# ENDOTHELIAL PERMEABILITY AND UPTAKE OF C-14-CHOLESTEROL, I-125-LABELED HIGH DENSITY LIPOPROTEINS AND I-131-LOW DENSITY LIPOPROTEINS IN AORTOCORONARY FEMORAL VEIN BYPASS GRAFT IN DOGS

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The purpose of this investigation was to evaluate the permeability of vein grafts with low molecular weight tracer cholesterol and high molecular weight tracer particles: low and high density lipoproteins. Scanning electron micrograph of vein graft indicates that due to surgical trauma, endothelial cells are lost from intima, thus graft permeability increases. At about one month graft intima re-endothelializes (1-8).

Thirty-eight dogs in eight groups underwent aortocoronary femoral vein bypass graft surgery (Figure 1). High, very low density and low density lipoproteins were separated from dog blood in 10% EDTA by KBr gradient flotation technique, 1.006, 1.063 and 1.210 gm/ml, respectively by centrifugation at 300,000 g for 20 hours each at 8°C (Beckman L8-55) and labeled with I-131 and I-125 radionuclides by the iodogen technique (9). 100 µg of iodogen dissolved in 100 µl of methylene chloride were added to a polypropylene tube, and the solvent was evaporated to dryness. 2 mg of purified low density or high density canine lipoproteins were added in 0.2 ml of 0.1 M phosphate buffer to the iodogen tube; reductant-free I-125 or I-131 iodide was added and incubated for 30 minutes at room temperature. Free iodide was separated from labeled lipoproteins by Sephadex G-25 column. Labeling efficiency of 50-70% was obtained for both lipoproteins; higher labeling (twofold) was obtained with iodogen than iodine monochloride method. 80 µCi of C-14-cholesterol and 100-150 µCi of I-131-iodomethyl norcholesterol were administered to 19 dogs; and all dogs were sacrificed 24 hours after injection and 1, 7, 30, and 90 days after aortocoronary femoral vein bypass graft surgery. After sacrificing the dogs, grafted veins and contralateral veins were harvested. Five sections of graft (proximal, distal anastomoses, and three midsections) and two sections of normal vessels were made. Beta and gamma radioactivity in grafted femoral vein, unoperated control femoral vein and blood was determined with a beta and gamma counter, respectively. The mean radioactivity ratios of grafted femoral vein/control femoral vein in dogs were tabulated as shown in Figure 2. Distribution of labeled cholesterol and lipoproteins was similar in all sections of the same graft. Scanning electron micrograph of grafted canine vein (magnified 300 times) at 7 (A) and 30 (B) days post-bypass grafting is shown in Figure 3.

Increased uptake of cholesterol in grafted femoral vein with respect to control femoral vein in the acute phase represents higher permeability due to graft de-endothelialization. Increased uptake of LDL and HDL 30 days after aortocoronary femoral vein bypass graft surgery might suggest higher uptake of lipoproteins by higher affinity and concentration of receptors on the regenerated endothelial cells of grafted femoral vein and higher uptake by newly-proliferated smooth muscle cells in media of vein graft.

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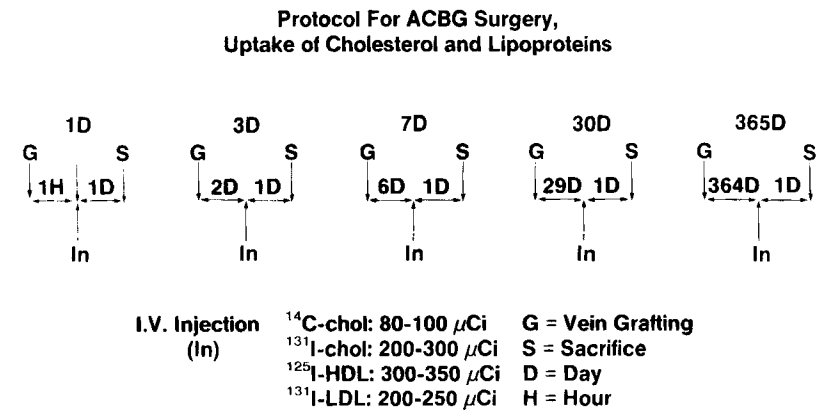


Fig. 1. Protocol for aortocoronary bypass surgery in dogs, injection of tracers and sacrifice for harvesting of vein and vein graft.

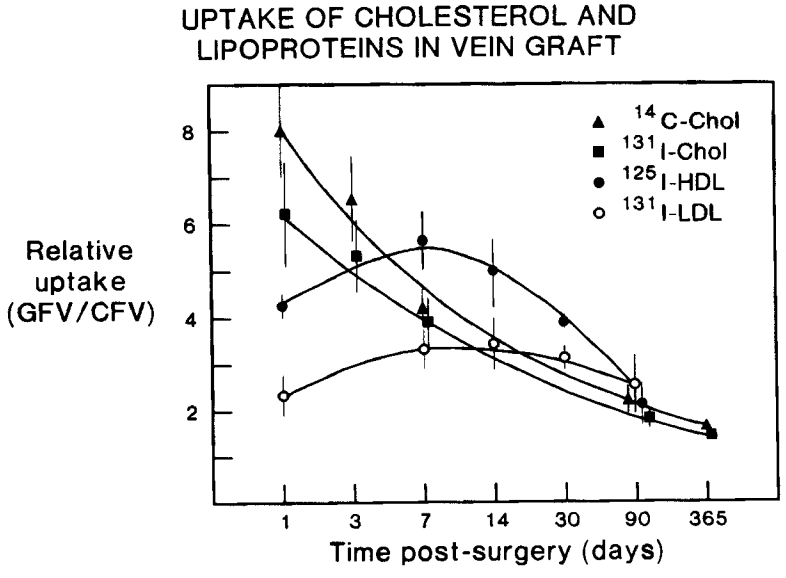


Fig. 2. Relative uptake of C-14- and I-131-cholesterol and I-125-high density and I-131-low density lipoprotein in grafted vein with respect to control unoperated vein at different times post-aortocoronary bypass graft surgery in dogs.

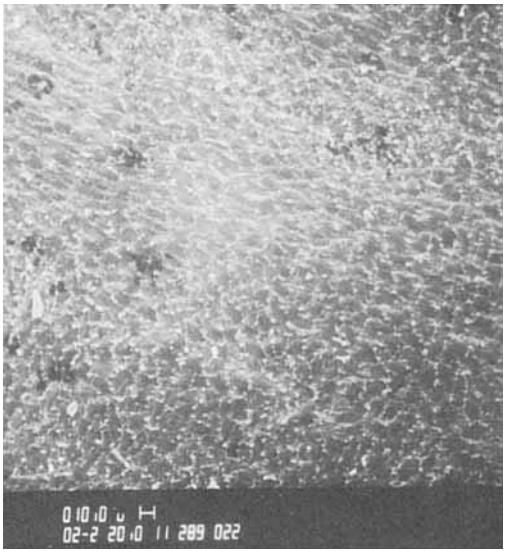

Fig. 3A.

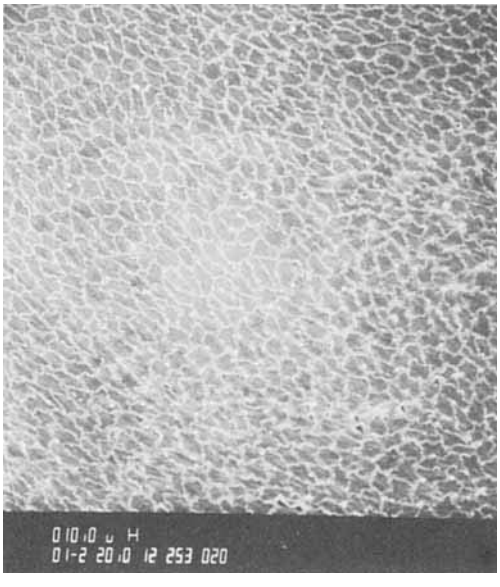

Fig. 3B.

Fig. 3. Scanning electron micrograph of grafted canine vein (magnified 300 times) at 7 (A) and 30 (B) days post-bypass grafting is shown. Note in Fig. 3A area of endothelial cell loss in SEM.

## DEVELOPMENT AND USE OF A MODEL SYSTEM TO EVALUATE METHODS FOR RADIOLABELING MONOCLONAL ANTIBODIES

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Recently there have been many reports describing the use of radiolabeled monoclonal antibodies for use in imaging studies. Most of these, however, have been done using radioisotopes of iodine even though the decay characteristics of the readily available nuclides (I-125 and I-131) are not ideal for use in imaging studies. In addition, rapid metabolism of the iodinated antibody leads to an increased uptake of radioactive iodine in the thyroid and other organs. Besides leading to an increased radiation dose to these organs this increased uptake also interferes with obtaining good images.

There have been several recent reports describing the use of bifunctional chelates to radiolabel monoclonal antibodies (1,2). Each of the reports has described conditions for radiolabeling different monoclonal antibodies but there has been no complete study comparing the various methods using the same antibody. Furthermore, some of the reports describe various systems which require the growth of tumors in animals. Because of the difficulties involved in using these systems for comparison studies we have developed a model system in which we can easily evaluate the different labeling methods for their efficiency in producing radiolabeled monoclonal antibodies that still retain their capacity to bind in vitro and in vivo.

In our system dinitrophenyl (DNP) is used as a model antigen, HDP-1 as a monoclonal antibody specific for DNP and Mopc-21 as a monoclonal antibody that has no specificity for this antigen. HDP-1 has been radiolabeled directly with I-125 or I-131 using the Iodogen method (3) or indirectly with In-111 via Deferoxamine (4), Diethylenetriamine pentaacetic acid (DTPA) (5) or 1-(para-Bromoacetomidobenzyl)-Ethylenediamine tetraacetic acid (BrEDTA) (6). We have evaluated the effects radiolabeling has on the ability of the antibody to bind in vitro using affinity chromatography. Samples of iodinated or In-111 labeled HDP-1 were placed in Hepes buffered saline or in plasma and then incubated at 37°C. Aliquots were removed at 0, 1, 4, 24 and 48 hours and then tested for their ability to bind to the affinity resin. All labeling methods resulted in labeled antibody that initially retained greater than 80% of its capacity to bind to the affinity resin. When DTPA was used in a 500:1 molar excess or Deferoxamine was used in a 20:1 molar excess (as compared to the amount of HDP-1 used) the amount of binding greatly decreased. The most stable molecules appear to be those labeled with I-125 or those labeled with In-111 via DTPA or BrEDTA.

The binding capacity of labeled monoclonal antibodies has also been tested in vivo. Agarose beads coupled with DNP were injected into the femoral veins of 250-300 gm rats. Because of the size of the beads (80-150$\mu$) they rapidly localized in the capillary beds of the lungs. Radiolabeled monoclonal antibody was injected 1-2 minutes after injection of the beads. Samples of blood, liver, lung , kidney, spleen and muscle tissue were removed from animals sacrificed 1, 4, 24 or 48 hours after injection of the antibody and counted in a gamma counter. For the development of the in vivo model the binding of (I-125)HDP-1 and (I-131)Mopc-21 antibodies were tested. (I-125)HDP-1 was observed to localize in the lungs while (I-131)Mopc-21 showed no specific uptake in the body. Maximum %ID/gm levels for HDP-1 in the lungs were seen at 24 hours after injection of the antibody after which they slowly declined. No such fluctuations were observed for Mopc-21.

The results obtained for HDP-1 labeled with In-111 using the bifunctional chelates have been compared with those obtained for the iodinated antibody. Preliminary studies indicate that HDP-1 labeled using DTPA localizes in the lungs almost as well as does

(I-125)HDP-1. Localization is also observed with HDP-1 labeled using BrEDTA but the %ID/gm levels are not as high. In both cases, however, there are large amounts of label in the liver throughout the course of the experiment. These levels did not decrease with time, a result quite opposite that observed when (I-125)HDP-1 was tested.

Experiments evaluating the use of several other bifunctional chelates for their ability to radiolabel monoclonal antibodies are currently in progress. Ultimately, we hope to determine which method is the most efficient in producing radioactive antibodies that have a high specific activity and yet still retain their ability to bind in vivo.

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## SYNTHESIS AND BIOLOGICAL DISTRIBUTION OF RADIOLABELLED 1-(2'-FLUORO-2'-DEOXY-β-D-RIBOFURANOSYL)-5-HALOURACILS

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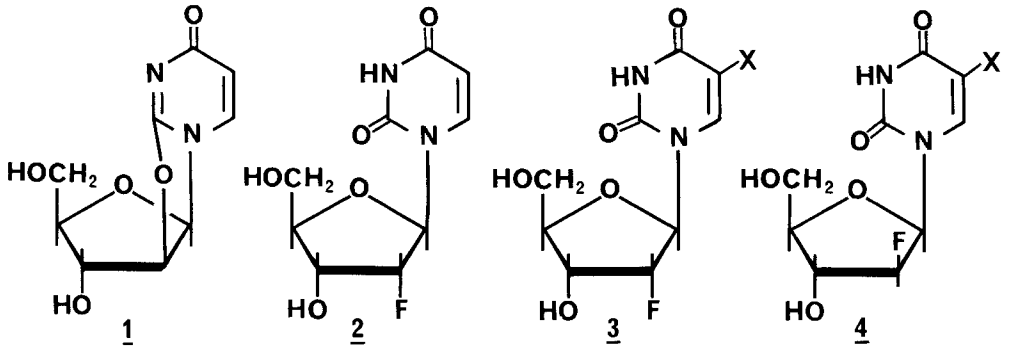
A number of recent studies have demonstrated the antiviral and antitumor effects of synthetic 2'-fluoro-2'-deoxy pyrimidine nucleosides having various halogen substituents at the C-5 position of the pyrimidine ring. The arabino nucleosides 4 (X=I,Br,Cl,F) have been reported to inhibit viral replication significantly and exhibit in vitro toxicity against mouse leukemic cell lines (1). The ribo nucleosides 3 (X=I,Br) also showed pronounced antiviral activity and cytotoxicity toward human lymphocytic cells in culture (2). This evidence of biological activity coupled with the potential of these compounds, as $^{18}F$ labelled analogs, to act as in vivo indicators of tissue proliferation prompted us to synthesise a series of compounds 3 (X=$^{131}I$,$^{82}Br$) and 3a (X=Cl,F). The tissue distribution of these compounds was examined in $BDF_1$ mice bearing a subcutaneous Lewis Lung tumor.




Reaction of 2 with Na$^{131}$I in dilute $HNO_3$ at 115°C for 1 h gave 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-5[$^{131}$I]-iodouracil 3 (X=$^{131}$I) in 90% radiochemical yield with a specific activity of 61 GBq/mmol (3). A similar no-carrier-added synthesis gave 3 (X=$^{131}$I) in 41% radiochemical yield after purification.

The reaction of 2 with reactor produced $NH_4{}^{82}Br$, in the presence of N-chlorosuccinimide, yielded 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-5-[$^{82}$Br]-bromouracil 3 (X=$^{82}$Br) in 75% radiochemical yield with a specific activity of 0.26 GBq/mmol.

The compounds [2-$^{14}$C]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-5-chlorouracil 3a (X=Cl) and the 5-fluoro analog 3a (X=F) were prepared from the 2,2'-anhydronucleoside 1a in two steps. Treatment of 1a with HF in dioxane yielded 2a in 40% yield. Reaction of 2a with a solution of the electrophilic $Cl_2$ or $F_2$ in acetic acid gave the required dihalo compounds 3a (X=Cl) and 3a (X=F) respectively in quantitative yield.

Compounds 3 (X=$^{131}$I,$^{82}$Br) showed rapid excretion in in vivo experiments and pronounced dehalogenation of the 5-substituent. Compounds 3a (X=Cl,F) exhibited significant tumor to blood ratios reaching a maximum of 2.5 to 1 for 3a (X=Cl) at four hours and a maximum of 7 to 1 for 3a (X=F) at two hours.

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# ELECTROPHILIC DESTANNYLATION: A VERSATILE AND EFFICIENT METHOD FOR RADIOHALOGENATION

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As part of our program to design and develop radiohalogenated radiopharmaceuticals, we have explored new methods for introducing the radionuclide into unactivated sites in organic compounds. We chose to evaluate the applicability of the trialkylstannyl moiety as a site-directing agent as opposed to boronic or silyl groups (1-3). Our rationale was based upon the well demonstrated synthetic versatility of the organotin reagents (4) and their ability to undergo facile electrophilic destannylation with retention of configuration (5).

During the past two years we have synthesized a variety of functionalized trialkylstannylvinyl and -aryl compounds utilizing the appropriate bis(trialkylstannyl) ethene or arene as the starting material. These compounds, which are chemically stable and readily characterized, were isolated in good yields (30-90%) using preparative HPLC. Radiohalogenation of the trialkylstannyl compound gave isolated radiochemical yields of 85-96% (Table 1). The yields were unaffected by using no-carrier-added I-123 or I-131 or by lowering the trialkylstannyl reagent: radionuclide ratio from 100-300:1 down to 10-20:1. The use of HPLC to analyze and purify the reaction mixture resulted in overall synthesis and purification times of 20-90 minutes. Generally a single pass through the column was sufficient for complete separation of the product from contaminants.

The results illustrate the versatility and efficiency of this method and suggest that it may be the preferred method for preparing a variety of aryl and vinyl radiohalides.

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Iodination of Trialkylstannylvinyl and -Aryl Substrates

| Trialkylstannyl Substrate | nmol Substrate | Substrate: I-125 | Yield (% Isolated) |
|---|---|---|---|
| Tamoxifen | 227 | 97 | 91.5 |
| 1-Thienylcyclo pentan-1-ol | 701 | 300 | 91.4 |
| 1-Thienylcyclo hexan-1-ol | 765 | 325 | 90.5 |
| 17α-Thienylestradiol | 640 | 274 | 95.7 |
| 17α-Thienylestradiol 3-O-Methyl Ether | 410 | 175 | 95.9 |
| 1-Vinylbutan-2-ol | 445 | 190 | 96.1 |
| 1-Vinylcyclohexan-1-ol | 765 | 325 | 90.5 |
| 17α-Vinylestradiol | 150-300 | 57-114 | 87 |
| 17α-Vinylestradiol 3-O-Methyl Ether | 320 | 140 | 94.6 |
| 17α-Vinyl-11ß-Methoxy Estradiol | 300 | 125 | 94.9 |
| 17α-Vinyl-11ß-Methoxy Estradiol 3-O-Methyl Ether | | | 53* |

*Substrate Contained Stannylated Impurity

## RADIOHALOGENATED (*E*)-5-(2-HALOVINYL)-2'-DEOXYURIDINES

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(*E*)-5-(2-Iodovinyl)-2'-deoxyuridine (IVDU, 1) and (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU, 2) are among the most potent compounds active against herpes simplex type-1 (HSV-1) (1). Their selective antiviral action is attributed to the preferential phosphorylation by HSV-1 encoded thymidine kinase (TK) (2). Therefore these compounds are expected to selectively localize in HSV-1 infected tissue. This selective uptake could be used as a basis for the development of radiohalogenated IVDU and BVDU as potential non-invasive probes for the diagnosis of herpes encephalitis.

[$^{125}$I, $^{131}$I) - IVDU (4) and [$^{82}$Br] - BVDU (5) were synthesized by the reaction of (*E*)-5-(2-carboxyvinyl-2'-deoxyuridine (1) with radiolabelled iodide or bromide in the presence of chloramine-T (routes C and D, Fig. 1). A "no carrier added" synthesis of [$^{131}$I] - IVDU was completed within 30 min. in 65% radiochemical yield (Table 1). The short reaction time makes it a useful method for the incorporation of the single photon emitter $^{123}$I ($t_{\frac{1}{2}}$ 13.26 h) into IVDU. Alternatively, radioactive iodine was incorporated into IVDU using a halogen isotope exchange reaction catalyzed by cuprous ion. The radiobromination of 1 by the chloramine T method (route D) was completed in < 10 min. (Table 2) and is suitable for the synthesis of BVDU labelled with the positron emitter $^{75}$Br ($t_{\frac{1}{2}}$, 95.5 min.). [$^{82}$Br] - BVDU was also prepared by direct neutron activation of unlabelled BVDU (route F).

The *in vitro* uptake studies showed that [$^{131}$I] - IVDU was selectively localized in HSV-1 (TK +ve) infected primary rabbit kidney (PRK) cells. No significant uptake was observed in HSV-1 (TK -ve) infected and mock infected PRK cell controls (Fig. 2). The brain imaging studies were carried out with the HSV-1 infected and uninfected rabbit models using [$^{131}$I] - IVDU. The radioactivity observed in the HSV-1 infected brain was not significantly higher than in the uninfected control. Preliminary tissue distribution studies showed that the concentration of the radiolabel in the blood was much higher than in the brain during a 6 h period (Table 3). However the total activity of the radiotracer in the HSV-1 infected brain was 3-8 times higher than in the uninfected brain 1 h after injection.

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TABLE I RADIOCHEMICAL SYNTHESIS OF RADIOIODINATED IVDU

| METHOD | SPECFIC ACTIVITY | TEMPERATURE (°C) | TIME | RADIO-CHEMICAL YIELD (%) | CHEMICAL YIELD, RECOVERY (%) |
|---|---|---|---|---|---|
| CHLORAMINE-T | 343.5 GBq $mmol^{-1}$ | 25 | 8 hrs | 67.2 | 69.0 |
| CHLORAMINE-T | 343.5 GBq $mmol^{-1}$ | 25 | 1 hr | 57.1 | 58.8 |
| CHLORAMINE-T | NO CARRIER ADDED | 25 | 30 min | 65.0 | — |
| HALOGEN ISOTOPE EXCHANGE | 16.7 GBq $mmol^{-1}$ | 70–80 | 20 hrs | 45.1 | 54.3 |

TABLE 2 PREPARATION OF [$^{82}$Br] – BVDU

| METHOD | SPECIFIC ACTIVITY | TEMPERATURE (°C) | TIME | RADIO-CHEMICAL YIELD (%) | CHEMICAL YIELD, RECOVERY (%) |
|---|---|---|---|---|---|
| DIRECT NEUTRON ACTIVATION | 31.82 MBq $mmol^{-1}$ | — | 4 h | 30 | 97 |
| RADIOCHEMICAL SYNTHESIS (CHLORAMINE-T/ $^{82}Br^-$) | 227 MBq $mmol^{-1}$ | 25 | <10 min | 69.8 | 72 |

TABLE 3 TISSUE DISTRIBUTION OF $^{125}$I-IVDU IN RABBIT EXPRESSED AS TISSUE TO BLOOD RATIO (DPM PER G. TISSUE/DPM PER G. BLOOD)

| TISSUE | 1 HOUR AFTER I.V INJECTION OF $^{125}$I-IVDU | | 6 HOURS AFTER I.V INJECTION OF $^{125}$I-IVDU | |
|---|---|---|---|---|
| | UNINFECTED CONTROL | HSV-1 INFECTED MODEL* | UNINFECTED CONTROL | HSV-1 INFECTED MODEL* |
| BRAIN | 0.090±0.029 | 0.418±0.087 | 0.046±0.005 | 0.083 ±0.024 |
| KIDNEY | 1.295±0259 | 1.675±0.445 | 0.914±0.002 | 0.930±0.117 |
| LIVER | 0.685±0.175 | 1.326±0.147 | 0.505±0.003 | 0.445±0.003 |
| THYROID | 0.768±0.152 | 0.798±0.016 | 0.661±0.067 | 0.669±0.017 |
| MUSCLE | 0.326±0.052 | 0.630±0.193 | 0.630±0.009 | 0.167 ±0.024 |

* $^{125}$I-IVDU WAS ADMINISTERED 5 DAYS AFTER THE INTRACEREBRAL INJECTION OF HSV-1 (JLJ)

FIG. 1. SYNTHESIS OF RADIOHALOGENATED IVDU (4) AND BVDU (5)

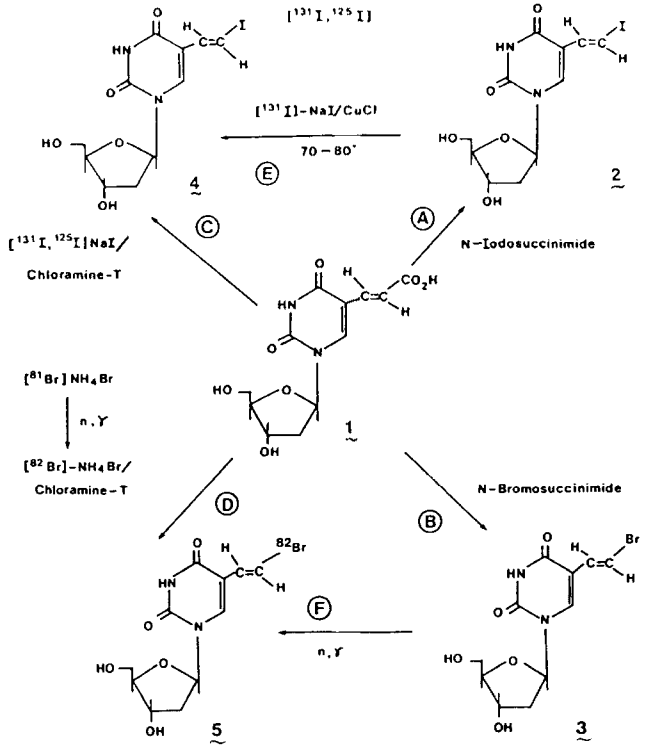
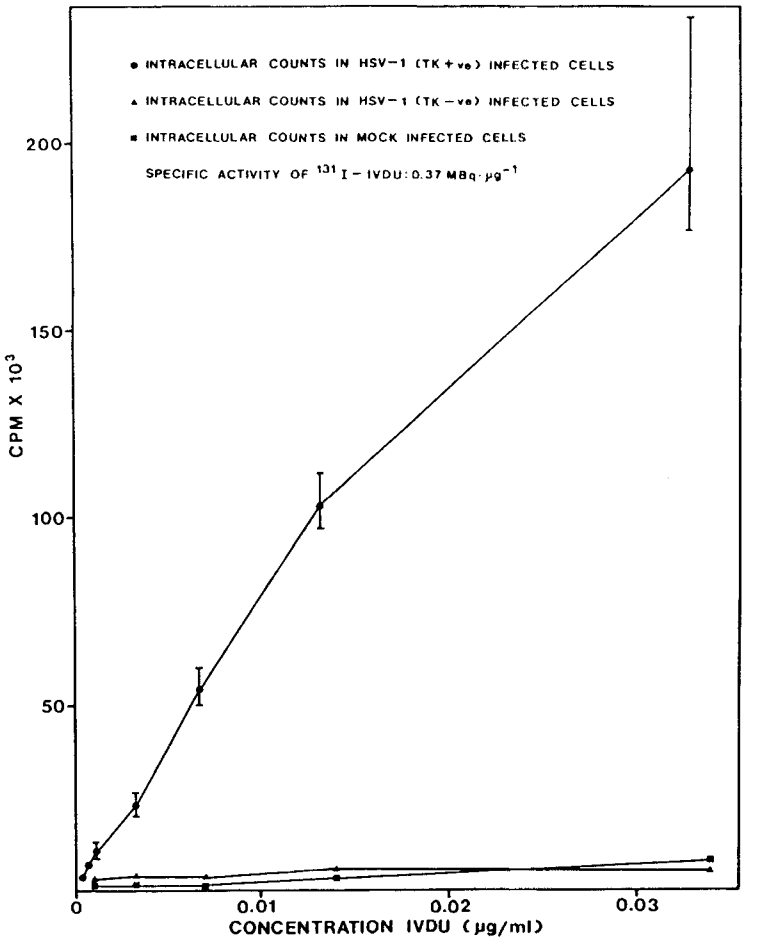



FIG. 2. SELECTIVE UPTAKE OF $^{131}I^-$ IVDU IN HSV-1 (TK+VE) INFECTED CELLS: DOSE RESPONSE CURVE



# SYNTHESIS AND BIODISTRIBUTION OF RADIOIODINATED NICOTINE AND NICOTINE ANALOGS

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Extensive studies on the biological effects of nicotine have been done due to its association with smoking. The central actions of this tobacco alkaloid are well documented (1). Nicotine labelled with C-14 or H-3 has been shown to accumulate in the brains of guinea pigs (2), rats, and mice (3) by various investigators. Furthermore, Hansson and Schmiterlow have shown that (C-14)-nicotine accumulated in the adrenal medulla of mice (3). It appears that nicotine and perhaps nicotine analogs labelled with a suitable single photon gamma emitter such as I-123 may prove to be a useful imaging agent for the brain as well as the adrenal medulla. We labelled nicotine (Figure 1) and four nicotine analogs (Figure 2) (4-9) with I-125 and studied their biodistribution in rats.

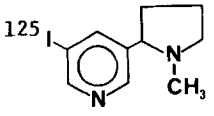
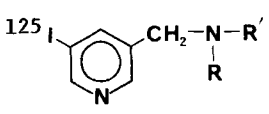



Fig. 1. (I-125)-nicotine




a. R = Me, R' = n-Pr (MP)
b. R = H, R' = i-Pr (IP)
c. R = R' = Et (DIET)
d. R = R' = i-Pr (DIP)

Fig. 2. (I-125)-nicotine analogs

I-125 labelled nicotine (NIC) and nicotine analogs were prepared from their respective bromine precursors (Figures 3 and 4). I-125 labeling was done via a halogen exchange reaction at elevated temperature in the presence of copper sulfate as a catalyst. The radiochemical yield and specific activity of the labelled compounds are shown in Table 1.

