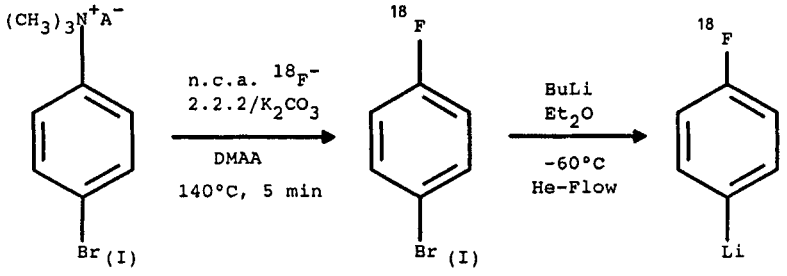
^{18}F-Fluoroarylation by 4-[^{18}F]fluorophenyl-lithium via 4-[^{18}F]fluorohalobenzenes.

GAIL, R.; COENEN*, H.H.; and STÖCKLIN, G. Institut für Nuklearchemie, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany. Nuklearchemie und Radiopharmazie, Klinik für Nuklearmedizin,Universitätsklinikum Essen IG 1, 4300 Essen, Germany.

4-[^{18}F]fluorophenyl-lithium can be regarded as a ^{18}F-fluoroarylation agent to label complex organic molecules with electrophilic moieties. The precursor of this labeled organometallic reagent are 4-[^{18}F]fluorohalobenzenes. 4-[^{18}F]Fluorohalobenzenes can easily be prepared in high radiochemical yields by a one-step ^{18}F-for-N$(CH_3)_3$ exchange on 4-halophenyl-trimethylammonium salts (Scheme1).



$A^- = TfO^-, TosO^-, MeOSO_3^-, I^-$

Scheme 1. Synthesis and transformation of 4-[^{18}F]fluorohalobenzenes into 4-[F]fluorphenyl-lithium

4-[^{18}F]fluorobromo- and 4-[^{18}F]fluoroiodobenzene have been prepared by a one-step reaction via nucleophilic exchange on corresponding 4-bromo- and 4-iodophenyl-trimethylammonium salts. Optimization studies of the reaction parameters revealed a marked sensitivity of the radiochemical yield of the 4-[^{18}F]fluorohalobenzenes on the reaction solvent, the anion activator, the counter ion of the trimethylammonium group, and the type of the halogen atom in para position to the leaving-group. In dimethylacetamide radiochemical yields of about 40% of 4-[^{18}F]fluorobromobenzene was obtained using 2.2.2/K_2CO_3, as anion activator at 140°C. The most effective anion activation was achieved with the 2.2.2/K_2CO_3-system or tetramethylammonium hydrogencarbonate, the latter leading to radiochemical yields of 50% ± 5 for 4-[^{18}F]fluorobromobenzene. The leaving ability of the trimethylammonium group increases strongly with decreasing basicity of the counter anion with the sequence : tosylate < methyl sulfate < iodide < triflate (Table 1) which is in good aggreement with the acidities of the corresponding acids. By an exchange of the bromo-substituent for a iodo-substituent in para position to the trimethylammonium leaving group radiochemical yield rises up to 80% for 4-[^{18}F]fluoroiodobenzene [1].

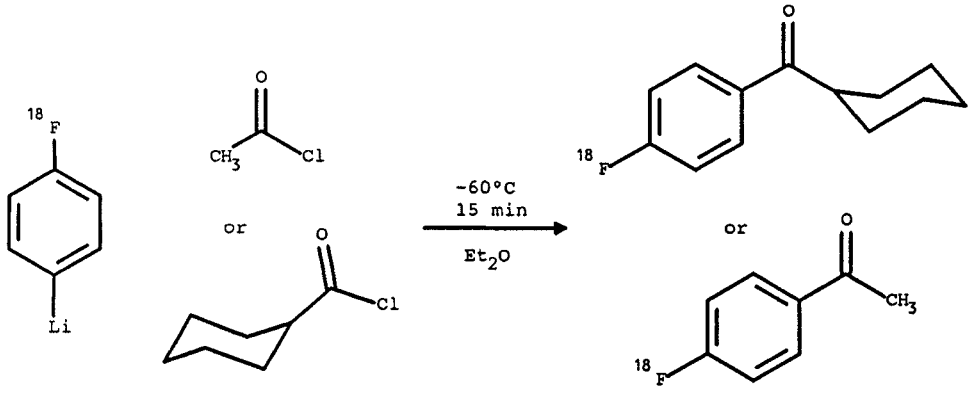
Table 3. Influence of the counter ion of the trimethylammonium group on the radiochemical yield of 4-[^{18}F]fluorobromobenzene

Anion	RCY (%)	time (min)
Tosylate	7 ± 1	5
	16 ± 3	15
Methyl sulfat	20 ± 4	5
Iodide	23 ± 3	5
Triflate	43 ± 3	5

Reaction conditions: [4-bromophenyl-trimethylammonium salt] = 35 mmol/L, [2.2.2] = 100 mmol/L, 140^{0}C, 500µL DMAA, molar ratio 2.2.2/K_2CO_3 = 2:1, n.c.a. [^{18}F]fluoride

The work-up-procedure to yield the pure, anhydrous 4-[^{18}F]fluorohalobenzene includes dillution of the reaction mixture with water, fixation of 4-[^{18}F]fluorohalobenzene on SEP-PAK C18, heating of the SEP-PAK C18 to 80°C, elution of the 4-[^{18}F]fluorohalobenzene from the heated cartridge by a helium gas flow of 100mL/min, drying of the carrier gas by a P_2O_5-cardridge and trapping of the 4-[^{18}F]fluorohalobenzene in a BuLi solution in ether at -60°C. A quantitative transformation of the 4-[^{18}F]fluorohalobenzene into the organo-lithium compound was observed in the case of 4-[^{18}F] fluorobromobenzene.

Until now, the behaviour of 4-[^{18}F]fluorophenyl-lithium was studied in reactions with acetylchloride and cyclohexylcarbonic acid chloride as model compounds (Scheme 2) at -60°C, and 15min, giving rise to non-optimized radiochemical yields for 4-[^{18}F]fluoroacetophenone and 4-[^{18}F]fluorophenyl-cyclohexyl-ketone of 20% ± 5 and 30% ± 5 respectivly, based on 4-[^{18}F]fluorohalobenzene.



Scheme 2. Reactions of 4-[^{18}F]fluorophenyl-lithium with acetylchloride and cyclohexylcarbonic acid chloride as model compounds

[1] Gail R. and Coenen H.H.,(1993) A one step Preparation of the n.c.a. Fluorine-18 Labelled Synthons : 4-Fluorobromobenzene and 4-Fluoroiodobenzene. *Appl. Radiat. Isot.* (in Press).

Radioiodinations of *nido*-Carboranes. Potential Hydrophilic Pendant Groups For Radiohalogenation of Biological Molecules.

WILBUR D.S., HAMLIN D.K. AND SRIVASTAVA R.R. Department of Radiation Oncology, University of Washington, Seattle, WA 98195

Pendant groups, such as functionalized aryl halides or vinyl halides, have been used for radiohalogenations of biomolecules (1-4). The use of such groups can provide a means of radiohalogenation where: 1) the biomolecule does not normally react with radiohalogens, 2) direct radiohalogenation of the biomolecule leads to an unstable attachment of the nuclide, or 3) direct radiohalogenation causes a decrease in desired biological properties (e.g. receptor binding). Our chemical studies with boron cage molecules have led to a preliminary investigation designed to evaluate the potential for *nido*-1,2-carboranes (see Scheme I, compound **3**) as pendant groups for radiohalogenation of biomolecules. These *nido*-(nest like)carborane moieties have been of interest for preparing water soluble compounds for application to boron neutron capture therapy (BNCT) (5-7). Hawthorne and co-investigators (7,8) have indicated that these compounds might be used for radioiodination of boronated molecules under study for BNCT, but there is little information on their reactivity with radioiodine, or other radiohalogens.

nido-1,2-Carboranes, **3**, can be prepared in two synthetic steps from substituted acetylenes, **1**, as depicted in Scheme I. The initially prepared substituted *closo*-1,2-carboranes, **2**, can be further modified (in most examples) using standard organic synthesis procedures (9-11). Formation of the *nido*-cage can be efficiently accomplished by removal of a cage boron (adjacent to the two cage carbons) by reaction with pyrrolidine at room temperature (12), and can also be accomplished by reaction with strong base (13) or with fluoride ion (14). This removal of a boron atom produces an asymmetric cage structure as depicted in Scheme I, compound **3**. Iodination of the *nido*-1,2-carborane cage is believed to occur at the boron atoms adjacent to the carbon atoms on the open face of the cage as depicted in Scheme I, compound **4** (15).

In our investigation, a variety of functionalized *nido*-1,2-carboranes were synthesized (**3a** - **3h**), and their reactivities in iodination reactions were evaluated. The results of the chemical and radiochemical yields are given in Table I. Initial studies were conducted by reacting the functionalized *nido*-1,2-carboranes with 1.5 equivalents of N-chlorosuccinimide (NCS) and 1 equivalent of NaI in MeOH containing 1% HOAc (v/v) for 5 min at room temperature. The iodination reactions were surprisingly rapid, with the reaction mixture becoming colorless within 1 min of the addition of NCS for most of the compounds. All iodination reactions of *nido*-1,2-carboranes resulted in the production of one major new species by HPLC analysis, but some reactions had a trace of unsubstituted starting material remaining and a trace of a di-iodo species being formed. The yields of iodinated *nido*-carboranes were very good (91-98% by HPLC). Without the addition of NaI, the NCS reacted with the *nido*-carboranes to (presumably) give the chlorinated products, although at a much slower rate of reaction. When two equivalents of iodination reagents were used, a mono-iodine substituted carborane was obtained prior to the addition of the second iodine. However, it was difficult to add a second iodine in most examples, perhaps indicating a difference in steric environment or electronic nature from the non-iodinated material.

Subsequent to the iodination studies, no-carrier-added (*nca*) radioiodinations of the *nido*-1,2-carboranes were examined. In those reactions, a quantity (300-400 μCi) of Na[^{125}I]I was mixed with 100 μg of *nido*-carborane and 20 μg of NCS in 125 μL solution for 5 min. The radiochemical yields were excellent (95-99%) for most compounds studied, with the exception of propionate **3e**, which had yields that could be considered very good (82% and 84% yields in repeat reactions). Additional studies were conducted to assess the quantities of reagents needed in the radioiodination reactions. The reactivity of methyl substituted **3b** with *nca* Na[^{125}I]I was studied by decreasing its quantity to 10 μg, 1 μg, and 0.1 μg. At the 10 μg level, a radiochemical yield of 94% was obtained, similar to the 99% yield obtained for 100 μg, but radiochemical yields of 77% and 20% were obtained for 1 μg and 0.1 μg, respt. Decreasing the quantity of NCS added, in a reaction containing 100 μg of **3b**, from 20 μg to 5 μg did not affect the radiochemical yields. It was also found that the reaction time could be shortened to 30 seconds without any affect on the radiochemical yields.

The relative reactivities of the *nido*-1,2-carboranes **3a-3h** were examined in competitive reactions with equimolar quantities of phenol. When the reactions were conducted as described for non-radioactive iodinations (above), only iodinated *nido*-carboranes were observed by HPLC. In the reaction of **3b** in the presence of an equimolar quantity of phenol as described for *nca* radioiodinations (above), the majority of the radiolabeled species was the radioiodinated *nido*-carborane, **4b**, with only a trace of iodinated phenol being formed (< 2 %). The high reactivity of *nido*-carboranes with electrophilic radioiodine may permit radiolabeling compounds having this pendant group, even in the presence of highly activated aromatic moieties such as a phenolic moiety.

Based on the results obtained, we believe that *nido*-1,2-carboranes may be effectively used as pendant groups for radiohalogenations of some types of biomolecules (e.g. peptides and proteins). Modification of *nido*-1,2-carboranes, such that they can be attached to biomolecules, is presently under study. The anionic character of these compounds makes them very water soluble (as Na salts). Perhaps more important is the fact that the boron-halogen bond is considerably stronger than carbon-halogen bonds (16), suggesting their use when labeling with astatine-211. As an example of the use of *nido*-1,2-carboranes as a pendant group, we have prepared a radioiodinated misonidazole derivative (Scheme II, **4i**) which will be evaluated for use in BNCT, and as a hypoxia imaging agent similar to iodovinyl misonidazole (17).

