DPBGABA 0.65; desmethyl analogue 0.55.

The final product was isolated by reverse-phase HPLC using an Ultrabase preparative column 250x12.5 mm (SFCC), acetonitrile/water 34/66, 3 ml/min, UV 254 nm and radioactivity detector, Rt 18'30 (desmethyl 17'). Radiochemical yield was 25-35 % in 40 min synthesis time. Specific activity was in the 100 mCi/μmol range, starting from 5' irradiation at 20 μA, and is expected to be in the usual range of 500-1500 mCi/μmol as for the other ligands produced with a full scale $^{14}N(p,\alpha)^{11}C$ irradiation (Diprenorphine, RO 15 1788).

Neuroanatomical work in animals is now in progress in order to assess the pharmacological properties and the usefulness of this new labelled GABA uptake inhibitor.

G. Antoni, B. Langstrom (1989): Synthesis of γ-Amino [4-^{11}C] Butyric Acid. J. Lab. Comp. Radiopharm., 27,5,571-576.
E. Falch, P. Krogsgaard-Larsen (1991): Gaba uptake inhibitors. Syntheses and structure activity studies on Gaba analogues containing diarylbutenyl and diarylmethoxyalkyl N-substituents. Eur. J. Med. Chem., 26, 69-78.

A GENERAL METHOD FOR PREPARING [^{18}F]FLUOROPHENETHYLAMINES. SYNTHESIS OF 4-[^{18}F]FLUOROFENTANYL.

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A new method for labeling alkylbenzenes with fluorine-18 has been reported recently.(1) This new procedure also provides a practical method for the preparation of [^{18}F]fluorophenalkyl halides which can be used for the synthesis of a variety of [^{18}F]-labeled phenalkylamines. We now report the successful application of this method to the synthesis of 4-[^{18}F]fluorofentanyl, [^{18}F]-**1**.

Fentanyl and its derivatives are a class of phenethylpiperidines with high affinity for the opiate mu receptor.(2,3) [^{11}C]-Labeled carfentanyl, a derivative of fentanyl, has been successfully used in conjunction with positron emission tomography (PET) for imaging mu opiate receptor in the human brain.(4,5,6) Previous attempt by Hwang et al to label fentanyl with fluorine-18 only yielded the corresponding 4-[^{18}F]fluorofentanol due to the limitation of labeling technique.(7) Although 4-fluorofentanol, an alcohol analog of fentanyl, was only slightly less potent than the parent compound, the rapid egress of the tracer from mouse brain limited its usefulness for PET studies of opiate receptor.

Now we report the first successful synthesis of [^{18}F]-**1** by a four-step procedure (Scheme 1): (a) the ^{18}F-for-NO_2 exchange reaction of 4-nitroacetophenone **2** to give 4-[^{18}F]fluoroacetophenone **3** (50%, EOS), (b) the bromination of **3**, using bromine in acetic acid, to yield 2-bromo-4'-[^{18}F]fluoroacetophenone **4** (35%, EOS), (c) the reduction of **4**, using triethylsilane-trifluoroacetic acid (TES/TFA), to 4-[^{18}F]fluorophenethyl bromide **5** (25%, EOS), and (d) the reaction of **5** and norfentanyl **6** to give [^{18}F]-**1** (> 80% alkylation yield, Table 1). The overall radiochemical yield of [^{18}F]-**1** was 10% (EOS) with an overall reaction time of 2.5 h. The specific activity was determined by HPLC to be greater than 500 mCi/mmol.

It was of interest to note that the reaction of the bromide **5** and norfentanyl proceeded slowly in refluxing toluene at various bath temperatures. The reaction finally proceeded in good alkylation yields when all toluene was removed at a reaction temperature of 160 °C. Our results suggested the heating of neat reactants may be the method of choice for those reactions proceeding poorly in solution. A similar finding was observed in our laboratory the successful preparation of a [^{18}F]-labeled 5-HT_3 antagonist (8).

Our successful synthesis of [^{18}F]-**1** clearly demonstrated that a general method has been developed for the preparation of [F-18]fluorophenethylamines. On-going investigations involve the optimization of the reaction conditions as well as the biodistribution studies of [^{18}F]-**1** and the corresponding 2-fluoro-derivative.

The authors would like to thank Dr. M.J. Welch for his helpful discussion and generous supply of all chemicals for the preparation of norfentanyl. The authors also thank the technical assistance of Mr. Brad Newcomer. This work was supportedby the Kettering Medical Center.

1. Hwang D-R, Dence CS, Gong J, Welch MJ. - Appl. Radiat. Isotop. 42:1043(1991).
2. Janssen PAJ, Niemegeers CJE, Dony JGH. - Arzneim.-Forsch. (Drug Res) 13:502(1963).
3. Van Daele PGH, De Bruyn MFL, Boey JM, et al. - Arzneim.-Forsch (Drug Res) 26:1521(1976).
4. Frost JJ, Wagner HN, Dannals RF, et al. - J. Comput. Assist. Tomogr.9:231(1985).
5. Frost JJ, Mayberg HS, Sadzot B, et al. - J. Cereb. Blood Flow Metabol. 10:484(1990).
6. Frost JJ, Douglass KH, Mayberg HS, et al. - J. Cereb. Blood Flow Metabol. 9:398(1989).
7. Hwang D-R, Feliu AL, Wolf AP, et al. - J. Label. Compd. Radiopharm. 23:277(1986).
8. Hwang D-R, Banks WR, Nagel AA et al. - J. Nucl. Med. 32:1841(1991).

Table 1. Reactions of amines and phenethyl bromides†. Effect of reaction temperature on the alkylation yields.

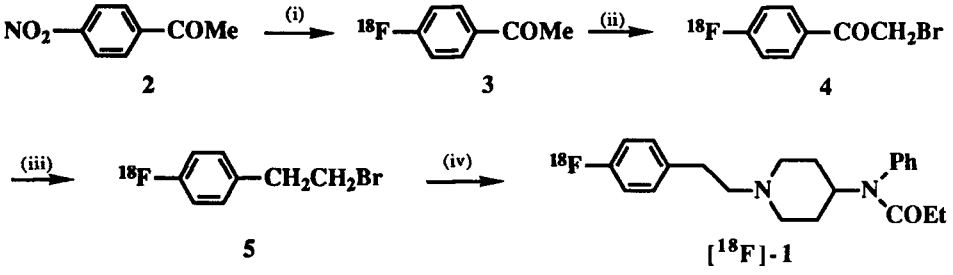
Bromides	Amine	Reaction Temp.(°C)	Reaction Time (min)	Yields (%)
PhEtBr	Pyrrolidine	120	30	50
PhEtBr	4-Benzylpiperidine	120	30	70*
4-F-PhEtBr	Norfentanyl	120	30	55
4-[^{18}F]PhEtBr	Norfentanyl	120	15	5
		120	30	16
		160	8§	80

† All reactions were carried out in 1 ml of toluene with a amine/bromide ratio of 1/1. For reactions involving 4-[^{18}F]FPhEtBr 5 mg of norfentanyl and 0.5 ml of toluene were used. All chemical yields were isolated yields, and all radiochemical yields were determined either by HPLC or TLC.

* Isolated as the corresponding hydrochloride salt which was not soluble either in water or ether.

§ All solvent was removed after heating.

SCHEME 1



Reaction conditions: (i) Compound 3, $K^{18}F$/Kryptofix 222, DMSO; microwave 5 min. (ii) Br_2/HOAc/HCl, $CHCl_3$, 80 °C, 9 min. (iii) TFA, Et_3SiH, 110 °C, 15 min. (iv) norfentanyl, toluene, 160 °C, 8 min.

SYNTHESIS OF FLUORINATED KAPPA OPIOID RECEPTOR LIGANDS.

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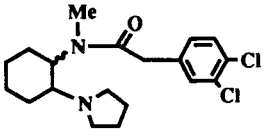
The presence of multiple opioid receptor subtypes has been characterized through the use of subtype specific opioid ligands (1). Recently, the opioid receptors in human brain have been successfuly imaged with positron emission tomography (PET) using positron-labeled opioid ligands, such as [^{11}C]-labeled diprenorphine (non-type-specific) (2,3) and carfentanyl (mu-specific) (2), and [^{18}F]-labeled cyclofoxy (non-type-specific) (4). We interested in the development of [^{18}F]-labeled opiate mu and kappa receptor specific ligands for mapping the receptors in vivo using PET. Recently, we have developed a rapid synthesis of [^{18}F]-labeled fentanyl (5), and its use in imaging mu-receptor in vivo is currently being evaluated. We report here our progress in the synthesis of [^{18}F]-labeled kappa specific ligands for mapping kappa receptor in vivo using PET.