Table 1

| Compound | Yield | Specific Activity |
|---|---|---|
| NIC | 253 µCi (25.3%) | 61.0 mCi/mmole |
| MP | 700 µCi (70.0%) | 170.1 mCi/mmole |
| DIET | 466 µCi (46.6%) | 113.2 mCi/mmole |
| IP | 750 µCi (75.0%) | 171.8 mCi/mmole |
| DIP | 660 µCi (66.0%) | 178.9 mCi/mmole |

Biodistribution studies performed in female Sprague-Dawley rats showed that all these compounds were taken up rapidly by the brain and the adrenal. The highest uptake of all these compounds in both organs occured at 2 minutes after tail vein injections. The organ:blood ratios at 2 minutes and the $T_{\frac{1}{2}}$ (min.) of radioactivity in these organs are shown in Table 2.

Table 2

| | NIC | MP | DIET | IP | DIP |
|---|---|---|---|---|---|
| Brain:Blood | 2.4 | 1.9 | 2.9 | 3.7 | 6.0 |
| Adrenal:Blood | 4.7 | 6.5 | 4.8 | 3.0 | 35.9 |
| $T_{\frac{1}{2}}$ Brain | 5.0 | 4.0 | 18.0 | 9.0 | 13.0 |
| $T_{\frac{1}{2}}$ Adrenal | 3.0 | 4.0 | 11.0 | 18.0 | 9.0 |

Biodistribution data of these five compounds were correlated with *in vitro* protein binding and lipophilicity studies.

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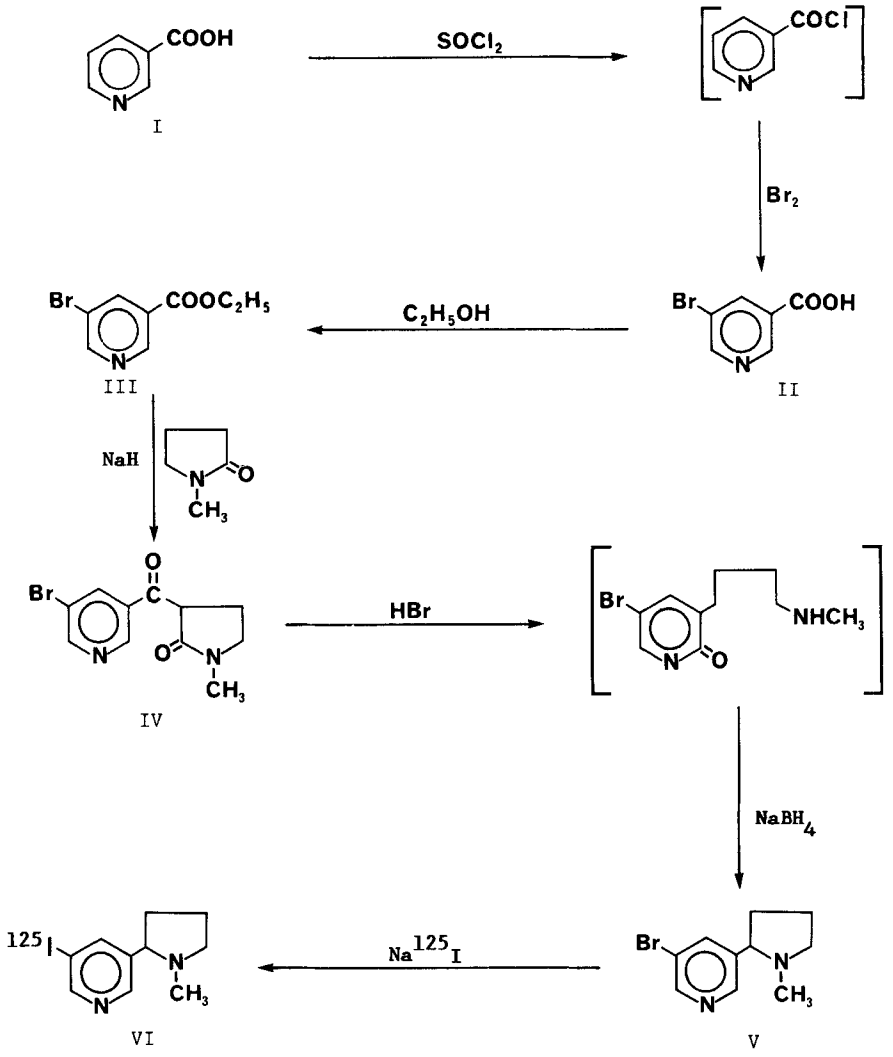


Figure 3. Preparation of $^{125}$I-5-iodonicotine.

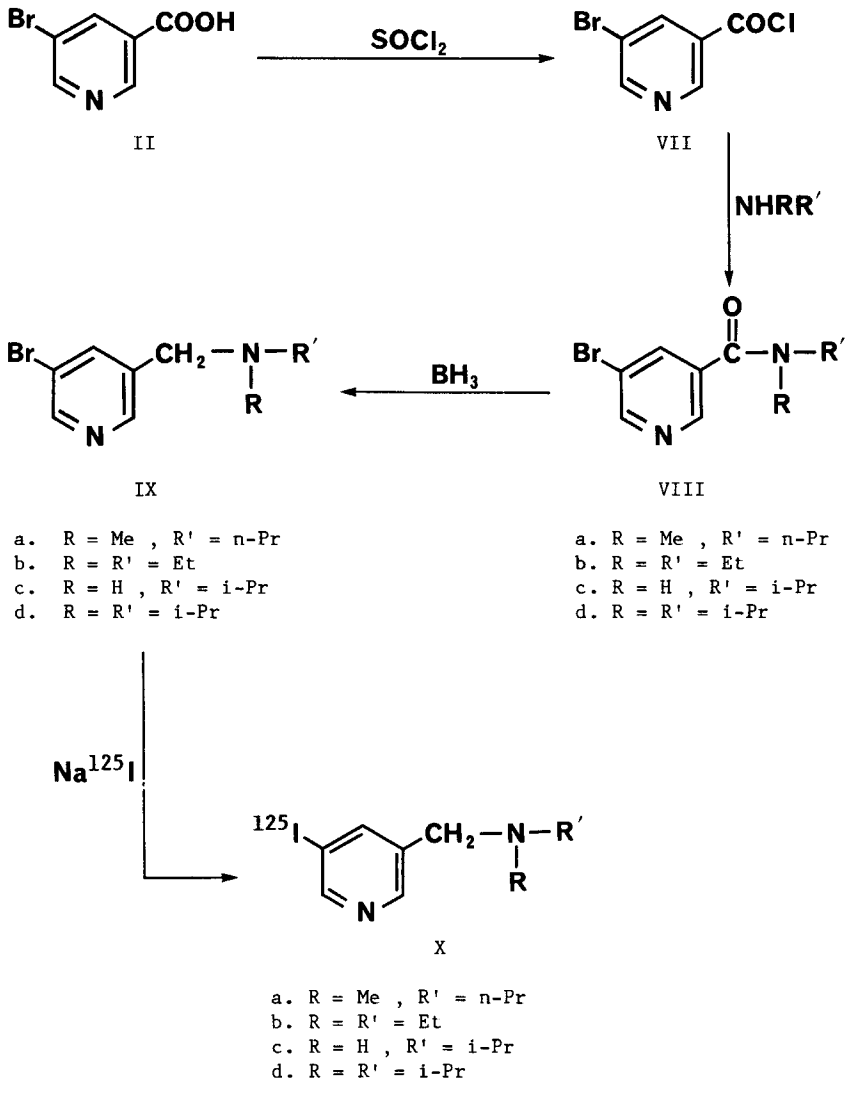


Figure 4. Preparation of $^{125}$I-3-(alkylaminomethyl)-5-iodo-pyridines.

## RADIOCHEMICAL AND ANALYTICAL ASPECTS IN OPTIMIZED PREPARATION OF I-123-N-ISOPROPYL-p-IODOAMPHETAMINE (I-123-pIAM)

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Since SPECT needs large doses of I-123-pIAM careful optimization of the labeling and purification methods was required for best utilization of the expensive I-123 and maximum target/nontarget ratios. Published procedures were not fully satisfactory in these respects (1-3).

pIAM is not activated for nucleophilic substitution: a comparison of methods for exchange radioiodination (using I-131) of pIAM (identity and purity established by H-NMR) revealed wide yield variations, summarized in table 1:

Table 1: Exchange radioiodination of pIAM (using I-131-NaI)

| method | reaction-temp. | -time | radiochem. yield |
|---|---|---|---|
| in solvent: | | | |
| EtOH | 120 °C | 60 min | 44 % |
| HOAc | 150 | 30 | 36 |
| in liquid phase: | | | |
| pure pIAM | 150 | 30 | 7 |
| in solid phase: | | | |
| | 150 | 30 | 58 |
| ammonium | 160 | 30 | 66 |
| sulfate | 160 | 60 | 72 |
| | 160 | 90 | 85 ± 7 |
| catalyzed: | | | |
| HOAc + $Cu^{++}$ | 160 | 60 | 79 ± 6 |

Subsequently the exchange in ammonium sulfate (4) was applied for labeling with (p,5n)I-123: about half of the results were equal to I-131 within exp. variability but for the rest yields dropped unpredictably to about 20-50 %. This was not observed when rechecking the method with I-131 (Fig.1). The oxidative conditions of the solid phase exchange and a similar drop in yield found after addition of $NaHSO_3$ lead us to assume a reducing agent in I-123. Its presence can be explained by target processing: the Xe-123 precursor decays by $\beta^-$-emission leading to positively charged energetic recoil I-123 atoms in the gas phase. To ensure iodide as final chemical form, $H_2S$ gas is added which, after degassing, can still leave traces of $S^{2-}$ in the aqu. I-123 solution (5).

Searching for a labeling system not sensitive to traces of reducing agent and since acidic, oxidizing conditions promote exchange of iodine in non-activated aromatics (4), we added to HOAc solvent $Cu^{++}$ ion as oxidative catalyst: it can generate reactive species ($I_2$,$I^+$) from iodide, removes free $I^-$ as CuI and can also oxidize traces of $S^{2-}$ (6). Using this system isolated yields of about 80 % were obtained consistently with I-123 within 30-60 min.

For purification after exchange reaction several methods were compared:
1. ether extraction of pIAM (amine) from basic aqu. solution.
2. ion exchange chromatography on a small column of anion exchange cellulose (DEAE) eluted with .01M HOAc-NaOAc removed $I^-$ to less than 1 % and was preferred for speed and handling.
3. preparative HPLC (described below and in Fig. 3).

Reaction mixtures and product solutions were analyzed by
1. TLC on silica gel with $CHCl_3$:MeOH:HOAc 85:15:1 achieved a better separation of free $I^-$(Rf 0) from pIAM(Rf0.3) than with EtOH:EtOAc 1:1.
2. Paper-Electrophoresis using .025M NaOAc-HOAc at 300 V for 8 min provided a fast quant. determination of free $I^-$, usually less than 1 %. The protonated amine group was documented by a slight migration towards the cathode and pIAM could be characterized by a migration ratio pIAM/NaI of 0.14.
3. HPLC: due to its secondary amine group pIAM could only be eluted in a sharp peak from reversed phase C18 columns by adding to EtOH:$H_2O$ solvent:
a) ion-pair reagent: e.g. sodium dodecyl sulfate (SDS), not suitable for i.v.inj.
b) amine modifier: alkyl amines have been used but would have to be removed before inj.. Therefore we used the amine with lowest MW, $NH_3$, and achieved a high resolution separation of $I^-$,$I_2$, unidentified by-products, ortho-IAM(?) and pIAM (Fig. 2). This analytical system can also be used preparatively but retention time (20 min) and peak volume (12 ml) were not optimum for preparation.
c) ammonium modifier: 1 % $NH_4OAc$ (and 1 % HOAc for pH 5 adjustment) provided rapid elution of pIAM in a low volume peak (2.5 ml) with efficient separation from $I^-$ and an unidentified impurity and was therefore used preparatively (Fig.3).

To evaluate the influence of HPLC-separated peaks on tissue distribution I-131-pIAM after purification by anion exchange and prep. HPLC resp. was compared in rats: uptake was similar in liver, lungs and brain (2.4 % dose/g after anion exchange vs. 2.9 after prep. HPLC). However blood activity was reduced to about half by prep. HPLC giving a significant improvement in target/nontarget ratio: brain/blood = 12 after anion exchange vs. 31 after prep. HPLC (Fig. 4). A similar trend was observed in ongoing patient studies.

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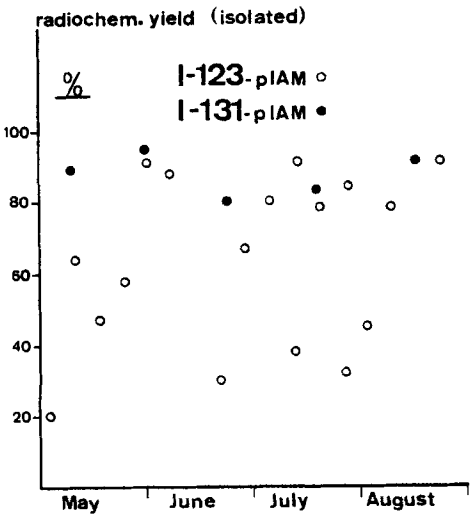
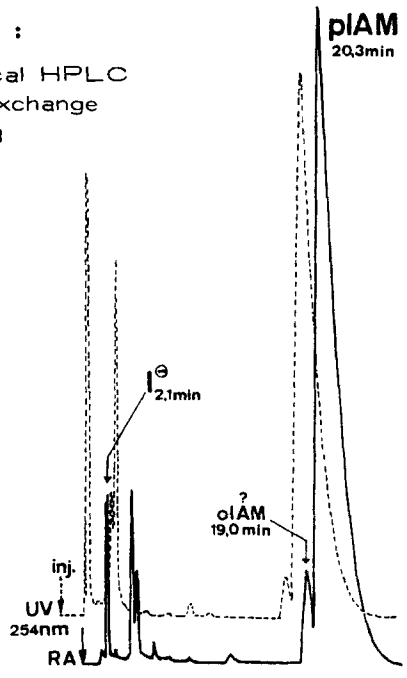


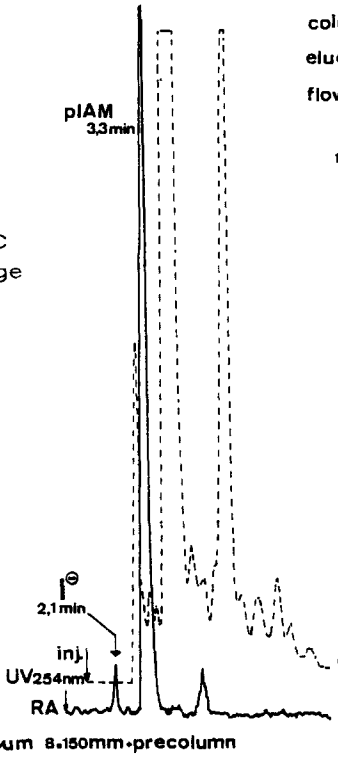

Fig. 1: Exchange labeling of 3,5 mg pIAM in 2 mg $(NH_4)_2SO_4$ 160°- 90 min

Fig. 2 : analytical HPLC after exchange labeling

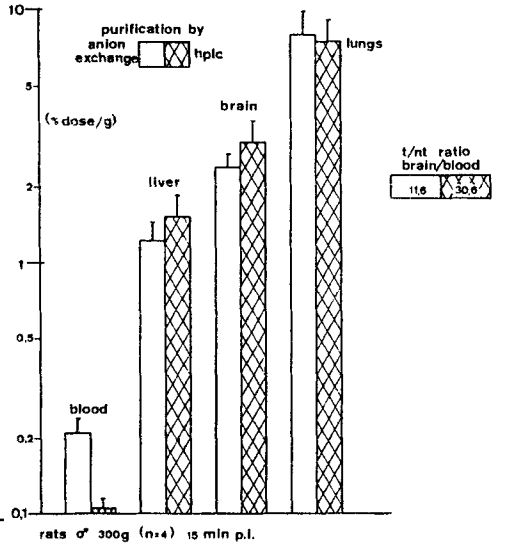



column: RP 18/7µm, 8.150mm-precolumn
eluent : EtOH: $H_2O$:$NH_3$ (60:40:1)
flow-rate: 2,5 ml/min

Fig. 3 : prep. HPLC after exchange labeling




column : RP 18/7µm 8.150mm-precolumn
eluent : 55% EtOH, 43% $H_2O$, 1% $NH_4OAc$, 1% HOAc
flow-rate: 2,5 ml/min




Influence of purification on tissue distribution

Fig. 4

# KINETICS OF RADIOIODINATION OF PHENYL PENTADECANOIC ACID (PPA) VIA PPA-THALLIUM INTERMEDIATE. A 'KIT' METHOD OF PREPARATION OF IPPA.

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Radioiodinated (I-123) fatty acids are likely to provide information regarding regional metabolic activity of the myocardium besides the regional flow. Terminally radioiodinated long chain alkyl fatty acids have been studied extensively in experimental animals and have shown promise in clinical assessments (1-4). However, the rapid deiodination of these tracers *in vivo* presents certain limitations. Various methods have been suggested for improving *in vivo* stability of these agents. Machulla et al. proposed a means of chemically stabilizing the carbon-iodine bond by replacing the alkyl carbon-iodine bond by an aryl carbon-iodine bond (5). They also showed that ω-(p-iodo)phenyl pentadecanoic acid could be utilized for myocardial imaging in experimental animals and in humans (6).

The methods proposed to radioiodinate phenyl pentadecanoic acid (PPA) include i) direct electrophilic substitution reaction with multiple HPLC separation (5) or single HPLC purification (7), ii) radio-iosotopic exchange reaction (8), and iii) triazene intermediate reaction (9). Each method has its own advantages and disadvantages. We had proposed and showed the feasibility of radioiodination utilizing an organothallium intermediate (10). The biodistribution of IPPA prepared via PPA-Tl intermediate was shown to be similar to the product prepared by the direct electrophilic substitution reaction. Here we report the kinetics of formation of IPPA, the simplified method of preparation, purification, and analysis.

We studied various parameters affecting the radiochemical yields: i) the amount of fatty acid, ii) the amount of thallium trifluoroacetate TlTFA), iii) reaction volume, iv) time of first incubation (reaction of Tl with PPA), v) time of the second reaction (radioiodination), and vi) temperature of the reaction. Once the reaction condtions were standardized we evaluated methods of purification and analysis of the final product.

Among the factors studied the time and temperature of the reaction were found to have profound effect on the radiochemical yields. The reaction equilbrium was reached at 3 hours at room temperature, 20-25 minutes at 50°C, and only 5 minutes at 100°C. The reaction could be carried out with only 100 μg of the fatty acid. The molar ratio of the fatty acid to TlTFA had no significant effect. The reaction volumes of 0.1 ml to 0.5 ml also had no effect. PPA-Tl intermediate once formed appears to be stable for several days either in liquid or solid phase. Thus a kit consisting of PPA-Tl intermediate in dry form could be prepared and stored at room temperature. Radioiodination is carried out by reconstituting the PPA-Tl intermediate with trifluoro-acetic acid, with radioioiodine and heating the reaction mixture. The product is purified by simple solvent extraction and silica gel column chromatography. The radiochemical analysis were performed with TLC,

HPLC, and chemical analysis for thallium was carried out with atomic absorption measurements.

IPPA prepared by our method, purified either by HPLC or by simple solvent extraction column chromatography showed similar biodistribution in rats. Heart to blood ratios were 12:1 in rats at one minute postinjection. Most of the activity in the heart was found to be in the lipid fraction and found in the triglyceride form. Similar pattern of incorporation was observed for $^{3}$H-palmitate (11). Our data confirm the observation of Reske et al for IPPA (12). I-123 PPA in conjunction with single photon emission computed tomography (SPECT) has been used to measure relative uptake and clearance of IPPA in canine myocardium under control conditions and after permanent and temporary coronary artery occlusion (13).

Radioiodination efficiency as determined by TLC or HPLC was >90% when i) amount of PPA was 200 μg or more, ii) amount of TlTFA was 50 μg or more, iii) the reaction temperature of 100$^{0}$, and iv) reaction time of 5 minutes or more. The radiochemical purity of the final product was >95% and the thallium level was <0.5 ppm in the final product even when 0.5 mg of TlTFA was used. Thus, it may be concluded that I-123 PPA, suitable for human investigation, can be prepared via PPA-Tl intermediate. "PPA-Tl" kit method is simple, convenient, efficient, and suitable for routine applications.

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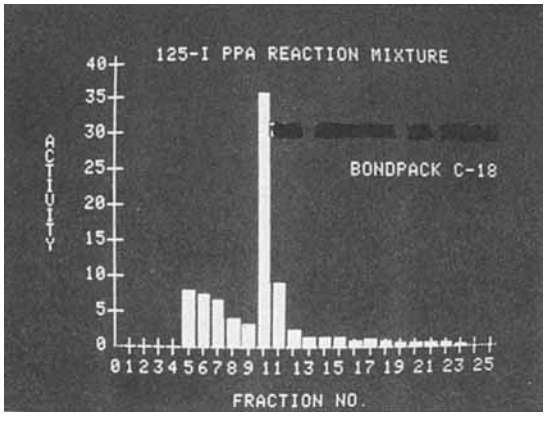
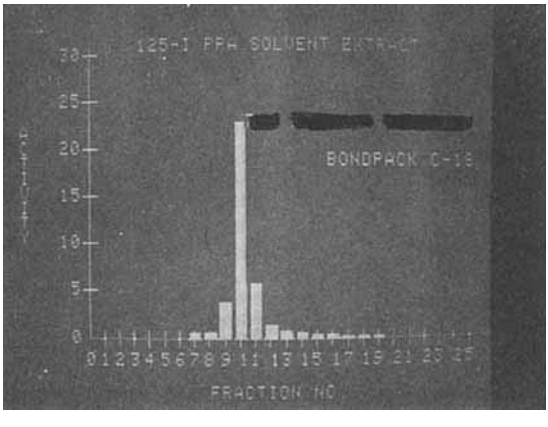
## SUPPORTING DATA

**High performance liquid radiochromatograms. IPPA prepared by the method of Kulkarni et al.**

**A. Reaction Mixture Before Purification**




**B. After Purification by Solvent Extraction.**



SUPPORTING DATA

I-PPA prepared utilizing thallium trifluoroacetate.
Purified by:

A) Solvent extraction/silica gel column chromatography
B) HPLC

% ID/g in Rats (mean ± SEM) 2 min postinjection

| Tissue | Method A X ± SEM (n = 6) | Method B X ± SEM (n = 6) |
|---|---|---|
| Blood | .28 ± .01 | .25 ± .01 |
| Liver | 1.24 ± .18 | 1.33 ± .05 |
| Spleen | .68 ± .04 | .75 ± .04 |
| Lung | .59 ± .04 | .58 ± .05 |
| Kidney | .44 ± .02 | .47 ± .04 |
| Heart | | |
| LV | 1.64 ± .95 | 1.81 ± .21 |
| RV | 1.71 ± .85 | 1.85 ± .23 |
| Base | 1.27 ± .22 | 1.54 ± .16 |
| Target-to-Nontarget Ratios | | |
| LV/Blood | 5.95 ± .50 | 7.51 ± 1.08 |
| LV/Liver | 1.39 ± .14 | 1.39 ± .97 |
| LV/Lung | 2.78 ± .09 | 3.18 ± .63 |

## THE DEVELOPMENT OF RADIOIODINATED ORGANIC CATIONS AS POTENTIAL MYOCARDIAL PERFUSION AGENTS

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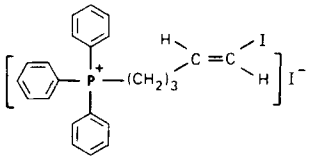
Thallium-201 is the most widely used cationic perfusion agent for the differentiation of ischemia from irreversible myocardial damage but has the disadvantages of inefficient detection of its low-energy X-rays and redistribution during the imaging period. A myocardial perfusion agent labeled with an isotope having more attractive radionuclidic properties would be an advantage. In addition, nuclear medicine techniques could be of even greater benefit to the cardiologist if agents were available for measuring early indices of myocardial disease as well as regional perfusion.

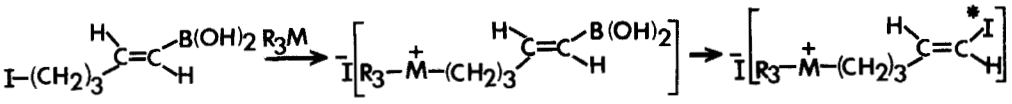
Recent studies have shown that the uptake of tetraphenylphosphonium bromide (IPP) in various cells grown in vitro is related to the cell membrane potential gradient (1-4). In addition, pronounced myocardial uptake of the [$^{3}$H]tetraphenylphosphonium and [$^{123}$I]iodobenzyl-dimethyl-phenylammonium cations (5) have suggested the potential use of such radiolabeled cations for evaluation of heart disease. A model radioiodinated phosphonium cation, E-1-[$^{123}$I]iodo-1-penten-5-yl-triphenylphosphonium iodide (I), shows high heart:blood ratios in Fischer rats. In dogs this agent shows both high myocardial and hepatobiliary uptake (6,7). Since a balance between charge, molecular size and polar and non-polar characteristics of the molecule are required for biliary secretion, modification of this type of phosphonium cation could potentially increase myocardial uptake and minimize hepatobiliary uptake. To study the relationship between tissue specificity and the structural features of this type of cation, several analogs of (I) have now been prepared and evaluated in rats.



(I)

The arsonium (As) analog of (I), E-1-[$^{125}$I]iodo-5-pentenyl-triphenylarsonium iodide (VII), and two phosphonium analogs, E-1-[$^{125}$I]iodo-5-pentenyl-dicyclohexylphenylphosphonium iodide (VIII) and E-1-[$^{125}$I]iodo-5-pentenyldimethyl-n-octylphosphonium iodide (IX), were prepared in the same manner described earlier for the parent (I) compound (6,7). The appropriate boronic acid substrates were synthesized by condensing 5-iodo-1-penten-1-ylboronic acid (II) with $R_3M$ and ioiodinated using NaI and chloramine T (CT) to yield the products VII-IX (Scheme I). All new agents and the intermediates were analyzed (TLC, NMR and C&H). The iodine-125 (*I)-labeled analogs were prepared by using Na*I-CT and evaluated in rats (5/group). All four agents show good heart uptake (Table 1) and retention (Figure 1) demonstrating that these structural changes do not greatly affect the myocardial specificity of these interesting new cations. Imaging studies (Figure 2) with (VIII) demonstrate that ligand modification can affect apparent hepatobiliary clearance.

Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.



| Substrate | Ligands, R | M | Product |
|---|---|---|---|
| III | triphenyl | P | I |
| IV | triphenyl | As | VII |
| V | dicyclohexylphenyl | P | VIII |
| VI | dimethyl-n-octyl | P | IX |

Scheme I

Table 1. Heart uptake and mean heart:blood ratios of radioiodinated cations in rats.

| | Range heart, % dose/gm (mean heart:blood) | | |
|---|---|---|---|
| $^{125}$I-Cation | 5 min | 30 min | 60 min |
| I | 3.7-4.7 (22) | 3.4-5.3 (38) | 3.8-4.5 (50) |
| VII | 1.7-3.6 (11) | 2.6-4.3 (13) | 3.0-3.7 (18) |
| VIII | 2.8-3.8 (9) | 2.7-3.7 (16) | 3.2-3.9 (21) |
| IX | 2.2-2.9 (6) | 1.4-2.4 (8) | 1.9-3.6 (19) |

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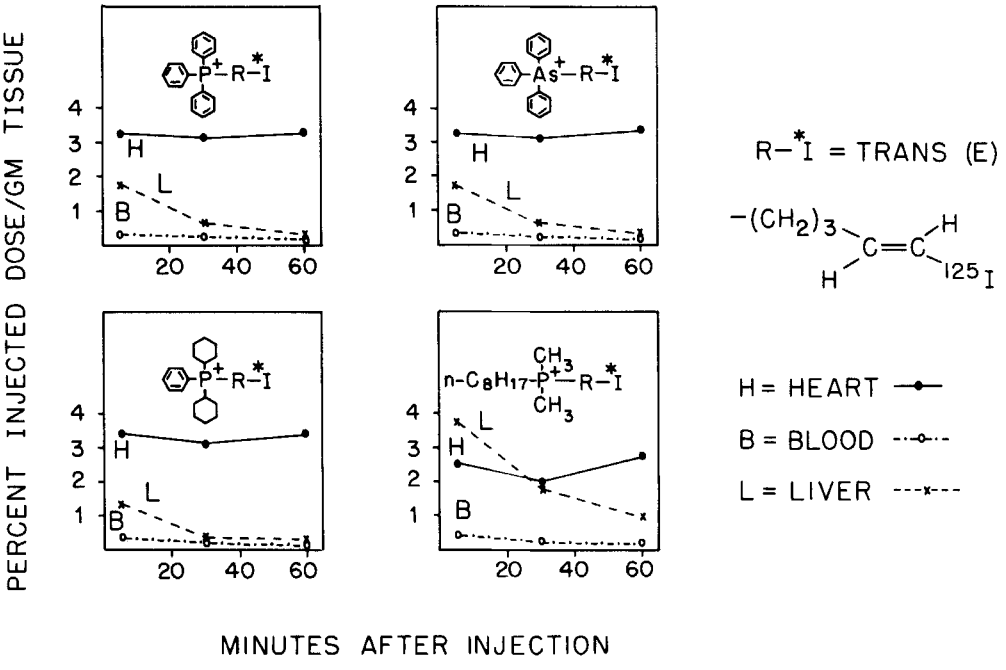


Figure 1. Relative heart, blood and liver uptake of $^{125}$I-labeled cations I, VII, VIII and IX in rats over a 60 min period.