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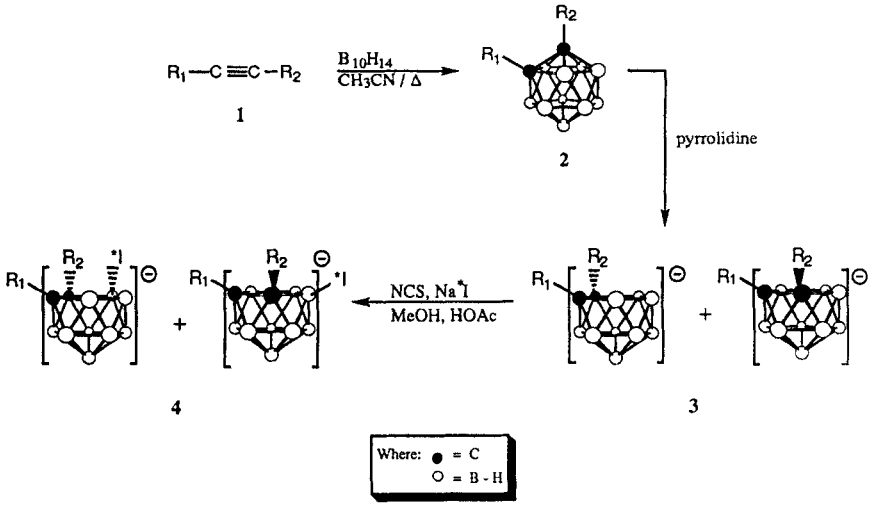
Table I: Iodination Yields of Substituted *nido*-1,2-Carboranes*

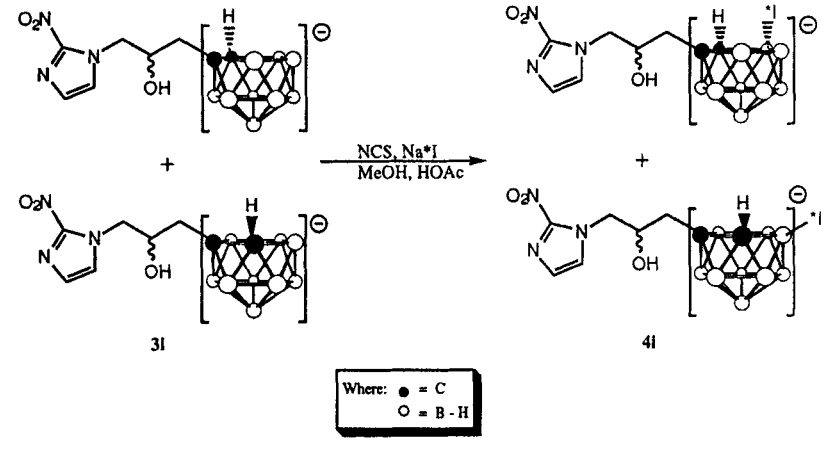
Cmpd	Substituents R_1	R_2	Iodination Yield (%)	Radioiodination Yield (%)
a	H	H	90	98
b	H	CH_3	92	99
c	CH_3	CO_2CH_3	91	95
d	H	$Si(CH_3)_3$	96	98
e	H	$CH_2CH_2CO_2H$	93	84
f	H	$CH_2CH_2CO_2CH_2CH_3$	95	96
g	H	$CH_2CH_2CO\text{-}N(CH_2)_4$**	98	98
h	H	$CH_2CH_2CH_2CN$	95	99

*Yields were obtained from HPLC analysis by UV and/or gamma detection. Compound designations are for the reaction of 3 with NCS/Na*I for 5 min at r.t. to form 4 (e.g. 3a → 4a, etc.) in Scheme I. ** $-N(CH_2)_4$ is pyrrolidine

Scheme I: Synthesis and Radioiodination of *nido*-1,2-Carboranes.



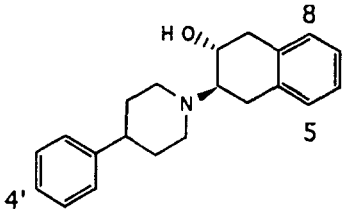
Scheme II: Radioiodination of a *nido*-Carboranyl Congener of Misonidazole.



Synthesis And In Vivo Evaluation Of Radioiodinated (+)-7-Iodobenzovesamicol: A High-Affinity Ligand For The Sigma Binding Site

Y-W. JUNG, D.L. GILDERSLEEVE, M.E. VAN DORT, D.E. KUHL AND D.M. WIELAND.
Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, MI. 48109-0552

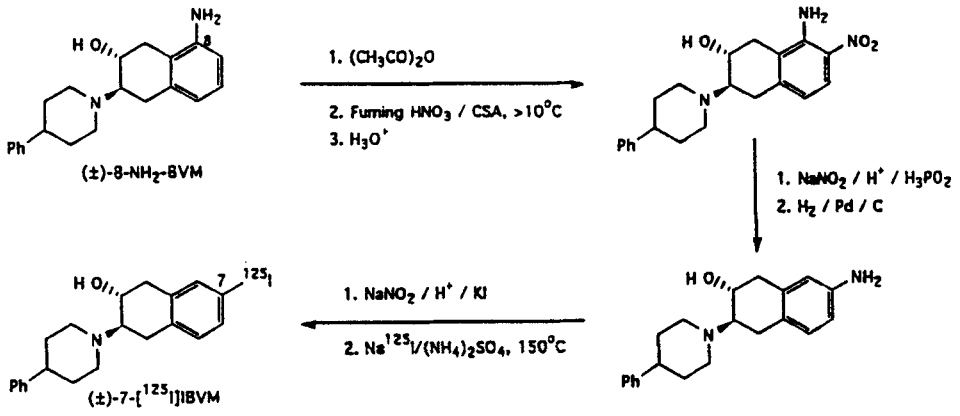
We have reported the synthesis and *in vivo* evaluation of ten radioiodinated derivatives of benzovesamicol (IBVM's) for external mapping of presynaptic cholinergic nerve density in brain by SPECT(1). Based on extensive *in vivo* testing of IBVM's in rodents, (-)-5-[^{123}I]IBVM was chosen for preclinical studies and subsequently approved by the F.D.A. for clinical evaluation in the detection of cholinergic lesions in Alzheimer's disease and other dementing disorders.(2) However, in a totally unexpected finding, SAR studies of the positional isomers of IBVM revealed that one of the isomeric IBVM's, 7-[^{125}I]IBVM, binds strongly to the sigma site *in vivo* in mouse and rat brain. Of the five IBVM isomers evaluated, ie., 5-, 6-, 7-,8- and 4'- IBVM, this strong sigma-site binding is unique to the (+)-enantiomer of 7-IBVM.



Racemic 7-NH_2-BVM was synthesized from 8-NH_2-BVM(3) via regioselective *ortho*-nitration followed by reductive deamination and conversion to the 7-iodo analog (Scheme 1). Solid-state radioiodide exchange labeling gave (±)-7-[^{125}I]IBVM in 65-85% yield and 98% radiochemical purity after Sep-Pak purification; specific activity was >140 Ci/mmol. The optical enantiomers of (±)-7-[^{125}I]IBVM were resolved by chiral HPLC using a Chiracel OD column. Figure 1 compares the regional brain distribution in mice 24 h after iv. administration of racemic 7-[^{125}I]IBVM and its enantiomers. The major difference between the distribution of the enantiomers is a striking 29-fold greater retention of the (+)-isomer in the cerebellum at 24 h. Notable also was the 24 h striatal concentration of the (+)-isomer which was only half that of the (-)-isomer.

Injection of mice with haloperidol (1mg/kg; i.p.), a potent blocker of sigma sites, one hour prior to tracer injection lowers the 4-h accumulation of (+)-7-[^{125}I]IBVM in all brain regions by 75-85% including the striatum (Figure 2). In contrast, haloperidol pretreatment increases the striatal accumulation of (-)-7-[^{125}I]IBVM by 150%, while other regional brain accumulations are unchanged. The (-)-enantiomer of 7-[^{125}I]IBVM shows a regional distribution pattern in the mouse brain nearly identical to that of the vesamicol receptor-mapping tracer (-)-5-[^{125}I]IBVM; the IC_{50} values for binding of racemic 5-IBVM and racemic 7-IBVM to the vesamicol receptor are both < 10 nM. Receptor screening of racemic 7-IBVM by the NovaScreen®/NIMH Drug Discovery Program using 26 different assays (vesamicol receptor assay not included) revealed potent binding only to sigma sites; Ki versus [^{3}H]di(2-tolyl)guanidine = 28 nM. Binding to dopamine-2 and serotonin-2 receptors was > μM.

Scheme 1. SYNTHESIS OF (±)-7-[^{125}I]IODOBENZOVESAMICOL



The enantioselectivities of the various IBVM's for binding to the vesamicol receptor and sigma site are presently being determined. Nonetheless, these preliminary studies lend support to the potential use of (+)-7-[^{123}I]IBVM as a mapping agent for sigma sites in the study of human brain disorders.

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Figure 1. The uptake of optical isomer of 7-[^{125}I]IBVM in mouse tissue at 24 h.

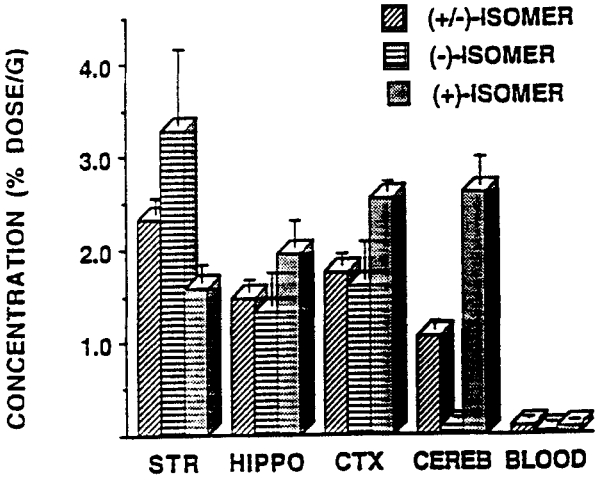
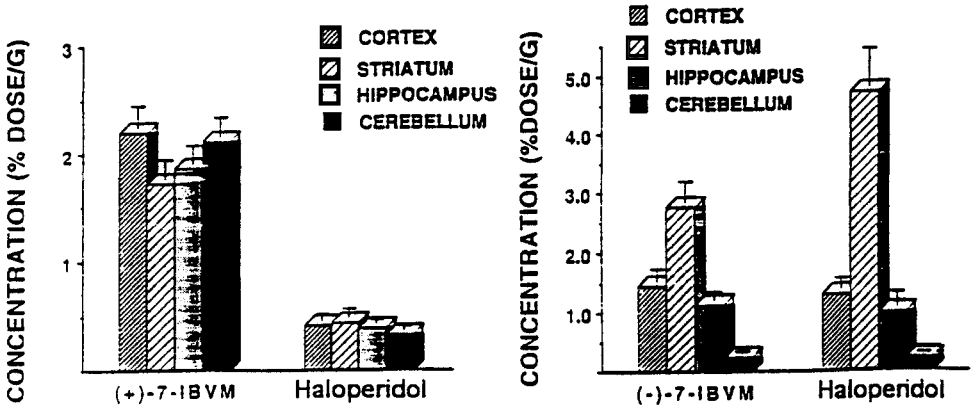


Figure 2. The uptake of (+)- or (-)-7-[^{125}I]IBVM in mouse tissue at 4 h. Haloperidol (1 mg/kg) was administered iv, 1 hour before injection of the radioligand.



Site-Directed Design and Synthesis of a Brain-Targeted Radioiodinated Nucleoside for Diagnosis of Herpes Simplex Encephalitis.

Morin, K.W.; Knaus, E.E.; and Wiebe, L.I. *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8*

Herpes Simplex Encephalitis (HSE) is the most common sporadic viral infection of the central nervous system. This is a devastating infection because without treatment, the mortality rate approaches 70% and neurological deficits among survivors occur frequently. HSE represents a challenge to the clinician since specific symptoms are lacking and results obtained from conventional diagnostic procedures such as EEG or CT can be mimicked by other neurological conditions. Brain biopsy remains the only definitive means of diagnosis. The etiologic agent in most patients is Herpes Simplex Virus Type-1 (HSV-1) and most often involves the orbital region of the frontal lobes and also the temporal lobes. Radioiodinated (E)-5-(2-iodovinyl)-2'-deoxyuridine (IVDU) has been studied previously as a non-invasive scintigraphic agent to image virus-infected tissue [1]. However, this nucleoside has limited ability to traverse the blood-brain barrier (BBB). Coupling IVDU to a dihydropyridine-pyridinium salt redox chemical delivery system (CDS)[2] via a cleavable ester linkage was carried out. The conceptual model involves permeation of the dihydropyridine prodrug across the BBB due to its high lipophilicity. Oxidation to the pyridinium salt in a manner analogous to the NAD ⇔ NADH redox system could occur in the brain. The highly polar salt species is retained, resulting in an elevated and sustained concentration within the brain. The salt is then slowly hydrolyzed to release the non-toxic trigonelline and IVDU, the active drug. A synthetic strategy was developed to facilitate the synthesis of a high specific activity labelled compound for *in vivo* investigation.