Since the development of U-50488, compound **1**, a kappa-specific opioid agonist which has potent analgesic properties (6), enormous efforts have been pursued for the preparation of highly kappa-specific agonists devoid of undesired side effects of the mu anlgesics. Recently, several compounds, e.g. **2** and **3**, have been reported to possess superior specificity for kappa receptor (high kappa/mu ratios). (7,8) We have evaluated a general method for the preparation of [^{18}F]fluorinated analogs of compounds **2** and **3**.and their potency will be evaluated in vivo.



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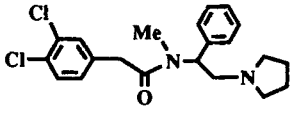

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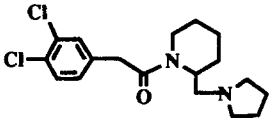


3

Our synthetic strategy was based on the reaction of [^{18}F]fluoride and sulfonate **5**, as outlined in the synthesis of compound **4** (Scheme 1). The alcohol **6** was prepared from 3-pyrrolidinol and phenylglycine according to a modified literature procedure (7). Alcohol **6** was successfully converted to the corresponding tosylates, **5a** and **5b**, as a pair of diastereoisomers which can be easily separated by HPLC (silica gel, EtOAc/hexane, retention time = 16 and 19 min). The results from the fluorination of tosylate **5a** and **5b**, using TBAF in THF, or TBA^{18}F in THF, or K^{18}F/Kryptofix222 in MeCN, are summarized in Table 1. The low yield of the fluorinated standard **4** by the initial approach also forced us to prepare **4** via a completely different synthesis as shown in scheme 2. However the multiple step synthesis could not be easily applied to the preparation of the [^{18}F]-labeled product.

Although the desired [^{18}F]-labeled compound **4** was not formed under the reaction conditions examined, the complete consumption of the starting tosylate and the formation of a labeled by-product (Rf=0.7, silica gel, EtOAc/hexane=3/1, > 40%) at 80^{o}C were observed. The labeled by-product has not been fully characetrized, but its low polarity (high Rf value) suggested it was not an amine but a compound with low molecular weight. A closer examination of the structure of compound **6** suggested the cleavage of the phenylacetamide group under the employed reaction conditions was the most probable side reaction. And hence the labeled by-product could be the labeled acyl fluoride. If the hypothesis is true, our results suggest the labeling could only be achieved at low reaction temperature with the use of a highly reactive substrate, such as triflate **5c**.

Initial attemps have failed to purify the highly reactive triflate **5c**, which was formed in situ at low temperature as evidenced by the formation of fluorinated standard **4** upon the addition of excess TBAF to the crude reaction mixture. This difficulty has prompted us to evaluate other sulfonates as the proper precursors for the [^{18}F]-labeling of **4**. The results of such evaluation were reported in the accompanied abstract.(9)

In summary several analogs (compounds **4** and **6**) of the kappa receptor agonist **2** have been prepared. Its receptor specificity and affinity will be determined in vitro. A one-step [^{18}F]-labeling approach was also evaluated, but the observed side reaction suggested a more reactive but stable precursor will be needed. On going works include the evaluation of suitable precursors and alternative synthetic approaches. Also under investigation is the preparation of a series of fluorinated sigma receptor ligands, which are closely related to **1**, by a similar approach.

The authors would like to thank the financial support of Kettering Medical Center.

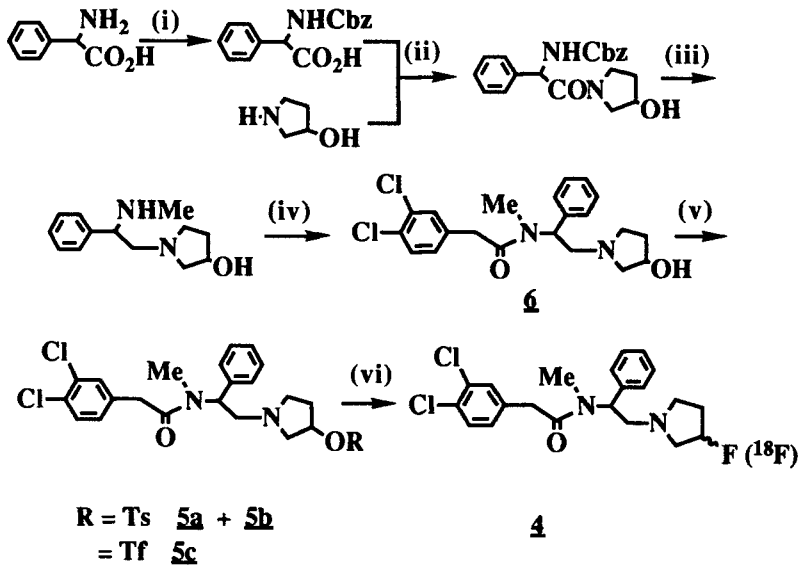
1. Opiate receptor subtypes and brain function. Brown RM, Clouet DH, Friedman DP Eds. NIDA Research Monograph 71, Roxkvillo, Maryland, 1986.
2. Mayberg HS, Sandzot B, Meltzer CC et al. - Annals Neurol. 30:3(1991).
3. Jones AKP, Qi LY, Fujirawa T et al - Neurosci. Lett. 126:25-28(1991).
4. Cohen RM, Carson RE, Channing M et al - J. Nucl. Med. 29:796(1988).
5. Hwang D.-R. Banks WR, Mantil JC. Abstract, Ninth International Symposium on Radiopharmaceutical Chemistry, 1992 (Submitted).
6. Szmuszkovicz J,Von Voigtlander PF. - J. Med. Chem. 25:1125(1982).
7. Costello GF, James R, Shaw JS et al. - J. Med. Chem. 34:181(1991).
8. Vecchietti V, Giordani A, Giardina G et al. - J. Med. Chem. 34:397(1991).
9. Hwang D.-R. Banks WR, Mantil JC Abstract, Ninth International Symposium on Radiopharmaceutical Chemistry, 1992, (Submitted).

Table 1. Fluorination of sulfonic esters 5 a-c.

Sulfonate	Base	Reaction Conditions Temp.(°C)	Time(h)	Yields† (%) **4**	By-product
5a + 5b	TBAF/THF	r.t.	12	5	
	TBAF/THF	80	0.5	3	
	KF/MeCN	r.t.	12	3	
	K^{18}F/MeCN	r.t.	0.5	0	< 1
		80	0.25	< 1	35
5b*	K^{18}F/MeCN	r.t.	0.5	0	< 2
		40	0.25	< 2	40
		80	0.25	< 6	40
5c§	TBAF/THF	-60 to r.t.Δ	1	5	
	K^{18}F/MeCN	r.t.	0.5	0	-
		40	0.25	0	-

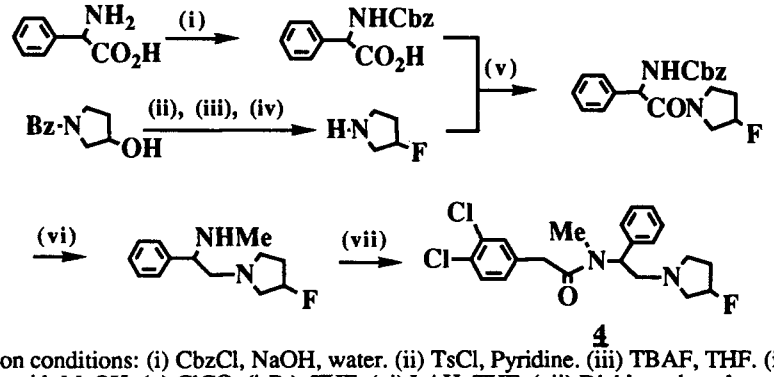
† All reaction yields were isolated yields. All radiochemical yields were determined by radioTLC. In all cases the starting sulfonates were completely consumed.
* The diastereoisomer with a HPLC retention time of 19 min.
§ Extremely unstable. In all cases a solution of 5c in EtOAc/hexane was used.
Δ Ten-fold excess of TBAF was added to the cold reaction mixture at -60 °C.
- In some cases excess of triflic anhydride was present in the solution of 5c, and the starting [^{18}F]fluoride was completely converted to a labeled by-product (>97%) which was believed to be the corresponding trifluoromethanesulfonyl [^{18}F]fluoride.

Scheme 1



Reaction conditions: (i) CbzCl, NaOH, THF-water. (ii) $ClCO_2$(i-Pr), THF. (iii) LAH, THF. (iv) dichlorophenylacetyl chloride, CH_2Cl_2. (v) Tosyl chloride, pyridine; or Triflic anhydride, CH_2Cl_2, pyridine. (vi) Tetrabutylammonium fluoride, THF; or $K^{18}F$/Kryptofix222 in MeCN; or TBA^{18}F,THF.

Scheme 2



Reaction conditions: (i) CbzCl, NaOH, water. (ii) TsCl, Pyridine. (iii) TBAF, THF. (iv) Pd/C, formic acid, MeOH. (v) $ClCO_2$(i-Pr), THF. (vi) LAH, THF. (vii) Dichlorophenylacetyl chloride, CH_2Cl_2.