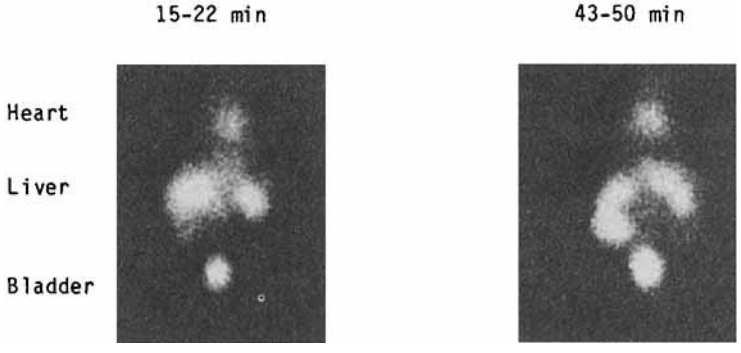


Figure 2. Early (15-22 min) and late (43-50 min) gamma camera images (anterior view) of $^{123}$I-labeled E-1-iodo-5-pentenyl-dicyclohexylphenylphosphonium iodide (VIII) in a rat demonstrating apparent hepatobiliary clearance.

## MITOCHONDRIA SPECIFIC RHODAMINE 123: RADIOIODINATION AND PRELIMINARY EVALUATION AS AN AGENT FOR SCINTIGRAPHY AND RADIOTHERAPY OF CERTAIN TUMORS

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Rhodamine 123 (methyl-0-(6-amino-3'-imino-3H-xanthane-9-yl) benzoate monochloride), a cationic Eastman Kodak laser dye, (Rhl23, Fig. 1) has been shown to localize in the mitochondria of living cells (1). From normal epithelial cells, however, the dye is released within a few hours but is retained for 2-5 days in a variety of carcinoma cells (2). Given i.p. (15 mg/kg, day 1,3,5) along with 2-deoxyglucose (0.5g/kg), Rhl23 prolonged the survival of mice implanted with Ehrlich ascites tumors or with MB-49 bladder carcinoma (3). The results have been attributed to the inhibition of glycolysis and mitochondrial adenosine triphosphate synthesis (3). This selectivity and the proposed mechanism prompted us to evaluate Rhl23 as an agent for scintigraphy and radiotherapy.

The mitochondrial specificity of the dye was ascertained by incubating Rhl23 with Chinese hamster ovary (CHO) fibroblasts and ovarian carcinoma cells in tissue culture, and by examining the cells with an ultraviolet fluorescence microscope (Fig. 2). The dye was labeled with iodine-131 by a 60 minute incubation at 22°C in the presence of iodogen and Sorenson's phosphate buffer. For optimal labeling (80%), 200 ug iodogen, 5-7 mCi I-131/ml phosphate buffer pH 7.5-9.0 and 1-5 mg dye dissolved in 1 ml buffer pH 7 are required.

An alkaline precipitation and a 0.3 ml, 0.9% NaCl wash eliminated unbound radioactivity. The labeled product was then dissolved in 30% DMSO in 0.9% NaCl. The radionuclidic purity was confirmed by paper chromatography and the cationic nature of the labeled product by electrophoresis.

Preliminary results of gamma camera imaging and tissue distribution studies in C3H/HeJ mice bearing approximately 1 cm diameter implanted KHT sarcoma indicated that i) initially the radioactivity was taken up by the intestine, urine, liver (3.4%), kidneys (1.7%), thyroid (1.7%), and tumors (1.2%), ii) the radioactivity from all these organs including the liver, kidneys, and thyroid, as well as tumors, cleared within 21 hrs., (0.67%, 0.2%, 0.2%, and 0.2% respectively), probably through the kidneys and that iii) at 18 hrs. the tumor/blood (0.95) and tumor/muscle (3.9) radioactivity ratios were low for scintigraphic delineation of tumors (Tables 1 and 2, Fig. 3).

Distribution and imaging studies in RIII/Imr female mice, bearing 1 cm or smaller diameter, spontaneous mammary adenocarcinomas also indicated similar clearance from all normal tissues but not from the tumors. (Table 2). In tumors, the radioactivity remained unchanged (1.5% at 4 hrs. and 1.64% at 21 hrs.) or slightly increased. This resulted in much higher tumor/blood (9.5) and tumor/muscle ratios (4.3) at 21 hrs. which allowed detection of the tumors scintigraphically. (Fig. 4).

Data from these preliminary studies were consistent with the mitochondrial specificity of Rhl23 and strongly suggested that, i) Rhl23 could be labeled efficiently with radioactive iodine, ii) Within 21 hours, little in vivo dehalogenation occurred, and iii) analogous to Rhl23, radioiodinated Rhl23 was specificallly retained in epithelial carcinoma cells but was rapidly cleared from all normal tissues.

We believe that these results are worthy of further investigation into the development of radioiodinated Rhl23 as an agent for scintigraphy and radiotherapy of spontaneous epithelial carcinomas.

(1) Johnson L.V., Walsh M.L., et al, Proc. Natl. Acad. Sci. USA, 77, 990 (1980).
(2) Summerhayes I.C., Lampidis T.J., et al, Proc. Natl. Acad. Sci. USA, 79, 5292 (1982).
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Table-1

| Tissue | KHT Sarcoma % Admin. dose/gram | | | Mammalian Adenocarcinoma % Admin. dose/gram | |
|---|---|---|---|---|---|
| | 2hr(N=2) | 4hr(N=2) | 18hr(N=3) | 4hr(N=2) | 21hr (N=3) |
| Blood | 2.45 (2.2-2.7) | 1.7 (1.4-2.0) | .27 (.2-.4) | .91 (.6-1.2) | .14 (.09-.2) |
| Tumor | 1.2 (1.1-1.3) | 1.05 (1.0-1.1) | .23 (.1-.4) | 1.59 (.56-1.6) | 1.64 (.44-3.8) |
| Muscle | .55 (.5-.6) | 0.7 (0.4-1.0) | .03 (0-.1) | .27 (.25-.28) | .33 (.14-.62) |
| Liver | 2.9 (2.8-3.0) | 2.0 (1.6-2.4) | .57 (.4-.8) | 1.09 (.78-1.4) | .25 (.2-.31) |
| Spleen | 1.0 | .65 (0.5-0.8) | .17 (.1-.3) | .41 (.2-.61) | .06 (.05-.07) |
| Thyroid | 10.9 (9.5-12.3) | 16.7 (11.0-22.4) | 3.13 (.8-6.8) | 3.64 (1.4-5.8) | .21 (.17-.23) |
| Kidneys | 4.35 (4.0-4.7) | 3.25 (3.1-3.4) | .53 (.2-.8) | 7.81 (7.1-8.54) | 5.3 (3.6-7.4) |
| Urine | 19.5 (18.6-20.4) | 32.65 (31.0-34.3) | 8.3 (3.3-17.6) | 52.29 (N=1) | - |
| Intestine | 19.45 (5.8-33.1) | 2.85 (2.4-3.3) | .27 (.2-.4) | 4.01 (.92-7.1) | .17 (.11-.24) |

Table-2

| Tissue | KHT Sarcoma % Admin. dose/whole organ | | | Mammalian Adenocarcinoma % Admin. dose/whole organ | |
|---|---|---|---|---|---|
| | 2h(N=2) | 4hr(N=2) | 18hr(N=3) | 4hr(N=2) | 21hr(N=3) |
| Liver | 3.4 (3.1-3.7) | 2.1 (1.7-2.5) | .67 (.5-.9) | 1.94 (1.8-2.1) | .5 (.40-.57) |
| Spleen | .15 (.1-.2) | .05 (0-.1) | .03 (.02-.04) | .05 (.03-.06) | .006 (.006-.007) |
| Thyroid | 1.7 (1.5-1.9) | 3.05 (1.3-4.8) | .23 (.1-.3) | .84 (.4-1.27) | .05 (.05-.05) |
| Kidneys | 1.7 (1.4-2.0) | 1.15 (1.1-1.2) | .2 (.2-.2) | 3.58 (3.4-3.76) | 2.29 (1.6-3.14) |
| Tumor / Blood | .47 (.46-.48) | .64 (.51-.76) | .95 (.81-1.02) | 1.91 (1.4-2.4) | 9.46 (4.65-18.9) |
| Tumor / Muscle | 2.17 (2.1-2.2) | 1.99 (1.1-2.9) | 3.98 (3.4-4.9) | 5.96 (5.7-6.2) | 4.31 (1.8-6.2) |

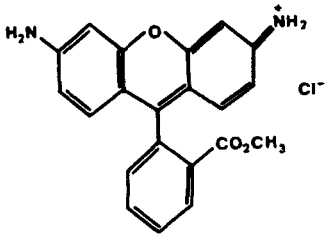



Fig. 1: Structural formula of Rh123

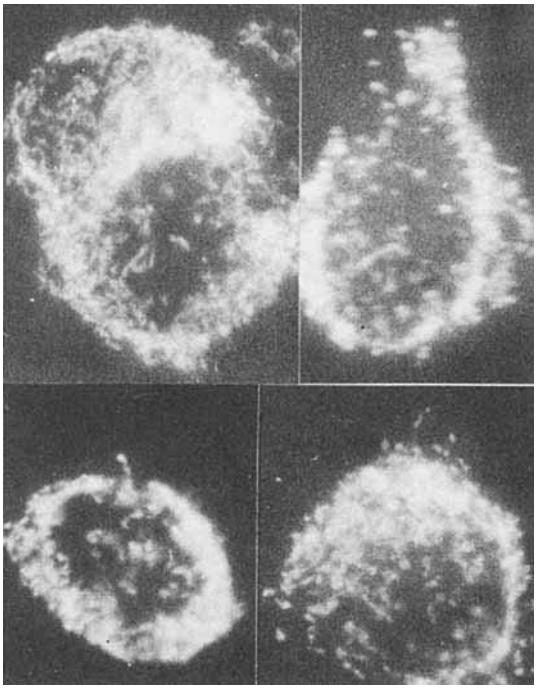
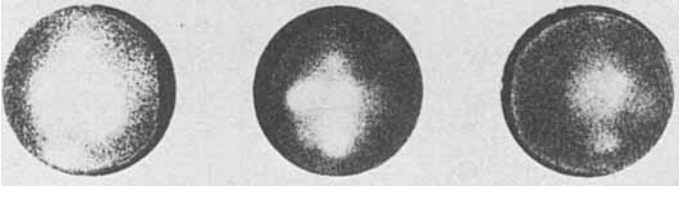


Fig. 2: $1 \times 10^4$ Ovarian carcinoma cells were grown on a plastic slide in Leighton tubes, incubated with 10ug Rh123 (1hr.,37°C), washed and photographed under a u.v. fluorescent microscope (x 90). The dye in cytoplasm is consistent with mitochondrial specificity. Unstained nucleus is also seen. (A composite of 4 cells.)

KHT SARCOMA



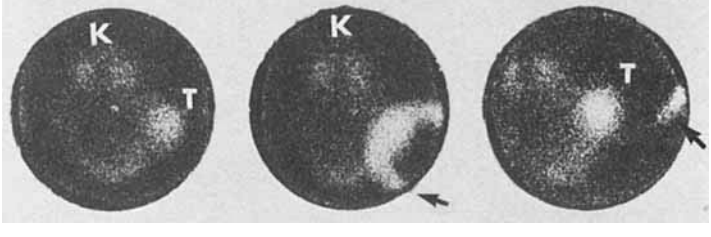
2 HR 4 HR 18 HR

POST INJECTION

Fig. 3: Note the clearance of radioactivity from the thyroid, liver, kidneys, and bladder of a mouse (Ant). However, KHT sarcoma in the flank is not detected.

Fig. 4: Spontaneous mammary adenocarcinoma tumor (T) is delineated. Faint radioactivity in the kidneys (K) is detectable. The arrows indicate a Tc-99m marker source above and adjacent to the tumor.

SPONTANEOUSLY GROWN CARCINOMA




21 HR POST INJECTION

(WITHOUT AND WITH TC-99M MARKER)

# A RAPID METHOD FOR HALOGEN EXCHANGE LABELLING OF 6-IODOMETHYL-19-NORCHOLEST-5(10)-EN-3β-OL WITH $^{82}Br$, $^{123}I$ AND $^{211}At$

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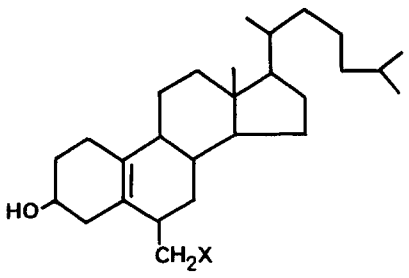
We have developed a new method for halogen exchange labelling of 6-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-I) with $^{82}Br$, $^{123}I$ and $^{211}At$ in the presence of crown ethers which plays an important role in the isotopic and nonisotopic exchange reactions. Benzo-12-crown-4 and 18-crown-6 were chosen as crown ether because of their low melting points. The substrate NCL-6-I and radioactive alkali halides ($Na^{123}I$, $Na^{82}Br$ and $Na^{211}At$) provided by Institute of Atomic Energy (Beijing) was directly dissolved in the crown ethers. A new astatine compound, $^{211}At$-6-astanomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-$^{211}At$) was obtained in a high yield at short reaction time by this labelling procedure.

The use of benzo-12-crown-4 and 18-crown-6 as solvent in the halogen exchange reactions of NCL-6-I resulted in the rapid formation of the labelled compounds. The naked radioactive halide ions have more reactive ability than those in the conventional conditions (1). Our result demonstrates that the rate of the exchange is very fast in comparison with the other method as shown in Table 1 (2-4). The result of the time dependence of the labelling yield indicated that the order of the rate of exchange reaction was At > I > Br. It may be expected that the present labelling method could also be used effectively in the preparation of some radiopharmaceuticals.

$^{211}At$ ($T_{1/2}$ = 7.2 hr, $E_\alpha$ = 5.866 MeV) is of potential interest for therapeutic application. The distribution of NCL-6-$^{211}At$ in rats was determined at different time intervals. The result is summarized in Table 2. The distribution profile of radioactivity from NCL-6-$^{211}At$ showed many similarities with $^{82}Br$-6-bromomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-$^{82}Br$)(3) and NCL-6-$^{131}I$ (2). This clearly demonstrates a considerable selective localization of NCL-6-$^{211}At$ in adrenals.

Table 1. Halogen exchanges of 6-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-I) with $^{82}Br$, $^{123}I$ and $^{211}At$

| Labelled compounds | Conditions | Reaction time | Yield (%) | Reference |
|---|---|---|---|---|
| NCL-6-$^{131}I$ | reflux in acetone | 4 hr | 70 | (2) |
| NCL-6-$^{123}I$ | reflux in acetonitrile | 80 min | 80 | (4) |
| NCL-6-$^{123}I$ | benzo-12-crown-4 70°C | 5 min | 80 | present work |
| NCL-6-$^{82}Br$ | reflux in acetonitrile | 6 hr | 72 | (3) |
| NCL-6-$^{82}Br$ | benzo-12-crown-4 70°C | 10 min | 80 | present work |
| NCL-6-$^{211}At$ | 18-crown-6 70°C | 2 min | 80 | present work |




X = I (NCL-6-I)
X = $^{131}I$ (NCL-6-$^{131}I$)
X = $^{123}I$ (NCL-6-$^{123}I$)
X = $^{82}Br$ (NCL-6-$^{82}Br$)
X = $^{211}At$ (NCL-6-$^{211}At$)

Table 2. Distribution of radioactivity in rats following injection of NCL-6-$^{211}At$[a)]

| Tissue | 6 hr | 24 hr |
|---|---|---|
| Adrenal | 104.65±20.23 | 123.15±21.61 |
| Liver | 20.60±1.27 | 6.65±1.02 |
| Kidney | 5.52±0.5 | 4.58±0.38 |
| Lung | 15.24±2.39 | 11.29±0.70 |
| Spleen | 18.46±2.37 | 10.62±0.53 |
| Blood | 8.11±0.2 | 2.65±0.39 |

a) Values represent mean % administered dose per gram of tissue for 3 rats with S.D. of mean.

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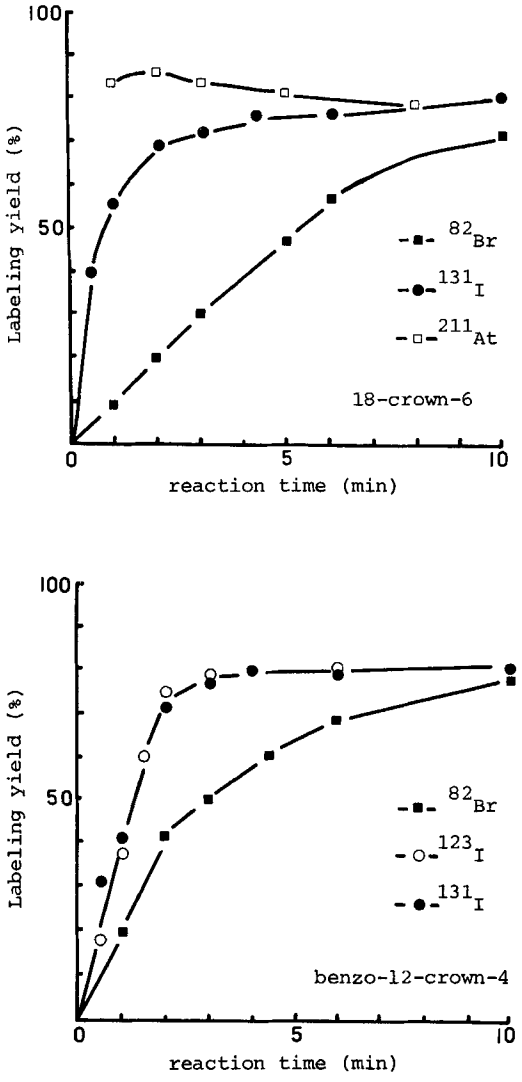


Figure 1. Time dependence of the halogen exchanges of 6-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-I) in crown ethers

## IODO-BEADS METHOD OF LABELING AND EVALUATION OF POTENTIAL TUMOR, BRAIN AND PANCREAS AGENTS

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In order to explore the possibility of Chloramine-T derivatized polystyrene beads or Iodo-beads method of labeling(1,2), potential agents for tumor, brain and pancreas were radioiodinated and the biodistributions were studied in mice.

The agents that were chosen are: a) Tumors: adriamycin, bleomycin, heparin, human serum albumin(HSA), insulin, and transferrin; b) Brain and pancreas: dipicolinic acid, ATP, methionine, tryptamine, dextrose, aminoethanethiol, and thymidine.

The labeling consisted of adding high specific activity $Na^{125}I$ to 1 mg of each substance dissolved in 1 ml of phosphate buffer at pH 7. To this mixture, 3 to 4 iodo-beads were added and incubated for 30 minutes at $\sim 20^{\circ}C$. The iodination was stopped by separating the solid beads from the reaction mixture.

The potential tumor specific agents were injected into S-180 solid tumor bearing ICR strain female mice and the biodistributions were studied at 2 days post intravenous injection. While the potential brain and pancreas agents were administered intravenously to ICR strain normal female mice and the biodistributions were studied at ½-hour post injection. The results were presented in Tables I and II.

The study showed that transferrin, adriamycin, and human serum albumin exhibit better localization in S-180 tumors when compared to bleomycin, insulin, and heparin.

Dipicolinic acid, ATP, thymidine, and dextrose showed relatively high uptake in brain while methionine, tryptamine, dipicolinic acid, ATP, aminoethanethiol, and thymidine indicate better uptake in the pancreas.

A logical extension of this work would be to label the promising tumor agents including the monoclonal antibodies with I-131 and the brain and pancreas agents with I-123 because of their suitable imaging qualities.

(1) "IODO-BEADS$_{TM}$: Bioresearch Products Technical Bulletin", PIERCE CHEMICAL CO., Rockford, IL., 61105, U.S.A.

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TABLE I. BIODISTRIBUTION OF RADICIODINATED TRACERS IN TUMOR-BEARING MICE

2-day post i.v. (Mean ± S.D. of 3 animals)

| %/Gram | ADRIAMYCIN | BLEOMYCIN | HEPARIN | HSA | INSULIN | TRANSFERRIN |
|---|---|---|---|---|---|---|
| Blood | 1.89 ± 0.34 | 0.24 ± 0.08 | 0.42 ± 0.22 | 1.80 ± 0.28 | 0.65 ± 0.16 | 4.30 ± 1.72 |
| Heart | 0.79 ± 0.10 | 0.12 ± 0.04 | 0.28 ± 0.08 | 0.53 ± 0.09 | 0.29 ± 0.07 | 1.35 ± 0.34 |
| Liver | 2.86 ± 1.34 | 0.62 ± 0.34 | 1.59 ± 0.21 | 0.51 ± 0.09 | 0.41 ± 0.06 | 1.62 ± 0.34 |
| Lung | 1.75 ± 0.07 | 0.29 ± 0.13 | 0.55 ± 0.07 | 1.11 ± 0.34 | 0.89 ± 0.09 | 2.76 ± 0.56 |
| Kidney | 1.34 ± 0.24 | 0.42 ± 0.06 | 0.89 ± 0.25 | 0.69 ± 0.10 | 0.50 ± 0.12 | 2.08 ± 0.45 |
| Stomach | 37.54 ±19.03 | 3.91 ± 2.26 | 6.71 ± 6.59 | 5.19 ± 3.97 | 16.54 ± 4.08 | 18.73 ±17.00 |
| Spleen | 1.79 ± 0.51 | 0.28 ± 0.06 | 0.76 ± 0.23 | 0.42 ± 0.12 | 0.37 ± 0.06 | 1.77 ± 0.86 |
| Muscle | 0.43 ± 0.10 | 0.06 ± 0.02 | 0.12 ± 0.04 | 0.30 ± 0.07 | 0.16 ± 0.04 | 0.74 ± 0.13 |
| Brain | 0.12 ± 0.006 | 0.02 ± 0.00 | 0.04 ± 0.03 | 0.08 ± 0.006 | 0.06 ± 0.03 | 0.24 ± 0.05 |
| Tumor | 1.18 ± 0.13 | 0.16 ± 0.05 | 0.42 ± 0.10 | 0.76 ± 0.08 | 0.42 ± 0.11 | 1.95 ± 0.49 |

TABLE II. BIODISTRIBUTION OF RADIOIODINATED TRACERS IN NORMAL MICE

½-hour post i.v. (Mean ± S.D. of 4 animals)

| %/Gram | DIPICOLINIC | ATP | TRYPTAMINE | METHIONINE | Am.Eth.Thiol* | DEXTROSE | THYMIDINE |
|---|---|---|---|---|---|---|---|
| Blood | 6.75 ± 0.69 | 8.23 ± 1.82 | 4.82 ± 0.42 | 5.22 ± 0.43 | 6.42 ± 0.83 | 5.13 ± 0.57 | 5.36 ± 0.34 |
| Heart | 2.84 ± 0.24 | 3.07 ± 0.72 | 1.96 ± 0.25 | 2.01 ± 0.13 | 2.49 ± 0.30 | 2.04 ± 0.22 | 2.12 ± 0.07 |
| Liver | 6.10 ± 1.09 | 4.11 ± 0.86 | 3.26 ± 0.21 | 2.05 ± 0.15 | 2.63 ± 0.20 | 4.54 ± 0.58 | 2.79 ± 0.23 |
| Kidney | 7.42 ± 1.03 | 9.44 ± 3.33 | 4.32 ± 0.24 | 3.15 ± 0.32 | 4.02 ± 0.71 | 8.20 ± 1.32 | 5.39 ± 0.41 |
| Stomach | 36.47 ± 7.83 | 24.62 ±12.88 | 18.96 ± 4.90 | 41.41 ± 5.43 | 68.91 ±15.84 | 27.83 ± 9.11 | 30.89 ± 2.85 |
| Spleen | 3.70 ± 0.32 | 3.69 ± 0.52 | 3.29 ± 0.19 | 3.84 ± 0.37 | 3.93 ± 0.36 | 2.83 ± 0.45 | 3.65 ± 0.31 |
| Pancreas | 4.43 ± 0.30 | 4.54 ± 0.47 | 3.74 ± 0.43 | 4.67 ± 0.73 | 5.11 ± 0.75 | 3.46 ± 0.42 | 4.86 ± 0.81 |
| Muscle | 1.67 ± 0.25 | 2.10 ± 0.55 | 1.44 ± 0.31 | 1.43 ± 0.27 | 1.59 ± 0.17 | 1.36 ± 0.08 | 1.40 ± 0.10 |
| Brain | 0.57 ± 0.07 | 0.53 ± 0.11 | 0.26 ± 0.02 | 0.30 ± 0.06 | 0.28 ± 0.05 | 0.42 ± 0.06 | 0.27 ± 0.02 |

| %/Organ | DIPICOLINIC | ATP | TRYPTAMINE | METHIONINE | Am.Eth.Thiol* | DEXTROSE | THYMIDINE |
|---|---|---|---|---|---|---|---|
| Blood | 11.06 ± 1.16 | 13.98 ± 3.06 | 8.08 ± 0.32 | 9.09 ± 0.82 | 8.02 ± 0.91 | 6.36 ± 0.55 | 6.78 ± 0.32 |
| Heart | 0.25 ± 0.02 | 0.27 ± 0.05 | 0.19 ± 0.04 | 0.20 ± 0.01 | 0.20 ± 0.02 | 0.16 ± 0.02 | 0.16 ± 0.01 |
| Liver | 8.65 ± 1.07 | 6.43 ± 1.39 | 4.96 ± 0.22 | 3.26 ± 0.13 | 2.39 ± 0.30 | 4.44 ± 0.42 | 2.85 ± 0.15 |
| Kidney | 1.93 ± 0.28 | 2.55 ± 0.98 | 1.20 ± 0.04 | 0.94 ± 0.10 | 0.89 ± 0.20 | 1.74 ± 0.29 | 1.16 ± 0.08 |
| Stomach | 17.27 ± 2.75 | 10.14 ± 3.48 | 13.15 ± 1.39 | 22.63 ± 3.73 | 23.23 ± 1.69 | 11.63 ± 2.16 | 16.30 ± 1.47 |
| Spleen | 0.47 ± 0.06 | 0.33 ± 0.07 | 0.38 ± 0.05 | 0.45 ± 0.03 | 0.36 ± 0.09 | 0.27 ± 0.02 | 0.33 ± 0.07 |
| Pancreas | 0.73 ± 0.15 | 0.81 ± 0.15 | 0.68 ± 0.06 | 0.92 ± 0.15 | 0.64 ± 0.18 | 0.41 ± 0.09 | 0.75 ± 0.26 |
| Muscle | 16.78 ± 2.49 | 22.02 ± 6.38 | 14.68 ± 2.36 | 15.25 ± 2.93 | 12.14 ± 0.75 | 10.34 ± 0.34 | 10.90 ± 0.68 |
| Brain | 0.26 ± 0.03 | 0.22 ± 0.03 | 0.11 ± 0.01 | 0.12 ± 0.01 | 0.12 ± 0.03 | 0.18 ± 0.03 | 0.12 ± 0.01 |

* represents Amino Ethane Thiol.

# $^{131}$I-LABELED PEANUT LECTIN FOR THE QUANTITATIVE DETERMINATION OF RENAL TUBULAR FUNCTION

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Current radiopharmaceutical methods for determining renal function rely on small molecular weight compounds such as $^{131}$I-hippuran, $^{99m}$Tc DMSA, and $^{99m}$Tc DTPA for the quantitation of renal plasma flow or glomerular filtration rate. To date, the use of receptor specific ligands or antibodies in the assessment of renal function has not been reported. We are now reporting on a novel approach for the assessment of renal tubular function via a receptor-specific radiopharmaceutical.

Peanut Lectin (PNA) is a plant protein of 105,000 MW which binds specifically to the β-D-galactosyl (1-3)α-N-acetyl-D-galactosamine moiety, the immunodeterminent group of the T-antigen.(1) Iodinated Peanut lectin ($^{131}$I-PNA) has recently been used as a tumor-seeking agent for the in-vivo detection of tumor expressing the T-antigen in animals and humans. (2,3) During preliminary animal and human studies, $^{131}$I-PNA was shown to be rapidly excreted by the kidneys and cleared into urine. This has prompted further investigation as to the pharmacokinetic and renal parameters of $^{131}$I-PNA.

PNA was iodinated with $^{131}$I by the iodogen method, and radiochemical purity achieved by gel column chromatography (Biogel P6 DG) and was injected intravenously into dogs and rabbits. Normalized plasma pharmacokinetics were best described by a two compartment exponential model ($A_o$ = 68.23 ± 7.41%, $B_o$ = 39.75 ± 7.81%, $t_{\frac{1}{2}\alpha}$ = 0.31 ± 0.03 hrs, $t_{\frac{1}{2}\beta}$ = 1.19 ± 0.16 hrs, $CL_{TB}$ = 17.52 ± 8.74 ml min$^{-1}$) Computer acquired gamma camera data of kidneys ( 1 frame/ 30 sec) was analyzed by exponential curve fitting ($t_{max}$ = 44.6 ± 4.8 min, $Activity_{max}$ = 21.8 ± 3.3%) and urine counts revealed 17.5 ± 2.8% of injected activity in the bladder at 1 hour P.I. Cumulative 48 h urine collection recovered 73.5 ± 9.8% of injected dose. Trichloroacetic acid precipitation, asialo $GM_1$ synsorb binding assay and gel column chromatography of urine confirmed the presence of intact $^{131}$I-PNA in the bladder.

Histological and autoradiographic studies of kidney slices indicate concentration on the basement membrane of renal tubules following in-vivo injection of FITC or $^{125}$I-labeled PNA.