Regiospecific reaction of 5-iodo-2'-deoxyuridine with *t*-butyldimethylsilyl chloride in the presence of imidazole yielded the 5'-O-*t*-butyldimethylsilyl derivative. Subsequent reaction with nicotinoyl chloride hydrochloride in pyridine afforded 5-iodo-3'-O-(3-pyridylcarbonyl)-5'-O-*t*-butyldimethylsilyl-2'-deoxyuridine. Deprotection with $n\text{-}Bu_4N^+ F^-$ yielded the 3'-O-nicotinoyl nucleoside. Coupling with (E)-*n*-Bu_3Sn-CH=CH-TMS in the presence of $(Ph_3P)_2Pd(II)$ chloride gave (E)-5-(2-trimethylsilylvinyl)-3'-O-(3-pyridylcarbonyl)-2'-deoxyuridine. Quaternization with iodomethane yielded the 1-methylpyridinium iodide salt. Attempts to synthesize the methylpyridinium derivative as the tosylate or sulfate salt was unsuccessful due to facile cleavage of the trimethylsilylvinyl moiety. Iodination of the reactive vinylsilane with iodine monochloride and reduction of the quaternary iodide salt with sodium dithionite in the presence of sodium bicarbonate was carried out as a one pot synthesis to afford (E)-5-(2-iodovinyl)-3'-O-(1-methyl-1,4-dihydropyridyl-3-carbonyl)-2'-deoxyuridine (**IVDUCDS**)(fig.1).

Radioiodination of IVDUCDS was performed by addition of $Na^{131}I$ to the iodine monochloride solution. Isolation of the labelled product was facilitated by the two phase reduction medium (H_2O/ethyl acetate) which allowed the lipophilic labelled product to be extracted into the ethyl acetate fraction in high purity. Further purification of the organic extract was performed by reversed phase HPLC using H_2O/acetonitrile (60/40) at 1.5 mL/min as mobile phase (fig. 2). Tracer quantities have been prepared at low specific activities (8.75 mCi/mmol) with 47% chemical and 22% radiochemical yield after purification by HPLC. Higher specific activities have been obtained with some reduction in chemical and radiochemical yield.

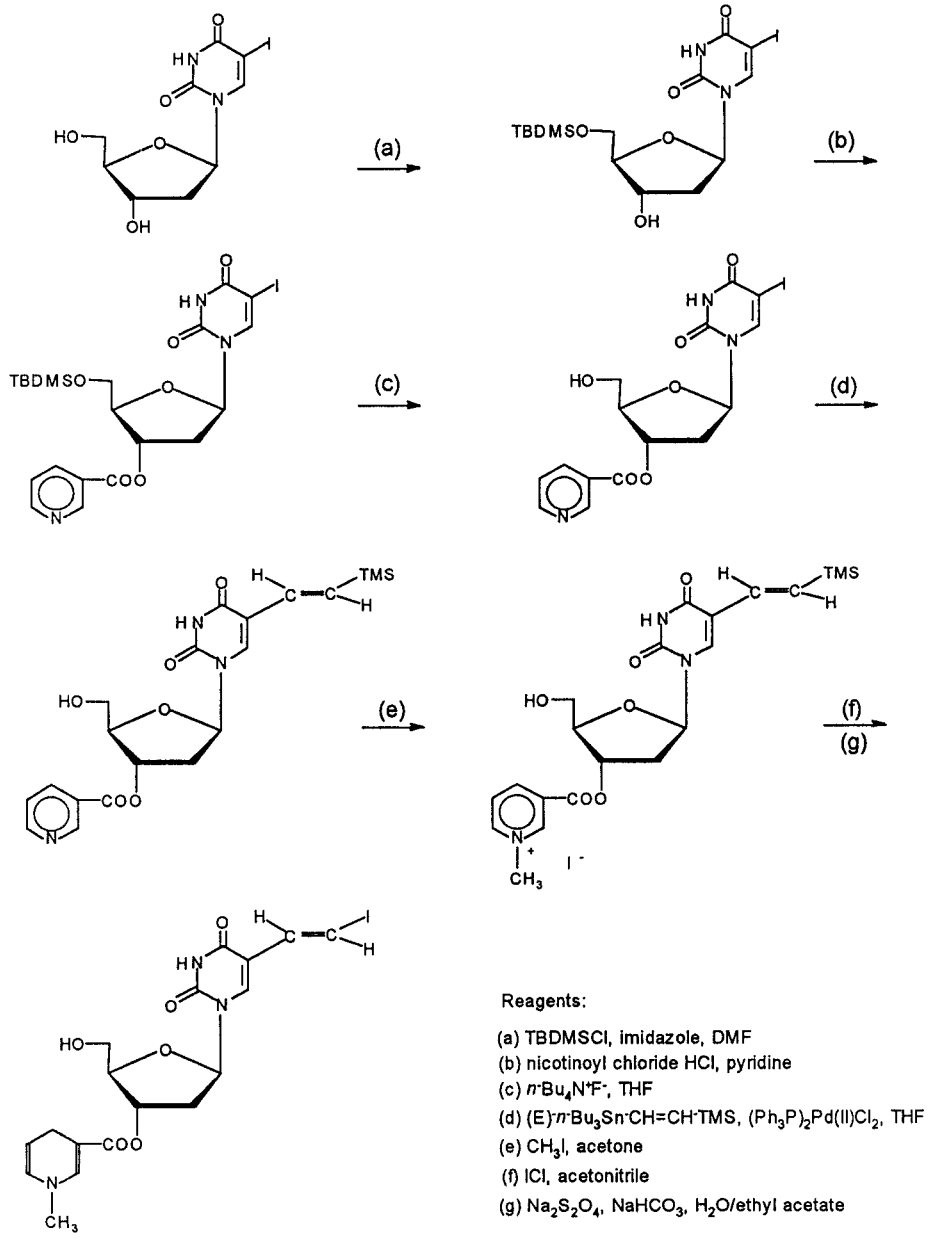


Figure 1 Synthesis of IVDUCDS

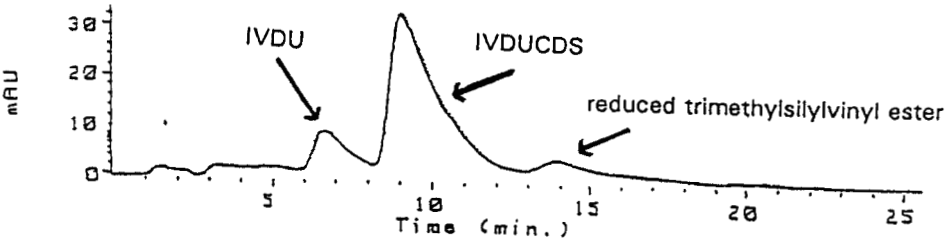


Figure 2 HPLC profile of the ethyl acetate fraction after radiolabelling IVDUCDS (retention time of 9.24 min).

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The Yield of F-18 from Different Target Designs in the $^{18}O(p,n)^{18}F$ Reaction on Frozen $[^{18}O]CO_2$.

Mahmoud L. Firouzbakht, David J. Schlyer and Alfred P. Wolf, Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973. USA.

The shortage of oxygen-18 enriched water has encouraged us to explore alternate methods of production of fluorine-18 where the recovery of the oxygen-18 enriched target material is extremely efficient. We have recently presented the results from a cryogenic target using carbon dioxide ice as the target (1). This is similar in design to a water ice target previously described (2,3). The amount of material required and the maximum beam current which can be put on the target are a function of the particular design. The effects of target cone length and number of cooling fins have been explored in order to optimize the target design.

Three different targets have been used to test these parameters. The three target are shown in Figures 1, 2 and 3. The first was the prototype target with a single heat sink at the rear of the target (Figure 1). The second is a target with several cooling fins and a short cone length which requires less target material (Figure 2). The third is a target with several cooling fins but a longer target length which allows for more efficient cooling of the material. The results from these studiers are summarized in Table 1. The target with four cooling fins could be run at a beam current of 18 μA with no perceptible volatilization of the target material while the target with the single cooling block showed volatilization at about 8 μA. The long and short cone targets did not show a difference in the volatilization of the target material at the beam current limit of the 60" cyclotron (18 μA).

The short target did maintain production with a lower amount of gas frozen into the target. The amount of gas frozen into the target in all these runs is two to four times that calculated to be necessary to form an ice layer thick enough to stop the beam in these targets. This is probably due to an uneven carbon dioxide layer on the surface of the cone. Experiments are currently under way to explore both the uniformity of the layer and the optimum angle for the cone.

This cryogenic target gives extremely efficient enriched target material recovery (>99%) and simplicity of material transfer. The production yields are similar to those obtained with the oxygen-18 enriched water target at beam currents up to 18 μA (our cyclotron limit, not necessarily the limit of the target design).

This work was carried out at Brookhaven National Laboratory under contract DE-AC02-76CH00016 with the U.S. Department of Energy, supported by its Office of Health and Environmental Research and grant No. NINDS, NS 15380.

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Table 1.
Production for Various Target Designs

Cone Length	Amount of CO_2 (liters)	Yield at EOB (mCi)	Percent of Theoretical (%)
Long	2.0	56±4	80
Short	2.0	52±3	74
Long	1.2	53±4	76
Short	1.2	50±3	71
Long	1.15	43±2	61
Short	1.15	44±3	63
Long	0.63	12±2	17
Short	0.63	26±4	37

All runs were carried out at 6μA for 10 minutes at an energy of 17.4 MeV incident on the carbon dioxide. Percent theoretical is based on the yield from 99% [^{18}O]CO_2. The dimensions of the long and short cones are given in the figures.

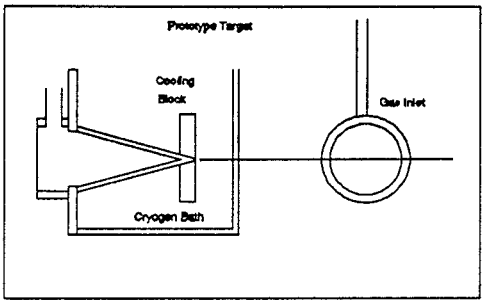

Figure 1. Prototype target design

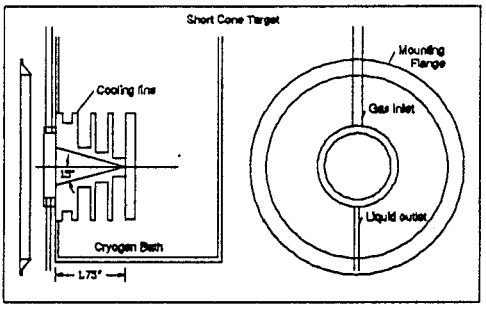

Figure 2. Short cone target

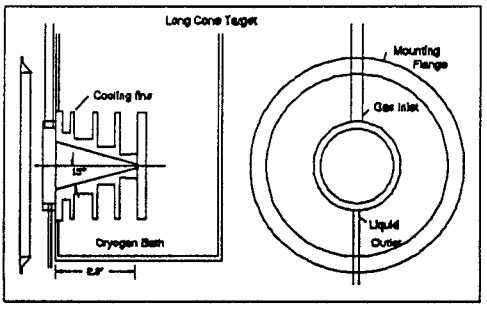

Figure 3. Long cone target

A New Method of Nitrogen-13 Ammonia Production Applicable with Low Energy Accelerator

DENCE, C.S., WELCH, M.J., HUGHEY*, B.J., KLINKOWSTEIN*, R.E., SHEFER*, R.E.
Mallinckrodt Institute of Radiology, Washington University Medical School, St. Louis, MO 63110. *Science Research Laboratory, Somerville, MA 02143.

Several new low energy accelerators have recently been developed for PET radioisotope production.[1-3] Conventional techniques for the production of nitrogen-13 ammonia are not applicable to these low energy accelerators.[4] We have developed a new technique for the production of nitrogen-13 ammonia suitable for very low energy deuteron beams.