A NEW APPROACH TO THE PRODUCTION OF NCA FLUORINE-18 LABELLED BUTYROPHENONE NEUROLEPTIC AGENTS. SYNTHESIS OF γ-IODO-p-[^{18}F]-FLUOROBUTYROPHENONE USING DIIODOSILANE.

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Fluorine-18 labelled butryophenone neuroleptics such as spiperone, benperidol, and their analogues have been widely exploited for non-invasive studies of the postsynaptic dopamine D_2 receptor function in man and primate. Several variations on the production of these useful agents center on the use of γ-chloro-p-[^{18}F]fluorobutyrophenone 1a as a key synthetic intermediate and include the production of 1a from cyclopropyl p-nitrophenyl ketone 2 using conventional heating (1), or using microwave facilitation to shorten reaction time (2).Yet another approach relied on the production of 1a from the reaction 4-[^{18}F]fluorobenzonitrile with cyclopropyl lithium halide, followed by acid catalyzed cleavage of the three membered ring (3). All of the above mentioned methods require the acid induced ring opening by a solution of concentrated hydrochloric acid in MeOH to the crude reaction mixture of 4-[^{18}F]fluorophenone 3 and heat at 110°C for approximately 10 min followed by aqueous work-up. The strongly acidic reaction conditions also have caused the release of a volatile ^{18}F species which presumably is hydrogen [^{18}F]fluoride.

The production of these agents had previously been optimized by Hwang et al., whose study involved the use of microwave technology during the exchange and alkylation steps (2). In an attempt to further optimize the production of these neuroleptics, we focused on the production of the γ-halo-p-[^{18}F]fluorobutyrophenone intermediate 1a-b. We also rationalized that the potential release of volatile fluorine-18 species may be avoided if the high temperature/strongly acidic conditions could be altered. Alkyl silyl iodide reagents such as trimethylsilyl iodide (TMSI) and its homologue diiodosilane (DIS) (SiI_2H_2) have previously been shown to affect ring opening of the cyclopropyl moiety (4,5). We report that DIS cleanly, quickly, and efficiently cleaves (Scheme 1) cyclopropyl p-[^{18}F]fluorophenyl ketone 3 at room temperature in methylene chloride or pentane solution to afford γ-iodo-p-[^{18}F]fluorobutyrophenone 1b. The cleavage reaction itself requires as little as 3-5 min to affect a near quantitative conversion of 3 into 1b.

The reaction conditions are summarized as follows. Cyclopropyl p-[^{18}F]-fluorophenyl ketone 3 is produced from the nitro-precursor 2 (1) using the method of Hwang et al. (2). After cooling, the exchange mixture was subjected to an aqueous solid phase extraction (C18) and elution with pentane (4-6 mL). Drying (Na_2SO_4) of the pentane mixture was followed by concentration to a volume of ~0.3 mL followed by reconstituting in CH_2Cl_2 (0.7-1.0 mL), if a solvent change is desired. DIS (10-20 μL) was introduced and the mixture stirred at room temperature (3-10 min). Excess reagent was destroyed by the addition of 10% sodium thiosulfate (1.0 mL), followed by dilution with CH_2Cl_2 (1.0 mL) and separation of the layers. The organic phase was dried by passing through a bed of Na_2SO_4 and Na_2CO_3 to afford 1b as a solution in CH_2Cl_2 (3-5 mL) (66.7±8.7 % radiochemical yield from 3 in preparation times of 10-30 min). The usefulness of γ-iodo-p-[^{18}F]fluorobutyrophenone 1b as a labelling precursor was investigated by the un-optimized preparation of [^{18}F]spiperone 5 as a model. The solution of 1b was concentrated to a volume of ~ 0.2 mL by heating at 80°C with an air cooled reflux condenser, followed by the addition of amine 4 (9.0 mg) as a solution in CH_3CN/DMF (7/3 v/v 1.0mL). The mixture was heated at 110°C in a sealed vessel for 20 min, cooled, and analyzed by radiomonitored tlc against authentic spiperone (36% conversion from 1b).

We will also present further modifications/optimizations to the above procedures which include: (a) the use of a silica gel facilitated purification of 3 to avoid an aqueous workup (b) the use of a solid phase resin based system to destroy excess DIS (6), again to avoid an aqueous work-up and (c) optimization of the final alkylation step.

SCHEME 1

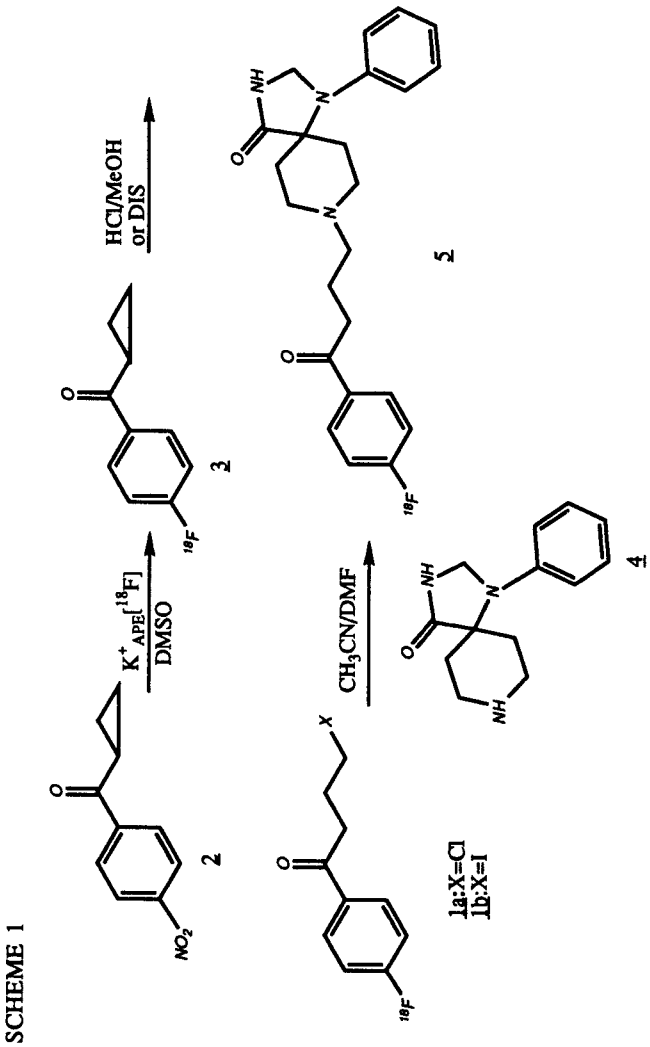

Table 1. Conditions and Yields for the Synthesis of γ-Iodo-p-[^{18}F]fluorobutyrophenone (1b) and [^{18}F]spiperone (5).

Reaction Step	Conditions	% Yield[a]
1	microwave 700 W 5 min	59.8±4.8 (n=6)
2	DIS/CH_2Cl_2 room temp	66.7±8.7% (n=5)
3	DMF/CH_3CN/110°C	36.1+12.8% (n=3)

a) Conversion yields of individual steps

The authors would like to acknowledge the generous financial support of the Kettering Medical Center.

1) Shiue, C.-Y., Watanabe M., Wolf A.P., Fowler J.S., Salvadori P.- J. Labelled Compd. Radiopharm. 21: 533 (1984)
2) Hwang D.-R., Moerlein S.M., Dence C.D., Welch M.J.- J. Labelled Compd. Radiopharm. 26: 391 (1989)
3) Shiue, C.-Y., Fowler J.S., Wolf A.P., Watanabe M., Arnett C.D.- J. Nucl. Med. 26: (2) 181 (1985)
4) Robertson D.W., Krushinski J.H., Beedle E.E., Leander J. D., Wong D.T. Rathbun R.C.- J. Med. Chem. 29: 1577 (1986)
5) Keinman E., Perez D., Sahai M., Shivly R.-J. Org. Chem. 55: 2927 (1990)
6) Hwang D.-R., Dence C.D., McKinnon Z.A., Mathias C.J., Welch M.J.- Nucl. Med. Biol. 18: (2) 247 (1991)

SYNTHESIS OF (±) 2-[^{11}C]METHYLAMINO-2-METHYL-3-PHENYL-1-PROPANOL, A POTENTIAL RADIOTRACER FOR PET IMAGING OF SEROTONIN UPTAKE SITES

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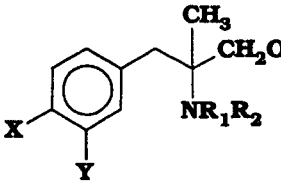
A number of drugs (fluoxetine, paroxetine, sertraline, citalopram, cyanoimipramine, and others) have been prepared in carbon-11 or fluorine-18 labeled forms as potential radioligands for *in vivo* studies of the neuronal serotonin uptake system. *In vivo* results have been disappointing, possibly due to high non-specific binding of these lipophilic amines. For all of these drugs, there have been few options for synthesis of analogues with varying lipophilicities. Cericlamine^{R} [2-(3,4)dichlorobenzyl)-2-dimethylamino-1-propanol, JO 1017], a highly selective and potent serotonin uptake inhibitor (1,2), is currently under clinical evaluation as a novel non-tricyclic antidepressant drug (3-5). The simple structure of Cericlamine^{R} offers numerous options for synthesis of radiotracers with varying lipophilicities (Table 1) which can be examined for brain uptake, pharmacological specificity, and *in vivo* PET imaging of serotonin transporters.