A receptor-mediated tubular endocytosis/exocytosis model is proposed for the excretion of $^{131}$I-PNA and suggests a potential use of this lectin in the in-vivo evaluation of renal tubular metabolic function.

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# LABELING OF $^{131}$I-mIBG WITH HIGH SPECIFIC ACTIVITY FOR RADIOTHERAPEUTICAL PURPOSES

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The scintigraphic imaging of pheochromocytoma (1) and neuroblastoma (2) using $^{131}$I-mIBG revealed an extraordinary high uptake of the injected radioactivity in most of the tumors. The calculation of the absorbed dose yielded up to 40 rd/mCi $^{131}$I-mIBG for pheochromocytoma and 26 rd/mCi $^{131}$I-mIBG for neuroblastoma which appeared to be sufficient for radionuclide therapy (2,3). A prerequisite for such a therapy with $^{131}$I-mIBG is a high specific activity of the radiotherapeutical. Unnecessary high mIBG concentrations could saturate the binding sites in the target tissue. Additionally mIBG which acts as an adrenergic neuron-blocking agent may cause side effects if applied in high doses.

We have radiolabeled mIBG more than 50 times for diagnostic purposes according to a modified iodine-isotope exchange procedure recently described in the literature (4). The enhancement of the radiochemical yield in presence of $(NH_4)_2SO_4$ could be confirmed. We used, however, higher temperatures in order to receive an exchange of >90%. At the temperatures described in the literature we received rather low yields. This effect may be due to our modification outlined below. The Table summarizes the results of some of the experiments dealing with high specific activity labeling which were performed according to the following procedure: Solutions of mIBG, $(NH_4)_2SO_4$, Na$^{131}$I and where indicated AcOH were transferred to a 5 ml ampoule. The solvents were evaporated on a rotavapor. Addition of $EtOH_{abs.}$ and evaporation to dryness again reduced the water content in the salt mixture to a minimum. Afterwards the glass ampoule was sealed and transferred to an oven. The exchange reaction was performed at 185°C for 20 minutes. The cold reaction mixture was then dissolved in isot. NaCl, filtrated over an anion exchange gel and filtrated sterile.

The following conclusions can be drawn from the labeling results in the Table:

1. The exchange labeling yield decreased with the amount of activity added. This effect was observed with reactions 1 - 5. The drop of $^{131}$I-mIBG formation seemed to depend on the amount of NaOH added with the radioiodine. $(NH_4)_2SO_4$ alone did not maintain the necessary low pH for optimal exchange conditions.
2. The radiochemical yield increased when AcOH was added to the exchange mixture prior to evaporation. Reactions 6 - 9 clearly demonstrated the improved labe-

ling yields with acceptable high specific activity concentrations. The loss of activity after evaporation was less than 2%.

3. The radiochemical yield dropped with the decrease of the mIBG/$^{131}I^-$ ratio. This effect may be interpreted with the mass action law (reactions 6 - 9).

4. Too much $(NH_4)_2SO_4$ was responsible for a further decrease of the exchange yield in reaction 1. Although only a 2.5 fold amount of $(NH_4)_2SO_4$ was applied by comparison with reaction 2 a by 15% smaller yield of $^{131}I$-mIBG was obtained. This must be interpreted by a dilution effect.

5. High dose labeling with a specific activity of more than 1o mCi/mg mIBG with resonable exchange yields may be obtained when AcOH is present for the neutralization of large amounts of alkaline $Na^{131}I$ solutions. Addition of 5 - 1o mg $(NH_4)_2SO_4$ as an exchange catalyst should be sufficient.

The question whether saturation of the binding sites in the target tissue occurs with the increase of carrier mIBG was determined by quantitative evaluation of the tumor-activity kineticts. Sequential scintigrams were taken of a patient suffering from a pheochromoblastoma of the right adrenal after the application of 2.1 mCi (o.8 mg) and 74.4 mCi $^{131}I$-mIBG (7.4 mIBG). The activity-time curves over the tumor are depicted in the Figure. The curves decayed parallel with a distance factor of 39.8 at 24 hr pi. The ratio of the injected activities was 35.4. The maximal activity uptake was 3.4% and 3.5% after application of 2.1 and 74.4 mCi $^{131}I$-mIBG, respectively. The effective half lifes amounted to 32.4 hr (2.1 mCi) and 3o.6 hr (74.4 mCi). The data clearly indicated that no saturation occured with the increase of carrier mIBG within the mass range of o.8 to 7.4 mg.

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(2) Kimmig B., Brandeis W.E., Eisenhut M., Bubeck B., Hermann H.J., zum Winkel K., Nuc Compact 14, 347 (1983)

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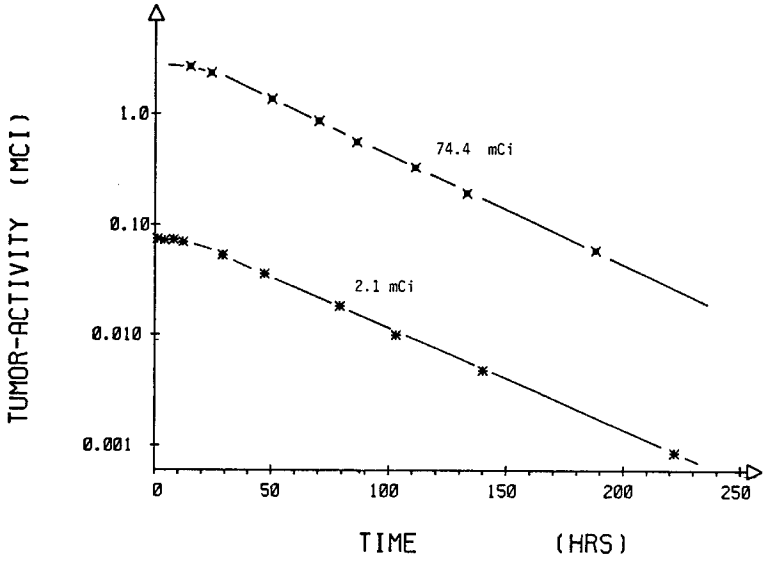
Table

| No. | m-IBG | $(NH_4)_2SO_4$ | AcOH | $Na^{131}I$ | yield | act. conc. |
|---|---|---|---|---|---|---|
| | mg | mg | /ul | mCi (/ul) | mCi (%) | mCi/mg |
| 1 | 19.5o | 65.o | - | 294 (3oo) | 72.o (24.5) | 3.7 |
| 2 | 1o.56 | 12.5 | - | 146 (15o) | 57.5 (39.5) | 5.4 |
| 3 | 19.71 | 12.3 | - | 15o (15o) | 66.5 (44.3) | 3.4 |
| 4 | o.85 | 12.o | - | 11.5 (2o) | 8.3 (72.2) | 9.8 |
| 5 | o.8o | 6.o | - | 1.6 (5) | 1.5 (93.8) | 1.9 |
| 6 | 1.12 | 12.o | 5 | 91.4 (4o) | 18.1 (19.8) | 16.2 |
| 7 | 1.85 | 12.o | 5 | 145 (18o) | 35.o (24.2) | 18.9 |
| 8 | 5.53 | 3o.o | 5 | 79 (13o) | 48.1 (6o.9) | 8.7 |
| 9 | 1.o8 | 11.o | 5 | 14.7 (2o) | 13.3 (9o.5) | 12.3 |



EVALUATION OF $^{75}$Se-LABELED CARAFATE AS A TRACER FOR DRUG KINETIC STUDIES

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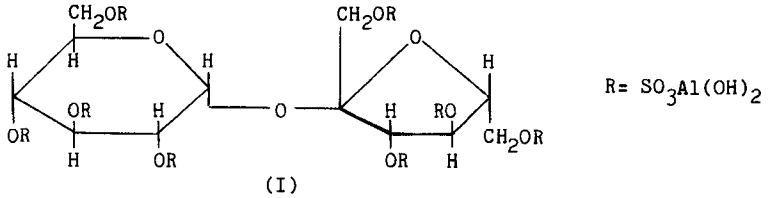
The drug Carafate, Basic Aluminum Sucrose Octasulfate (I), is administered orally for the treatment of peptic ulcers. Among its other actions, it polymerizes in dilute acid and binds protein in vitro, and is believed to physically coat ulcers to protect them from digestive action to allow healing to occur (1,2). A suitable gamma-emitting radiolabel was sought for Carafate to permit in vivo measurements of kinetics of gastric emptying rates and binding to gastric and duodenal ulcers. Although $^{99m}$Tc-HSA-Carafate has been reported (3), an intermolecular label was felt to be necessary to accurately monitor the behavior of the core of the molecule.




(I)

Radiolabeling of Carafate with Se-75 involved substituting trace quantities of Se-75 for sulfur atoms in the molecule. The first step of the synthesis was the sulfation/radioselenation of sucrose by a complex prepared from Se-75 selenic acid, sulfuric anhydride and dry pyridine. The product was crystallized as the potassium salt (20% labeling yield), which was then converted to the basic aluminum salt of Se-75-sucrose octasulfate by addition of excess $Al(OH)_2Cl$ solution (48% yield). The final specific activity was 100 uCi/g. When mixed with 0.1N HCl, the material polymerized and >90% of the Se-75 was associated with the polymer. HPLC analysis of the material revealed that it is composed of a mixture of levels of sulfation (octa, hepta and hexa), but otherwise resembled standard carafate.

$^{75}$Se-Carafate was administered orally to rats with acetic acid-induced gastric ulcers. Gamma camera imaging was used to follow the emptying of the material from the stomach, and showed that Se-75 activity remained bound to the ulcer site after the stomach had emptied. At 5.5 hours post dosing, the rats' stomachs were removed and rinsed. The ratio of Se-75 uptake at the ulcer site compared with normal stomach tissue (by weight) was 15.4 $\pm$ 6.1. When the experiment was performed with Tc-99m-HSA-Carafate or In-111-Carafate (In-111 substituted for Al atoms in the molecule), the uptake ratios were 5.6 and 6.3, respectively.

Biodistribution studies of Se-75-Carafate and C-14-Carafate in normal rats showed that about 13% of Se-75 and 10% of C-14 activity is absorbed from the GI tract. This represents far less absorption than was observed with Tc-99m-HSA-Carafate (3), which may be broken down in the intestine.

It is concluded that $^{75}$Se-Carafate is a better marker for Carafate than the Tc-99m-HSA- or In-111-labeled Carafates, is similar in biodistribution to the C-14-labeled material, has chemical and physical characteristics similar to unlabeled Carafate, and is therefore a suitable gamma-emitting tracer for following the kinetics of the drug Carafate in vivo in human subjects.

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SUMMARY OF BIODISTRIBUTION STUDY OF LABELED CARAFATE IN RATS

$^{75}$Se-CARAFATE
% administered dose/organ

| | 3 hr | 5 hr | 24 hr | 48 hr | 1 week |
|---|---|---|---|---|---|
| GI tract | 91.2 | 93 | 42.6 | 2.78 | 0.68 |
| feces* | 0.0 | 0.02 | 44.1 | 81.3 | 82.3 |
| blood | 2.2 | 4.98 | 3.8 | 2.7 | 0.12 |
| kidneys | 0.68 | 0.78 | 1.3 | 1.4 | 0.51 |
| urine* | 1.65 | 1.64 | 6.0 | 9.1 | 13.3 |
| liver | 1.94 | 1.83 | 2.2 | 2.4 | 1.37 |
| spleen | 0.36 | 0.13 | 0.17 | 0.07 | 0.08 |
| lung | 0.19 | 0.18 | 0.32 | 0.25 | 0.27 |

$^{14}$C-CARAFATE
% administered dose/organ

| | 3 hr | 5 hr | 24 hr | 48 hr | 5 days |
|---|---|---|---|---|---|
| GI tract | 97.1 | 5.86 | 5.45 | 0.988 | 0.194 |
| feces* | 0.057 | 0.0 | 83.7 | 90.0 | 91.6 |
| blood | 0.27 | 0.074 | 0.092 | 0.025 | 0.024 |
| kidneys | 0.66 | 0.104 | 0.227 | 0.159 | 0.071 |
| urine* | 1.36 | 4.15 | 9.31 | 8.21 | 7.82 |
| liver | 0.482 | 0.060 | 0.255 | 0.250 | 0.006 |
| spleen | 0.012 | 0.116 | 0.019 | 0.016 | 0.006 |
| lung | 0.020 | 0.016 | 0.046 | 0.095 | 0.028 |

*cumulative
Each value is the mean based on three animals

# DEVELOPMENT OF HPLC METHODS FOR THE ANALYSIS OF RADIOIODINE AND FOR THE PURIFICATION AND QUALITY CONTROL OF IODINATED RADIOPHARMACEUTICALS

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A wide variety of iodination techniques are employed for preparing radiopharmaceuticals based on iodine-123 and other iodine nuclides. The success of many of these methods depends on the chemical nature of iodine in the radioiodine solutions. This study was undertaken to develop HPLC methods for the analysis of iodine in various chemical forms, and also for the characterization and quality control of a number of iodinated compounds of interest to nuclear medicine. Although various paper and thin-layer chromatographic systems have been used, HPLC offers a better alternative because of its higher sensitivity, speed, and resolving capabilities. Materials that were studied included BLIP-produced (p,5n) iodine-123, commercial radioiodine samples, and radiolabeled iodoamphetamine (IMP), HIPDM, and deoxyuridine (IUDR).

A number of reverse-phase columns ($C_2$, $C_8$, $C_{18}$) and solvent mixtures were evaluated for optimal separation efficiency. RP8 Lichrosorb columns and an eluting solution (pH 7) containing 0.05 M phosphate, 0.002 M $NBu_4OH$, and 10% acetonitrile provided the best separation of various iodine species. Using well characterized standards, elution factors (k' = Elution volume − column volume/column volume) were established for $IO_3^-$, $I^-$, $I_3^-$, $IO_4^-$, $I^+$, $CH_3I$, and $CHI_3$. Typical results are summarized in Table 1. Separation of iodate, iodide, and periodate in a synthetic mixture is depicted in Figure 1. Analysis of BLIP-produced I-123 samples (using optimized processing chemistry (1) - decay of Xe-123 precursor in an ampoule in the presence of 0.5 atm. $H_2S$, evacuation of the ampoule, rinsing out with base) consistently gave >95% iodide and almost no iodate.

Less than 5% of other unidentifiable species were also occasionally separated in some I-123 solutions. When the samples were subjected to cycles of oxidation and reduction using hypochlorite, hydrogen peroxide, chloramine T, thiosulfate, metabisulfite, $H_2S$, $SO_2$, etc., formation of a number of different species was demonstrable. A method was also developed for determining the carrier iodine content of radioiodine solutions by monitoring the uv absorption of HPLC fractionated samples at 225 nm. The standard curve showed a linear relationship between 0.01 and 10 μg iodide.

Iodoamphetamine was iodinated with I-123 using an exchange reaction in refluxing glacial acetic acid. This reaction was found to be somewhat sensitive to iodate impurity in radioiodide solutions. Carrier-free IMP was prepared by iodinating bromoamphetamine (BrMP) followed by separation of IMP and BrMP on a $C_{18}$ reverse-phase column using pH 7, 0.05 M phosphate in 40% → 60% methanol. Both radioactive detection and uv detection at 265 nm were utilized. Animal experiments demonstrated the purity of the HPLC fractionated product. HIPDM was iodinated with I-123 using the kit method of Kung and coworkers (2). High labeling yields (∿95%) were obtained even when considerable amounts of iodate were present in iodide solutions. A satisfactory HPLC separation was achieved by using a $C_{18}$ reverse-phase column and a solvent mixture containing 0.05M phosphate in 30% acetonitrile, pH 5.0. The effect of pH on the HIPDM labeling reaction was followed by HPLC: at pH 2, only one major product peak was seen whereas at pH 3.6, 2-5% of an additional product was formed. The HPLC results correlated well with the TLC analysis.

Iodinated deoxyuridine was separated on $C_{18}$ reverse-phase columns using a pH 5, 0.05 M phosphate buffer in 10% methanol. The elution factors were as follows: 0 (Uracil); 0.40 (UDR); 1.30 (iodouracil); and 2.50 (IUDR). Unbound iodide eluted much later with considerable tailing.

Results of this study demonstrate the effectiveness of HPLC in allowing a fast and reliable separation of various chemical forms of iodine as well as of a number of iodinated radiopharmaceuticals. The developed methods provide excellent resolution and appear superior to the commonly used thin-layer and paper chromatographic techniques.

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Table 1. Analysis of Various Radioiodine Species (k' values)[a] by $C_2$, $C_8$ and $C_{18}$ Reverse-Phase High Performance Liquid Chromatography[b]

| | 100% Aqueous | | | 10% Acetonitrile | | | 40% Acetonitrile | | |
|---|---|---|---|---|---|---|---|---|---|
| Species | $C_2$ | $C_8$ | $C_{18}$ | $C_2$ | $C_8$ | $C_{18}$ | $C_2$ | $C_8$ | $C_{18}$ |
| $IO_3^-$ | 0.09 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $I^-$ | 1.55 | 4.86 | 3.29 | 0.73 | 1.21 | 0.43 | 0.09 | 0 | 0 |
| $IO_4^-$ | 11.2 | - | R[c] | 3.55 | 5.36 | 6.57 | 1.36 | - | 0 |
| $CH_3I$ | 1.64 | - | 3.71 | 1.55 | 4.14 | 2.86 | 1.0 | 1.57 | 1.0 |
| $CHI_3$ | R | - | R | R | R | R | 3.0 | 10.7 | 3.71 |

[a] $k' = \frac{\text{elution volume (Ve)} - \text{column volume (Vo)}}{\text{Vo}}$

[b] Elution buffer was a 0.05 M pH 7 phosphate solution containing the stated amount of aqueous or organic phase and 0.002 M tetrabutylammonium hydroxide.

[c] Retained

Acknowledgments. The labeling procedures and ligands used in this study were kindly provided by Medi-Physics, Inc. (iodoamphetamine), and H. Kung of SUNY, Buffalo (HIPDM). This research was supported by the U.S. Department of Energy under Contract #DE-AC02-76CH00016.

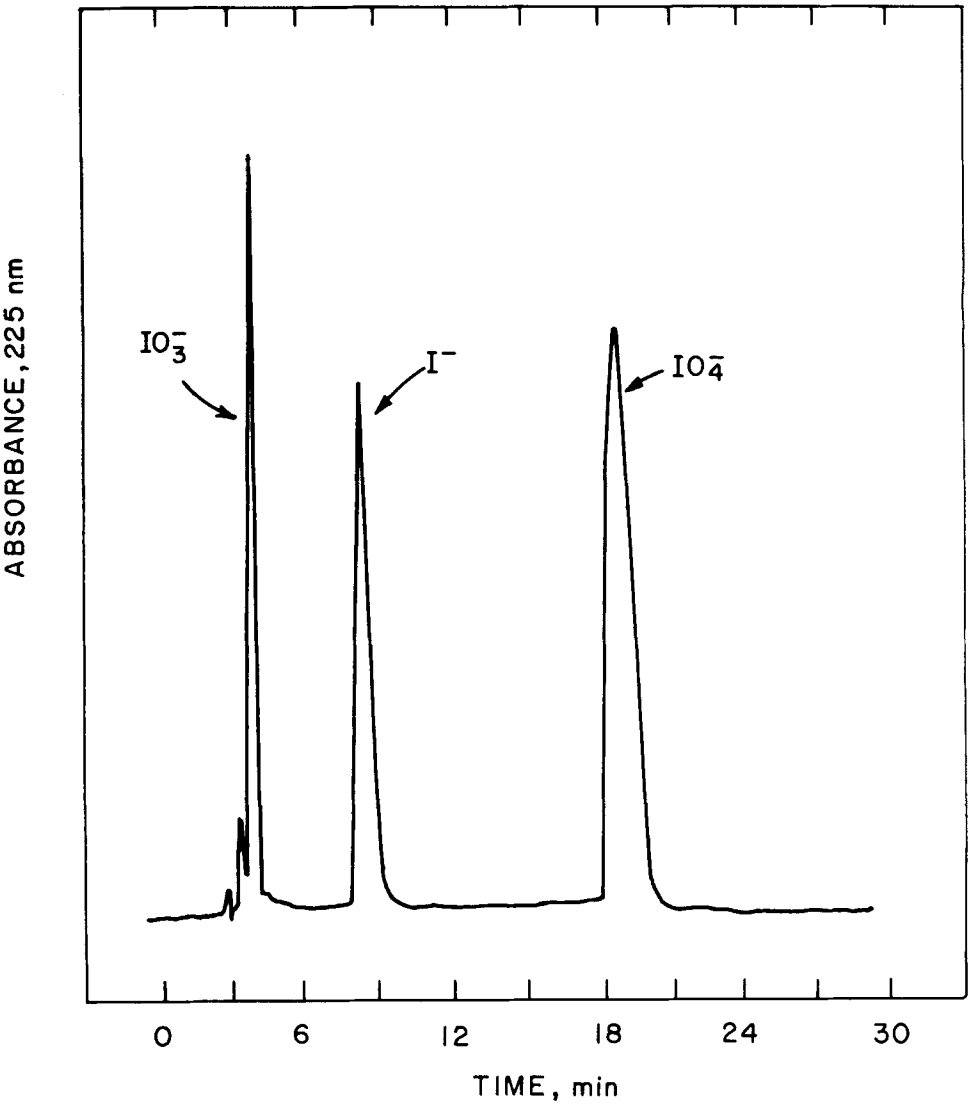


Figure 1. HPLC fractionation of a freshly prepared synthetic mixture containing iodate, iodide, and periodate. (Upon aging the iodide gets oxidized by periodate to produce iodate). Column: RP8 Lichrosorb 4.6x250 mm. Eluting buffer: 0.05 M phosphate and 0.002 M $Bu_4NOH$ in 20% methanol, pH 7. Temp. 22°, flow rate 1 ml/min.

SOURCES OF CARRIER BROMINE IN HIGH SPECIFIC ACTIVITY RADIOBROMINATION REACTIONS AS DETERMINED BY NEUTRON ACTIVATION ANALYSIS.

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Radiobrominated compounds are of interest as receptor-binding radiopharmaceuticals (1). However, obtaining the radiobrominated compounds in high specific activity as needed for non-saturation of the specific receptors has been a difficult task even though high specific activity radiobromine has been used. The radionuclide of bromine which has been most often employed for high specific activity radiobrominations is bromine-77. Production of this nuclide (2) by 800 MeV spallation reactions on a molybdenum target at the Los Alamos Meson Physics Facility has yielded curie quantities, which have specific activities > 5000 Ci/mmole. Despite this, specific activities of labelled compounds using bromine-77 have been considerably lower (3).

The decrease in specific activity observed when labelling with bromine-77 led to an investigation of the concentration of the stable nuclides of bromine in the different components of some radiobromination reactions. The investigation used neutron activation analysis as a sensitive method of determining the concentration of bromine.

We chose to analyze some commonly employed components of radiobromination reaction mixtures. Thus, the reaction mixtures were divided into four general components for the purposes of our evaluation. The analyses of the components of the reaction mixtures, the solvent(s), the reagents, and the substrates are shown in Table 1, 2 and 3 respectively. More analyses are presently being carried out and these will also be presented.

[+] Medical Radioisotope Research (CNC-3), Los Alamos National Laboratory, Los Alamos, New Mexico.

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TABLE I

Stable Bromine in Solvents

| Solvent | Conc. (ng/ml) |
|---|---|
| MeOH | 154 |
| HOAc | 8 |
| $H_2O$ (single dist.) | 40 |
| $H_2O$ (Barnstead) | 4 |
| EtOH (USP Brand A) | 8 |
| EtOH (USP Brand B) | 40 |

TABLE II

Stable Bromine in Reagents

| Reagents | Conc. (ug/g) |
|---|---|
| NCS (Alfa) | 3.0 |
| NCS (Aldrich) | 189 |
| CAT (Eastman) | 24.3 |
| CAT (MCB) | 5.2 |
| HCl (Allied) | 0.42 |

TABLE III

Stable Bromine in Substrates

| Substrates | Conc. (ug/g) |
|---|---|
| Phenol | 0.09 |
| Estradiol (Sigma) | 2.5 |
| p-MePhSi(Me) | 651 |
| p-MePhSn(n-Bu)$_3$ | 65.1 |

A RAPID SOLID-PHASE RADIOIODINATION KIT FOR THE SYNTHESIS OF IODINE-123 LABELED HIPDM.

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Radioiodinated brain-imaging agents (amines and diamines) are presently being developed to study and diagnose brain physiology (1,2). Because of the need for good imaging resolution of brain scans, and to reduce radiation dose burden to the patient, high purity "I-124 free" I-123 radiopharmaceuticals must be used (3). In addition, rapid and simple-to use radioiodination kits are desirable to maximize the use of the limited supply of pure I-123. A useful characteristic of a radiopharmaceutical kits, is that it should provide a fast and efficient labeling so as to minimize radionuclide losses due to decay and to time-consuming radiochemical purifications.

A solid-phase I-127-for-I-123 isotopic exchange reaction for the rapid and efficient labeling of HIPDM, using (p,5n)-made I-123, was investigated. Several parameters, i.e. carrier HIPDM, solvents, pH, temperature, inert atmosphere, and time-of exchange, were found to affect to some extent labeling efficiency, in spite of the fact that even at room temperature, an appreciable exchange in the liquid phase was measured. The method consists of preparing a buffered solution of HIPDM (5-10 mg/mL, pH 5-6) to which an I-123 NaI solution is added. The solution is quickly evaporated to dryness (preferably by means of a dry-heating block), and the resulting solid mixture is heated for 30-60 sec at HIPDM's submelting temperatures (m.p. HIPDM 180-182°C). Using pH= 5.5 adjusted 0.9% NaCl isotonic saline solution, the labeled HIPDM is dissolved, and filtered. Greater than 98% labeling was measured for several of the conditions studied. A summary of the results obtained under a variety of experimental conditions is given in Table 1. The radiochemical purity of I-123 HIPDM was measured using TLC and paper chromatography methods. Radiochromatograms were scanned using a computerized system and radioactivities were also checked against UV detection of HIPDM. An example of this assay is shown in figure 1.

This method was found to be similarly efficient for the synthesis of small, single-dose batches (5 to 30 mCi), as well as large, multi-dose batches (up to 1.5 Ci). Optimum conditions to yield > 98% labeled HIPDM, were found to be 140 +/- 2°C, I-123 NaI in aqueous (0.1N NaOH or 0.1N HCl) or in organic (EtOH or MeOH) solvents, and using a minimum of 3.7 mg of HIPDM (> 98%, n=30). Radiochemical impurities commonly associated with NCA I-123 NaI, (i.e. I-123 iodate and/or I-123 periodate), had no effect on the HIPDM labeling yield or its radiochemical purity. Therefore, we concluded that this method reduces the I-123 oxidized forms prior to the exchange, making unnecessary the addition of reducing agents (i.e. thiosulfate) to the I-123 NaI solutions. Several analytical techniques (i.e. NMR, HPLC, UV, IR) have shown no detectable chemical modifications to the I-123 HIPDM. I-123 HIPDM prepared by this method also has shown similar in vivo distribution and chemical stability as HIPDM prepared by other methods (2). Because of the simplicity and reliability of this labeling technique, a radioiodination kit has been prepared and is presently being tested.

The development of a similar radioiodination kit for other promising brain-imaging agents, i.e. I-123 n-isopropyl p-iodoamphetamine (1) and I-123 4-iodo- 2,5 dimethoxy-N, N-dimethyl phenylisopropylamine (4), is presently under investigation.