In the production system, nitrogen-13 is produced using the nuclear reaction $^{12}C(d,n)\ ^{13}N$ in a solid graphite target. After irradiation, the target was heated in a stream of oxygen and it was found that heating the graphite to a temperature of approximately 850°C, released 80% of the activity as oxides of nitrogen. The remaining activity was in the form of ^{13}NN. Several traps were utilized to determine the one with the best "trapping efficiency" and it was found that a solid phase trap containing 5% of sodium hydroxide absorbed on silica gel was the most efficient, trapping greater than 95% of the available oxides of nitrogen. The activity could be readily washed from the trap by saline and the radioactivity was found by ion chromatography to be greater than 99% nitrogen-13 labeled nitrite (^{13}N-NO_2). This nitrite could be readily reduced to ammonia with an overall decay corrected conversion efficiency from activity induced in the target to ^{13}N-ammonia of 44%.

A solid phase trapping medium containing a reducing agent was also utilized. This consisted of a 5% sodium hydroxide silica gel mixed with Raney-nickel. The system used to study this process is shown in the figure. This mixture was placed in a quartz tube as shown in Figure 1. The graphite could be combusted in the first furnace (A) and trapped in the second furnace (B) utilizing a stream of oxygen. The trap containing the Raney-nickel was then isolated. A flow of 5% hydrogen in nitrogen saturated with water was established and furnace B was heated to 250° to 300°C. The ammonia produced was collected in vial C. A series of experiments were performed, yielding an overall conversion efficiency to ammonia (from the amount of nitrogen-13 induced in the carbon to final activity present as ammonia) of 37 ± 9% with a radiochemical purity > 99% and a synthesis time of < 17 minutes.

This ammonia synthesis technique was tested with a novel graphite target installed on the Science Research Laboratory Tandem Cascade Accelerator. In this case, the oxides of nitrogen are rapidly released in situ by inductive heating of the graphite. Extraction of activity takes approximately 3 minutes and the target is reusable for multiple batches of nitrogen-13. Trapping yields similar to those described above were obtained and in situ inductive heating reduced to less than 10 minutes the total time required for extraction, trapping and synthesis.

Acknowledgements: Work supported by National Institutes of Health Grant #2R44CA53953, #1R43HL48969 and Strategic Defense Initiative Organization Grant #SDIO84-89-C-0049.

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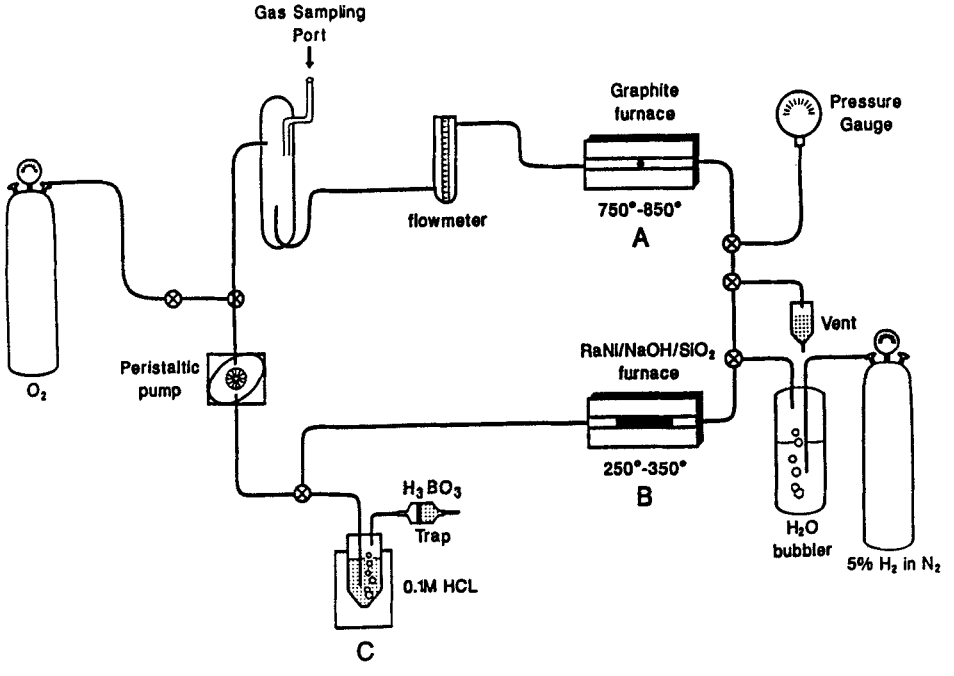

FIGURE 1

A New Ge-68/Ga-68 Generator System Prepared from N-methylglucamine Type Organic Polymer.

Koiso, T.; Ishibashi, O.; Harada, K.; Nakayama, M.; and Sugii, A. Faculty of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi 5-1, Kumamoto 862, Japan.

The $^{68}Ge/^{68}Ga$ generator system is one of the most important sources for preparing radiopharmaceuticals for use with positron emission tomography. Inorganic materials such as alumina and tin dioxide are used as adsorbents in the $^{68}Ge/^{68}Ga$ generator system.[1,2] In this study, we have seeked chemically modified organic polymers as effective and stable adsorbents for ^{68}Ge. The chemical modification may result the high selective adsorption for ^{68}Ge and the elution of ^{68}Ga from the adsorbent under mild conditions, although the stability against radiolysis should be investigated.

Several kinds of organic polymers were chosen to examine the affinity for Ge and Ga. As shown in Table 1, a commercially available macroporous N-methylglucamine type resin (R-MGlu) containing 1-deoxy-1-(methylamino) sorbitol group (Fig. 1) had a high adsorption capacity (1.2 mmol/g-resin) for Ge(IV), and showed a low affinity for Ga(III). This resin can be synthesized from styrene-divinylbenzene copolymer beads which have the high physical and chemical stability, and the polymer surface become very hydrophilic by the introduction of hydrophilic hydroxyl groups to hydrophobic polymer matrix.

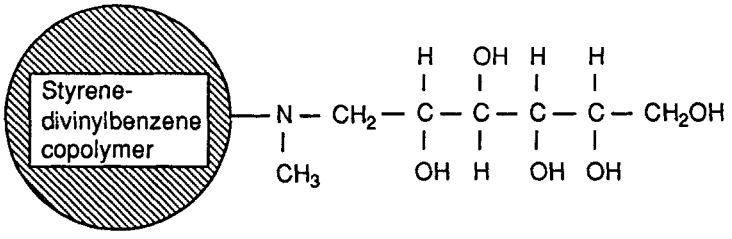

Figure 1. Schematic Structure of R-MGlu

Carrier free ^{68}Ge was rapidly and effectively adsorbed on the R-MGlu in the solution adjusted at pH 7 by batch method. After ^{68}Ga was built up in the resin by radioactive decay of ^{68}Ge, several complex-forming agents were used as eluting agent to prevent the formation of hydrolyzed ^{68}Ga at neutral pH. The low affinity of the resin for ^{68}Ga resulted in the elution of ^{68}Ga from the resin at high yield. (Table 2) ^{68}Ge was also effectively adsorbed on the resin(350 mg) packed into a mini-column (5 mm diameter). When 0.2 M trisodium citrate solution as eluting agent was passed through the column at a flow rate of 0.5 ml/min, $85 \pm 5\%$ of the ^{68}Ga in the column was recovered in 10 ml of the effluent, in which the radioactivity based on the breakthrough of ^{68}Ge was little detected. (Fig. 2) Although ^{68}Ga-citrate is avairable for PET radiopharmaceuticals, the eluate can be used for another radiopharmaceutical preparations with simple ligand exchange reaction.

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Table 1. Adsorption of Ge and Ga on Various Organic Polymer

Functional group	Ge(IV) Adsorbed[1] (mmol/g-resin)	Ga(III) Adsorbed[2] (mmol/g-resin)
-COOH	0.0	1.4
$-PO_2H$	0.0	1.4
$-PO_2H$ and $-PO_3H$	0.0	2.2
$-N(CH_3)CH_2(CHOH)_4CH_2OH$	1.2	0.3
$-N(CH_2COOH)_2$	0.0	0.3
$-NHCH_2PO_3H_2$	0.0	1.1
$-C(NH_2)NOH$	0.4	0.5
-CONHOH	0.0	0.5

[1]Batch method : 0.01 M GeO_2 in pH7 Phosphate buffer
[2]Batch method : 0.01 M $Ga(NO_3)_3$ in H_2O
Room temp,5hr

Table 2. Desorption of ^{68}Ga on R-MGlu by Batch Method

Complex-forming agent	Desorption of ^{68}Ga (%)
0.005 M EDTA[1]	88
0.005 M DTPA[2]	82
0.005 M CyDTA[3]	88
0.005 M trisodium citrate	23
0.01 M trisodium citrate	39
0.1 M trisodium citrate	90
$4x10^{-5}$M DF[4]	85
DF conjugated HSA	85
Phosphate buffer(pH7)	34

[1] Ethylenediaminetetraacetic acid
[2] Diethylenetriaminepentaacetic acid
[3] Cyclohexanediaminetetraacetic acid
[4] Deferoxamine mesilate
Shaking time : 1hr

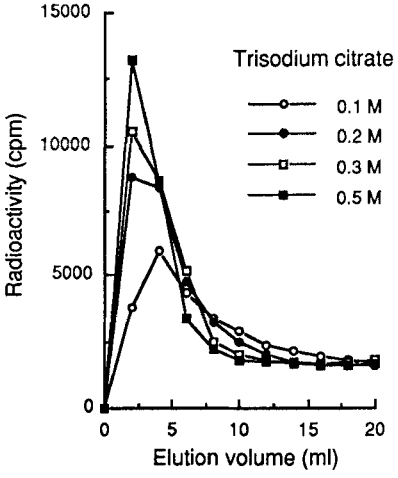

Figure 2. Elution Curves of ^{68}Ga by Column Method
Column : 5x50 mm
Flow rate : 0.5 ml/min

Production of ^{186}Re with high specific activity by the Szilard-Chalmers reaction

P.A. SCHUBIGER, R.M. CAHN, R.A. ALBERTO, P. BLÄUENSTEIN,
Radiopharmacy Division, Paul Scherrer Institute, CH-5232 Villigen-PSI, Switzerland.

Rhenium-186 is a radionuclide with very favorable properties for radioimmunotherapy: half-life = 90.6 h, β_{max} = 1.1 MeV, γ = 137keV (9 %). For this purpose the radionuclide should be carrier free or at least of high specific activity. Production by neutron irradiation yields a sufficent specific activity only when using a high flux reactor. Using the reactor SAPHIR at PSI ($\varnothing$ = 1.5 $\cdot 10^{13}$ n $\cdot$ cm^{-2} $\cdot$ sec^{-1}) the irradiation of natural Re yields a poor A_{sp} of 2MBq/μg (^{186}Re) at saturation.

Therefore, the Szilard-Chalmers reaction (1,2) was investigated as a way to improve the specific activity. A very promising compound pentamethyl cyclopentadienyl - rheniumtrioxide (Cp^*ReO_3) has been selected for this purpose (see Fig. 1). Cp^*ReO_3 is soluble in organic solution and perrhenate - water soluble - is already preformed. During irradiation of Cp^*ReO_3 with neutrons the recoil energy of γ-emissions destroys the activated compound to ReO_4^- or ReO_x, while the inactive one remains basically intact.

Based on the different solubility of educt and products three different separation techniques have been tried:

- Liquid-liquid extraction (toluene-water)
- Filtration through a polycarbamide membrane (adsorption-desorption)
- Column chromatography (toluene, acetonitile etc.)

Extensive evaluation clearly favoured the third method. The best condition using a gradient elution is given in Table 1. By this method Cp^*ReO_3 could be separated from ReO_4^- and ReO_x, (as ReO_4^-). Typical results of short time irradiations are given in Table 2.

The "enrichment" factor is, under these conditions, between 400 and 800 for ^{186}Re. From longer irradiations - done for the method evaluation - we assume we can extrapolate up to a few hours irradiation time. We expect to reach at least the specific activity of the Missouri high flux reactor (up to 150 GBq/mg). To long irradiation decreases the specific activity probably due to an increased amount of Cp^*ReO_3 destroyed by the γ-radiolysis of the reactor.

In conclusion, the Szilard-Chalmers reaction gives very encouraging results concerning a highly improved specific activity of ^{186}Re. Therefore, the method will now be validated for higher activities.