Table 1.



	X	Y	R1	R2	
	Cl	Cl	CH_3	CH_3	(Cericlamine, JO 1017)
1	H	H	H	CH_3	
2	H	H	CH_3	CH_3	
3	Cl	H	H	CH_3	
4	Cl	H	CH_3	CH_3	
5	Cl	Cl	H	CH_3	

We have successfully prepared the first in this series, 2-[^{11}C]methylamino-2-methyl-3-phenyl-1-propanol (**1**), by the simple methylation of the free amine of the hydroxyamine derivative using [^{11}C]methyl iodide (Fig. 1). (±) α-Methylphenylalanine was treated with $LiAlH_4$ in dry THF at room temperature for 3 h; after work-up, the hydroxyamine derivative was recrystallized from ethyl acetate as a white powder (86% yield). The N-[^{11}C]methyl product **1** was prepared by bubbling [^{11}C]CH_3I into a solution of the hydroxyamine derivative (1.0 mg) in dry DMF (200 μL) cooled at - 40 °C; the vessel was then sealed and heated at 90 °C for 5 min. The crude product mixture was purified by semi-preparative reversed phase C_{18} HPLC to afford **1** in high radiochemical yield (> 55%, decay corrected). After evaporation of the HPLC solvent, the product was formulated for i.v. injection with sterile solution of isotonic phosphate buffer (pH 6.0) followed by filtration. Total synthesis time was 45 minutes from EOB. Analytical HPLC analysis of the formulated compound showed a high radiochemical purity (>95%) and a specific activity >1500 Ci/mmol (end of synthesis). HPLC and

TLC analyses of the C-11 labeled product were identical to authentic **1** prepared by N-methylation of the hydroxyamine derivative with CH_3I (1 equiv.).

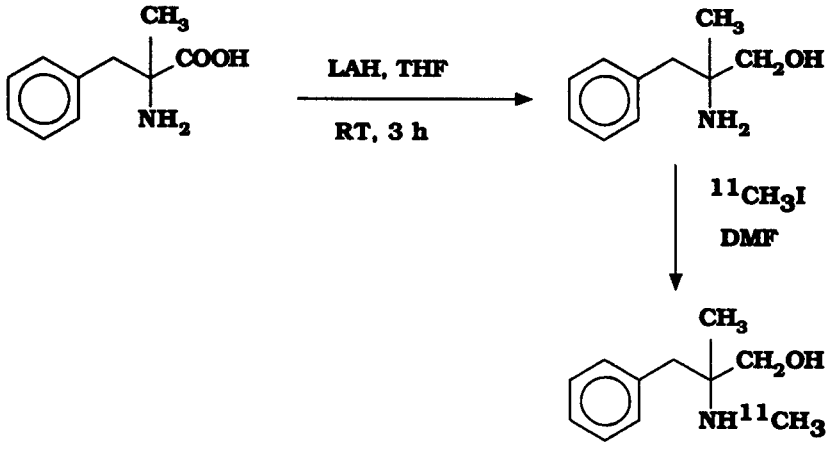


Fig. 1.

Synthesis of **1** has demonstrated the feasibility of preparing the complete series of compounds in Table 1, including Cericlamine^R, by a simple and high yield [^{11}C]methylation reaction. Preliminary *in vivo* experiments in CD-1 mice indicated that this first derivative, the simplest in the series, showed good brain penetration and, interestingly, higher whole brain levels at 30 minutes than at 2 minutes. The pharmacological specificity of the various derivatives lacking methyl and chloro substituents remains to be established.

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1. Wettstein J.G., Gouret, C.J. and Junien, J.L. - Eur. J. Pharm., 183, 1477 (1990).
2. Gouret C.J., Porsolt R., Wettstein J.G., Puech A., Soulard C., Pascaud X. and Junien J.L. - Arzneim.-Forsch./Drug Res., 40, 633-640 (1990).
3. Gouret C.J., Wettstein J.G., Porsolt R.D., Puech A. and Junien J.L. - Eur. J. Pharm., 183, 1478 (1990).
4. Jolas T., Adrien J., Lanfumey L., Hai-Dahmane S. and Hamon M. - Soc. Neurosci. Abst. 17, 1488 (1991).
5. Earley B., Burke M., Leonard B.E., Gouret C.J., and Junien J.L. - Psychopharm. 101 (Suppl), S17 (1990).

SYNTHESIS OF A RADIOTRACER FOR STUDYING SIGMA RECOGNITION SITES USING POSITRON EMISSION TOMOGRAPHY: [^{11}C]L-687,384

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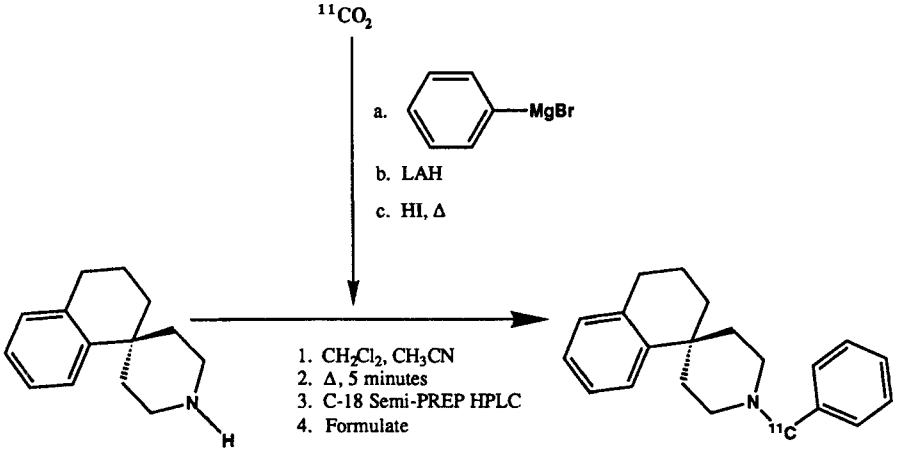
The sigma recognition site may be associated with psychosis (1) and is related to the estrogen/progesterone axis (2). In addition, the sigma recognition site has been observed in human brain tumors (3). Recently, a novel ligand that has high affinity and selectivity for the central sigma recognition site, L-687,384 (1-benzylspiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine]), has been developed. L-687,384 was found to be a potent and specific displacer of [^{3}H]DTG from the sigma recognition site with an IC_{50} of 3.9 nM.

We have synthesized [^{11}C]L-687,384 with the label incorporated in the benzylic methylene from [α-^{11}C]benzyl iodide (see figure).



The synthesis of [^{11}C]L-687,384 required approximately 38 minutes from end of bombardment and involved five major steps: Grignard reagent carboxylation, hydride reduction, iodination, alkylation, and HPLC

purification. The radiochemical yield based on [α-^{11}C]benzyl iodide was approximately 10% while the overall radiochemical yield was approximately 5% based on the initial activity of [^{11}C]CO_2 delivered. The average specific activity was 2760 mCi/μmole, calculated at end-of-synthesis. Radiochemical purity was > 99 %. All samples were determined to be sterile and apyrogenic.

Preliminary *in vivo* studies in mice indicate that [^{11}C]L-687,384 binds selectively to the sigma recognition site. Subsequent studies in baboons indicate that this radiotracer is suitable for imaging the site with PET.

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1. Weissman AD, Casanova MF, Kleinman JE, London ED, DeSouza EB: Selective loss of cerebral cortical sigma but not PCP binding sites in schizophrenia. Biol Psychiatry 29: 41-54, 1991.
2. Walker JM, Bowen WD, Walker FO, Matsumoto RR, DeCosta B, Rice KC: Sigma Receptors Biology And Function. Pharmacol Rev 42: 355-402, 1990.
3. Thomas GE, Szucs M, Mamone JY, Bem WT, Rush MD, Johnson MD, Coscia CJ: Sigma and opioid receptors in human brain tumors. Life Sci 46: 1279-1286, 1990.

Paper G19

EVALUATION OF RADIOIODINATED DESMETHYL DIAZEPAM AS RADIOTRACER FOR IN VIVO QUANTITATIVE STUDIES OF BENZODIAZEPINE RECEPTORS.