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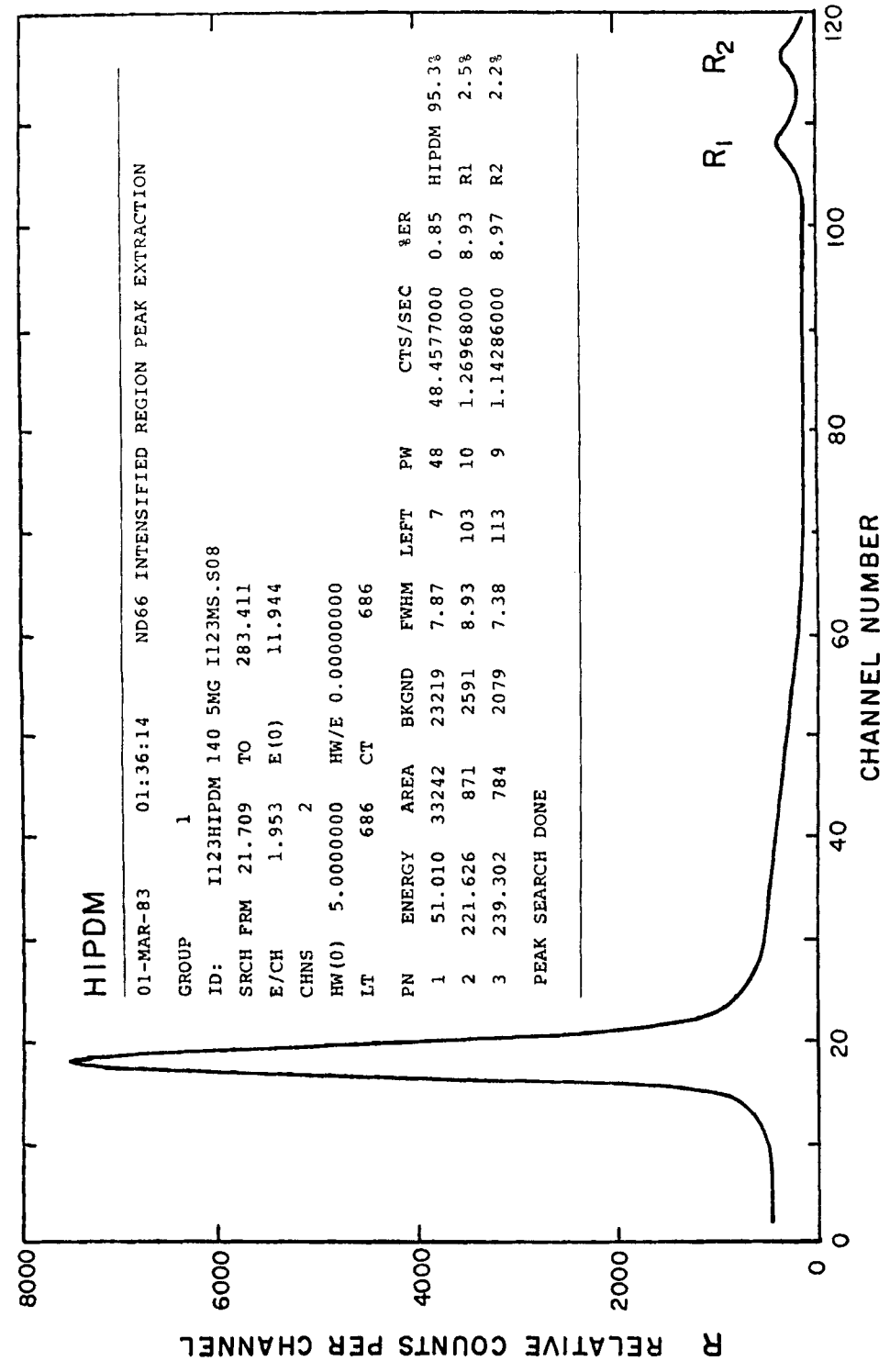
Table 1. Experimental Conditions Studied for Labeling I-123 HIPDM

| HIPDM/Solv. (mg) | I-123 Solv. | pH | I-123 HIPDM (%) | Others (%) | Samples |
|---|---|---|---|---|---|
| Temperature Range: 22-26°C | | | | | |
| 10/$H_2O$ | 0.1N NaOH/$S_2O_3^=$ | 5-6 | 50-80 | 20-50 | 12 |
| 10/$H_2O$ | 0.1N NaOH | 5-6 | 50-80 | 20-50 | 12 |
| Temperature Range: 138-142°C | | | | | |
| 5/$H_2O$ | 0.1N NaOH | 5.5 | > 98 | < 2 | 4 |
| 5/$H_2O$ | 0.1N NaOH/$S_2O_3^=$ | 5.5 | > 98 | < 2 | 4 |
| 10/$H_2O$ | 0.1N NaOH | 5.5 | > 98 | < 2 | 5 |
| 10/$H_2O$ | 0.1N NaOH/$S_2O_3^=$ | 5.5 | > 98 | < 2 | 5 |
| 10/$H_2O$ | 0.1N HCl | 3.0 | > 99 | < 1 | 4 |
| Temperature Range: 148-155°C | | | | | |
| 5/$H_2O$ | 0.1N NaOH | 4-6 | > 95 | < 5 | 11 |
| 0.5/$H_2O$ | Ethanol | 10 | 0 | 100 | 3 |
| 3.7/$H_2O$ | 0.1N NaOH | 5-6 | 97-98 | 3-2 | 3 |
| Temperature Range: 170-172°C | | | | | |
| 5/$H_2O$ | 0.1N NaOH | 6-7 | > 96 | < 4 | 5 |
| 5/$H_2O$ | 0.1N NaOH | 5.0 | > 97 | < 3 | 5 |
| 5/$H_2O$ | 0.1N NaOH/$S_2O_3^=$ | 6-7 | > 96 | < 4 | 6 |
| 5/$H_2O$ | 0.1N NaOH/$S_2O_3^=$ | 5.0 | > 98 | < 2 | 6 |
| 10/$H_2O$ | 0.1N NaOH | 5.0 | > 98 | < 2 | 4 |
| 10/$H_2O$ | 0.1N NaOH/$S_2O_3^=$ | 5.0 | > 98 | < 2 | 4 |
| Temperature Range 178-182°C (melting) | | | | | |
| 10/$H_2O$ | 0.1N NaOH | 8.0 | ~ 70 | ~ 30 | 2 |
| 10/$H_2O$ | 0.1N NaOH | 5.0 | ~ 65 | ~ 35 | 2 |


HIPDM
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ID: I123HIPDM 140 5MG I123MS.S08
SRCH FRM 21.709 TO 283.411
E/CH 1.953 E(0) 11.944
CHNS 2
HW(0) 5.0000000 HW/E 0.00000000
LT 686 CT 686
PN ENERGY AREA BKGND FWHM LEFT PW CTS/SEC %ER
1 51.010 33242 23219 7.87 7 48 48.4577000 0.85 HIPDM 95.3%
2 221.626 871 2591 8.93 103 10 1.26968000 8.93 R1 2.5%
3 239.302 784 2079 7.38 113 9 1.14286000 8.97 R2 2.2%
PEAK SEARCH DONE
R1 R2
R RELATIVE COUNTS PER CHANNEL
CHANNEL NUMBER
0
2000
4000
6000
8000
0
20
40
60
80
100
120

FIGURE # 1

## VALIDATION OF RECEPTOR BINDING RADIOTRACERS

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Receptors have been characterized by an operational definition that involves its binding properties. Kahn(1) has defined a receptor as exhibiting the following properties: (1) binding is rapid and reversible (2) binding is saturable (3) binding is with a high affinity and (4) the radioligand distribution can be correlated with biologic or pharmacologic effects. We have recently prepared 3-quinuclidinyl 4-iodobenzilate using R-quinuclidinol (R-4-IQNB) and validated its binding to the muscarinic acetylcholine receptor (mAChR) using in vitro systems(2,3). The distribution of R-[$^{3}$H]QNB and R-4-IQNB in rats also has been published(4).

Proof of R-4-IQNB binding to mAChR in the intact animal or man is more difficult than proof in vitro because of the many additional variables such as protein binding, transport, and metabolism. This proof is the goal of our most recent efforts. The test for saturable binding for R-[$^{3}$H]QNB and R-4-IQNB show that the binding in the lung, heart, pancreas, cerebellum and corpus striatum is saturable to varying degrees (Tables 1,2). Since the coinjection is carried out with a single amount of nonradioactive QNB, the saturable binding observed for each tissue is not the percentage of the radioligand binding to the receptor. The tissues contain various concentrations of receptor; also, the efficiency of ligand transport to the receptor is different for each ligand. The % saturable binding is the result of a complicated interaction described mathematically by Ekins et al. for equilibrium systems(5). Nevertheless saturable binding is indicated.

Another test for receptor binding is stereoselectively in those cases where the ligand has a pharmacologically active form and inactive form. We have prepared the inactive form of 4-IQNB, S-4-[$^{125}$I]IQNB and studied its distribution in rats (Table 3). Radioactivity is cleared rapidly from organs containing mAChR as expected for the inactive form. In addition to coinjection of non-radioactive ligand, postinjection or "chase" experiments can be used to validate the saturability of the binding sites and test whether the radioactivity is associated with the receptor ligand. Chase experiments were carried out by injecting nonradioactive QNB at 0.5 hours after the injection of R-[$^{3}$H]QNB (Table 4). Again, less radioactivity was observed in the target tissue after the "chase" dose of QNB. The percentage chased is a complicated function of the off rate, the receptor concentration, and other factors mentioned above.

These data and the observation that R-4-[$^{123}$I]IQNB does not localize in the cerebellum of man(4) supplies sufficient support to suggest that the distribution of R-4-IQNB is receptor mediated.

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Table 1. In Vivo Test for Saturable Binding Sites of R-[$^3$H] QNB in Rat

| | time (h)+ | | | | | |
|---|---|---|---|---|---|---|
| Tissue | 1.5 (Control) | 1.5(QNB) | % * | 3(Control) | (QNB) | % * |
| Blood | .060 ± .006 | .064 ± .004 | 0 | .065 ± .006 | .093 ± .005 | 0 |
| Lung | .495 ± .067 | .404 ± .228 | 18 | .397 ± .059 | .278 ± .045 | 29 |
| Heart | 3.56 ± .319 | .206 ± .015 | 94 | 2.90 ± .223 | .278 ± .023 | 90 |
| Liver | .212 ± .020 | .272 ± .038 | 0 | .199 ± .027 | .308 ± .033 | 0 |
| Pancreas | .801 ± .110 | .198 ± .011 | 75 | .704 ± .141 | .227 ± .035 | 67 |
| Cerebellum | .532 ± .091 | .126 ± .017 | 76 | .522 ± .159 | .189 ± .031 | 64 |
| Corpus Striatum | .658 ± .137 | .351 ± .024 | 47 | .647 ± .158 | .558 ± .067 | 13 |

Table 2. In Vivo Test for Saturable Binding Sites of R-4-[$^{125}$I] IQNB in Rat.

| | time (h)+ | | | | | |
|---|---|---|---|---|---|---|
| Tissue | 1.5 (Control) | 1.5 (QNB) | % * | 3(Control) | 3(QNB) | % * |
| Blood | .073 ± .057 | .091 ± .018 | 0 | .136 ± .233 | .234 ± .013 | 0 |
| Lung | 3.66 ± 4.80 | 1.62 ± .303 | 55 | .722 ± .259 | .717 ± .139 | 7 |
| Heart | .505 ± .113 | .195 ± .038 | 61 | .212 ± .108 | .128 ± .013 | 39 |
| Liver | .503 ± .107 | .628 ± .084 | 0 | .446 ± .174 | .496 ± .058 | 0 |
| Pancreas | .851 ± .416 | .514 ± .157 | 40 | .417 ± .251 | .231 ± .049 | 45 |
| Cerebellum | .157 ± .142 | .051 ± .011 | 67 | .116 ± .100 | .024 ± .006 | 79 |
| Corpus Striatum | .302 ± .157 | .190 ± .052 | 37 | .422 ± .129 | .110 ± .011 | 70 |

All values are % Dose/gram wet tissue ± standard deviation for $\geq$ 5 rats per value.

*% = $\frac{\text{Control value - coinjected QNB value}}{\text{control value}}$ x 100 = % saturable binding

25 nmol of R QNB coinjected with R-4-[$^{125}$I] IQNB

Table 3 Distribution of S-4-[$^{125}$I] IQNB in Rat.

| | Time (h)+ | | | | |
|---|---|---|---|---|---|
| Tissue | 0.5 | 1 | 2 | 4 | 6 |
| Blood | .090± .010 | .063 ± .013 | .090 ± .014 | .057 ± .010 | .045 ± .008 |
| Lung | 3.21 ± .556 | 1.92 ± .435 | 1.10 ± .283 | .491± .113 | .421 ± .147 |
| Heart | .242 ± .039 | .175 ± .025 | .123 ± .021 | .055 ± .011 | .026 ± .021 |
| Liver | .708 ± .092 | .632 ± .143 | .686 ± .131 | .416 ± .072 | .440 ± .097 |
| Pancreas | 1.43 ± .271 | 1.14 ± .358 | 1.35 ± .188 | .661 ± .193 | .563 ± .189 |
| Cerebellum | .090 ± .013 | .045 ± .011 | .028 ± .007 | .010 ± .002 | .009 ± .004 |
| Corpus Striatum | .115 ± .016 | .064 ± .018 | 0.041 ± .009 | .014 ± .003 | .010 ± .004 |

+ All values are % Dose/gram wet tissue ± standard deviation for ≥ 5 rats per value

Table 4 Displacement of R-[$^{3}$H] QNB by Postinjection of QNB*

| | Sacrifice Time(h) after injection of R-[$^{3}$H] QNB | | | | | |
|---|---|---|---|---|---|---|
| Tissue | 1.5 (control) | 1.5 (QNB)[a] | % | 1.5 (QNB)[b] | % | 2.5 (QNB)[b] |
| Blood | .060 ±.006 | .122 ± .020 | 0 | .094 ± .016 | 0 | .087± .017 |
| Lung | .495 ± .067 | .899 ± .253 | 0 | .817 ± .208 | 0 | .388 ± .072 |
| Heart | 3.56 ± .319 | 2.51 ± .470 | 29 | 2.57 ± .875 | 28 | 1.41 ± .610 |
| Liver | .212 ± .020 | .341 ± .043 | 0 | .340 ± .092 | 0 | .237 ± .037 |
| Pancreas | .801 ± .110 | .692 ± .182 | 14 | .807 ± .253 | 0 | .267 ± .226 |
| Cerebellum | .532 ± .091 | .366 ± .116 | 31 | .265 ± .078 | 50 | .173 ± .051 |
| Corpus Striatum | .658 ± .137 | .579 ± .161 | 12 | .361 ± .103 | 45 | .317 ± .093 |

*50 nmol QNB[a] or 500 nmol QNB[b] injected 0.5 hr after injection of R-[$^{3}$H] QNB in rat; % Dose/g wet tissue ± standard deviation in ≥ 5 animals per value.

# RADIOSYNTHESIS OF A DOPAMINE RECEPTOR-BINDING RADIOTRACER FOR POSITRON EMISSION TOMOGRAPHY: [$^{11}$C METHYL]-3-N-METHYLSPIPERONE

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Abnormalities in dopaminergic neurotransmission have been implicated in several neurologic and psychiatric disorders, such as Parkinson's disease (1), tardive dyskinesia (2-3), Huntington's disease (4), and schizophrenia (5-10). Neuroleptics, such as spiperone (Figure 1.I), a potent member of the butyrophenone series, elicit extrapyramidal Parkinsonian side effects by blocking dopamine receptors in the corpus striatum and also exert antischizophrenic actions by blocking these receptors (11).

Over the past several years, there has been considerable interest in the development of radiotracers for the external detection and quantification of neuroreceptors *in vivo*. Several laboratories have synthesized drugs labeled with positron-emitting isotopes with the goal of *in vivo* non-invasive measurement of dopamine neurotransmitter receptors in the brain (12-16). As part of our neuroreceptor program to identify and radiolabel high affinity, highly specific ligands for the dopamine receptor, we have selected and synthesized a derivative of spiperone, 3-N-methylspiperone (Figure 1.II), labeled with carbon-11.

Although the synthesis of 3-N-methylspiperone had been previously reported in a patent (17), the known route for its preparation was not suitable for the incorporation of $^{11}$C. Therefore, we rapidly incorporated $^{11}$C into the molecule by the methylation of the amide nitrogen with $^{11}$C methyl iodide in a biphasic reaction mixture (Figure 2). Carbon-11 methyl iodide was prepared from $^{11}$C carbon dioxide (18) and bubbled through a methylene chloride solution of spiperone. Aqueous tetrabutylammonium hydroxide was added with mixing by sonication. The product was isolated by preparative reverse phase high performance liquid chromatography (C-18 HPLC) and analyzed for radiochemical purity by analytical C-18 HPLC.

In 108 consecutive production runs of this radiotracer, the average radiochemical yield has been approximately 35% with an average synthesis time of 36 minutes and an average specific activity of approximately 290 mCi/μmole E.O.S. (1000 mCi/μmole E.O.B.).

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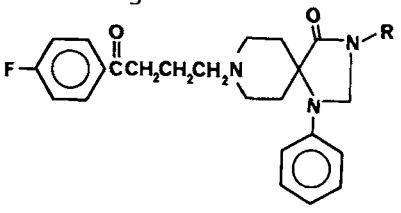
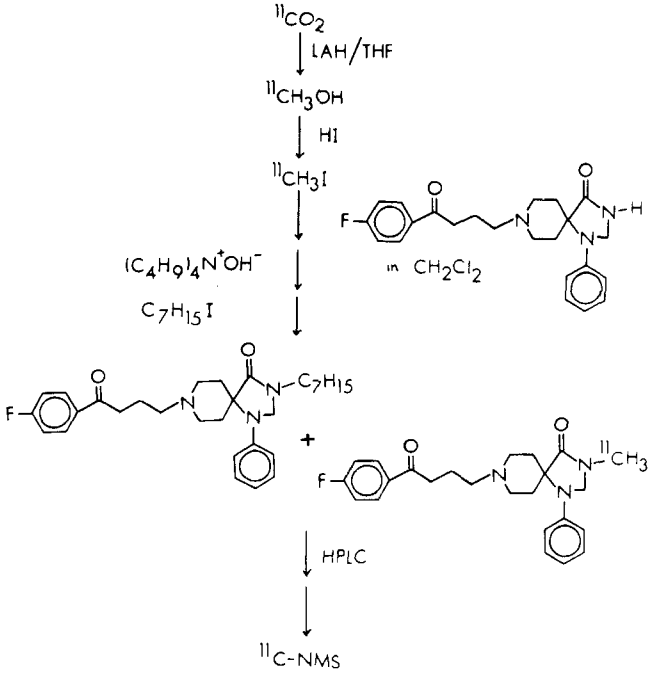
Fig. 1



I. Spiperone R = H
II. 3-N-Methylspiperone R = $CH_3$

Fig. 2 SYNTHESIS OF $^{11}C$-3-N-METHYLSPIPERONE



## A METHOD FOR THE PREPARATION OF 2-($^{11}$C)METHYL-SPIPERONE* — AN AGENT FOR STUDYING DOPAMINE RECEPTOR DISTRIBUTION IN VIVO

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Recently Wagner et al. (1) have shown that 2-($^{11}$C)methyl-spiperone (1) is a good ligand for the dopamine receptor, and can be applied to the determination of the spatial distribution of such receptors in human brain by means of positron emission tomography (PET). Thus 2-($^{11}$C)methyl-spiperone is likely to be of considerable value in the investigation of those neuropsychiatric disorders, such as Parkinson's disease and schizophrenia, that are possibly associated with abnormal dopamine systems. To date no detailed method for the preparation of 2-($^{11}$C)methyl-spiperone has been published. Here we report a satisfactory method for the preparation of 2-($^{11}$C)methyl-spiperone in a form acceptable for clinical use.

($^{11}$C)Iodomethane (prepared from cyclotron-produced ($^{11}$C)carbon dioxide (2)) is collected in cold (0°C) acetone (0.5 mL) and added to a solution of spiperone (2) (2 mg) in acetone (0.5 mL) and aqueous sodium hydroxide solution (10 M; 5μL). This solution is sealed within a reaction vessel (vol. ca 3 mL) and heated at 80°C for 4 min with magnetic stirring. Then the reaction mixture is cooled to room temperature, diluted with pentane (10 mL) and loaded onto a silica gel cartridge (Sep-pak; Waters Associates). The desired product is subsequently eluted with chloroform-methanol (9:1 v/v; 4 mL). The eluate is reduced to ca 0.5 mL and injected onto a silica gel column (30 cm x 0.7 cm i.d.; "Porasil", Waters Associates) eluted with chloroform-methanol (9:1 v/v) at 2 mL/min. 2-($^{11}$C)Methyl-spiperone (retention time, 9.5 min) elutes ahead of unreacted spiperone (retention time, 11.5 min).

2-($^{11}$C)Methyl-spiperone, prepared in this manner, has been shown to be radiochemically and chemically pure by analytical hplc and tlc, using authentic 2-methylspiperone (3) (kindly supplied by Janssen Pharmaceuticals) as reference material. Furthermore, it has been shown that the conditions of the labelling reaction, when applied on a macroscale, afford 2-methylspiperone in good yield from non-radioactive iodomethane. High resolution fourier transform $^{1}$H nmr and $^{13}$C nmr were used to achieve an unambiguous determination of the structure of the prepared 2-methylspiperone. The behaviour of the prepared 2-methylspiperone in hplc and tlc was found to be identical to that of the supplied authentic material.

2-($^{11}$C)Methyl-spiperone is formulated for intravenous injection by rotary evaporation to dryness, solubilisation in ethanol (0.1 mL) and human serum albumin solution (10% v/v; 5 mL), and finally millipore filtration (pore size 0.22 μm; Millex GS, Millipore Corporation).

The procedure requires 40 min from the end of radionuclide production and provides 2-($^{11}$C)methyl-spiperone for injection in 12% radiochemical yield (from cyclotron-produced ($^{11}$C)carbon dioxide, corrected for decay). Analytical hplc using detectors for both radioactivity and mass (by absorbance at 240 nm) indicates that the specific activity of 2-($^{11}$C)methyl-spiperone prepared by the above procedure is 1.9 — 3.7 GBq/μmol (50 — 100 mCi/μmol) prior to formulation.

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*In reference 1 this compound is named as 3-N-($^{11}$C)methylspiperone. Our nomenclature accords with that in U.S. Patent 3155670 (1964), the original description of the preparation of 2-methylspiperone.

The data presented by Wagner et al. (1) indicates that the specific activities of our preparations are high enough to enable the distribution of dopamine receptors in human brain to be measured by PET. Preliminary results (3) from investigations of the binding of 2-($^{11}$C)methyl-spiperone to dopamine receptors in vitro and in vivo are encouraging in this respect.

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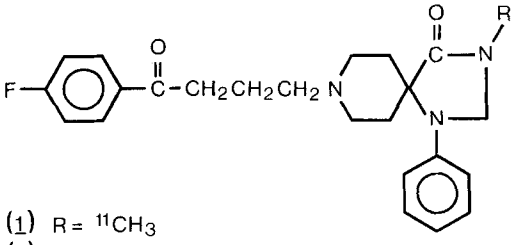

(1) R = $^{11}CH_3$
(2) R = H
(3) R = Me

FLUORINE-18 SPIROPERIDOL; SIMPLE, ONE-STEP SYNTHESIS, PURIFICATION AND DOSIMETRY

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The preparation of spiroperidol, or a derivative thereof, labeled with a positron-emitting radionuclide has been the goal of the many investigators. This interest has led to the synthesis of neuroleptics labeled with carbon-11 (1,2) and fluorine-18 (3). We have for many years sought a simple method for the preparation of fluorine-18 labeled spiroperidol. Although promising at first the triazene method of synthesis has been abandoned, due to the low yields, problems with F-18 HF production, and difficulties in product purification.

We report here a simple, rapid, one-step synthesis of F-18 spiroperidol, using nucleophilic aromatic substitution. Large amounts of F-18 fluoride ion are easily obtained by irradiation of O-18 enriched water (4), and this fluoride can be converted rapidly to reasonably dry and reactive F-18 labeled tetra-n-butylammonium fluoride (carrier-added by exchange with TBAF, and no-carrier-added by reaction with tetra-n-butylammonium hydroxide). The F-18 TBAF is then added to a solution of 2 mg nitro-aromatic precursor (I) in 100 microliters of dimethylsulfoxide, and the solution heated for five minutes at 150 C. The dimethylsulfoxide solution is then simply cooled, diluted with chloroform, and immediately injected onto an HPLC. Separation of the spiroperidol from the nitro-aromatic precursor is achieved on a preparative silica gel column using a methylene chloride:methanol:ammonium hydroxide eluant. The desired spiroperidol is obtained with a retention time of 20 minutes.

The synthesis is done using a remote apparatus in a shielded hood, with all manipulations and reagent additions done from outside. The entire synthesis (from EOB) takes less than 60 minutes. The product is obtained in sterile, pyrogen-free form by evaporation of the HPLC solvent, dissolving the residue in dilute acidic saline, and Millipore filtration.

Analysis of the final product by HPLC indicates a radiochemical purity of 100%. Two cold products are observed: 0-10 micrograms of spiroperidol, and 0-10 micrograms of nitro-aromatic precursor. The cold spiroperidol arises from cold fluoride in the fluorine-18 obtained from the cyclotron irradiation plus that from the TBAF (if done CA). The latter impurity arises from incomplete separation on the HPLC: the nitro compound elutes first, and a small amount (<1% of the total amount) inevitably trails into the following spiroperidol peak.

The overall yield of this reaction is low (<2%, not corrected for decay). Although not that much better than previous methods of synthesis of F-18 spiroperidol, this synthesis has proven amenable to the production of quantities for human studies (5-6 mCi): large amounts (500 mCi) of F-18 TBAF can be easily prepared, the synthetic manipulations are simple and easily remoted, and the product obtained in suitable radiochemical and chemical form by one quick HPLC purification. The specific activity of the final product ranges from 200-1000 Ci/mmol (determined by quantitative HPLC), depending on whether the synthesis is done carrier-added, and on the overall yield.

We have studied the biodistribution of this compound in both rats and monkeys. The rat data is similar to that which has been reported for C-11 labeled spiroperidol, showing high uptake in lungs, liver and kidneys. However, F-18 spiroperidol is rapidly metabolized, and after 30 minutes <25% of the injected dose could be recovered, the rest presumably lost via excretion. The determination of route and amount of excretion in the rat is very difficult. The calculation of human dosimetry (using the MIRD data) showed the liver (.106 rads/mCi) and kidney (.067 rad/mCi) as the critical

organs. As it was felt that the rat is not a suitable model for human dosimetry of F-18 spiroperidol, the biodistribution was studied in monkeys, using an Anger camera and pinhole collimator. Calculation of the human dosimetry from this data gave the bladder wall (1.4 rads/mCi, assuming no voiding) and kidneys (0.68 rads/mCi) as the critical organs. This data has been used to calculate the permissible human dose of F-18 spiroperidol.

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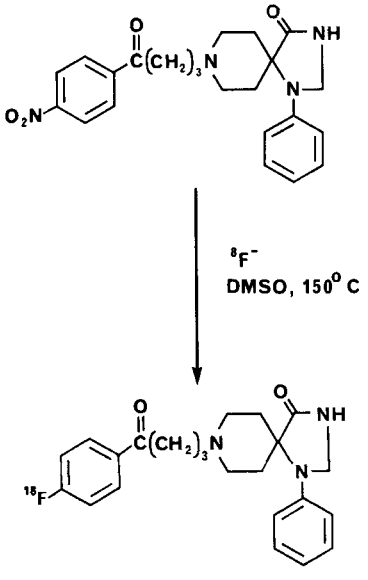

# $^{11}$C-LABELED INDOLEALKYLAMINES AS POTENTIAL SEROTONIN$_1$ RECEPTOR MAPPING AGENTS : SYNTHESIS AND BIODISTRIBUTION

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Indolealkylamines such as N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine, and bufotenine are known to have hallucinogenic properties and they are found in the serum or the urine both of normal subjects and of the psychotic patients(1.2). Indolealkylamines are more selective toward 5-hydroxytryptamine binding sites($S_1$ receptors) than $S_2$ receptors(3). $^{11}$C-labeled indolealkylamines are expected as potential serotonin$_1$ receptor mapping radiopharmaceuticals.

$^{11}$C-dimethyltryptamine($^{11}$C-DMT), $^{11}$C-5-methoxy-N,N-dimethyltryptamine($^{11}$C-5-OMe DMT), $^{11}$C-N-methyltryptamine, and $^{11}$C-bufotenine were prepared by the reaction of $^{11}$C-$CH_3I$ (Figure 1). The labeled products were separated by high performance liquid chromatography on a Radialpack silica column (Waters) with a solvent system of chloroform and methanol containing 1% dimethylamine (90:10). The overall radiochemical yields of $^{11}$C-DMT, $^{11}$C-OMe DMT, $^{11}$C-N-methyltryptamine, and $^{11}$C-bufotenine were about 46%, 18%, 2.2%, and 8.9%, respectively. The specific activity of $^{11}$C-DMT was 50 - 76 Ci/mmol at the time of use (average 63 Ci/mmol).

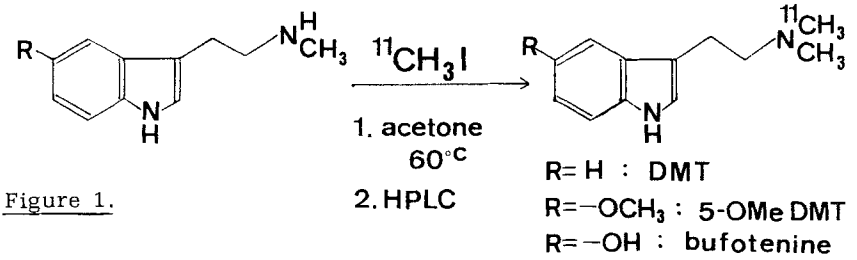


Figure 1.

Tissue distributions of these indolealkylamines were summerized in Figure 2. The brain uptake of $^{11}$C-DMT was the highest of the three indoleamines and that of $^{11}$C-bufotenine was the lowest. It is suggested that 5-hydroxylation of indolealkylamine inhibits the transport through the blood-brain barrier. The regional accumulation of $^{11}$C-DMT was examined in the rat brain in vivo by means of autoradiography and punch sampling procedure(Figure 3). The accumulation of $^{11}$C-DMT was higher in the cerebral cortex, caudate putamen, and amygdaloid nuclei than in the cerebellum and medulla oblongata. It is reported that a large quantity of 5-hydroxytryptamine binding is more enriched in the the crude mitochondrial fraction (3). Therefore we examined the subcellular distribution of

$^{11}$C-DMT in the brain and the effects of monoamine oxidase inhibitor pargyline and reserpine on the subcellular distribution(Figure 4). The radioactivity in the nuclear (N), crude mitochondrial(CM), and microsomal(MIC) fractions was increased with the decrease in the loading dose of $^{11}$C-DMT. Pargyline pretreatment greatly enhanced the radioactivity in the three fractions. On the contrary reserpine had no influences on the subcellular distribution. These data suggest that the inhibition of $^{11}$C-DMT metabolism by pargyline increases the binding of $^{11}$C-DMT to the postsynaptic serotonin receptors. Figure 5 illustrates the images of $^{11}$C-DMT and $^{45}$Ti-DTPA in a dog obtained by the positron emission tomography(ECAT II , Ortec). $^{11}$C-DMT was markedly accumulated in the brain in contrast with the image of $^{45}$Ti-DTPA which represented the blood volume in the brain.