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Figure 1: Cp^*ReO_3

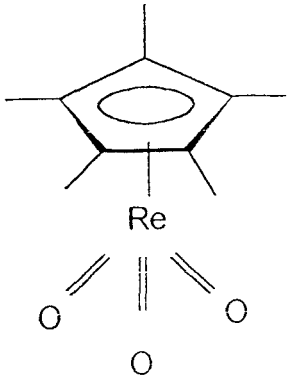


Table 1:

Elution of Silicagel 40-column with

1) Toluene/Acetonitrile 3:1 → retention (Cp^*ReO_3)

2) Toluene/Acetonitrile 1:1 → ReO_4^-

3) Acetonitril / H_2O_2 (1 %) → ReO_x (as ReO_4^-)

Table 2:

Separation yield: Typical values for a 10 min. irradiation

	Activity (MBq)	Amount (μg)	Spec. Activity (MBq/μg)
Cp^*ReO_3	0.4	340	0.001
$[ReO_4]^-$	2.4	3	0.8
ReO_2	0.4	0.1	4
not eluted	0.8	9	-
Sum	4.0	350	0.01

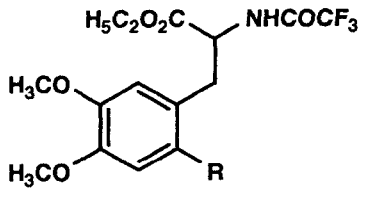
Synthesis and Halodemercuration of Polymer-Bound 6-Thiolatomercury and 6-Mercuric Sulfonate DOPA Precursors.

KAWAI, K.*; CHANNING, M.A.; KIESEWETTER, D.O.; ECKELMAN, W.C.

Department of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12 Ichigaya Funagawara-machi, Shinjuku, Tokyo 202, Japan* and PET Department, Warren Grant Magnuson Clinical Center, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892, U.S.A.

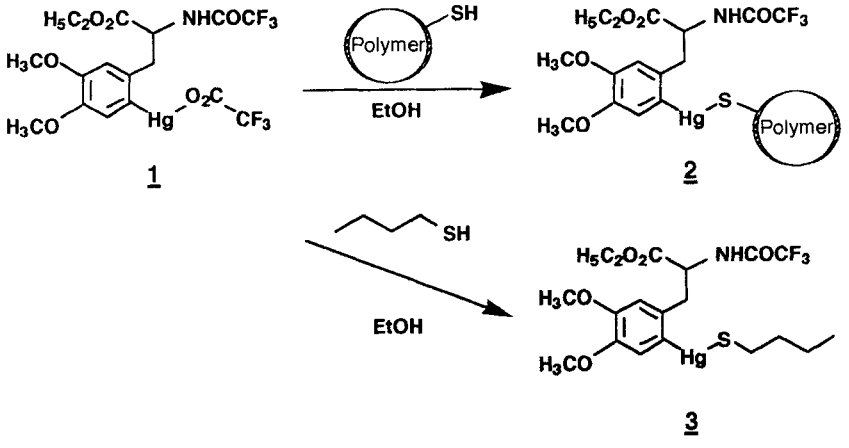
6-[^{18}F]Fluoro-3,4-dihydroxy-L-phenylalanine (6-[^{18}F]-DOPA) has been shown to be a useful radiopharmaceutical for evaluating cerebral dopamine metabolism in humans with positron emission tomography (1) and as a consequence much attention has been given toward improvements in its radiosynthesis (2). Among the various electrophilic methods reported for the preparation of 6-[^{18}F]-DOPA, the fluorodemercuration has been given the greatest utility (3). Radiolabeling procedures of halodemercuration require separating the very small amount of radiopharmaceutical from unreacted mercury precursor and other mercury-containing compounds. To remove them, chromatographic purification procedures preceding the final HPLC purification are needed. One approach to minimizing this problem is the development of a polymer-bound mercury precursor. In principle, the unreacted mercury precursor would remain bound to an insoluble polymer and the radiopharmaceutical would be released into solution. Although a similar approach has recently been reported for the preparation of N-isopropyl-4-[^{123}I]iodoamphetamine (4) and 4-[^{18}F]fluoroanisole (5) via a halodestannylation reaction, this modality is not easily adaptable for the preparation of polymer-bound DOPA precursor.

N-Trifluoroacetyl 3,4-dimethoxy-6-trifluoroacetoxymercurio-L-phenylalanine ethyl ester **1**, a 6-mercuric trifluoroacetate protected DOPA, is a regiospecific labeling precursor that reacts with ^{18}F acetyl hypofluorite exclusively in the 6 position (2). In this study, polymer-bound 6-thiolatomercury **2** and polymer-bound 6-mercuric sulfonate **4** protected DOPA derivatives were synthesized from **1**. In order to evaluate the effect of polymer binding on the halodemercuration reaction, monomeric analogs of **2** and **4**, the 6-butanethiolatomercury **3** and the 6-mercuric p-toluene sulfonate **5** protected DOPA derivatives were prepared.



1 R=HgOCOCF$_3$
2 R=HgS-polymer
3 R=HgSC$_4$H$_9$
4 R=HgOSO$_2$C$_6$H$_4$-polymer
5 R=HgOSO$_2$C$_6$H$_4$CH$_3$
6 R=F
7 R=I

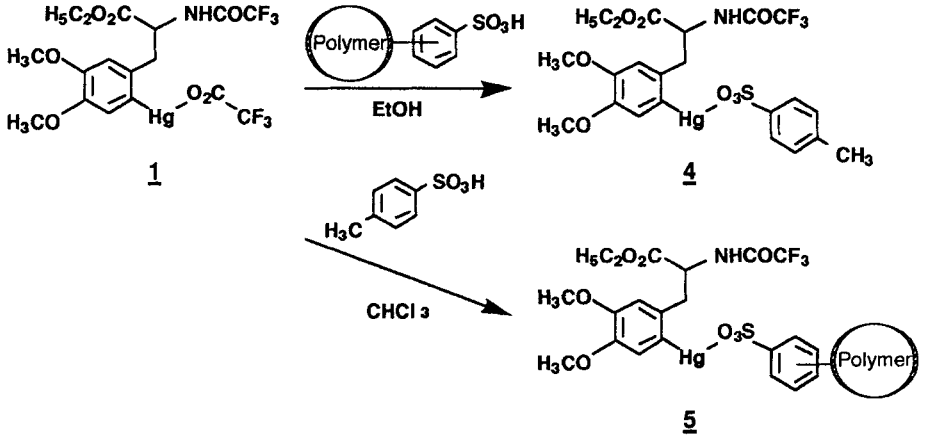
Chelite S (0.3-0.8mm), a macroporous polystyrene resin modified with thiol groups, was selected. An ethanol solution containing 2 equivalents (eq.) of 1 was added to Chelite S, the resin was filtered to obtain the polymer-bound thiolatomercury DOPA 2 with 25% coverage of the available thiol groups on the resin. The butanethiolatomercury compound 3 was prepared in high yield using the method of Canty and Kishimoto (6), by addition of 1 eq. of butanethiol in ethanol at room temperature.



Both fluorodemercuration and iododemercuration were carried out in $CHCl_3$ at room temperature using 2/3 eq. of acetyl hypofluorite and I_2 according to the method of Luxen et al. (3). After preliminary purification by flash column chromatography, the products were analyzed by HPLC, GC/MS and NMR. All analytical systems were calibrated with authentic compounds. The protodemercuration product was a major impurity.

Fluorination of compound 1 provides 6-fluoro protected DOPA 6 in 30-34% yield, but in the case of 2 and 3, no 6-fluoro protected DOPA 6 was observed. Iodination of compounds 1 and 3 produced the same yield of 6-iodo protected DOPA 7, but in the case of the thiol polymer 2, the yield decreased by about 1/3. The results obtained from the fluorination of the thiolatomercury compounds 2 and 3 suggested that thiolatomercury protected DOPA was not a viable means of obtaining the desired 6-fluoro derivative.

As a results, we selected AG50W-X2 resin which is composed of sulfonic acid groups attached to a styrene divinyl-benzen polymer. AG50W-X2 resin is a strong acid cation exchange resin that has higher ion selectivity for mercury than hydrogen. The exchange reaction of AG50W-X2 (H^+ form) with 1 showed 85-90% coverage of the available sulfonate groups and gave the mercuric sulfonate polymer 4. The monomeric analog was synthesized by the addition of p-toluene sulfonic acid to 1 followed by evaporation to obtain the tosyl mercury compound 5 in high yield.



Although the mercuric sulfonate compounds 4 and 5 were mercury salts similar to 1, in the case of polymer 4, no fluorinated product 6 was detected. Fluorodemercuration of monomeric analog 5 gave half the yield of 6 while iododemercuration gave the same yield of 7 when compared with 1. It appears that mercuric sulfonate can undergo halodemercuration, but that the structure of the resin is not suitable for the fluorodemercuration reaction.

These results indicated the possibility of developing polymer-bound halodemercuration precursors. Future work is directed towards optimizing the polymer-bound precursors to produce radiohalogenated radiopharmaceuticals.

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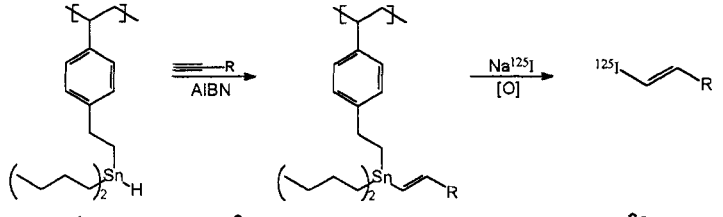
Synthesis of Radioiodinated Vinyl Iodides

KABALKA, G. W.; GOODMAN, M. M.; SRIVASTIVA, R. S.; BOWERS, K. R.; MARKS, R. C., Departments of Chemistry and Radiology, University of Tennessee, Knoxville, Tennessee, USA 37996-1600

Organometallic reagents based on boron, tin, mercury, and silicon have been utilized to successfully prepare a number of important medical imaging agents.[1] Polymer supported organometallic reagents have been used as precursors to radiopharmaceuticals, eliminating the problems involved in removing reaction impurities following radioisotope incorporation. Typical reaction impurities include unreacted isotopically labelled precursors, co-reactants, and the pharmaceutical precursors. Culbert and Hunter recognized that the use of polymer bound organotin precursors was advantageous in the preparation of iodoaryl radiopharmaceuticals due to the high rate of iododemetallation of tin reagents.[2,3]

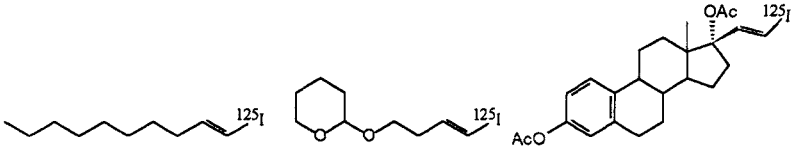
Early studies demonstrated that vinyl iodides exhibit enhanced stability when compared to the corresponding alkyl iodides.[4,5] The unique capabilities of organotin polymers prompted us to apply the methodology to the preparation of radioiodinated biologically active vinyl iodides. Radiolabeling of the polymeric precursor **2a-c** gave (*E*)-1-[^{125}I]-iododecene **3a**, the DHP ether of (*E*)-3-[^{125}I]-iodobuten-1-ol **3b**, and (*E*)-17α-[^{125}I]iodoethenylestra-1,3,5(10)-triene-3,17β-diacetoxy **3c** in yields ranging from 20 - 70%.



1 **2a** R = C_8H_{17} **3a - c**
2b R = DHP Ether of 2-Ethanol
2c R = 17α-Estra-1,3,5(10)-triene-3,17β-diacetoxy

The synthesis of the DHP ether of (*E*)-3-[^{125}I]-iodobuten-1-ol **3b** is representative of the general procedure for radioiodination. Polymer **1** was prepared, using the method of Gerigk, *et. al.*, from divinyl benzene and dibutyl[2-(m-/p-vinylphenyl)ethyl]tin chloride.[6] Following removal of unreacted starting material and solvent, the tin chloride polymer was reduced to the corresponding hydride using diisobutylaluminum hydride in THF. The hydride polymer **1** (500 mg, 0.816 mmol hydride/g, 0.408 mmol hydride) and 5 mg 2,2-azobisisobutyronitrile was placed in a 100 mL round-bottom flask under an inert atmosphere. The flask was fitted with a reflux condensor and magnetic stir bar, and the apparatus sealed while maintaining the inert atmosphere. Dry toluene (20 mL) was added to the flask followed by 74.3 mg (0.408 mmol) of the DHP ether of 2-ethanol. The slurry was heated in an oil bath at 80°C for 12 hours, filtered, and the polymer **2b** washed with methanol and ether.