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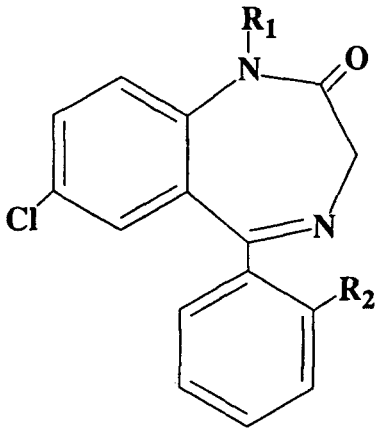
We recently developed 2'-iododiazepam (2'-IDZ), a diazepam derivative iodinated at the ortho position in the C-5-phenyl ring, as a radioligand for imaging benzodiazepine receptors in the brain (1). This iodination at the 2'-position generated an agent with high affinity for benzodiazepine receptors; however, the occurrence of various metabolites in the brain makes the quantitative study of in vivo receptor binding difficult. So, a metabolically stable benzodiazepine derivative is most desirable. We have focused our interest on the 2'-iodo-desmethyl diazepam (2'-IDMDZ), a derivative iodinated at the same 2'-IDZ position. In the present work, 2'-IDMDZ was synthesized, radioiodinated and its receptor binding affinity and in vivo biodistribution were studied.In the synthesis of 2'-IDMDZ, p-iodobenzonitrile was first reacted with p-chloroaniline; the produced aminobenzophenone was condensed with bromoacetyl chloride, followed by amination and cyclization.The 125-I labeled 2'-IDMDZ (125-I-2'-IDMDZ) was synthesized by exchange reaction of the brominated derivative with Na125-I (specific activity: 200 - 300 Ci/mmol).The affinity of 2'-IDMDZ for benzodiazepine receptor was measured by the competitive inhibition with 3-H-diazepam binding to rat cortical membranes. The 2'-IDMDZ showed an almost similar high affinity as the 2'-IDZ, approximately 3 times that of diazepam. In mice distribution studies, 125-I-2'-IDMDZ entered rapidly into the brain, reaching a peak at 5 min, and then cleared along with the time; at this maximum, the brain/blood ratio reached a value of 1.13. For the study of the in vivo stability of 125-I-2'-IDMDZ, the methanol extract of brain homogenate, at various post injection time, up to 60 min, were analyzed by HPLC. More than 90 % of the brain radioactivity was detected in the first 20 min, at the same retention time of the original 125-I-2'-IDMDZ;

during this interval, no dehalogenation was registered.Thus, the metabolically stable nature of this newly developed compound, 2'-IDMDZ, offers great potential for future SPECT quantification of benzodiazepine receptor in the brain.

1. H.Saji et al., J.Label.Compd.Radiopharm. 30, 238 (1991)



	R_1	R_2
Diazepam :	CH_3	H
2`-IDZ :	CH_3	I
nor-2`-IDZ :	H	I

Paper G20

PREPARATION OF NO-CARRIER ADDED META-IODOBENZYLGUANIDINE AND META-ASTATOBENZYLGUANIDINE

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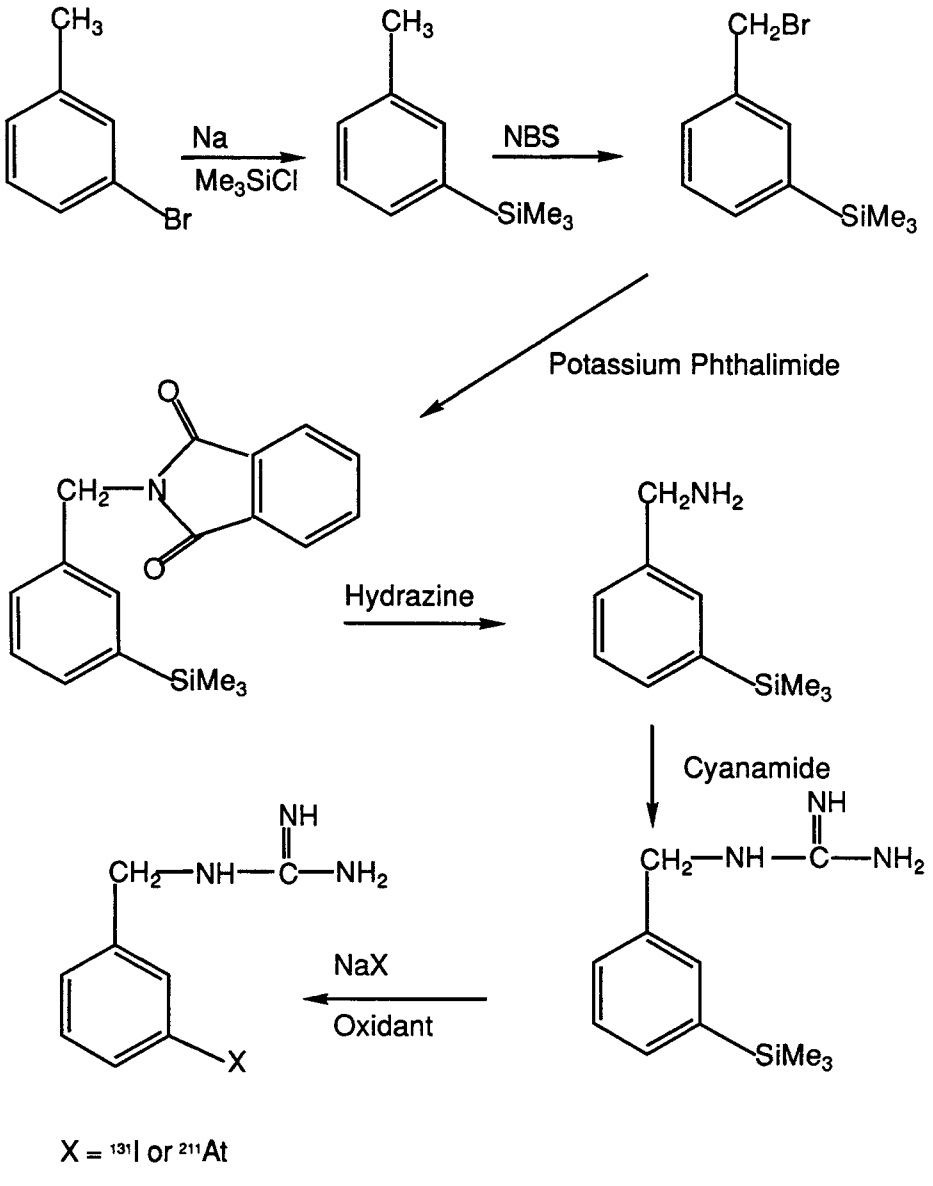
Meta-iodobenzylguanidine (MIBG) has an uptake and storage mechanism similar to that of norepinephrine. For this reason, *m*-[^{123},^{131}I]IBG has been used in the diagnosis and therapy of neuroendocrine tumors such as pheochromocytomas, neuroblastomas and carcinoids. Currently, MIBG is labeled by isotopic exchange, resulting in a maximum specific activity on the order of 100 Ci/mmole with ^{131}I (2). In vitro studies have shown that the cytotoxic effect of *m*-[^{125},^{131}]IBG on SKN-SH human neuroblastoma cells increased with increasing specific activity (1), suggesting that it might be possible to improve the therapeutic utility of MIBG if a no-carrier-added synthesis were available. Our goal was to develop a method for the no-carrier-added synthesis of MIBG that would also be suitable for producing the analog *m*-[^{211}At]astatobenzylguanidine (MABG). For therapeutic applications, ^{211}At is of interest because its alpha particles are radiation of high linear energy transfer, resulting in a higher relative biological effectiveness than the beta particles of ^{131}I.

A method was developed for the no-carrier-added labeling of MIBG and MABG via halodesilylation as illustrated in Scheme 1. The *m*-(trimethylsilyl)benzylguanidine radiohalogenation precursor was synthesized in 5 steps starting with *m*-bromotoluene. The reaction conditions were optimized using ^{131}I. The dependence of yield of *m*-[^{131}]IBG (determined by HPLC) on the oxidant, solvent, temperature and amount of substrate were studied. As shown in Figure 1, there was a marked effect of temperature on yield with *N*-chlorosuccinimide (NCS) as the oxidant, (69 ± 3.1% at 70°C *vs* 24.9 ± 2.3% at 20°C). The use of peracetic acid as the oxidant resulted in decreased yields compared to NCS. The yield after a 5 min reaction was not much different than that seen after 30 min (61.2 ± 8.4% *vs* 68.9 ± 3.1%; Figure 2). As illustrated in Figure 3, yield of *m*-[^{131}I]IBG increased with increasing amounts of trimethylsilyl precursor. Changing the solvent from acetic acid to trifluoroacetic acid (TFA) increased yields dramatically and minimized dependence on other parameters. Yields of 85-90% were obtained in TFA after only a 5 min reaction with 0.5 µmole of precursor at room temperature. Using similar conditions, yields of 85% were observed when pertrifluoroacetic acid was used as the oxidant. The best conditions for the synthesis of *m*-[^{211}At]ABG involved a 30 min reaction at 70°C with NCS and 2 µmole precursor, resulting in a yield of 60-80%; however, experiments are in progress to further optimize this reaction.