Acknowledgment: This work was supported by a Grant-in-Aid for Scientific Research No.58870064, Ministry of Education, Science and Culture, Japan.

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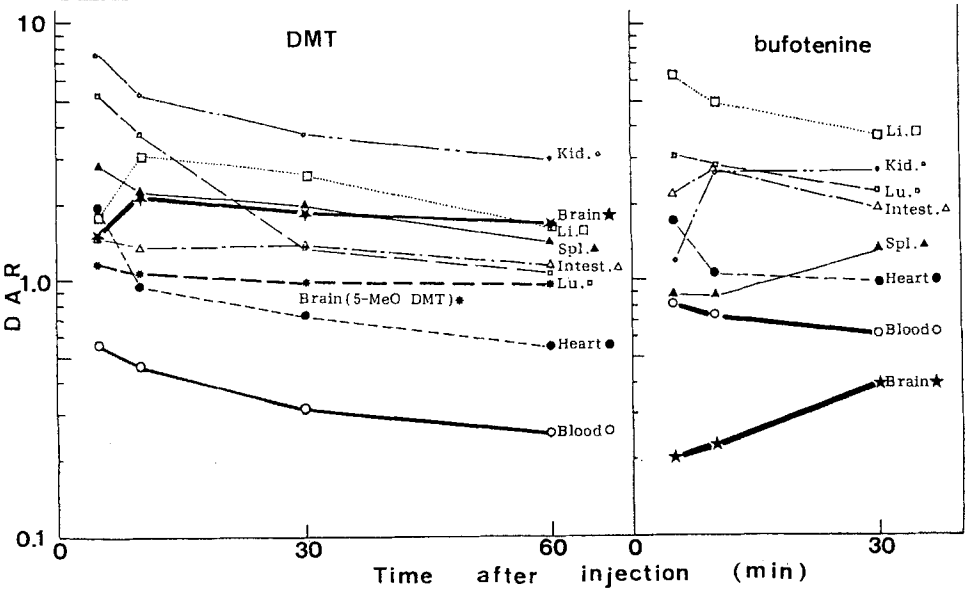
Figure 2

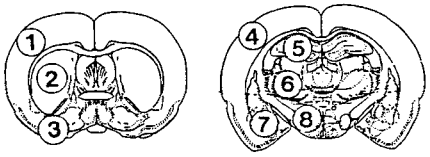



Abbreviation Kid; Kidney. Li; Liver. Spl; Spleen. Lu; Lung. Intest; Small intestine.

Figure 3

| Region | | Region | | Region | |
|---|---|---|---|---|---|
| 1 | 2.1±0.1 | 5 | 1.6±0.2 | 9 | 1.2±0.1 |
| 2 | 1.9±0.1 | 6 | 1.6±0.1 | 10 | 1.2±0.1 |
| 3 | 1.8±0.2 | 7 | 1.8±0.1 | serum | 0.21±0.03 |
| 4 | 2.2±0.2 | 8 | 1.5±0.1 | | |

unit: %dose/g N=4 wistar rats




9 CEREBELLUM
10 MEDULLA OBLONGATA

Figure 4

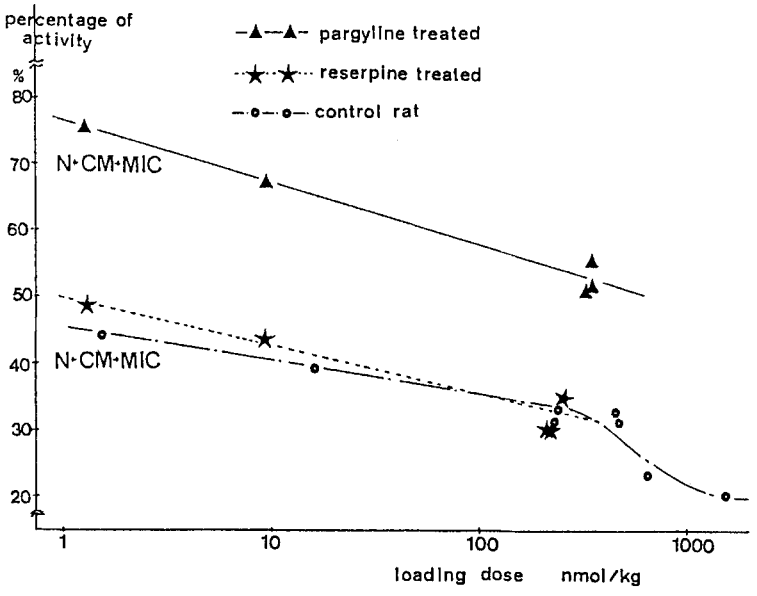
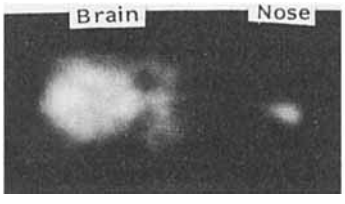



Figure 5




$^{11}$C-DMT

$^{45}$Ti-DTPA

# $^{11}$C-MESULERGIN, A POTENTIAL AGENT FOR MAPPING THE SEROTONIN RECEPTOR: SYNTHESIS AND ANIMAL EXPERIMENTS.

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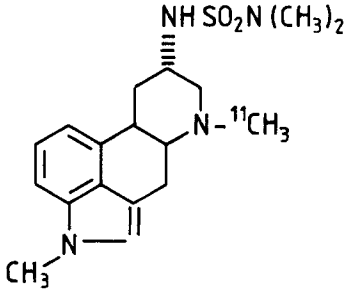
Effects of serotonin have been hypothesized in a number of neurological diseases like sleep disorders, affective disorders and migraine. Thus, ligands for in-vivo mapping of the serotonin receptor seem to be useful radiopharmaceuticals when labelled with short-lived radionuclides. Furthermore, many dopamine receptor antagonists have high affinity towards the serotonin receptor as well. Thus, selective serotonin receptor antagonists may also be useful agents for differentiation in dopamine receptor studies.

Mesulergin (I) was shown (1) to be a selective ligand for the serotonin-2 receptor (2). Affinity for other neurotransmitter receptors was lower, the next highest to the dopamine-2 receptor is 50 times weaker than to the serotonin-2 receptor (1). Selectivity, thus, seems higher than for ketanserin (3), which has previously been labelled with $^{11}$C (4).




I

Mesulergin (I) was labelled with $^{11}$C on N-6 by reaction of $N_6$-desmethyl-(I) with $^{11}CH_3I$. Briefly, 2 µmole of $N_6$-desmethyl-(I) and 4.5 µmole of N-ethyl-di-isopropylamine were dissolved in 0.6 ml freshly distilled dimethylformamide. To this, $^{11}CH_3I$ (∿ 100 mCi) was added as a gas by a constant stream of He (cooling in dry ice). The mixture was heated at 100 °C for 10 min. After that, the mixture was evaporated to dryness, taken up in 300 µl methanol : water : triethylamine 70:30:0.25 (v/v/v) and chromatographed on a Lichrosorb RP 18 10 µm column (25 x 0.4 cm) using the same mixture as eluent (flow 2.1 ml/min). The $N_6$-desmethyl-(I) and (I) were easily separated (k' = 1.56 for $N_6$-desmethyl-(I) and 3.44 for (I)). The radiochemical yield after chromatography was 40-50% (decay corrected) after a synthesis time of 30 min. Up to 12 mCi of (I) have been prepared in injectable solution (after sterile filtration) at a specific activity of ∿ 10 Ci/mmole.

Previous studies (5) in rats had shown that $^{3}H$-(I) was rapidly accumulated in the brain with a distribution suggestive of a serotonin-2 receptor ligand. Preliminary in-vivo experiments in monkeys with $^{11}C$-(I) and PETT seem to indicate that $^{11}C$(I), despite of the low specific activity, was distributed in the brain like a serotonin-2 receptor antagonist (2). The distinct distribution, furthermore, was abolished after displacement with a pharmacological dose of unlabelled ketanserin.

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# $^{11}$C-BENZODIAZEPINE (BDZ) LIGANDS FOR POSITRON EMISSION TOMOGRAPHY (PET) STUDIES

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In order to study "in vivo" by PET two different types of BDZ receptors (central and peripheral) three ligands which can be labelled with carbon 11 (20.4 min half life) using a methylation process, are proposed :

a) 5-(2-fluorophenyl)-1,3-dihydro-1-$^{11}$C-methyl-7-nitro-2H-1,4-benzodiazepine-2-one (Flunitrazepam-$^{11}$C) which binds to central and peripheral sites (1).

b) Ethyl 8-fluoro-5,6-dihydro-5-$^{11}$C-methyl-6-oxo-4H-imidazo (1,5-a)(1,4) benzodiazepine-3-carboxylate (RO 15 1788-$^{11}$C), a selective BDZ antagonist which exerts its central effects by competitive high affinity interaction with central type BDZ receptors (2).

c) N-($^{11}$C)-methyl, N-(methyl-1 propyl), (chloro-2 phenyl) -1 isoquinoleine carboxamide-3 (PK 11 195-$^{11}$C), the specific binding of which to target tissues, concern peripheral type benzodiazepine receptors (3).

These three compounds are labelled by methylation of their nor-derivatives using the same radioactive precursor I$^{11}$CH$_3$. But, in order to reach the best radiochemical yield, different parameters conditionning the synthesis (solvent-temperature-reaction time-mass of compound involved) and purification conditions had to be very carefully studied. Results obtained for each synthesis are shown in figure 1.

After purification of the labelled compound by high performance liquid chromatography, identities of the radiopharmaceuticals were checked, using different methods (U.V. absorption, refractometry, HPLC, mass spectra, melting point measurements) against reference drugs.

The total $^{11}$C-synthesis required a mean time of thirty minutes (from the end of bombardment to the obtention of the solution ready for use).

A 30 minutes irradiation of nitrogen (air liquide N 60) by a 20 MeV proton beam of intensity 30 µA under 8 bars pressure, yields an average of 100 mCi (maximum : 185 mCi) with a mean specific activity of 1 curie (3-7 $10^7$BQ)/µmole (maximum : 1.9 Curie/µmole).

An I.V. administration of 10 mCi of the labelled drug into the baboon Papio papio, for PET studies, corresponds to a mass of 1 nmole/kg. This quantity is in agreement with the one used for "in vitro" characterization of BDZ receptors. The first PET results will show the difference in the "in vivo" uptake and kinetics of these various drugs in different parts of the body known to be rich in central or peripheral type BDZ receptors.

Acknowledgement :
This work was partially supported by grants of C.N.R.S. (ATP n° 031927). We wish to thank Hoffmann-La Roche and Pharmuka Laboratories for kindly supplying drugs used in this study.

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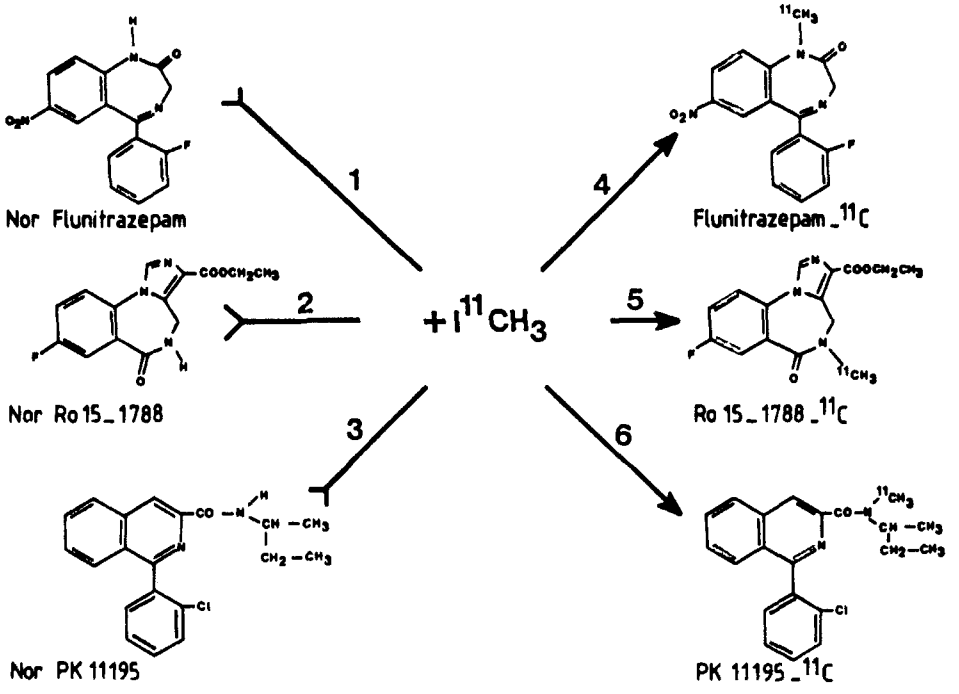


Reaction conditions

1) Nor-flunitrazepam : 2 µmoles - NaOH : 2 µmoles - 15 crown 5 : 2 µmoles - TBP : 100 µl - temperature : 90°C - reaction time : 3 min.
2) Nor-RO 15 1788 : 3 µmoles - TMBA : 3 µmoles - TBP : 300 µl - temperature : 70°C - reaction time : 3 min (4).
3) Nor PK 11 195 : 1.2 µmole - KOH : 5 mg - DMSO : 175 µl - Room temperature (5).

Purification conditions (HPLC)

4) Column : Whatman M9 PAC (Partisil with amino cyano groups) - Eluent : $CH_2Cl_2$/hexane/B : 49-49-2 flow rate : 6 ml/min - Tr : 8 min.
5) Column : Whatman M9 silica - Eluent : $CH_2Cl_2$/B : 98-2-flow rate 8 ml/min - Tr : 9 min.
6) Column : Whatman M9 Partisil ODS-2 - Eluent ETOH/$H_2O$ : 75-25 - Flow rate : 4 ml/min - Tr : 4 min.
TMBA : Trimethyl benzyl ammonium
TBP : Tri butyl phosphate
DMSO : Dimethylsulfoxyde
B : Ethanol-$H_2O$-Ethylamine : 96 -2.5 - 1.5.

# PREPARATION AND EVALUATION OF F-18-LABELED LIGANDS FOR ESTROGEN RECEPTORS

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We have prepared 2R*,3R*-1-(F-18)fluoro-2,3-bis(4-hydroxyphenyl)pentane, [(F-18)fluoro-norhexestrol (I)], 2R*,3S*-1-(F-18)fluoro-3,4-bis(4-hydroxyphenyl)hexane, [(F-18)-fluoro-hexestrol (II)], (F-18)-16$\alpha$-fluoro-17$\beta$-estradiol (III), and (F-18)-16$\beta$-fluoro-17$\beta$-estradiol (IV). The fluorononorhexestrol was prepared as described in the literature[1] and the other three compounds were prepared using F-18 fluoride produced by the 0-18(p,n)F-18 nuclear reaction[2] and the schemes shown in Figure 1. All four compounds were prepared with yields of 10-50% and specific activities >100Ci/mmole. Uptake studies in immature rats showed receptor mediated uptake of all compounds in the uterus, however, the 16$\alpha$-fluoro-17$\beta$-estradiol(III) showed considerably greater uterus to blood ratios (>80) at 2 hours than the other compounds (ratios (I) 22.1, (II) 37.4, (IV) 17.6).

Studies using compound III in mature DMBA induced mammary tumor bearing rats showed receptor-mediated uptake in the tumors; however, the tumor to blood ratios exhibit large variation (range 2:1 to 40:1). As this could be an effect of differing blood flows and/or blood volumes, tumor bearing rats were administered compound (III) (3 hours prior), Tc-99m-labeled red blood cells (0.5 hours prior), and I-125-4-iodoantipyrine (one minute ramp infusion one minute prior). Tumor blood flow was then determined as described by Sakurada et al.[3] The partition coefficient for compound (III) was determined in tumor rats with the hepatic portal vein, hepatic artery, mesenteric vein and artery, and renal vein and artery occluded; utilizing I-125-iodoantipyrine and obtaining tumor to blood ratios at 3 hours. The measured partition coefficient was 0.842 $\pm$ 0.094; blood volume varied from 1% to 25%; and blood flow varied from 4 to 200ml/100g/minute. The tumor uptake of compound (III) did not correlate with either flow or volume. The correlation of uptake of compound (III) with estrogen receptor levels will be presented.

Compound (III) was administered to 3 cynamologus monkeys positioned under a large field of view gamma scintillation camera fitted with a 6mm pinhole collimator to measure clearance of the compound from the major organs which allowed time activity curves to be calculated. From these data estimation of the radiation dosimetry was calculated, showing that the critical organ is the bladder wall with a dose of 0.68 $\pm$ 0.12 rads/millicurie. This dose should allow administration of ~10mCi to humans to study estrogen receptor levels in mammary tumors using PET.

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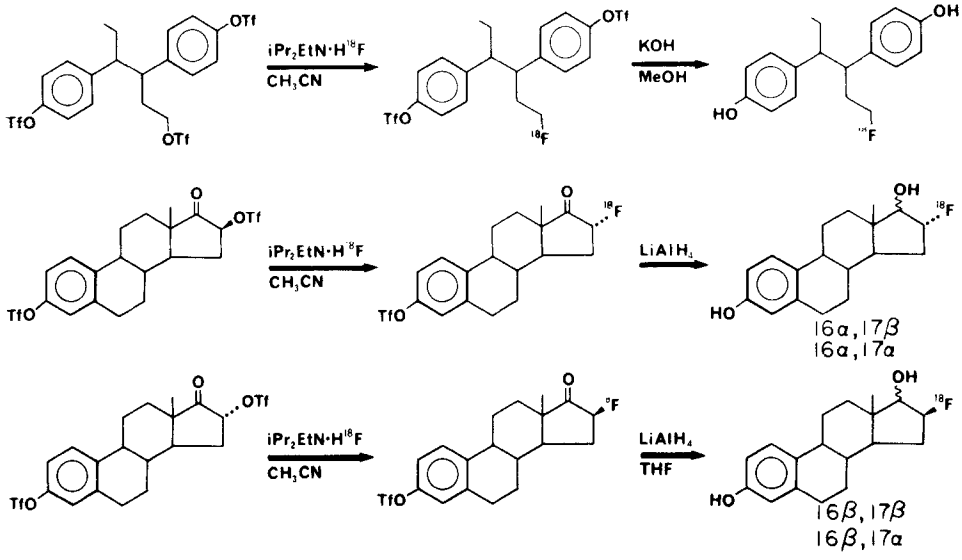


Figure 1. Synthetic schemes for the preparation of compounds (II), (III), and (IV).

## C-11-LABELLING OF LIGANDS FOR PET STUDIES OF DOPAMINE AND BENZODIAZEPINE RECEPTORS

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The possibility of performing regional in vivo binding studies of different receptors in the human brain using positron emission tomography (PET) has recently been demonstrated by several authors. Thus both cerebral dopamine receptors (1-5) and benzodiazepine receptors (6,7) have been visualized. In this presentation the synthesis, purification and preliminary PET results using a C-11-labelled benzamide, FLB 542, a dopamine receptor antagonist and C-11-labelled Ro 15-1788 a benzodiazepine receptor antagonist are described.

$^{11}CO_2$ was produced using a baby cyclotron, scanditronix RNP 16, and the $^{11}CO_2$ was converted to C-11-methyl iodide (8); time: 10 minutes, yield: 60 - 80 %. In the synthesis of $^{11}C$ - FLB 542, S(-)-2- [(5-chloro-3-ethyl -2-hydroxy-6-metoxybenzamid)-methyl] -1-methyl-pyrrolidine, the $^{11}CH_3I$ was trapped in a solution of the corresponding secondary amine in 500 µl of acetonitrile at -42 °C. The solution was sucked into the loop of an HPLC-injector which was closed and the temperature raised to 130 °C for 5 min. The solution was diluted with 400 µl of water and injected on to a µBondapak C-18 column, 300 x 7.8 mm (waters) and the $^{11}C$-FLB 542 was separated using acetonitrile: $H_3PO_4$ 0.01 M (27:73) as the mobile phase; flow rate: 5 ml · $min^{-1}$. The labelled compound was collected between 18 - 20 minutes. The solution was evaporated to dryness in a rotary evaporator and the $^{11}C$-FLB 542 was dissolved in 10 ml sterile isoton saline and sterilized by filtration through a Millipore filter, 0.22 µm. Synthesis time: 50 - 60 minutes; yield: 20 %; specific activity: 150 Ci / mmole.

In the synthesis of $^{11}C$-Ro 15-1788 the $^{11}CH_3I$ was trapped in a solution of 1 µmole Ro 15-5528 ( the corresponding secondary amide) in 200 µl of anhydrous DMSO and 10 µl dimsylsodium (1 g NaH in 10 ml anhydrous DMSO) at room-temperature. After 5 minutes the reaction was terminated by adding 500 µl water and the $^{11}C$-Ro 15-1788 separated on the HPLC-column described above. The labelled compound was collected between 8 - 10 minutes, evaporated to dryness in a rotary evaporator and dissolved in a solution of 3.5 ml of propylene glycol and 1.5 ml of ethanol. Water (5 ml) was added and the solution was sterilized by Millipore filtration. Synthesis time: 40 minutes; yield: 40 %; specific activity: 150 Ci/mmole.

The N-alkylated benzamide FLB 542 has been shown to be a selective dopamine antagonist with high binding affinity for dopamine receptors both in vitro and in vivo on rats (9). We have done PET-studies on monkeys with the C-11-labelled FLB 542 and found a ratio of striatum: Cerebellum = 10:1. Administration of a high dose of haloperidol before the investigation with $^{11}C$-FLB 542 on monkeys resulted in a complete displacement of the activity from the striatal area. PET-studies on both monkeys and humans will be presented. PET-studies using $^{11}C$-Ro 15-1788 in the monkey has also been performed. This showed an uptake similar to that previously reported for flunitrazepam and Ro 15-1788 (7) in the baboon.

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# NEW SYNTHESIS OF NO-CARRIER-ADDED $^{18}$F-HALOPERIDOL *

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CANADA

A change in the concentration of dopaminergic receptor sites has been observed in several diseases in the human brain, particularly in Parkinson's disease(1). However, since the analyses were carried out post-mortem, we still do not know what happens to receptor sites during the progress of disease or therapy.

Dopaminergic receptor sites were recently visualized in living human brain by means of $^{18}$F-labelled 6-fluorodopa(2) and $^{11}$C-N-methyl spiroperidol(3) in conjunction with positron emission tomography (PET). Since spiroperidol also binds to 5-TH$_2$ serotonergic receptor sites(4) we chose to investigate the synthesis of no-carrier-added $^{18}$F-labelled haloperidol (pure dompamine antagonist) as a marker for dopaminergic receptor sites.

Haloperidol has already been labelled with $^{18}$F by both the triazine(5) and the Balz-Schiemann reaction(6). Both reactions provide relatively low yield; the latter does not yield a product of high enough specific activity for PET studies.

We developed two syntheses for "no-carrier-added" haloperidol, both of which use heterogenic exchange as a mode of labelling. Heterogenous exchange of chlorine and the nitro group in activated benzene rings has been reported earlier in some simple systems(7,8). Recently the synthesis of no-carrier-added spiroperidol(9) by means of heterogenous exchange in p-nitro-benzonitrile was also reported. In our synthesis of $^{18}$F-haloperidol we used heterogenous exchange in the p-nitro-benzonitrile, p-chloro-benzonitrile, and the chloro-analog of haloperidol (4-chloro-4-[4-hydroxy-4-(4-chlorophenyl)-piperidine]-butyrophenone(3)) in order to synthesize no-carrier-added $^{18}$F-haloperidol.

Fluorine-18 was produced by irradiating $^{18}$O-enriched water with protons in a stainless steel target box. After irradiation water was added to a platinum crucible containing a 200$\mu$l solution of tetrabutylammonium hydroxide (50$\mu$mol) in water. The water was then evaporated to dryness in a sand bath kept at 120-130°C. Evaporation and drying took about 20 min, after which the crucible was cooled to room temperature and the residue dissolved in 500$\mu$l of dry DMSO and transferred into a small reaction vessel containing 50$\mu$mol of compounds 1, 2 (Fig.1) or compound - 3 mentioned earlier. The exchange reaction was carried out for 15-20 min at a bath temperature of 145°C. When compound-3 was used, $^{18}$F-haloperidol was isolated by adding 1 ml of 0.1 M KF and then extracted with chloroform and haloperidol isolated by HPLC. In experiments where exchange was done on p-nitro-benzonitrile and p-chloro-benzonitrile, $^{18}$F-labelled haloperidol was synthesized by the reaction sequences outlined in figure 1 and the final product purified by HPLC.

The compounds prepared by those procedures were identified by $^{1}$H and $^{19}$F-NMR, IR, and melting point and were identical to an authentic sample of haloperidol extracted from a drug. $^{1}$H and $^{19}$F-NMR and MS supplied proof that the chlorine on the benzene ring next to the carbonyl group was exchanged with $F^-$.

$^{18}$F-labelled haloperidol was identified by comparing $R_f$-values obtained in two different solvent mixtures. $R_f$ values were 0.42 and 0.48 in $CHCl_3$-MeOH (9:1) and $CHCl_3$-MeOH-$NH_4OH$ (95:5:1) respectively, and were identical to those of an authentic sample extracted from the drug Haloperidol-Haldol. The elution volume of $^{18}$F-radiopharmaceutical was identical to that of an authentic sample.The overall radiochemical yield of all three synthesis was comparable at 10%-15%, yielding a product of high radiochemical purity ( $\sim$ 97%). The specific activity, as measured by HPLC, was about 10,000 Ci/mmol.

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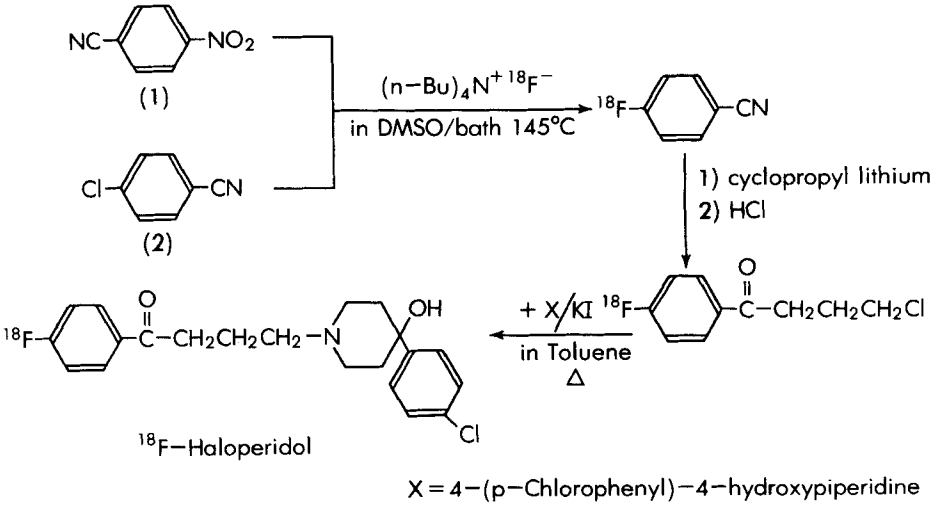


**FIGURE 1**

# SYNTHESIS OF RADIOIODINATED ANALOGS OF 2-PHENYLPYRAZOLO[4,3-c]QUINOLIN-3(5H)-ONE BY TRIAZENE APPROACH

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Recent publications have shown that 2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one (I) antagonizes the effect of benzodiazepines whereas its chloro derivative (II) has benzodiazepine-like activity (1). The unique pharmacological properties of the pyrazoloquinolines (I and II) made it attractive to develop the pyrazoloquinoline analogs labeled with suitable gamma and/or positron emitting nuclides in order to determine their tissue distribution properties and then correlate them with structure and pharmacological activity. As part of a current program to develop the radiolabeled analogs of (I), we initially interested in radioiodinated derivatives of this class of compounds which might retain biological activities associated with the pyrazoloquinoline structure.

The present method chosen for introduction of iodine into the para-position of the phenyl ring and the $C_8$-position of (I) involved the decomposition of the aryltriazene intermediates (III) and (IV)(2). Although a variety of efficient methods for the preparation of radioiodinated aromatic compounds with high specific activity have been recently reported (3), the triazene approach was chosen because the decomposition reaction can generally be performed rapidly and the relative high yield has been observed in the case of radioiodine-labeling when compared with $^{18}$F-labeling (4).

The aromatic amines obtained in five synthetic steps were converted into the triazenes (III) and (IV) by conventional method. Treatment of the piperidinyl triazene (III) in acetonitrile with four equimolar amount of sodium iodide and three equimolar amount of methanesulfonic acid under reflux for 50 min gave the (2-p-iodophenyl)pyrazoloquinoline (V) in 64% yield together with (I)(34%). The use of tetrahydrofuran as solvent resulted in the decreased yield of (V). The pyrrodinyl triazene (IV) in acetonitrile under the same conditions was more effectively converted into the 8-iodo compound (VI)(85%). In the triazene decomposition reactions the longer time was required for the complete decomposition of these diazonium salt under the absence of sodium iodide. The initial step appears to be an electron transfer process from the iodide ion (5).

The basic procedure used to prepare the non-radioactive (V) and (VI) was adapted to the synthesis of the $^{131}$I-labeled compounds using no-carrier-added Na$^{131}$I. Analytical thin layer chromatography using reversed phase plates showed that the dediazoniation process for the two triazenes in acetonitrile under reflux at 40-60 min gave the required $^{131}$I-labeled analogs (VII) and (VIII) in about 40% of labeling yields. The labeling yield was dependent of the amount of the acid. Using 1-2 mg of the triazenes and 1-2 mCi of Na$^{131}$I, chemically and radiochemically pure no-carrier-added (VII) and (VIII) were obtained with 12 and 5.4% radiochemical yield, respectively, after purification by high pressure liquid chromatography (HPLC) using a normal phase column and a reverse phase column. Identification of the products was performed by comparative HPLC with the authentic samples. Methods of further improvement are being investigated.