Polymer bound organotin precursor **2b** (40 micromoles) was slurried in 1 mL of dry THF in a 25 mL round-bottom flask containing a magnetic stir bar and fitted with a septum. A charcoal filter constructed from a 3 mL syringe was inserted into the septum to prevent leakage of radioiodine. The flask was shielded from direct light and immersed in an ice-water bath. Sodium [^{125}I]iodide (400 microcuries, no carrier added) was added in 0.1 mL of 0.1 N NaOH. The oxidant, chloramine-T (50 microliters of 0.5 M in 50% aqueous THF), was then added and the solution stirred for 10 minutes. The reaction was quenched with 0.1 mL of saturated aqueous sodium thiosulfate. Separation of the THF solution from polymer was followed by radioassay in a Capintec CRC12. The solution was then treated with 10 mL of water and extracted with 2 x 5 mL of diethyl ether. The aqueous and ether layers were assayed using the Capintec CRC12 and the radiochemical purity monitored by TLC on silica gel (5% ethyl acetate in hexanes on silica). RadioTLC was performed using a Bioscan System 200 imaging scanner and indicated a 47% radiochemical yield.



3a **3b** **3c**

Acknowledgement:
We wish to thank the United States Department of Energy for research support.

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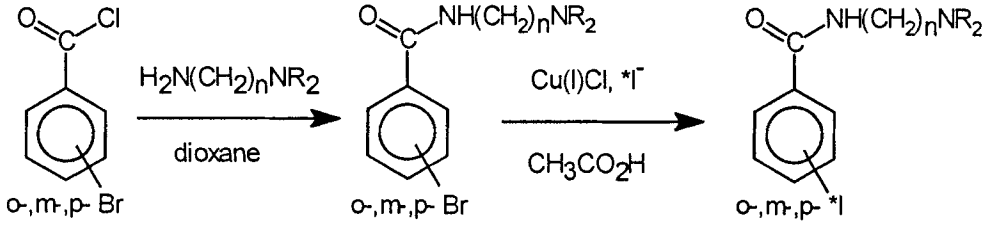
N.c.a. Cu(I)-assisted iodine-exchange on N-alkylated ortho-, meta-, and para-bromobenzamides

COENEN, H. H.; DUTSCHKA, K. P.; BRANDAU*, W. Nuklearchemie und Radiopharmazie, Universitätsklinikum, 45122 Essen; *Nuklearmedizin, Universitätsklinikum, 48129 Münster, Germany

Radioiodinated N-aminoalkyl-benzamides show very high uptake in experimental melanoma in mice [1,2] and proved useful for melanoma detection in patients [3]. The n.c.a. radioiodination of these compounds was studied and improved using Cu(I)-assisted non-isotopic exchange. So far, an isomeric effect of ortho-, meta- and para-substituted derivatives on pharmacokinetic behaviour and on Cu(I)-mediated reactions was not systematically examined. The synthetic sequence including a new one-step precursor preparation is illustrated in the Scheme.



Scheme: One-step synthesis of N-aminoalkylated o-, m-, and p-bromobenzamides and subsequent Cu(I)-assisted radioiodine exchange (n = 2, 3; R = CH_3, C_2H_5)

The ortho-, meta-, and para- bromo-precursors and analogous iodo-standards of N-(dialkylaminoalkylene)-benzamides were prepared by one-step condensation of the isomeric benzoic acid chlorides with corresponding amines at room temperature within 30 minutes and subsequent vacuum distillation with 85 to 90 % yield [cf. ref. 3]. The non-isotopic exchange with n.c.a. $^{123,131}I^-$ proceeded on 250 μg of bromo-precursor in presence of 5 μg CuCl in 55 μl acetic acid with high radiochemical yield and specific activity of > 5000 GBq/μmol. The reaction was optimized with respect to educt concentration, reaction time and temperature. Radiochemical yields obtained at 180°C and 20 minutes, for example, are listed in the Table. The total synthesis time required for radiopharmaceutical preparation, including HPLC-separation and sterile filtration, about 90 minutes. The synthesis developed here is superior to

previous attempts via isotopic exchange [1,2], iododestannylation [4], and non-isotopic exchange in benzoic acid melt [3] with respect to ease of precursor preparation, specific activity, and radiochemical yield.

Table: Radiochemical yields of Cu(I)-assisted iodine-exchange on o-, m-, and p-bromobenzamides in acetic acid at 180°C and 20 minutes reaction time

n	2	2	3	3
R	CH_3	C_2H_5	CH_3	C_2H_5
ortho	94	87	89	96
meta	44	88	85	92
para	68	88	49	45

± 5 - 10 % standard deviation

A faster reaction was always observed with the ortho-derivatives, and with compound n = 2, R = C_2H_5 a radiochemical yield of 85% was still obtained at 100°C within 40 minutes. On the other hand, reaction temperatures of 200°C with the para-bromo derivative n = 3, R = CH_3 accelerated the reaction but did not increase the maximum labelling yield of about 70 % due to decomposition processes at extended reaction times. The high yields obtained with meta-derivatives contradict somewhat the generally accepted concept of a nucleophilic halogen-exchange via a tetracoordinated Cu-complex [5-7], since strong nucleophilic activation is only expected to occur in ortho- and para-position to the carbonyl substituent. This supports recent observations on the Cu(I)-assisted iododebromination in DMSO [7] where also at least a partial contribution of iodine radicals was suggested. In addition, also the type of the N-alkyl side chain seems to interfere with the exchange process (cf. Table).

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Synthesis And Radioiodination Of A Phenylalanine Derivative For Use In Peptide Synthesis.

WILBUR D.S.[1], HAMLIN D.K.[1], SRIVASTAVA R.R.[1] AND BURNS H.D.[2]
[1]Department of Radiation Oncology, University of Washington, Seattle, WA 98195
[2]Merck Research Laboratories, West Point, PA 19486

There are a large number of biologically active peptides, such as growth factors (1,2) and neuropeptides (3,4), that are being investigated as potential new pharmaceuticals. The investigations of naturally occurring peptides and/or synthetically modified peptides often requires labeling with an easily detectable tracer such as radioiodine, to evaluate their *in vitro* and *in vivo* properties, as the peptides are generally pharmacologically active in very small quantities (i.e. react with receptor proteins at the cellular level). A method of radioiodine labeling of peptides which might be expected to cause only minimal alteration in the peptide's native structure is to prepare an amino acid analog that can be incorporated into the peptide chain. Using this method one could either incorporate a precursor molecule into a peptide and later radiolabel it (perhaps after protecting any tyrosine phenols), or radioiodinate the derivatized amino acid prior to its incorporation through peptide synthesis (5,6). While a number of different amino acids could be radioiodinated, incorporation of a radioiodine atom in the *para*-position of the phenyl ring of phenylalanine was particularly attractive as this should result in minimal alteration in a peptide and provide a stable radiolabel for both *in vitro* and *in vivo* evaluations. Preparation of a phenylalanine derivative which could be used directly in peptide synthesis requires protection of the α-amino group and activation of the carboxylate group. Based on previous studies, radioiodine labeling of the aromatic ring in phenylalanine could best be accomplished using organometallic intermediates (7-9). Thus, an investigation was conducted in which a phenylalanine derivative, N-Boc-*p*-(tri-*n*-butylstannyl)-L-phenylalanine tetrafluorophenyl ester, **5**, was synthesized and radioiodinated to prepare N-Boc-*p*-[^{125}I]iodo-L-phenylalanine tetrafluorophenyl ester, [^{125}I]**6**.

A number of synthetic routes to **5** were attempted before the synthesis was finally accomplished as depicted in Scheme I. The requisite aryl stannylation reaction was found to be best conducted using the phenylalanine methyl ester. Thus, N-Boc-*p*-iodo-L-phenylalanine methyl ester, **2**, was synthesized in 91% yield from commercially available N-Boc-*p*-iodo-L-phenylalanine, **1**, by reaction of methyl iodide and $NaHCO_3$ in DMF. Following its preparation, **2** was stannylated using bistributyltin and tetrakis(triphenylphosphine)palladium(0) in refluxing toluene to prepare N-Boc-*p*-(tri-*n*-butylstannyl)-L-phenylalanine methyl ester, **3**, in 81% yield. De-esterification of **3** with aqueous base was accomplished in nearly quantitative yield to give N-Boc-*p*-(tri-*n*-butylstannyl)-L-phenylalanine, **4**. Preparation of the targeted stannylphenylalanine tetrafluorophenyl (TFP) ester **5** was then accomplished using 2,3,5,6-tetrafluorophenol and 1,3-dicyclohexylcarbodiimide in anhydrous THF giving an overall yield of 33% (after purification). Iodination and radioiodination reactions of **5** were conducted in MeOH/1% HOAc at room temperature over a 3 min period to yield 83-95% of the desired N-Boc-*p*-[*I]iodo-L-phenylalanine TFP ester, **6**.

Racemization of the L-isomer was a concern in the synthesis, so two synthesized compounds (**4** and **5**) were analyzed by chiral HPLC analysis. Attempts were made (on two different chiral columns) to separate the D- and L- isomers of N-Boc-stannylphenylalanine **4**, but this was unsuccessful. However, separation of D- and L-isomers of N-Boc-*para*-iodophenylalanine, **1**, was achieved on a chiral cyclodextrin column (Cyclobond I RSP). Thereafter, arylstannane **4** was iodinated (using NCS/NaI) to form **1**, then analyzed by chiral HPLC. Only a trace of the D-isomer was observed, providing strong evidence that minimal racemization had occurred in the stannylation, de-esterification, and iodination reactions. Similarly, **5** was iodinated to form **6**, and was de-esterified with treatment of base to yield **1**. Chiral HPLC analysis of the crude product mixture again indicated only a trace of the D-isomer was present, indicating that it was likely introduced in the stannylation or de-esterification of the methyl ester, **2**, not the iodination reaction or de-esterification of the TFP ester.

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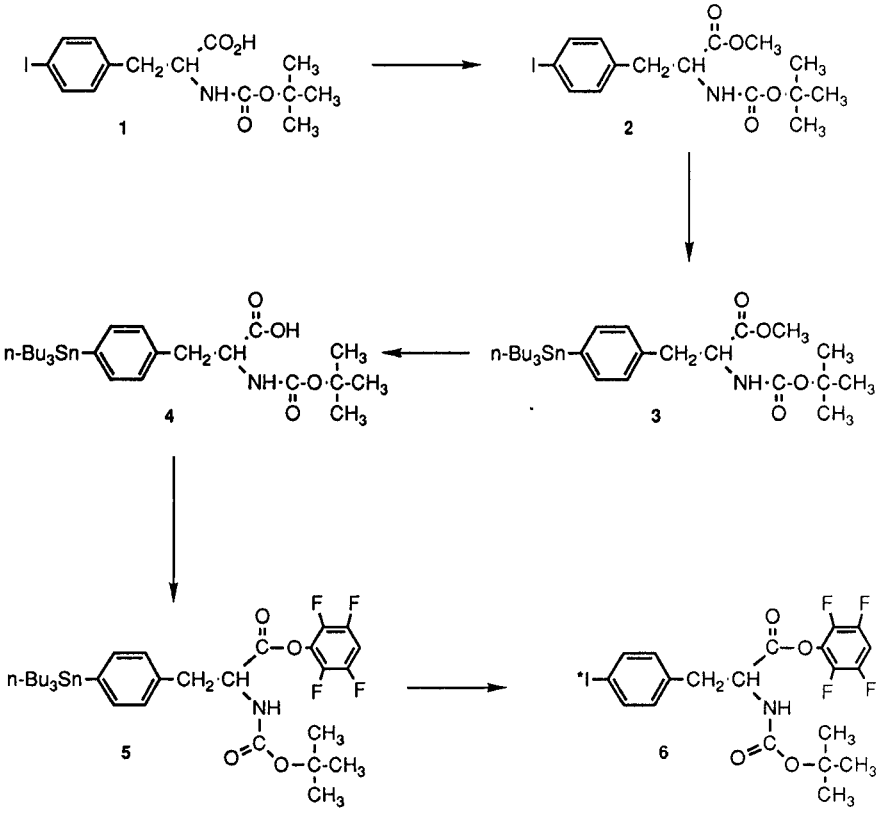
Scheme I: Synthesis and Iodination of Phenylalanine Derivatives



Preparation of ^{123}I Labeled Metoprolol with Iodine Monochloride Process
JIN DU; XIAOHAI JIN; SONGYING YUAN Isotope Department, China Institute of Atomic Energy, P.O. Box 275 (58), Beijing, China.

Metoprolol derivative (MPD), a kind of cardioselective β_1-adrenergic blocking reagents was labeled with ^{123}I. ^{123}I-metoprolol may be used for specific organ localization based on its special drug-receptor interaction, meanwhile, the β_1-adrenergic receptor system appears to be sucessful for myocardial imaging.