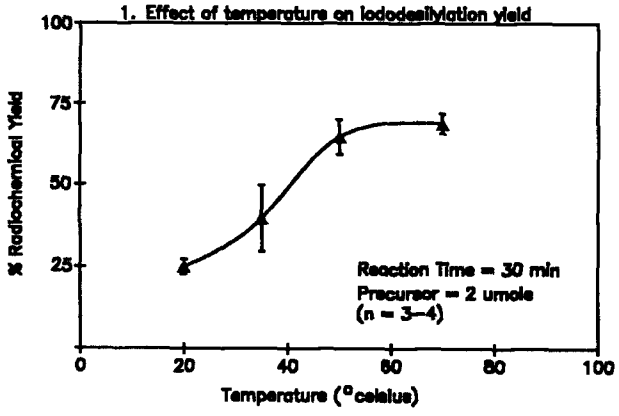
The *in vitro* binding properties of *m*-[^{131}I]IBG and *m*-[^{211}At]ABG were evaluated using SKN-SH human neuroblastoma cells with the SKN-MC line serving as a negative control. Using 4 x 10^5 cells, MIBG labeled using our new method generally maintained a constant level of binding over a 2-log activity range while the binding of MIBG labeled by isotopic exchange decreased by a factor of three-to five-fold. Binding of *m*-[^{211}At]ABG was similar to that observed for *m*-[^{131}I]IBG.

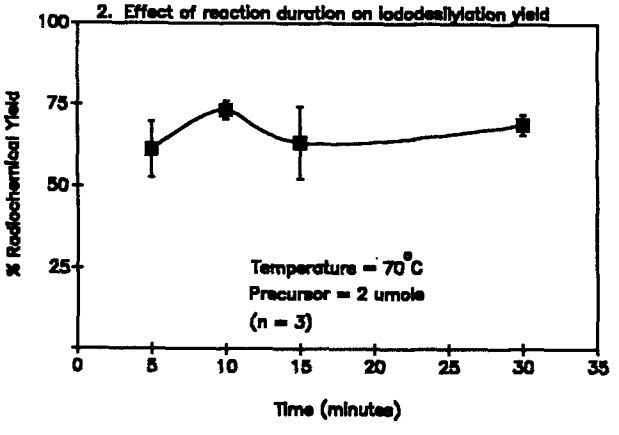
Tissue distribution studies were performed in normal mice to assess uptake in adrenals and heart, tissues expected to concentrate these norepinephrine analogs. At 1 hr, myocardial uptake was slightly higher for the no-carrier-added preparation (15.8 ± 1.8% ID/g) than that labeled by isotopic exhange (11.5 ± 1.9% ID/g) while the adrenal accumulation was 13.1 - 14.8% ID/g for both methods. Unexpectedly, uptake of *m*-[^{211}At]ABG in heart, 26.8 ± 4.0% ID/g, and adrenals, 20.2 ± 5.8% ID/g, was even higher suggesting that substitution of astatine for iodine may result in an even better norepinephrine uptake/storage analog.

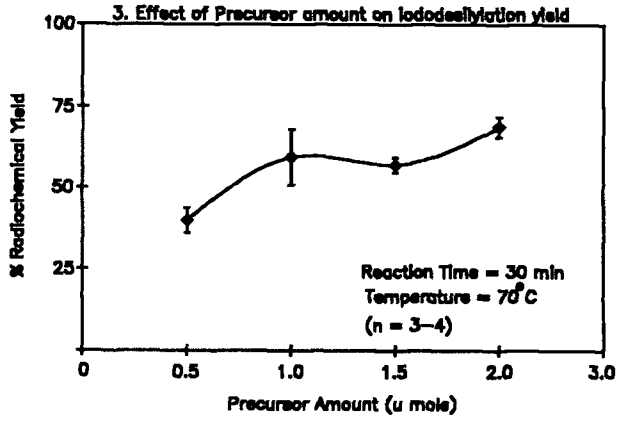
1. Bruchelt G., Girgert R., Buck J., et al. Cancer Res. 48, 2993-2997 (1988).
2. Mangner T.J., Wu J.-I. and Wieland D.M. J. Org. Chem. 47, 1484-1488 (1982).


CH3
Br
Na
Me3SiCl
CH3
SiMe3
NBS
CH2Br
SiMe3
Potassium Phthalimide
O
CH2—N
O
SiMe3
Hydrazine
CH2NH2
SiMe3
Cyanamide
NH
CH2—NH—C—NH2
SiMe3
NaX
Oxidant
NH
CH2—NH—C—NH2
X
X = 131I or 211At

Scheme-1


1. Effect of temperature on iododesilylation yield
% Radiochemical Yield
100
75
50
25
0
20
40
60
80
100
Temperature (°celsius)
Reaction Time = 30 min
Precursor = 2 umole
(n = 3–4)


2. Effect of reaction duration on iododesilylation yield
% Radiochemical Yield
100
75
50
25
0
5
10
15
20
25
30
35
Time (minutes)
Temperature = 70°C
Precursor = 2 umole
(n = 3)


3. Effect of Precursor amount on iododesilylation yield
% Radiochemical Yield
100
75
50
25
0
0.5
1.0
1.5
2.0
3.0
Precursor Amount (u mole)
Reaction Time = 30 min
Temperature = 70°C
(n = 3–4)

Paper G21

SYNTHESIS OF A NEW RADIOLIGAND FOR EXPLORATION OF 5-HT RE-UPTAKE SITES : 4'-[125I]-IODO-5-METHOXYVALEROPHENONE O-(2-AMINOETHYL) OXIME.

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To explore 5-HT re-uptake, we synthesized a radioiodinated analog of fluvoxamine : 4'-iodo-5-methoxy valerophenone 0-(2-aminoethyl) oxime (4a). Affinity for 5-HT re-uptake was studied on human platelets, a wellknown model of serotoninergic transporter.

Synthesis, purification and characterization (schemes 1 and 2)

The iodinated ligand (4a) was synthesized in two steps from 4-iodobenzonitrile (1) according to a method described for 4'-bromo-5-methoxy valerophenone 0-(2aminoethyl) oxime (1). Its radioiodinated analog (4b) was prepared in two steps from 4'-bromo-5-methoxyvalerophenone (3). Iodide for bromide nucleophilic exchange to obtain 4'-[^{125}I]-iodo-5-methoxy valerophenone (2b) from 4-bromophenylketone (3) was realized by a procedure described by Mertens (2). The radiochemical yield of radiotracer (4b) was 50 %. The optimization of radiolabeling will be performed. Reaction products were purified with flash chromatography, TLC and HPLC methods. These compounds were identified with proton NMR, IR spectroscopy and mass spectrometry.

Biological experiments

Biological properties of (4a) was studied on human platelets by incubation with [^{3}H]-5-HT without inhibitor or with fluvoxamine (inhibitor reference compound). The 50% inhibition of [^{3}H]-5-HT uptake was obtained for a 10^{-8}M concentration for the fluvoxamine analog (4a).

Conclusion

We demonstrated that synthesis of 4'-iodo-5-methoxy valerophenone 0-(2-aminoethyl) oxime (4a) and its [^{125}I]radioiodinated analog (4b) could be realized by convenient pathways.

Preliminary biological experiments with iodinated ligand (4a) showed expected affinity for serotoninergic transporters. Biological studies of radioligand (4b) is in progress on animal models.

References

1 - N.V. Philips'glooeilampenlabrieken, French Pat, 76 08017 (March, 19, 1976).

2 - J. Mertens, W. Vanryckeghem, M. Gysemans, J. Eersels, E. Finda-Panek, L. Carlsen, *Eur. J. Nucl. Med.*, **13**, 380 (1987).

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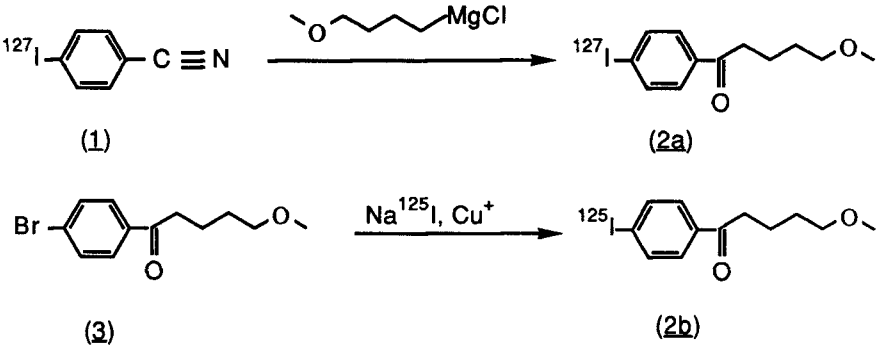
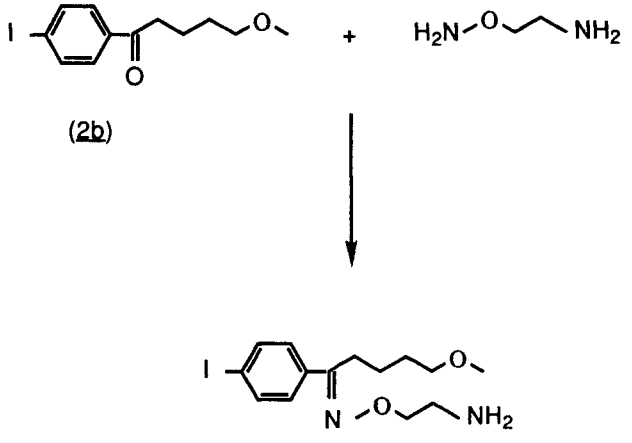
The authors are grateful to Duphar's help.