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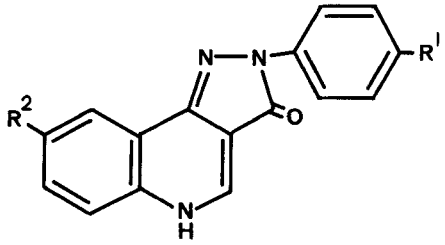


(I) $R^1=R^2=$ H

(II) $R^1=$ Cl $R^2=$ H

(III) $R^1=$ N=N-N⟨ ⟩ $R^2=$ H

(IV) $R^1=$ H $R^2=$ N=N-N⟨ ⟩

(V) $R^1=$ I $R^2=$ H

(VI) $R^1=$ H $R^2=$ I

(VII) $R^1=$ $^{131}$I $R^2=$ H

(VIII) $R^1=$ H $R^2=$ $^{131}$I

# SYNTHESIS OF LIGANDS FOR IMAGING OPIATE RECEPTORS BY POSITRON EMISSION TOMOGRAPHY: CARBON-11 LABELED DIPRENORPHINE

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The pharmacological actions of the opiates, which range from analgesia and the perception of pain to respiratory depression, have been linked to the regional concentration of opiate receptors in the brain (1). The opiate receptors in animals can be successfully labeled preferentially *in vivo* using high affinity, high specific activity radiotracers (2). In contrast, localization of opiate receptors in the human brain has been limited to *in vitro* studies of post-mortem tissue (3). The visualization and quantification of opiate receptors in living brain would yield valuable and unique information. Toward this end, we are investigating the syntheses of carbon-11 labeled ligands of high binding affinity suitable for the study of opiate receptors using positron emission tomography (4).

Diprenorphine, a potent and pharmacologically well-defined opiate antagonist (2,5), is a particularly useful ligand for such studies. The short half-life of carbon-11 (20.4 min) and the high levels of radiation involved require that the label be incorporated in a rapid step near the end of the synthetic sequence. A suitable route for the synthesis of radiolabeled diprenorphine **1** is the addition of carbon-11 methyllithium to compound **2**. Precedent for this transformation is the recently reported addition of carbon-11 methyllithium to estrone, a steroidal phenolic ketone, to provide 17-α-methylestradiol (6). However, addition of alkyllithiums to ketomorphinans similar to **2** is complicated by enolization or rearrangement (7), and it was therefore necessary to explore the feasibility of this radiolabeling procedure.

Carbon-11 methyllithium was prepared in high radiochemical yield by the rapid halogen-metal exchange reaction between carbon-11 methyl iodide and an excess of n-butyllithium in diethyl ether/hexane at -78°C (6,8). Addition of a THF solution of known ketone **3** to this mixture provided **4**, as verified by HPLC comparison to an authentic sample (7). The synthesis time required from end-of-bombardment was 20 minutes and the product which resulted was determined to be approximately 200 mCi/μmole at end-of-synthesis. The radiochemical yield based on carbon-11 methyl iodide was calculated to be 8%.

Since known syntheses of diprenorphine and similar compounds are not entirely applicable to the preparation of precursor **2**, a new synthetic strategy was developed. Ketoamide **5** was prepared in five steps (32% yield) from thebaine as described by Bentley and Hardy (7). Hydrogenation (10% Pd-C/45°C/45 psi; 94%) followed by reduction (LAH/THF/reflux; 98%) and subsequent selective cleavage of the aromatic methyl ether (PrSNa/DMF/reflux; 63%) afforded **6** as a mixture of diastereoisomers. Protection of the phenolic moiety of **6** as the benzyl ether (NaH/DMF;BzBr; 83%) was required in order to effect oxidation of the secondary alcohol by means of pyridinium chlorochromate buffered with sodium acetate. Subsequent hydrogenolysis liberated **2** which exhibited spectral characteristics in full accord with its structure. Most notably, the proton NMR spectrum shows the absorption of H-5β at 4.80 ppm indicative of the preservation of α-stereochemistry at C-7 throughout the sequence (9). Thus, the required precursor was obtained from thebaine in eleven steps, 2% overall yield.

As a result of the availability of precursor **2** and the successful incorporation of carbon-11 into the morphinan **4**, the preparation of carbon-11 labeled diprenorphine is possible.

This work was supported in part by DHHS grand Nos. CA-32845 and NS-15080. We wish to thank the Penick Corporation (Lyndhurst, New Jersey, USA) for a generous gift of thebaine.

Note that diprenorphine is listed as: (17-CYCLOPROPYLMETHYL)-4,5-EPOXY-18,19-DIHYDRO-3-HYDROXY-6-METHOXY-α-METHYL-α,α-DIMETHYL-6,14-ETHENOMORPHINAN-7-METHANOL-(5α,7α) in Chemical Abstracts, 10th Collective Index; [14357-78-9].

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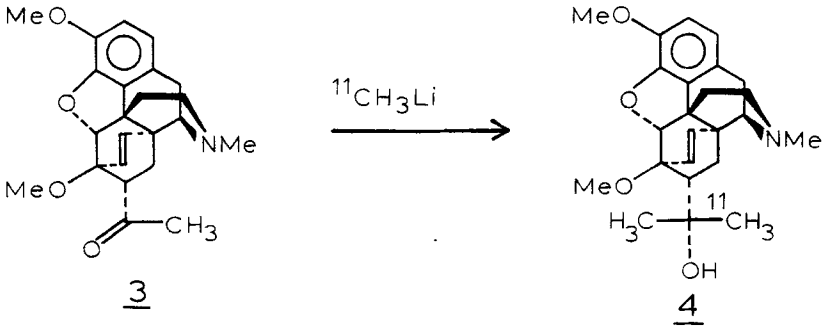
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MeO
NMe
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11
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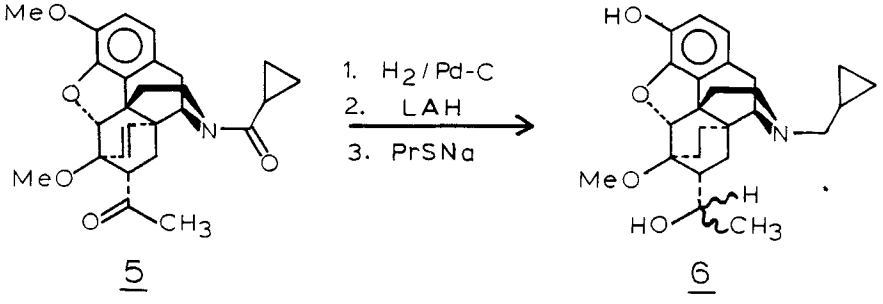
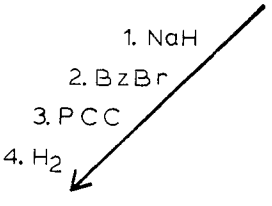
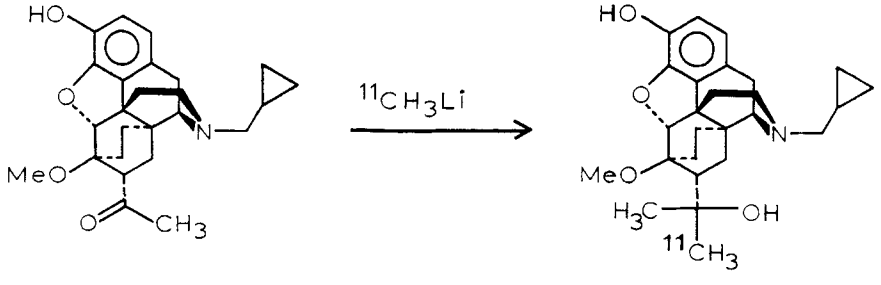
CH3
OH

3 4


MeO
N
MeO
CH3
1. H2 / Pd-C
2. LAH
3. PrSNa
HO
N
MeO
H
HO
CH3

5 6


1. NaH
2. BzBr
3. PCC
4. H2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MeO
CH3
11CH3Li
HO
N
MeO
H3C
OH
11CH3

2 1

# RADIOSYNTHESIS OF AN OPIATE RECEPTOR-BINDING RADIOTRACER FOR POSITRON EMISSION TOMOGRAPHY: [$^{11}$C METHYL]-METHYL-4-[N-(1-OXOPROPYL)-N-PHENYLAMINO]-4-PIPERIDINE CARBOXYLATE ($^{11}$C 4-CARBOMETHOXYFENTANYL)

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Non-invasive measurement of drug and neurotransmitter receptors in the brain may someday enable researchers to find out if changes in receptor concentrations and/or distributions are associated with selected disease states. Aside from simply providing new basic information, these results may suggest causes as well as treatments for the diseases, eliminate the uncertainty associated with postmortem receptor binding studies, and monitor the natural changes in receptor levels with the progression of certain neuropsychiatric diseases.

The development of high affinity, high specific activity tritium labeled neurotransmitter receptor ligands has made it possible to determine the relative concentration and spatial distribution of some neuroreceptors by means of in vivo receptor labeling techniques in experimental animals (1-11). These techniques involve the injection of a tritium labeled ligand into experimental animals and determination of the ligand's distribution in the brain by autoradiography. Recently, these techniques have been extended to mapping the spatial distribution and concentration of dopamine receptors in man in vivo (12) using a receptor ligand for dopamine receptors labeled with carbon-11 and positron emission tomography.

As part of a continuing program to identify and radiolabel high affinity, highly specific ligands for the opiate receptor for positron emission tomographic studies, we have selected two derivatives of fentanyl (Figure 1a), a well-known analgesic, as candidates for radiolabeling: R-31,833 (4-carbomethoxyfentanyl, Figure 1b) and R-34,995 (lofentanil, Figure 1c) (13,14).

Carbon-11 labeled R-31,833 was synthesized by the methylation of the appropriate carboxylate with $^{11}$C methyl iodide (15) in dimethylformamide at room temperature and purified by reverse phase high performance liquid chromatography (see Figure 2). The average synthesis time from end-of-bombardment (E.O.B.) was 30 minutes. The specific activity was determined by ultraviolet spectroscopy to be 890 mCi/μmole end-of-synthesis (approx. 2500 mCi/μmole E.O.B.).

This work was supported in part by DHHS grant nos. CA-32845 and NS-15080. A sample of the protected carboxylate precursor was generously supplied by Dr. P. Laduron of Janssen Pharmaceutica.

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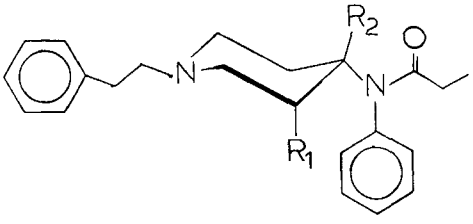
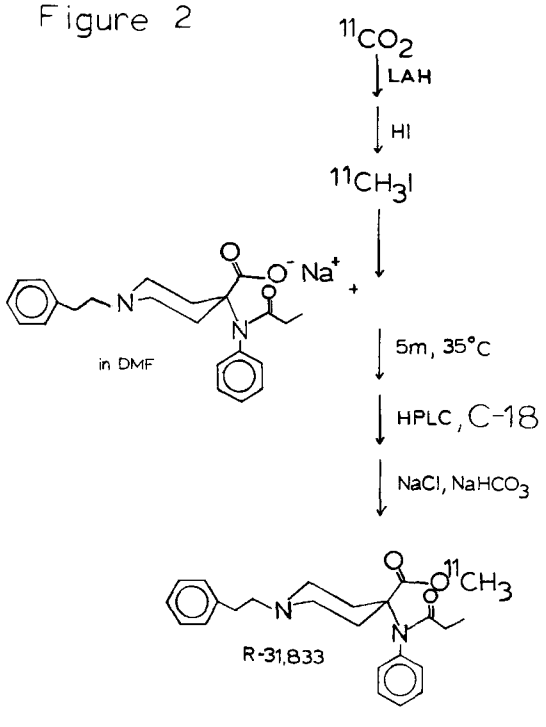
Figure 1




| | $R_1$ | $R_2$ |
|---|---|---|
| a. fentanyl | -H | -H |
| b. R-31,833 | -H | $-CO_2CH_3$ |
| c. R-34,995 | $-CH_3$ | $-CO_2CH_3$ |

Figure 2



PREPARATION AND EVALUATION OF 17α-[I-125]VINYL ESTRADIOL DERIVATIVES AS ESTROGEN RECEPTOR-SEEKING AGENTS

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Recent studies (1-6) have demonstrated that radiolabeled derivatives of estradiol possess an avidity and selectivity for estrogen receptor-containing tissues. As such they have potential value as imaging agents for ER-containing breast tumors. We have been involved in research directed toward the preparation of such agents in which radioiodine, ultimately iodine-123, can be affixed to an unsaturated moiety at the 17α-position. Such a functionality would retain a high affinity to the receptor as well as impact stability to the carbon-iodine bond.

We have synthesized a series of five estrogens having a variety of groups attached to the estradiol nucleus with the objective of identifying those features that provide target tissue uptake, selectivity and carbon-iodine bond stability (Figure 1). The compounds were labeled by electrophilic destannylation at the no-carrier-added level and isolated by HPLC in 85-95% radiochemical yields. The compounds were initially evaluated in immature rats (Table 1). The 11β-methoxy agents showed the best uptake and retention while the 11-unsubstituted and 11α-methoxy derivatives had much poorer characteristics. It was of interest to note that the 3-methyl ether which has little receptor affinity in vitro possessed marked uterine uptake in vivo. A subsequent evaluation in normal adult female rats and in rats bearing DMBA-induced mammary tumors provided similar results. The 11β-methoxy substituted estradiol derivatives again showed the greatest uptake and selectivity. Their ease of preparation, selectivity of uptake and in vivo selectivity makes them promising candidates for evaluation as breast imaging agents.

This work was supported in part by PHS grant 5RO1CA31624 and DOE Contract DE-AC02-76EV04115.

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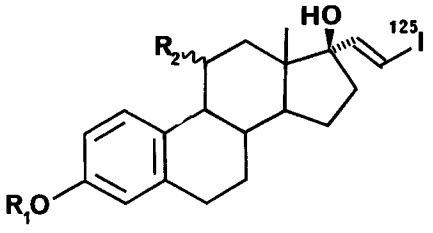
Figure 1




| | $R_1$ | $R_2$ |
|---|---|---|
| 1 | H | H |
| 2 | $CH_3$ | H |
| 3 | H | β-$OCH_3$ |
| 4 | $CH_3$ | β-$OCH_3$ |
| 5 | H | α-$OCH_3$ |

Table 1

Tissue Distribution (% ID- k/g) in Immature Female Rats

| Compound | Time (Hrs) | Uterus | Liver | Blood | Uterus / Blood |
|---|---|---|---|---|---|
| 1 | 1 | 0.434 | 0.144 | 0.027 | 16.1 |
| | 2 | 0.465 | 0.105 | 0.023 | 20.2 |
| | 4 | 0.200 | 0.074 | 0.026 | 7.7 |
| 2 | 1 | 0.198 | 0.262 | 0.031 | 6.4 |
| | 2 | 0.286 | 0.162 | 0.022 | 13.0 |
| | 4 | 0.220 | 0.131 | 0.035 | 6.3 |
| 3 | 1 | 0.821 | 0.146 | 0.019 | 43.0 |
| | 2 | 0.751 | 0.117 | 0.013 | 56.1 |
| | 6 | 0.704 | 0.085 | 0.012 | 60.7 |
| 4 | 1 | 0.264 | 0.117 | 0.011 | 24.0 |
| | 2 | 0.425 | 0.103 | 0.012 | 35.4 |
| | 6 | 0.760 | 0.105 | 0.011 | 69.1 |

SOLID PHASE SYNTHESIS OF $^{11}C$-LABELLED COMPOUNDS

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The use of solid phase syntheses as a general approach to the labelling of compounds such as dopa, with $^{11}C$, will be presented. Solid phase synthesis has recently become a very important area in organic chemistry (1) and offers many advantages as a radiolabelling technique. Since reagents are immobilized work up procedures can be accomplished simply by filtration and washing. Thus, rapid, multistep syntheses are more feasible with short lived radioisotopes and all reactions can be carried out in one vessel. Automation and shielding of such a system is also much more manageable.

The synthesis of $^{11}C$ labelled dopa using solid phase supports is illustrated below. Discussion of the appropriate choice of solid phase and immobilized precursors and technical aspects of such syntheses will be given.

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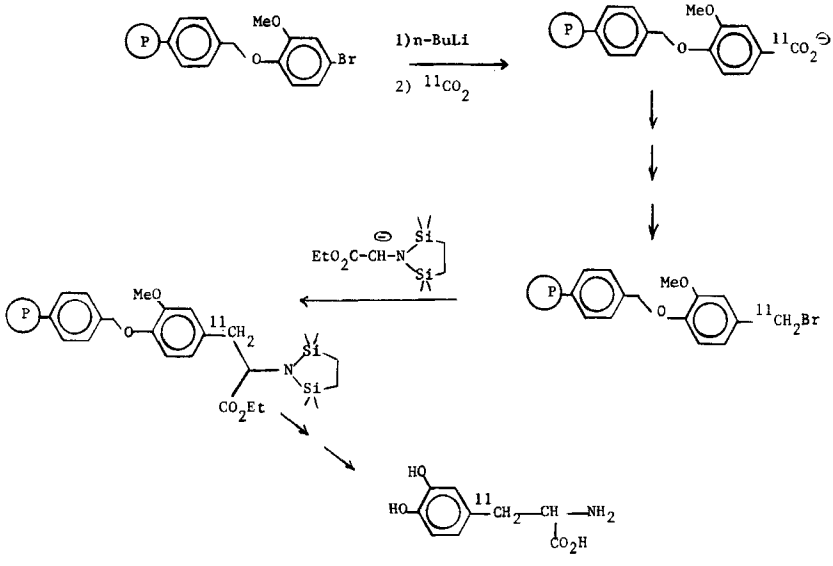

# THE SYNTHESIS OF SOME $^{11}$C-LABELLED ALIPHATIC AMINO ACIDS

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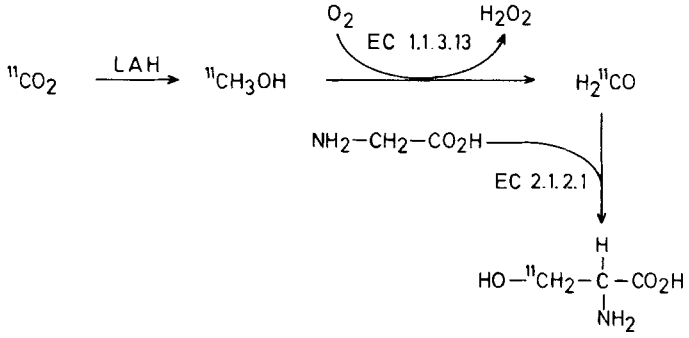
In this paper new approaches to the synthesis of some aliphatic amino acids labelled with $^{11}$C is reported.

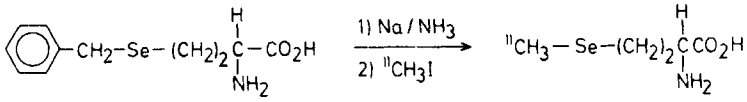
By use of alcoholoxidase EC 1.1.3.13, $^{11}$C-formaldehyde was prepared (1) and used in the synthesis of [3-$^{11}$C]serine according to Scheme 1.




Scheme 1

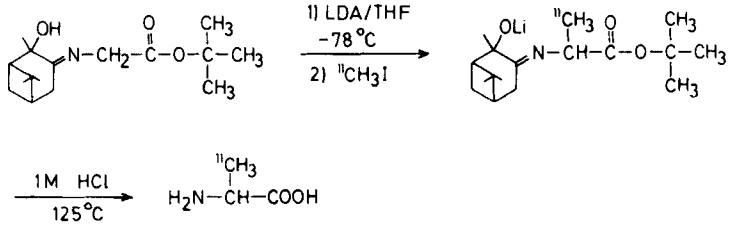
The $^{11}$C-formaldehyde was obtained in 85 % radiochemical yield within 9 min. timed from the release of $^{11}$C-carbon dioxide. The produced formaldehyde was then used with glycine and serinehydroxymethyl synthetase EC 2.1.2.1 in the synthesis of [3-$^{11}$C]serine, which so far has been obtained in 10 % radiochemical yield within 10 min. Work is now progressing with respect to immobilisation of enzymes and optimising reaction conditions.

[$^{11}$C-Methyl]-selenomethionine (3) was prepared using $^{11}$C-methyl iodide in an alkylation of the selenol anion of selenohomocysteine generated from the benzyl-Se-homocysteine (2) with sodium in liquid ammonia, (Scheme 2) in almost quantitatively radiochemical yields within 20 min. counted from the start of the synthesis of $^{11}$C-methyl iodide. The radiochemical purity of the crude product was better than 99 %.



Scheme 2

Finally in an asymmetric synthesis, [3-$^{11}$C]alanine (4) was prepared using another route than described previously (5) by alkylation of the dianion of the N-((+)-2-hydroxypinanyl-3-idene)glycine tert. butyl-ester using $^{11}$C-methyl iodide as shown in Scheme 3 (6). The reaction is very fast and the amino acid is obtained after acid hydrolysis within 3 min. in 80 % radiochemical yields. The enantiomeric purity is 80 e.e (L/D is 90/10). It might however be possible to obtain the labelled amino acid enantiomeric pure by LC separation of the diastereomeric alkylation-products. The radiochemical purity of 3-$^{11}$C-alanine is better than 90 %. This reaction seems to be of potential interest for the synthesis of other aliphatic and aromatic amino acids.



Scheme 3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# CARBON-11 LABELLED TYROSINE FOR INVESTIGATION OF TUMOR METABOLISM BY POSITRON EMISSION TOMOGRAPHY

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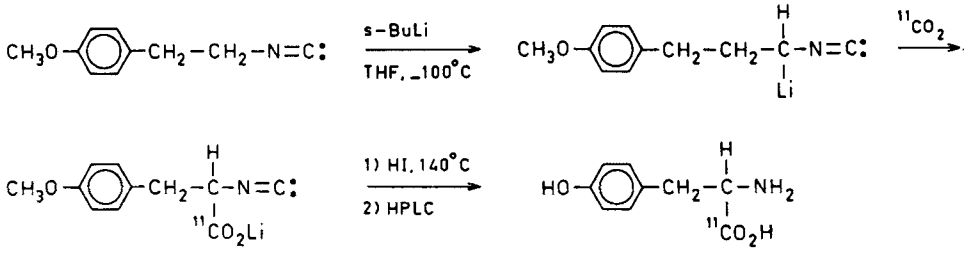
Quantitative measurement of the extent of amino acid utilization in tumor tissue by positron emission tomography may be of importance for the differential diagnosis and staging of tumors. Moreover in-vivo quantification of alterations in amino acid demand may be of interest to estimate tumor response to therapy. The aim of this communication is to present:

- -the preparation of D,L-(1-$^{11}$C)-tyrosine (I)
- -the separation of I in its enantiomeric isomers
- -distribution studies in rats with L-(1-$^{14}$C)-tyrosine and the measurement of the incorporation of the radioactivity into protein
- -accumulation studies by positron emission tomography using D- and L-(1-$^{11}$C)-tyrosine in rats bearing transplanted Walker 256 carcinosarcoma.

Carboxylic labelled D,L-(1-$^{11}$C)-tyrosine was prepared by carboxylation of the appropriate α-lithiated isocyanide by $^{11}CO_2$ as shown in the reaction scheme.




Within 40 minutes after EOB a yield up to 16%, not corrected for decay, was obtained. The separation of I into its stereoisomers was accomplished by HPLC using a chiral stationary phase column and a phosphate buffer as eluent. The separation was achieved within 10 minutes.

It is well documented that after administration of $^{3}$H- or $^{14}$C-labelled tyrosine the radioactivity is rapidly distributed over the extracellular and intracellular free amino acid pool. Within the tissue the amino acid is incorporated into protein, transaminated or decarboxylated. Scarce information is available on the radioactivity distribution after i.v. administration of L-(1-$^{14}$C)-tyrosine. Because of our aim to measure protein synthesis in-vivo with carbon-11 labelled tyrosine, we investigated the radioactivity distribution in rats, bearing Walker 256 carcinosarcoma, 10, 15 and 30 minutes after administration of this carbon-14 labelled amino acid. It was found that 15 minutes after injection the radioactivity in plasma was less than 1.5% of the injected dose. For several tissues (brain, liver, spleen, heart, muscle, kidneys) also the intra-cellular protein/non-protein bound radioacivity ratio was measured.

Using our dual-headed positron camera we also measured the distribution of D- and L-(1-$^{11}$C)-tyrosine in rats bearing the experimental Walker 256 carcinosarcoma. Both enantiomers were found to accumulate in the tumor, however, the uptake of the D-isomer showed to be much higher than the uptake of the L-isomer.

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

## SYNTHESIS OF 2- OR 3-$^{11}$C-LABELLED AROMATIC AMINO ACIDS

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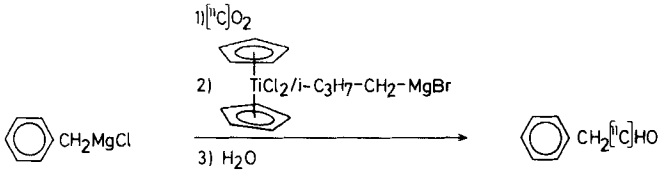
The synthesis of the aromatic amino acids phenylglycine, phenylalanine, tyrosine and DOPA, labelled in the 2- or 3-position, using various routes will be presented. In all the syntheses the $^{11}$C-carbonyl aldehydes were the key-substrates. The trapping of $^{11}$C-carbon dioxide in a solution of the appropriate Grignard reagent gave the salt of the corresponding $^{11}$C-carboxylic acid. In the preparation of the aldehydes two routes were used:

a) a selective ion-pair oxidation of the corresponding $^{11}$C-alcohol obtained after lithium aluminum hydride reduction of the acid salt (details presented elsewhere (1)).
b) a selective reduction of the $^{11}$C-acid salt according to Scheme 1 using dichlorobis ($\pi$-cyclopentadienyl)titanium and isobutylmagnesium bromide (2). The radiochemical yields were in both reactions 60-95 % with reaction times of the order of 5 min.

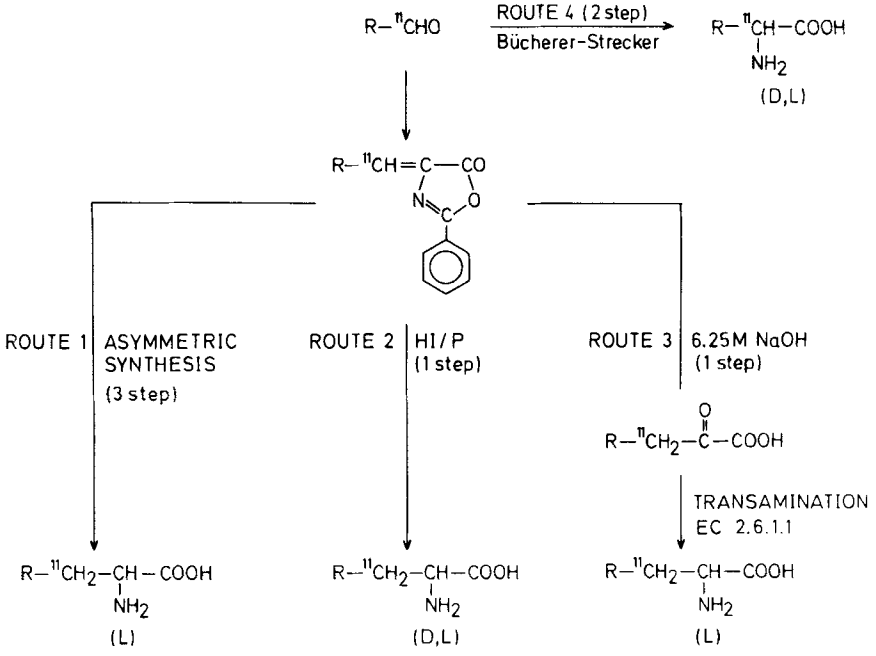



Scheme 1

The [3-$^{11}$C]labelled racemic phenylalanine, tyrosine and DOPA have all been prepared by route 2 in Scheme 2 by a condensation of the appropriate $^{11}$C-aldehyde and various 2-substituted 5-oxazolones. The hydrogenation was either carried out using hydroiodic acid and phosphorous, yielding the racemic [3-$^{11}$C]amino acid (3) in 8-30 % radiochemical yields within 50-60 min. or by hydrogenation reactions using chiral rhodium catalysts (4). In the latter case (route 1, Scheme 2) the oxazolone ring had to be opened before hydrogenation. Using chiral rhodium complex of (R)-Prophos or (+)-DIOP the hydrogenation gave [3-$^{11}$C]phenylalanine in 80 % e.e and 60% e.e respectively (i.e. L/D = 90/10 and 80/20) in 10-15 % radiochemical yield within 60 min.

By use of an enzymatic transamination reaction, [3-$^{11}$C]phenylalanine was prepared from [3-$^{11}$C]phenylpyruvic acid. The $^{11}$C-ketoacid was obtained by basic hydrolysis of the [$\alpha^{11}$C]-4-arylene-2-aryl-5-oxazolone as seen in route 3, Scheme 2 and converted by EC 2.6.1.1 to [3-$^{11}$C]phenylalanine in 20 % radiochemical yield within 10 min. (5).