In here, we describ a method of ^{123}I labeled metoprolol with iodine (^{123}I)monochloride for radioiodination. Frist, metoprolol was added to ^{123}I-monochlroide solution and allowed to stir for radioioiodination. Secondly, the reaction mixture was evaporated at 35℃ under reduced pressure and then the final products was seperated by ether or isopropyl ether extraction method. A series of labelling condition of this method, such as the effect of pH value of reaction medium, the quantities of metoprolol, reaction temperature and time on the labelling efficiency were studied in detail and optimum values were obtained. The effect of pH values was very important. The radiochemical purity of ^{123}I-metoprolol depends on the pH of the reaction mixture during the extraction process.

The product of ^{123}I-metoprolol has been proved by radiochromatography method and spot method of UV light(254nm). Rf value of ^{123}I-metoprolol is 0.9. Free iodine is at original.The radiochemical analysis indicated that the labelling efficiency of ^{123}I-metoprolol is more than 85%, the radiochemical purity of the product is more than 95% after seperation. The initial results of clinical experiments have shown that the dynamic studies of metoprolol drug in vivo was very satisfied.

Preparation and Biodistribution of [^{76}Br]-N-Methyl-4-Bromodexetimide: A Potential Radiotracer for Myocardial Muscarinic Receptors. KASSIOU, M.*; LOC'H, C.#; LAMBRECHT, R.M.*; VALETTE, H.#; KATSIFIS, A.*; FUSEAU, C.#; MAZIÈRE, M.#; and MAZIÈRE, B.# Service Hospitalier Frédéric Joliot, CEA, F-91406 Orsay, France# and Biomedicine and Health, ANSTO, Lucas Heights, Australia*

Myocardial muscarinic cholinergic receptors are important in cardiac function, and changes in receptor number and affinity occur in various physiological, pharmacological and clinical conditions. Attempts to use analogues of dexetimide, a high affinity antagonist for in vivo studies of myocardial muscarinic receptors, proved unsuccessful due to high lipophilicity of the molecule which was evident by the high lung uptake[1]. We have reported a bromine-76 labelled derivative of dexetimide, [^{76}Br]-BDEX, suitable for in vivo PET brain imaging of muscarinic receptors. Here we describe the preparation of the hydrophilic quaternary ammonium derivative: [^{76}Br]-N-methyl-4-bromodexetimide ([^{76}Br]-MBDEX).

The radiosynthesis involves the preparation of [^{76}Br]-4-bromodexetimide ([^{76}Br]-BDEX) first by electrophilic bromodesilylation as described, followed by treatment with CH_3I (fig 1). The reaction was carried out by dissolving [^{76}Br]-BDEX (50 MBq) in tributyl phosphate (100µL) followed by an addition of an excess of CH_3I (200µL). Allowing the reaction mixture to stand at room temperature for 10 min resulted in the formation of the quaternary salt in a 34% radiochemical yield. When the temperature is increased to 90°C, radiochemical yields reached 95%. After evaporating excess methyl iodide and cooling the reaction mixture, isolation and purification of the radiopharmaceutical was carried out by HPLC on a µ-Bondapak C18 column (300 x 7.8 mm) while the UV absorption was measured at 239 nm. With acetonitrile and 0.1M ammonium acetate buffer (45:55, v:v) as mobile phase and a flow rate of 2.5 mL/min, the retention times of [^{76}Br]-MBDEX and [^{76}Br]-BDEX were 27 and 37 min respectively. Radiochemical and chemical purities assessed by radio-TLC and HPLC were 99% and the specific activity of [^{76}Br]-MBDEX was identical to that of [^{76}Br]-BDEX (11 GBq/µmol).

In vivo biodistribution studies in rat showed a high uptake in the heart (2 % ID/g) that decreased with a half-life of ~3-4 min; 5 min after injection, the heart to lung radioactivity concentration ratio (H/L) was 3.5. No uptake of [^{76}Br]-MBDEX was observed in the brain. When the peripheral muscarinic recptors were blocked by the co-injection of a load of cold methyl-quinuclidinyl benzylate (MQNB, 1 mg/kg), the heart uptake was reduced to 0.12% ID/g (H/L = 0.27) (fig. 2). Radio-TLC analysis indicated that [^{76}Br]-MBDEX was rapidly metabolised in plasma (20% of unchanged tracer 10 min after injection) and showed that the radioactive metabolites were essentially polar by-products. In PET studies in dogs, [^{76}Br]-MBDEX accumulated rapidly in the heart; the uptake remained in a plateau for 10 min and then decreased with a half-life of 20 min. Due to a rapid wash-out in the lungs, 3 min after injection, the heart to lung ratio reached a value of 8. The uptake of [^{76}Br]-MBDEX was displaced by MQNB (30µg/kg) that demonstrated the specificity and saturability of the binding of [^{76}Br]-MBDEX to muscarinic receptors in the heart. From these preliminary animal studies it appears that [^{76}Br]-N-methyl-4-bromodexetimide has the potential of being developed as a useful PET radiotracer for the characterisation of heart muscarinic receptors.

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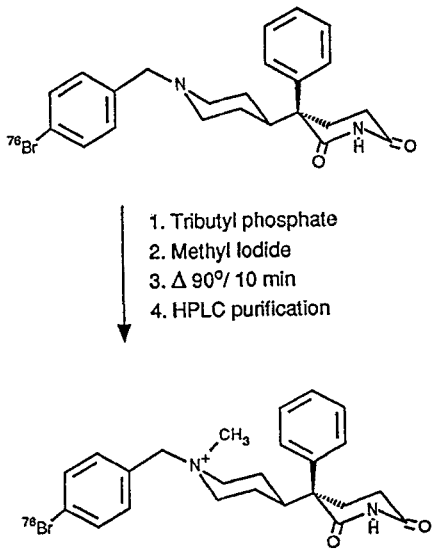

Figure 1

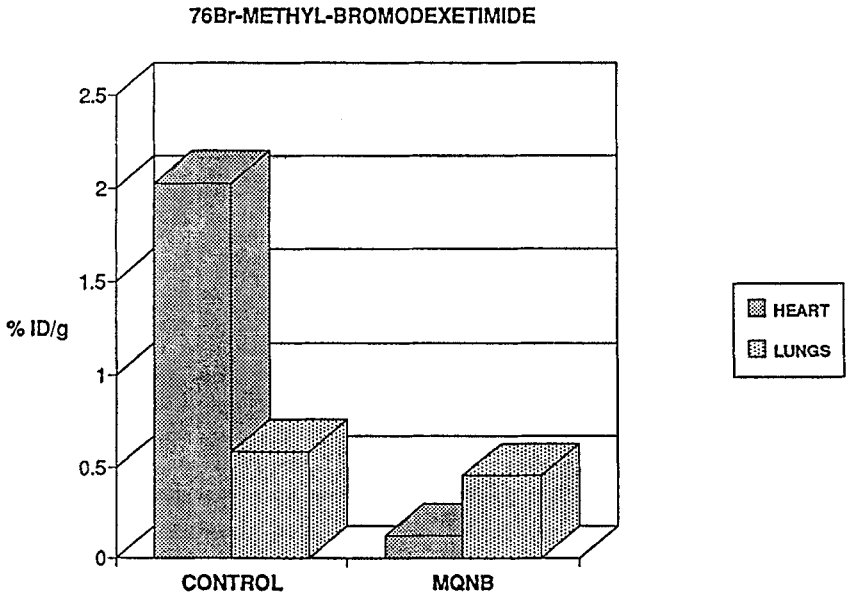

Figure 2

Preparation and Pharmacological Evaluation of a New Central Muscarinic Cholinergic Receptor Imaging Agent: [^{76}Br]-4-Bromodexetimide. KASSIOU, M*; LOC'H, C. #; BOTTLANDER, M#; LAMBRECHT, R.M.*; KATSIFIS, A.*; SCHMID, L.#; OTTAVIANI, M.#; MAZIERE, M.#; MAZIERE, B.# Service Hospitalier Frédéric. Joliot, CEA, Orsay, France# and Biomedicine and Health, ANSTO, Lucas Heights, Australia*.

Muscarinic cholinergic receptors (mAChR) play an important role in a number of physiological and behavioural responses. The putative role of muscarinic receptors in neurodegenerative disorders such as Alzheimer's disease, Huntington's disease and dementias associated with Parkinson's disease has generated considerable interest for the non invasive mapping of mAChR. Potential muscarinic imaging agents include ^{11}C- and ^{123}I-QNB analogs, ^{11}C-scopolamine and ^{11}C-benztropine while radiolabelled dexetimide derivatives have shown exciting potential[1-4]. Simpler methods for the preparation of dexetimide derivatives incorporating longer lived isotopes suitable for PET studies is required. We are reporting the synthesis and the pharmacological characterisation of a bromine-76 derivative of dexetimide suitable for PET studies.

The radiosynthesis of [^{76}Br]-4-bromodexetimide ([^{76}Br]-BDEX) was carried out by bromination via electrophilic bromodesilylation with no carrier added [^{76}Br]NH_4. During the preparation of this radiopharmaceutical a number of reaction conditions and reagents were examined. Oxidising agent such as peracetic acid and dichloramine-T were evaluated and found inefficient while chloramine-T appeared the reagent of choice. Peracetic acid reactions were carried out in acetic acid with radiochemical yields of 6% obtained. Dichloromine-T reactions were conducted in both methanol and TFA solvent with radiochemical yields of 10% and 24% respectively. The choramine-T reactions were most efficient but were also concentration and solvent dependent. The optimum labelling conditions were found to be the use of chloramine-T (10^{-3}M) in 0.1N HCl at room temperature for 15 min followed by addition of a sodium metabisulfite solution (fig. 1). Under these conditions the radiochemical yield reaches 80%. The purification and isolation of the radiotracer from the reaction mixture was carried out by HPLC on a μ-Bondapak C18 column (300 x 7.8 mm) with a mixture of acetonitrile and ammonium acetate buffer (45:55) as the mobile phase and a flow rate of 2.5 ml/min while UV absorption was measured at 239 nm. Radiochemical and chemical purities assessed by radio-TLC and HPLC were 98% with a specific activity of 11 GBq/μmol.

The affinity and selectivity of [^{76}Br]-BDEX for muscarinic receptors was investigated in vitro and in vivo in rats and non human primates. In vitro, the binding parameters of this new radioligand were evaluated on homogenates of rat cerebral cortex membranes. Analysis of saturation data using a non linear least square regression method revealed a single population of binding sites (nH = 0.99 B_{max} = 130 ± 7 pmol/g tissue) with an apparent dissociation constant of 1.9 ± 0.3 nM. In vivo, the biodistribution of this new tracer was studied in rats. In the brain, 2 h post injection, the highest concentrations (about 5 x 10^{-3} of the injected dose per gram) were found in the frontal cortex, the hippocampus and the striata while the cerebellar radioactive concentration was very similar to that of the plasma. Ex vivo autoradiographic studies have shown that this preferential accumulation was totally prevented by the co-administration of a cold load (0.1 mg/kg) of a muscarinic antagonist, scopolamine. A metabolite study performed on cortex, cerebellum and plasma samples showed that 1 hr post injection, 98, 79 and 20 % of the radioactivity respectively was related to unchanged [^{76}Br]-BDEX.

The time course of this radioligand was studied in a baboon during a preliminary PET investigation (fig 2) 10 min after the administration of 33 MBq of [^{76}Br]-BDEX, 2.3 x 10^{-4} of the injected dose was found per ml of frontal cortices. This concentration, which correspond to a receptor occupancy lower than 1 %, remained in a plateau and was rapidly displaced by an I V injection of a load of 1 mg/kg of cold dexetimide. This data suggests that [^{76}Br]-BDEX is a potential probe for investigating in vivo by PET in humans the status of central muscarinic cholinergic receptors.