Scheme 1 : Preparation of 4'-Iodo-5-methoxy valerophenone (2a) and its radioiodinated analog (2b)



Scheme 2 : Synthesis of 4'-iodo-5-methoxy valerophenone O-(2-aminoethyl)oxime (4a) and its radioiodinated analog (4b)



Paper G22

STUDIES ON RADIOIODINATION OF MIBG IN AQUEOUS AND SOLID PHASES.

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The radioiodination of mIBG with iodine-131 for diagnostic purposes was studied both in aqueous and solid phases.
The radiochemical yields were determined by TLC and HPLC analysis of the final products (see fig. 1).

The radioiodination of mIBG in aqueous solution is usually performed using copper cations as catalysts [1-3]. With these catalysts a rather low yield was obtained,this is due to the formation of different labelled intermediary products. Other metallic cations were also studied as possible catalysts of the exchange reaction.
Palladium chloride was found to be more effective than copper salts. In order to determine the optimal labelling conditions with this catalyst, various parameters affecting the radiochemical yields were determined (see table1).
Among the factors considered, pH and temperature were found to have more profound effect on the reaction.
The exchange rate increases with temperature and becomes stable at 80°C.
Better yields were obtained at pH 5 or less and for concentration of $PdCl_2$ 10 µmole/ml.
The exchange occurs only when $PdCl_2$ is added before $Na^{131}I$ to mIBG solution. $PdCl_2$ forms probably with mIBG an organo-complexe which facilitates the exchange.
Hydrolysis of $PdCl_2$ occurs at high pH and temperature and the formed products have not catalytic effect.
The labelling efficiency is more than 90 %, however $PdCl_2$ presents high toxicity and the insoluble palladium iodide formed during the reaction must be separated.

In solid state, we radioiodinated the mIBG according to a modified method described in [4].
The exchange in ammonium sulfate was applied, however higher temperatures and an inert atmosphere (nitrogen gas) were used. In optimal conditions, the radiochemical yields were always more than 95% (see table 2).
The maximal amount of ammonium sulfate needed for the reaction is 5 mg/mg mIBG. At higher amounts, the yield decreases. This is probably due to a dilution effect.
Better yields were obtained at temperatures higher than 170°C (melting point of mIBG). The exchange occurs in acidic conditions created by the in-situ decomposition of ammonium sulfate but not necessary in oxydant environment (use of an inert atmosphere).

REFERENCES :

1 - Mertens J.J.R., Vanrucheghem W. and Carlsen L. - J. Nucl. Med. 26 : 123 (1985).
2 - Mertens J.J.R. et al, - J. Lab. Comp. Radioph. XXII, 1 : 89 (1984)
3 - Mertens J.J.R. et al, - IAEA-CN-45/1 : 303 (1984).
4 - Mangner T.J., Wu T.L. and Wieland D.M. - J. Org. Chem. 47 : 1484 (1982).

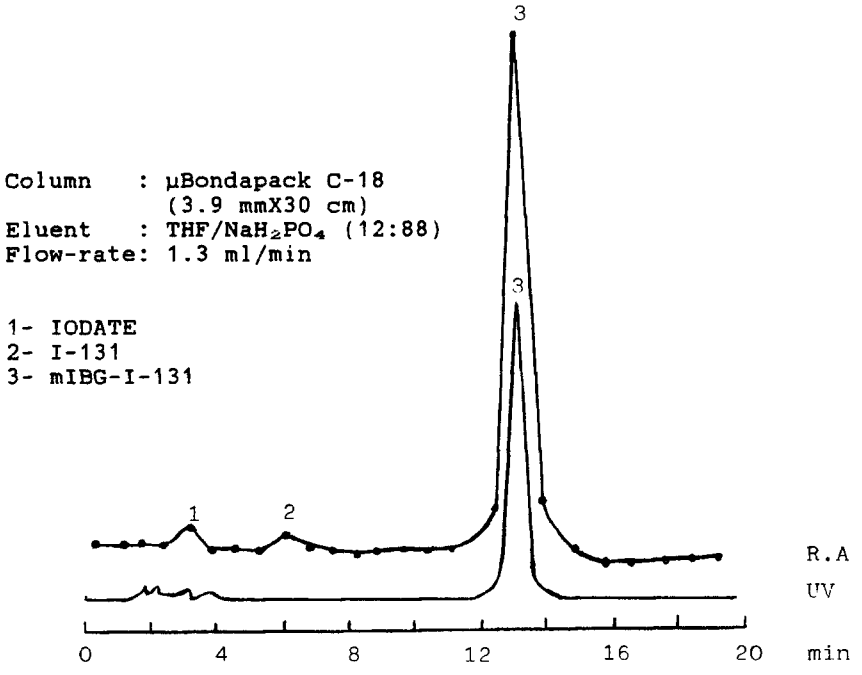

Fig.1. HPLC Analysis after exchange radioiodination.

Table I. Exchange radioiodination of mIBG in aqueous solution (OHAc) catalyzed by $PdCl_2$.

N°	Amount of $PdCl_2$ (µmole/ml)	pH	Temperature (°C)	Time (min)	Radiochemical yield (%)
1	00	4,2	80	30	11,43
2	10	4,2	80	30	98,97
3	10	5,0	80	30	96,03
4	10	4,2	80	15	87,62
5	10	3,7	105	60	79,31

Table II. Exchange radioiodination of mIBG in solid phase (Ammonium sulfate).

N°	Amount of $(NH_4)_2SO_4$ (mg/mg mIBG)	Temperature (°C)	Radiochemical yield (%)
1	00	180	91,63
2	5	200	99,49
3	5	180	98,58
4	15	180	85,01
5	5	130	31,19

Paper G23

Radiosynthesis of [O-^{11}C-methyl]CP-96,345, a Nonpeptide Substance P (NK_1) Receptor Antagonist.

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CEA, DSV/DPTE Cyceron and INSERM U.320, 14021 Caen ; Unité de Recherche Associée au CNRS, Institut des Sciences de la Matière et du Rayonnement, 14050 Caen ; Laboratoire de Pharmacologie, CHU, 14033 Caen, France

Substance P (SP), an undecapeptide of the family of the structurally related peptides tachykinins, is widely distributed in the central nervous system, where it presumably has a role as a neurotransmitter-neuromodulator interacting with others neurotransmitters (1). A loss in substance P receptors has been reported in neurodegenerative diseases (2). Peptidic fragments of SP have been labelled with carbon 11 in order to study the functional role of SP *in vivo* by positron emission tomography (PET) (3). If these are known to retain the biological activity of SP, their ability to cross the blood brain barrier has not been reported. CP-96,345[cis-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine]**1**,a non peptide SP antagonist is highly selective for the tachykinin NK_1 receptors (1). [^{3}H]-CP-96,345 binds to guinea pig striatal membrane homogenates with K_D value of 0.22 nM and an IC_{50} for SP of 90 nM : successful *in vitro* autoradiography has been reported with this compound on guinea pig brain slices (4); the labelling of CP-96,345 with a positron emitter (carbon-11) would allow the invesgation *in vivo* by PET of the brain NK_1 receptors in physiological and disease states in humans.

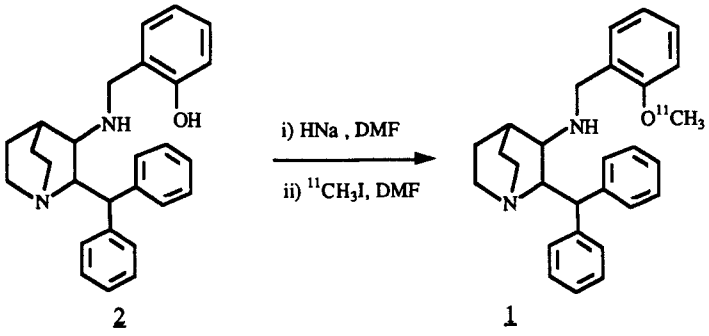
The alkylation of appropriate desalkyl compounds with an alkyl halide is an useful method for synthesizing ligands labelled with ^{11}C (5). The presence of two nucleophilic centres in the same molecule usually requires the selective protection of one function (6). With the aim of a simple and rapid synthesis, we have studied the direct [^{11}C] methylation of desmethyl CP-96,345 **2** in different conditions using [^{11}C] iodomethane prepared as previously described (7). The best results were obtained when the reaction was carried out on the sodium salt of **2** in dimethylformamide (DMF) at 80°C for 10 min. Typically, the precursor **2** (2 mg, free base) in DMF (300 µl) was added to a suspension of sodium hydride (5 mg) in DMF (100 µl). The mixture was stirred at room temperature for 5 min then a solution of [^{11}C] iodomethane in DMF (200 µl) was added. After reaction, the crude product was quickly prepurified by passing through a C_{18} Sep-pak. The expected compound **1** (90 % pure) eluted with 1.5 mL of acetonitrile, was then injected onto a reverse phase HPLC column (eluent : CH_3CN / phosphate buffer, pH 4, v/v, 60/40) and isolated in 20-30 % yield (from $^{11}CH_3I$, 40 min EOB,98 % pure). The structure of **1** was based on its acido-basic properties and the comparison of its R_f in radio-TLC and retention time in HPLC with those of an authentic sample. Work is now in progress to label the (2S, 3S) CP-96,345 which is the most potent enantiomer (1).