Using $^{11}$C-benzaldehyde and $^{11}$C-phenylacetaldehyde, racemic [2-$^{11}$C]-phenylglycine and [2-$^{11}$C]phenylalanine were prepared in a modified Bucherer-Strecker synthesis according to route 4, Scheme 2 in a 20-30 % radiochemical yield within 40 min. (not including LC-purification) (6).




Scheme 2

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## DEVELOPMENT OF NEW TYPE OF RADIOTRACERS FOR IN VIVO ESTIMATION OF BRAIN MAO ACTIVITY : N-METHYL LABELED PHENETHYLAMINE DERIVATIVES

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Monoamine Oxidase(MAO) has a significant functions in regulating levels of neurotransmitter amines and neuromodulating amines in central nervous system,and alterations in brain MAO activity have been implicated in some kinds of central nervous disorders(1). Direct estimation of human brain MAO activity may be greatly contributed on fundamental or clinical research of such diverse disorders.

We designed metabolically-trapped radiotracers for brain MAO function study as shown in Fig-1,and evaluated these labeled compounds on their properties as a tracer(2,3).C-14 MPEA and C-14 DMPEA rapidly and highly entered into mouse brain,and then deaminated by brain MAO to C-14 methylamine or dimethylamine which were trapped by the blood-brain barrier because of cationic charge.On the other hand,when brain MAO activity was completly inhibited with MAO inhibitors, brain radioactivity rapidly decreased(see Fig-2),which suggested unmetabolized tracer itself was rapidly eliminated from the brain. Therefore, the deamination rate of radiotracer in the brain can be estimated from the disappearance curve of brain radioactivity.

The most important problems in this tracer method are the specificity against objective enzyme and the detectable range of changes in brain enzyme activity.By the theoretical consideration using simplified model, both the specificity and the range were found to be much dependent upon the enzymatic and physicochemical properties of substrate used as a tracer. For example, MAO is classified into two forms;MAO-A and MAO-B with different substrate specificity and physiological functions(4). These three kinds of PEA derivatives have different enzymatic properties against each forms of MAO, and in fact C-14 MPEA was found to be metabolized by both forms of MAO in mouse brain,whereas C-14 MPEOA and C-14 DMPEA were deaminated by only MAO-B.

Radioactivity in the brain and blood after injection of C-14 DMPEA into mice were summarized in Fig-2. Very high brain radioactivity just after injection and considerable long-term retention in the brain were observed. Radioactivity in the blood was very low and declined fast. When brain MAO-B activity was selectively inhibited with a differnt dosage of l-deprenyl(0.01-10 mg/kg i.v.), brain radioactivity at 1 hr after injection of C-14 DMPEA significantly decreased in a dosage-dependent way. On the other hand, no significant difference of brain radioactivity between control and clorgyline (a specific MAO-A inhibitor) pretreated mice was observed. This reduction of radioactivity seemed to be due to the decrease of production rate of labeled metabolite in the brain, because pretreatment with MAO inhibitors did not affect on the incorporation of the tracer into the brain.

We synthesized C-11 DMPEA by one step reaction with C-11 methyliodide and preliminaly animal study was performed. Fig-3 showed the relation between brain radioactivity of C-11 DMPEA at 1 hr after injection and brain MAO-B activity remaining under MAO-inhibition experiment. These data indicates C-11 DMPEA is a specific radiotracer for in vivo estimation of brain MAO-B activity with a fair sensitivity.

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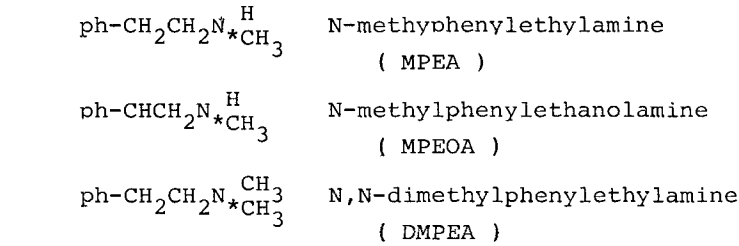
Fig-1 Metabolically-trapped radiotracers



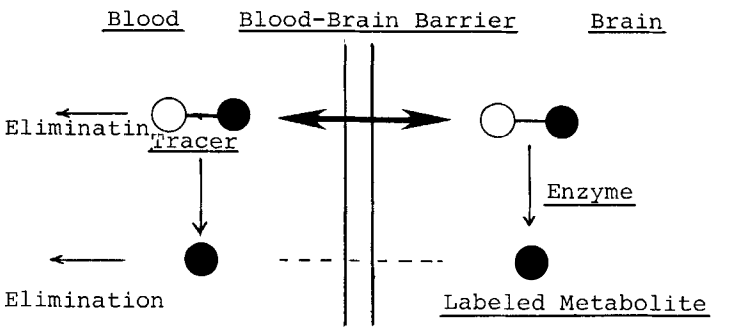



Fig. 2 Radioactivities in brain and blood after injection of $^{14}$C-DMPEA

(Three mice in each point)

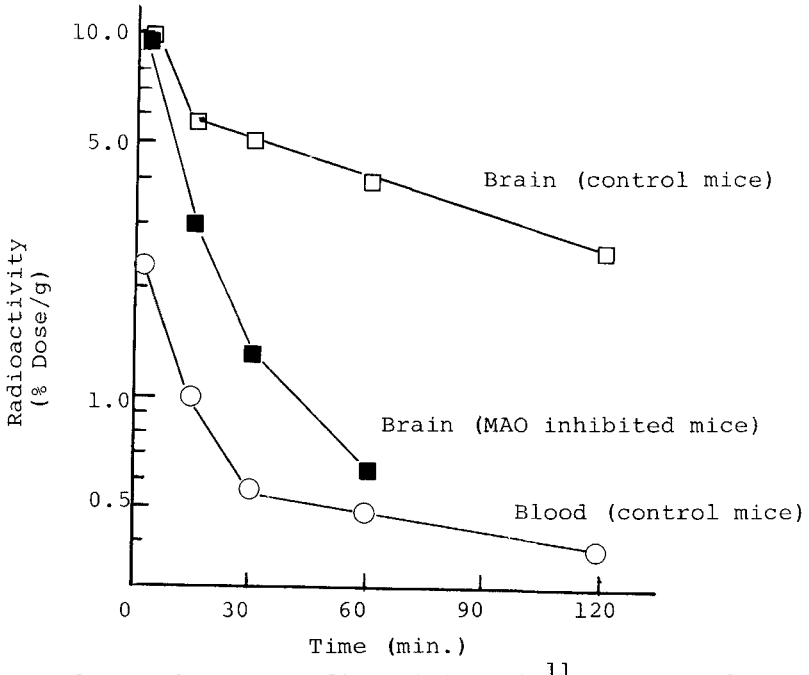
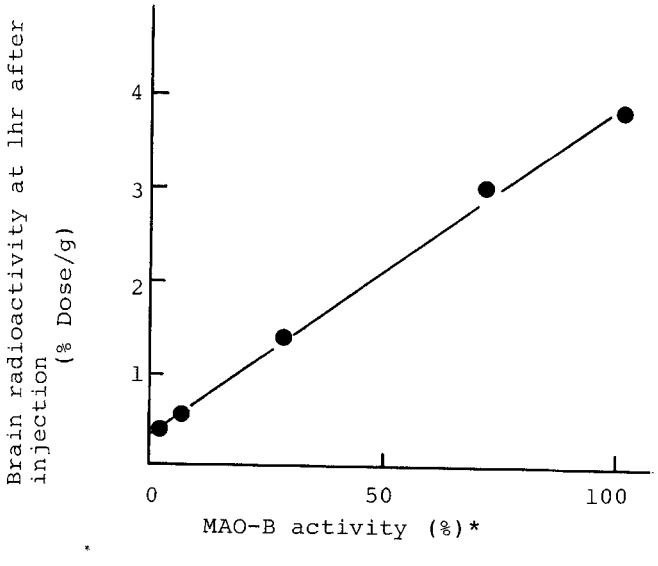



Fig. 3 Relation between radioactivity of $^{11}$C-DMPEA and MAO-B activity remaing in the brain

* Each MAO activity in the brain was measured by radiometricmethod.



## CARBON-11 LABELLED ALBUMIN MICROSPHERES FOR CLINICAL USE

F. Brady, D.R. Turton, V.W. Pike, J. Deanfield, C.M. DeLandsheere, M.J. Shea, R.A. Wilson, A. Maseri, A.P. Selwyn

MRC Cyclotron Unit and Royal Postgraduate Medical School, Hammersmith Hospital, London U.K.

Microspheres labelled with suitable positron emitters are required for the quantitation of regional myocardial blood flow (RMBF) and cardiac output by positron emission tomography. Microspheres labelled with gallium-68 have been used to study RMBF in animals (1). Their use however suffers from a number of disadvantages. The microspheres generally require lengthy pre-modification with chelating agents such as DTPA (2,3) or EDTA (4) derivatives. The resultant gallium-chelate complex is still liable to disruption by plasma components. The half life of gallium-68 (68 mins) may also lead to high radiation doses to critical organs.

Carbon-11 (half life 20.4 mins) is an attractive alternative positron label for microspheres. A stable covalent bond can be made between the microsphere and carbon-11 which would be unaffected by plasma components. No pre-modification of the microspheres would be required. The radiation dose from carbon-11 labelled microspheres would be only ca. one fifth of that from the same injected activity of gallium-68.

Consequently we have labelled human serum albumin microspheres with carbon-11 by reaction with carbon-11 iodomethane. Carbon-11-iodomethane is prepared in ethanol solution and rapid labelling of the microspheres is achieved by reaction of an alkaline suspension (pH 10) for ten minutes at 50°C. Efficient separation of labelled microspheres is readily achieved by millipore filtration allowing preparation of sterile, apyrogenic suspensions for clinical use. The overall preparation time is short, 30 minutes from the end of radionuclide production. Satisfactory radiochemical yields are achieved, ca. 20% based on $^{11}CO_2$ used and decay corrected.

Carbon-11 labelled microspheres have been evaluated against gamma labelled spheres in dog experiments. The carbon-11 label exhibits extremely high in-vivo stability and excellent correlation of blood flows with those obtained using reference long lived gamma (scandium-46) labelled microspheres.

The carbon-11 labelled microspheres are now in routine clinical use for the measurement of RMBF and cardiac output in patients.

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## CYCLIC SULFATES AS SUBSTRATES FOR NUCLEOPHILIC SUBSTITUTION

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Cyclic sulfates have been used as precursors to produce 2-fluoro-2-deoxyglucose (1) and 16-α-fluoroestradiol (2) rapidly and in high yield by nucleophilic attack of no-carrier-added fluoride. The success of this approach to fluorine-18 labeling is encouraging for further application, but the chemistry of cyclic sulfates has not been systematically studied. In order to provide a base for rational application of cyclic sulfate displacements to F-18 labeling we have studied a series of model cyclic sulfates and their products of substitution by fluoride and phenoxide ions.

The model sulfates are shown in the table. They were synthesized when possible by reaction of the corresponding diol with sulfuryl chloride. With the simple unhindered diols this route gave no desirable product and it was found necessary to produce the cyclic sulfite by reaction with thionyl chloride and then to oxidize it to the cyclic sulfate with potassium permanganate. The yields of cyclic sulfates by this route were about 60% based on diol.

The nucleophilic substitutions were carried out in acetonitrile and were generally rapid. The sulfate products of phenoxide substitution were hydrolyzed and oxidized to yield alpha-substituted ketones and carboxylic acids from primary and secondary nucleophile attack, respectively. These products were identified by physical and spectroscopic methods.

The results of the displacements indicate that steric hindrance is very important in determining the position of substitution. Reaction of both fluoride and phenoxide was predominantly at the the primary position even on relatively unhindered diols. The comformationally unfavorable glucose-2-3-cyclic sulfate gave nucleophile attack at sulfur as the only detectable result. The implications of these results for use of cyclic sulfate precursors will be discussed.

Substitution Results

| | Nucleophile (% Primary/Substitution) | |
|---|---|---|
| Cyclic Sulfate | Phenoxide | Fluoride |
| 1,2 Propane | 75 | 50-60 |
| 1,2 Butane | 100 | 100 |
| 1,3 Butane | 100 | - |
| 1,4 Butane | N/A good product yield | N/A |
| 1,2 Phenylethane | - Sulfate not stable - | |
| 2,3 Glucose 4,6 Benzylidine | Attack on sulfur | |

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# A NEW MILD AND SELECTIVE [$^{18}$F]-FLUORINATION METHOD

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Only a few practical methods are available for the controlled regiospecific introduction of [$^{18}$F]-fluorine into an aromatic compound. These methods are all based on a displacement by $^{18}F^-$ such as the Balz-Schieman reaction, the triazene decomposition and the nucleophilic displacement of activated halogen- or nitrogroups (1). During the last decade a target system was developed for the production of high amounts of [$^{18}$F]-$F_2$. However, up to now the methods used for the electrophilic introduction of $^{18}F_2$ into aromatic compounds often suffer from overreactivity and give low yields (2).

Based on the more or less controlled electrophilic monofluorination of activated aromatic rings on reaction with $CH_3COOF$ (3) and our previous results with radiohalogenation ($^{131}I$, $^{211}At$) through mercury compounds (4) we have investigated the displacement of the mercury group by [$^{18}$F]-$CH_3COOF$ as a new approach to regiospecific [$^{18}$F]-fluorination.

The reaction of an arylmercury compound with [$^{18}$F]-$CH_3COOF$ in $CH_3COOH$ at room-temperature gave within 5 min. satisfactory radiochemical yields (45-65%) of the corresponding [$^{18}$F]-fluorodemercuration products (Table 1).
It is interesting to note that the reaction of $CH_3COOF$ with benzene or toluene results in very low yields (3), but good results are obtained with the mercurated analogs, which indicates that unwanted side reactions are suppressed by this approach. However, it appears to be very critical in which solvent the demercuration is performed: in other solvents than $CH_3COOH$ like e.g. $CH_3CN$, MeOAc or EtOAc no or very low yields of $^{18}$F-compounds are obtained.

Given the relative ease with which mercury can be attached to aromatic systems this approach accounts well for the synthesis of regiospecific fluorinated aromatic compounds. In the context of positron emission tomography the method is suitable for studies that require medium to low specific activities ($<$ 370 GBq/mmol i.e. $<$ 10 Ci/mmol).

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Table 1. $[^{18}F]$-fluorinated aryl compounds from the displacement of a mercury group by $[^{18}F]$-$CH_3COOF$.

| Substrate | Product [a] | Yield(%) [b] |
|---|---|---|
| p-HgOAc-anisol | p-$^{18}$F-anisol | 65 ± 3 |
| p-HgOAc-acetanilide | p-$^{18}$F-acetanilide | 60 ± 3 |
| o-HgCl-phenol | o-$^{18}$F-phenol | 53 ± 3 |
| p-HgCl-phenol | p-$^{18}$F-phenol | 47 ± 3 |
| HgOAc-benzene | $^{18}$F-benzene | 58 ± 3 |
| HgCl-*toluene* [c] | $^{18}$F-toluenes o:m:p= 3:1:13 | 57 ± 3 |

a Identified by GCMS and by comparison of the retention times in reversed phase HPLC with those of authentic samples.

b Based on $[^{18}F]$-$CH_3COOF$; $[^{18}F]$-$CH_3COOF$ was prepared from $[^{18}F]$-$F_2$ in 80-90% chemical yield, so the radiochemical yields based on $[^{18}F]$-$F_2$ are 20-30%.

c Mixture consisting of 18% ortho, 6% meta and 76% para HgCl-toluenes(5).

# USE OF SILANE FOR INTRODUCTION OF $^{18}F$ INTO ORGANIC COMPOUNDS*

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Fluorine-18, a positron-emitting radionuclide used for studies with humans, is particularly valuable because its relatively long half-life of 110 minutes allows the radiochemist to perform quite complicated syntheses. Another advantage is its molecular size: since it is almost equal in size to hydrogen, fluorine can replace it without drastic change in the biological behaviour of the fluorinated compound.

The introduction of fluorine-18 into organic molecules was extensively investigated after it was first reported by Nozaki and Tanaka (1). The syntheses could be divided into two general categories: those involving introduction at the no-carrier-added level, and those involving introduction with the addition of a carrier. The former synthesis was carried out via triazine (3) and heterogeneous exchange reaction (4,5); the latter via the Balz-Schiemann reaction (1,5), the isotopic exchange reaction (7), cleaving of the carbon-tin bond (8), acetylhypofluorite (9) and some other.

We have developed a new technique to introduce fluorine-18 into organic compounds via silanes and have proved its feasibility on some simple compounds (10). The silanes are generally stable compounds and can be prepared easily from inexpensive, commercially available substances. The reaction of silanes with $^{18}F$-fluorine is very fast and the entire synthesis usually takes less than 30 minutes(10).

In general, silanes were reacted with $^{18}F$-fluorine in $CFCl_3$ or $CCl_4$ kept at -78°C or -32°C. After bubbling of $^{18}F$-$F_2$ is finished the solvent is evaporated and the $^{18}F$-labelled compound separated by high performance liquid chromatography. The radiochemical yields achieved after irradiation varying in length from 5 to 30 minutes were 15%-20% expressed relative to $^{18}F$-$F_2$ extracted from a low-pressure target (11). The synthesis of $^{18}F$-labelled 4-fluoroantipyrine (a blood flow tracer), and 6-fluorodopa (a tracer for dopaminergic receptor sites) will be discussed and compared with published syntheses. The radiochemical yield of $^{18}F$-4-fluoroantipyrine was about 20%; the radiochemical yield of $^{18}F$-6-fluorodopa was about 15%. The radiochemical purity of both compounds exceeded 98%, and the specific activity at the end of irradiation was abtout 830 mCi/mmol when 30

minutes irradiation was used. The syntheses reported here have higher radiochemical yields, and simpler purification procedures than those described in the literature (12,13).

Radiopharmaceuticals were identified by comparing the HPLC eluston volumn and $R_f$-value to those of authentic samples identified by IR, $^1H$ and $^{19}F$-NMR, and MS.

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*This investigation was supported in by the Medical Research Council of Canada, the Cone Memorial Research Fund of the Montreal Neurological Institute and a Killam Scholarship to M.D.. We are specially grateful to Dr. Victoria Lees for editing this manuscript. The Faculty for Graduate Studies and Research, McGill University, provided financial support toward the purchase of an HPLC system.

# THE SYNTHESIS OF $^{18}F$-LABELED ARYL FLUORIDES BY ELECTROPHILIC FLUORINATION OF ARYLTRIMETHYLSILANES AND ARYLPENTAFLUOROSILICATES

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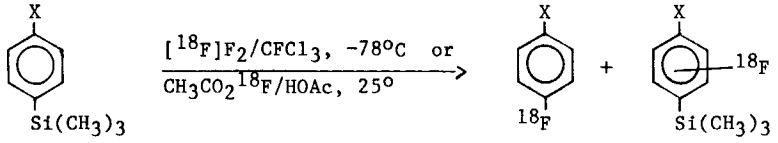
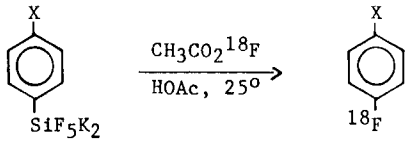
Fluorine-18 substituted aromatic rings are important structural components of a number of $^{18}F$-labeled radiopharmaceuticals (1,2). To date, the preparations of $^{18}F$-labeled aryl fluorides have relied mainly on the Schieman Reaction, the triazene decomposition and nucleophilic aromatic substitution (3-10). The application of electrophilic fluorination to the synthesis of $^{18}F$-labeled aryl fluorides has recently been reported for aromatic compounds (11) ($^{18}F$ for H substitution) and for aryl tin compounds (12) ($^{18}F$ for Sn substitution).

In order to extend the scope of the electrophilic aromatic substitution reaction, we have investigated the electrophilic radiofluorination of aryltrimethylsilanes (1) and arylpentafluorosilicates (2), substrates which have previously been used in other electrophilic halogenated reactions (13-17). We report here that 1 and 2 are fluorinated with either $[^{18}F]F_2$ or $CH_3CO_2{}^{18}F$ to give the corresponding $^{18}F$-labeled aryl fluorides (3) and aryltrimethylsilyl fluorides (4) in overall radiochemical yields of 10-20% depending on the reaction conditions (Eq. 1, Table 1).




1 3 4 (Eq. 1)

Compounds 3 and 4 can easily be separated by HPLC or GLC. The structures of compounds 4 were elucidated by treating the reaction mixture with $Br_2$ (18) or MeOH-$HClO_4$ solution (19) to give the corresponding aryl bromide or hydrogen substituted product. Fluorination of arylpentafluorosilicates (2) with $CH_3CO_2{}^{18}F$- gave the corresponding aryl $[^{18}F]$fluorides (3) in 10-20% radiochemical yield (Eq. 2).




2 3 (Eq. 2)

The yields of compounds 3 and 4 depend on the reaction conditions. Lower temperature favors the formation of aryl fluorides (Tables 1 and 2), $[^{18}F]F_2$ gives a better yield of aryl fluorides (and a higher ratio of C-Si to C-H substitution) than $CH_3CO_2{}^{18}F$, and the electron-withdrawing groups on the aromatic ring favor silicon substitution over hydrogen substitution.

In summary, rapid regiospecific incorporation of $^{18}F$ into simple aromatic molecules can be accomplished by fluorination of aryltrimethylsilanes or arylpentafluorosilicates with $[^{18}F]F_2$ or $CH_3CO_2{}^{18}F$ under very mild conditions. This method thus provides an alternative route to $[^{18}F]$-labeled radiopharmaceuticals such as p-$[^{18}F]$fluorohippuric acid which is otherwise difficult to prepare, and other $[^{18}F]$-labeled radiopharmaceuticals which are not required to be NCA and contain an aromatic ring without activating groups.

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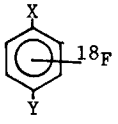
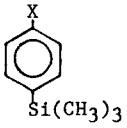
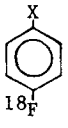
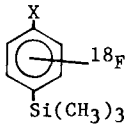
Table 1
Radiochemical Yields of F-18 Aryl Fluorides From the Fluorination of Aryltrimethylsilanes or Arylpentafluorosilicates with $CH_3CO_2{}^{18}F$ in $CH_3CO_2H$ at 25°C

| Substrate | | Radiochemical Yield (%) | | $(\frac{C\text{-}Si}{C\text{-}H})$ subst. |
|---|---|---|---|---|
|  | |  |  | |
| X | Y | | | |
| H | $Si(CH_3)_3$ | 5 | 6 | 0.83 |
| Cl | $Si(CH_3)_3$ | 7 | 4 | 1.75 |
| Br | $Si(CH_3)_3$ | 14 | 11 | 1.27 |
| $CH_3$ | $Si(CH_3)_3$ | 5 | 7 | 0.71 |
| $OCH_3$ | $Si(CH_3)_3$ | 2 | 6 | 0.33 |
| $COCH_3$ | $Si(CH_3)_3$ | 8 | 6 | 1.33 |
| O-$COCH_3$ | $Si(CH_3)_3$ | 3 | 10 | 0.30 |
| $Si(CH_3)_3$ | $Si(CH_3)_3$ | 10 | 4 | 2.50 |
| $CONHCH_2CO_2H$ | $Si(CH_3)_3$ | 12 | a | ---- |
| H | $SiF_5K_2$ | 12 | a | ---- |
| $CH_3$ | $SiF_5K_2$ | 18 | a | ---- |

[a] Undetermined.

Table 2
Radiochemical Yields of F-18 Aryl Fluorides From the Fluorination of Aryltrimethylsilanes with $[^{18}F]F_2$ in Freon-11 at -78°C

| Substrate | Radiochemical Yield (%) | | $(\frac{C\text{-}Si}{C\text{-}H})$ subst |
|---|---|---|---|
|  |  |  | |
| X | | | |
| H | 12 | 13 | 0.92 |
| $COCH_3$ | 27 | 8 | 3.38 |
| Br | 29 | 8 | 3.63 |

# IS THE ADDITION OF [$^{18}$F]-ACETYLHYPOFLUORITE TO GLUCALS REALLY STEREOSELECTIVE ?

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Of all positron emitting radiopharmaceuticals 2-deoxy-2-[$^{18}$F]fluoro-D-glucose (FDG) has generated the most widespread interest since it can be used for both myocardial and cerebral glucose metabolism as for the detection of tumors. As a result, many centres do research towards simple and efficient synthesis of this compound.
The synthesis, using elemental fluorine, has the main disadvantage of giving a mixture of [$^{18}$F]-gluco-and mannopyranosyl fluorides (1).Recently, some methods have appeared which seem to give exclusively [$^{18}$F]-FDG by the addition of [$^{18}$F]-$CH_3COOF$ to either triacetylglucal in acetic acid (2,3) or glucal in water (4). However, from simple CPK-models such a high degree of stereoselectivity seems rather peculiar.

For this reason it was decided to reexamine all products formed in the above mentioned reactions. Therefore, the reaction mixtures were analysed by HPLC either directly or after hydrolysis and reacetylation. After separation eight products were identified by $^1$H-NMR spectroscopy.
For the main products ($\alpha$ and $\beta$ anomers of FDG and FDM) the results are summarized in Table 1.

In addition, a TLC system was developed for the separation of deoxy-fluoro-glucose and deoxy-fluoro-mannose after hydrolysis, using phosphate loaded silica gel plates. Work is in progress to use this support for preparative HPLC column separation.

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Table 1. Yields(%) of [$^{18}$F]-fluoro-deoxy-glucose(FDG) and [$^{18}$F]-fluoro-deoxy-mannose(FDM) from reaction of [$^{18}$F]-$CH_3COOF$ with glucals.

| Substrate | Method [a] | Solvent | α-FDG | (α+β) | β-FDG | α-FDM | (α+β) | β-FDM |
|---|---|---|---|---|---|---|---|---|
| TAG | A | AcOH | 73 | (76) | 3 | 5 | (17) | 12 |
| TAG | B | AcOH | 80 | (83) | 3 | 5 | (14) | 9 |
| Glucal | B | 0.5 M NaOAc | 18 | (42) | 24 | 26 | (56) | 30 |
| Glucal | B | 0.025 M NaOH | 14 | (43) | 29 | 38 | (53) | 15 |
| Glucal | B | 0.025 M AcOH | 24 | (42) | 18 | 35 | (55) | 20 |
| Glucal | A | AcOH | 15 | (28) | 13 | 38 | (69) | 31 |

[a] A; $CH_3COOF$ prepared in acetic acid (2) or
B; by a gas-solid-phase reaction (4,5)

## DETAILED STUDY OF NUCLEOPHILIC AROMATIC SUBSTITUTION USING CARRIER-FREE FLUORINE-18

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The subject of nucleophilic aromatic substitution using carrier-free fluorine-18 fluoride acquired the status of a well defined field of labelling reactions. Two approaches have been followed up at present: the addition-elimination mechanism for reactions involving substrates activated by electron withdrawing substituents, and the unimolecular mechanism for the thermal decomposition of diazonium ions. The latter has been applied to prepare most of the aromatic derivatives labelled with fluorine-18, but the yield or the specific activity of the final product were rather low. Unlike that, nucleophilic aromatic substitution on activated substrates was established now to be the most efficient method of introducing the carrier-free fluorine-18 anion into aromatic compounds (1). Even the preparation of the neuroreceptor ligand molecules spiro= peridol and haloperidol makes the no-carrier-added state imperative if neurochemistry has to be tested in vivo.

Substitution reactions using the F-18 fluoride were studied on the basis of our former results obtained from the exchange of F-18 for aromatic iodine (1). The reactions leading to the results given in the table below were all carried out within 15 min at 180°C using dimethyl sulphoxide as solvent. The activated substrates listed were chosen with regard to the eventual application to the final synthesis of F-18 spiroperidol. The study offered yet encouragement for the preparation of this important receptor imaging molecule. It may be synthesized in acceptable radiochemical yields of 15-20% from several of the precursors listed in the table.(2) However work is continued to find precedent for the most suitable preparation. Substrates which were not reported in the literature have been characterised by n.m.r., i.r. and mass spectroscopy, and h.p.l.c. has been used for mapping the reaction conditions.

Since the displacement of aromatic nitro groups by F-18 fluoride in various substrates was reported recently (3), the labelling reactions were compared with F for $NO_2$ exchange in the derivatives of interest. The effects of temperature, reaction time, and substrate concentration were examined.

Anhydrous HF-18 served as a source for the no-carrier-added F-18 fluoride. It was produced via the Ne-20(He-3,He-4 n)Ne-18 → F-18 reaction as described (4). The average yield by this method was 5.4 mCi/μAh.

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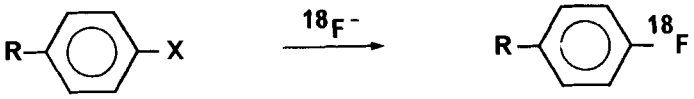
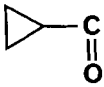
TABLE: YIELD FROM NO-CARRIER-ADDED F-18 FOR X EXCHANGE.




| R / X | CN | $CH_3-C(=O)$ | $Cl-(CH_2)_3-C(=O)$ |  |
|---|---|---|---|---|
| Cl | 8% | 3% | 1% | / |
| Br | 65% | 50% | 10% | 80% |
| I | 50% | / | / | / |
| $NO_2$ | 70% | 55% | substantial formation of side products | exists as allylic derivative* |

*The yield of the F-18 labelled product was 65-80%. Only one radioactive product was detected on t.l.c (silica; hexane/ethyl acetate 80/20) which showed clearly the substitution of the nitro group. Unfortunately the molecule was not suited for the final reaction with 1-phenyl-1,3,8-triazaspiro(4,5)decan-4-one to yield the spiroperidol.