Acknowledgments :The authors are grateful to Dr. Lowe III J.A. for useful discussion and provision of O-desmethyl CP 96,345 and CP 96,345.

References

1. Snider R.M., Constantine J.W., Lowe III J.A., Longo K.P., Lebel W.S., Woody H.A., Drozda S.E., Desai M.C., Vinick F.J., Spencer R.W., Hess H.J. - *Sciences*, 251 : 435 (1991).
2. Tenovuo O., Kohlinen O., Laihinen A., and Rinne U.K. - *Adv Neurol.* 53 : 145 (1990).
3. Franzén H.M., Ragnarsson Ulf, Nagren K., Langström B. -*J. Chem. Soc. Perkin Trans.* 1, 2241 (1987).
4. McLean S., Ganong A.H., Speeger T.F., Bryce D.K., Pratt K.G., Reynolds L.S., Siok C.J. , Lowe III J.A., Heym. J. -*Sciences*, 251 : 437 (1991).
5. Fowler J.S., and Wolf A.P. - "Positron Emission Tomography and Autoradiography" Phelps M., Mazziotta J. and Schelbert H., Raven Presse, N.Y. (1986).
6. Pascali C. ,Turton D.R., Hume S.P., and Pike V. W. -*J. Labelled Compd. Radiopharm*, 30 : 390 (1991).
7. Crouzel C., Langström B., Pike V.W. and Coenen H. -*Appl. Radioat. Isot.*, 38 : 601 (1987).

Scheme 1 : Preparation of [O-^{11}C-methyl] CP 96-345



2 1

Paper G24

SYNTHESIS OF A NEW ANALOG OF PCP : [^{18}F]-3-FLUOROMETHYL-TCP, A POTENT LIGAND FOR THE NMDA GLUTAMATERGIC RECEPTOR.

*PONCHANT.M, *CROUZEL.C, **KAMENKA. J.M and ***PAPPATA.S.
* Service Hospitalier Frédéric-Joliot,DRIPP, DSV, CEA, 91406 ORSAY (FRANCE).
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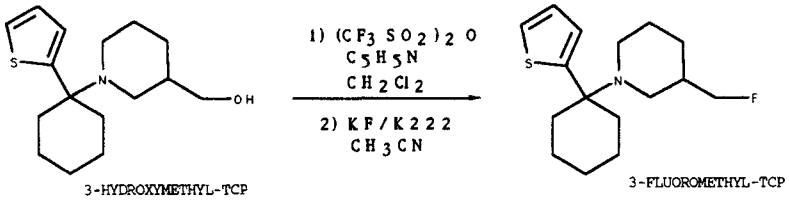
*** Service Hospitalier Frédéric-Joliot,INSERM U 334, 91406 ORSAY (FRANCE).

As previous attempts with [^{18}F]- MK 801 PET studies have not been successful (Denis et al.,1989) and as N-[1-(2-thienyl)cyclohexyl]-piperidine (TCP) is now a well known analog of phencyclidine (PCP) to specialy bind the NMDA gated ionic channel, cold and [^{18}F] analogs have been developped in order to visualize the NMDA receptors by positon emission tomography (PET).
Starting material, 3-hydroxymethyl-N-[1-(2-thienyl)cyclohexyl]-piperidine (3-hydroxymethyl-TCP) was selectionned as a good chemical precursor to obtain 3-fluoro-TCP via the triflate pathway; two reasons for this choice:

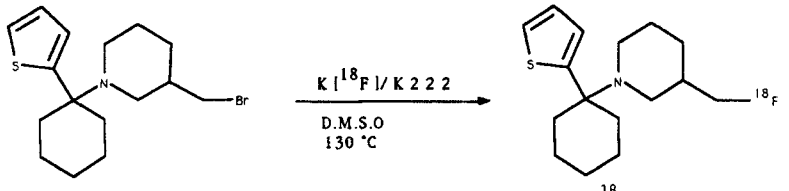
- elimination product was obtained by triflate pathway when the hydroxy group was directly bind to the piperidine ring (Maeda et al., 1991).
- to avoid the blockage of the TCP analog metabolism we have choosen the 3-position on the piperidine ring.

Cold fluoro compound was obtained in two steps via the triflate pathway with a 50% yield.



3-HYDROXYMETHYL-TCP 3-FLUOROMETHYL-TCP

After two unsuccessful attempts to obtain [^{18}F]-3-fluoromethyl-TCP via the triflate pathway, radiosynthesis was carried out by nucleophilic substitution using [^{18}F$^-$] produced by a (p.n) reaction with 16 Mev protons on 50% [^{18}O] enriched water.



3-BROMOMETHYL-TCP [^{18}F]-3-FLUOROMETHYL-TCP

[^{18}F]-3-fluoromethyl-TCP was usefuly synthesized by direct exchange from 3-bromomethyl-TCP with K[^{18}F] /K 222 in DMSO at 130°C for 15 min after parameter studies (solvent, temperature,time).
12 mCi (about 10% yield, decay uncorrected) with a specific radioactivity of 728 mCi/µmol was obtained after filtration on silica Sep-Pack cartridge and two HPLC purifications on reverse phase column (µ-Bondapack C 18 using ethanol:70%, water:30%, triethylamine:0.05% as eluant).

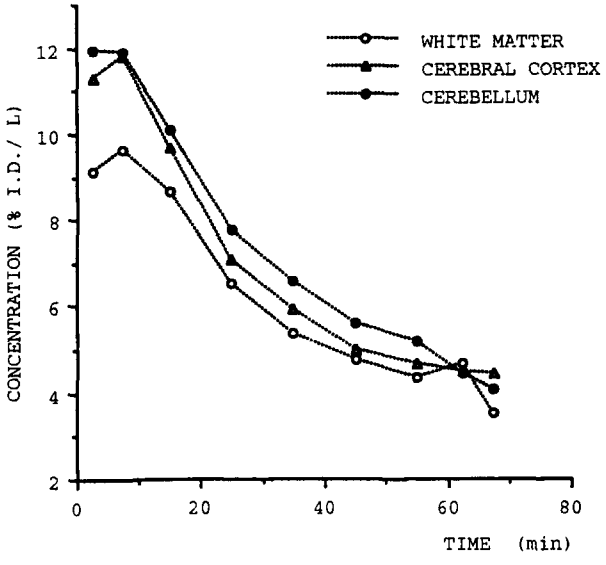
In preliminary PET studies on baboon brain, [^{18}F]-3-fluoromethyl-TCP readily cross the blood brain barrier, however, the regional distribution observed in vivo did not match the in vitro distribution of TCP binding sites.

TABLE OF PARAMETER STUDIES FOR RADIOLABELLING OF 3-BROMO-TCP

TCP-CH_2-Br mg	K 222 mg	K_2CO_3 mg	Solvent	Time min	Temperature °C	*Yield %
base 3.68	14.7	3	CH_3CN	10	85	2.6
base 4.61	15.3	3.2	DMSO	15	125	30.8
base 5.92	15.2	3	DMSO	10	120	12.85
HCl 4.8	14.6	2.5	DMSO	10	120	-

* yield corrected

[^{18}F]-3-FLUOROMETHYL-TCP UPTAKE ON BABOON BRAIN : REGIONAL DISTRIBUTION



Denis, A., Crouzel, C.: Synthesis and labelling with [^{18}F] of an MK 801 analog : [^{18}F]-5-(b-fluoroethyl)-10,11-dihydro-5H-dibenzocycloheptene-5,10-imine. J. Label. Comp. radiopharm. 27: 1007-1013, 1989.

Maeda, M., Tsukiyama, S., Fukumara, T., Orita, K. and Kojima, M. : Positon labeled analogs of TCP: Synthesis of 1-[4-[^{18}F]-fluoromethyl-1-(2-thienyl)cyclohexyl]piperidine. Appl. Radiat. Isot. 42: 563-570, 1991.