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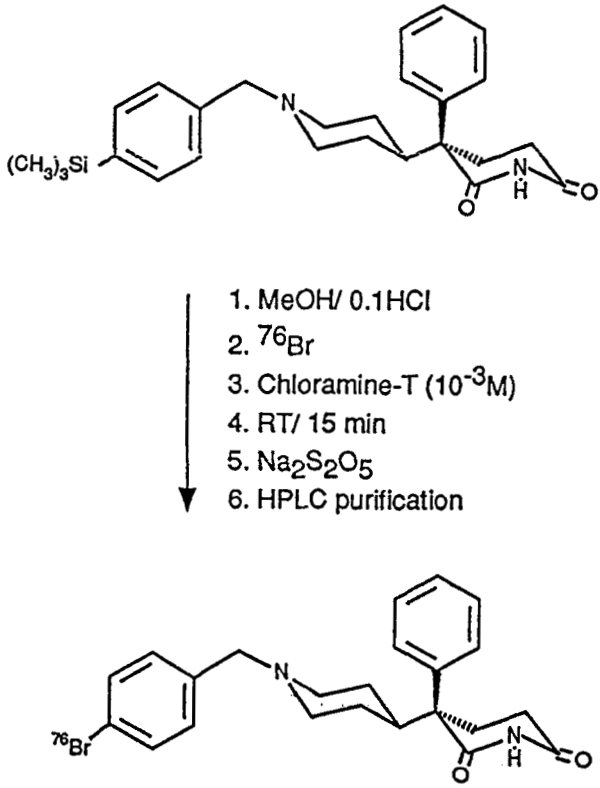

Figure 1

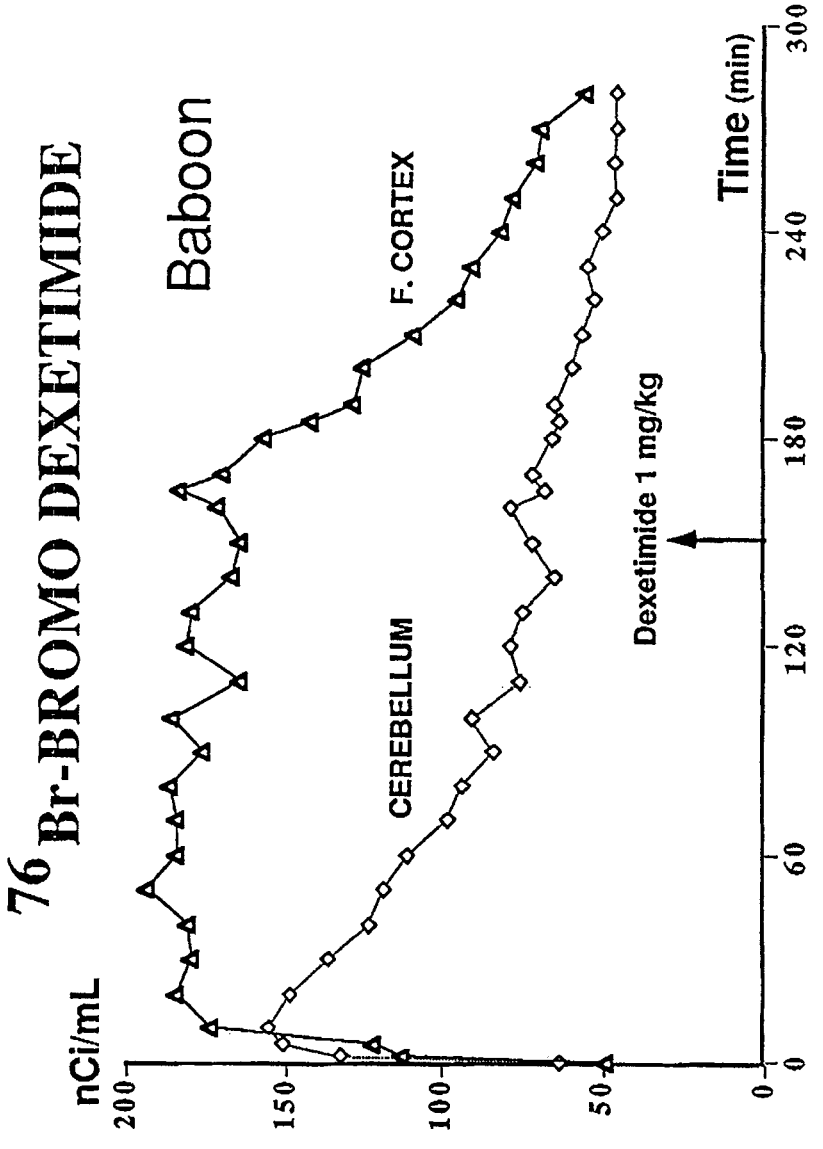
76 Br-BROMO DEXETIMIDE
Baboon
nCi/mL
200
150
100
50
0
F. CORTEX
CEREBELLUM
Dexetimide 1 mg/kg
0
60
120
180
240
300
Time (min)

Figure 2

[^{123}I-N-Methyl-4-Iododexetimide: A Radioiodinated Ligand for SPECT Studies of Myocardial Muscarinic Receptors. KASSIOU, M.*; KATSIFIS, A.*; LAMBRECHT, R.M.*; EU, P.#; HICKS, R.# Biomedicine and Health, ANSTO, Lucas Heights, Sydney N.S.W* and Heidelberg Repatriation Hospital, Melbourne VIC, Australia#.

Muscarinic cholinergic receptors (mAChR) mediate a slowing in the rate of contraction of the heart and a decrease in the force of contraction, while changes in receptor density occur in various physiological, pharmacological and clinical conditions. Altered muscarinic receptor distribution in the heart may be a substrate for cardiac arrhythmias and lead to cardiac arrest. Attempts to image myocardial mAChR involves use of radiotracers such as [^{11}C]MQNB and [^{11}C]MTRB with limited SPECT radiotracers avaliable. Recently [^{123}I]-4-iododexetimide ([^{123}I]IDEX), a potent mAChR antagonist was used for in vivo studies of myocardial mAChR but proved unsuccessful due to its high lung uptake.[1] We are reporting the preparation and evaluation of the hydrophilic quaternized derivative: [^{123}I]-N-methyl-4-iododexetimide ([^{123}I]MIDEX).

The radiosynthesis involves firstly preparation of [^{123}I]IDEX by electophilic iododesilylation using trifluoroacetic acid as solvent and chloramine-T as the oxidising agent as described elsewhere,[2] followed by treatment of [^{123}I]IDEX with excess CH_3I (fig 1). The methylation reaction is carried out by dissolving [^{123}I]IDEX (10 mCi) in tributyl phosphate (50 μL), a solvent known to promote formation of quaternary ammonium salts, followed by addition of CH_3I (300 μL). The reaction mixture was tightly stoppered and heated at 90°C for 15 minutes. After evaporating the excess CH_3I and cooling the mixture, isolation and purification of the radiopharmaceutical was carried out by preperative HPLC. A μ-Bondapak C18 column (300 x 7.8 mm) was used while the UV absorption was measured at 239 nm and radioactivity measured on a Berthold system. The mobile phase consisted of acetonitrile and 0.1M ammonium acetate buffer (45:55 v.v) and a flow rate of 2.5 mL/min. The retention times of [^{123}I]IDEX and [^{123}I]MIDEX were 38 and 26 minutes respectively. Radiochemical yields of 80% were reached while radiochemical and chemical purities assessed by HPLC were 97% and the specific activity of [^{123}I]MIDEX was identical to [^{123}I]IDEX >2000 mCi/μmol.

Rat biodistribution studies were performed and showed high heart uptake (2.4 %ID/g) 10 minutes after injection with a heart to lung radioactivity concentration ratio (H/L) of 5.1. The H/L ratio decreased rapidly to 2.2 after 30 minutes and reached unity at 60 minutes. No uptake of [^{123}I]MIDEX was observed in the brain. The specificity and stereoselectivity of [^{123}I]MIDEX binding at 10 minutes was demonstrated by coinjecting a cold load of levetimide (LEV 0.15 mg/kg), dexetimide (DEX 0.15 mg/kg) and methyl-quinuclidinyl benzylate (MQNB 1 mg/kg) (fig 2). With DEX and MQNB the heart uptake was reduced to 0.20 and 0.13 %ID/g displacing 92% and 95% of the activity respectively while LEV maintained high heart uptake (2.2 %ID/g). Interestingly, the kidney uptake was 21% ID/g and remained constant over a period of 30 minutes. Preliminary SPECT studies carried out on rabbit and dog will also be described. The carbon-11 methylation of dexetimide will also be mentioned. These results suggest that [^{123}I]MIDEX has the potential of being developed as a SPECT radiotracer for the characterisation of myocardial muscarinic receptors.

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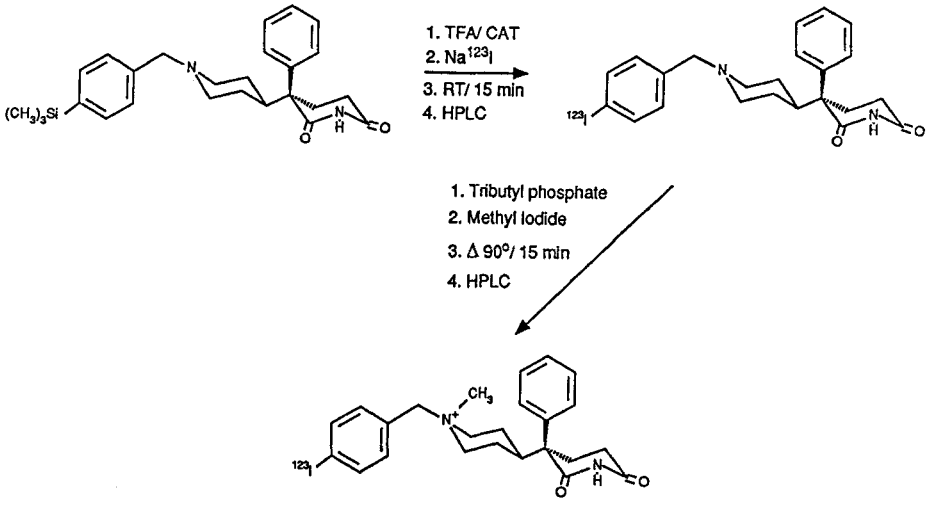

Figure 1

[^{123}I]-N-Methyl-4-Iododexetimide

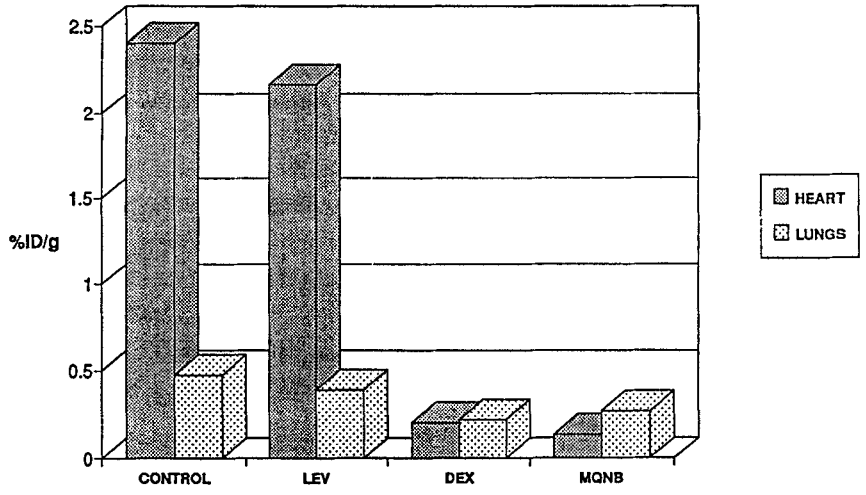

Figure 2

RADIOFLUORINATED CLORGYLINE DERIVATIVE FOR MAPPING MAO-A ACTIVITY IN BRAIN WITH PET

HIRATA, M.; MAGATA, Y.; OHMOMO, Y.*; SAJI, H.; TANAKA, C.*; and YOKOYAMA, A. Kyoto University, Sakyo-ku , Kyoto, 606, Japan, *Osaka University of Pharmaceutical Sciences, Matsubara, Osaka, 580, Japan

For direct and non-invasive mapping and functional studies of MAO-A activities in the living human brain, the carbon-11 labeled suicide inhibitor, clorgyline, has been investigated as a positron ligand for PET[1]. It has been indicated that the radioactivity of ^{11}C-clorgyline in the brain at 1 hr post-injection manifests high non-specific bindings[2]. Thus, such non-specific binding would diminish at a longer post-injection time if an appropriate radionuclide, such as F-18, was available. On the other hand, since clorgyline has two chloride atoms in its molecule, substitution of one of these chloride atoms with a radiohalogen offered a novel approach to explore F-18 labelled clorgyline derivatives for MAO-A studies in brain with PET. Of the previously reported fluorinated clorgyline derivatives[3], 4-chloro-2-fluoro-clorgyline and 2-chloro-4-fluoro-clorgyline have shown similar inhibitory potency and selectivity toward MAO-A compared with clorgyline.

In this study, radiofluorination and in vivo characteristics of these clorgyline derivatives were examined. In consideration of the introduction yield of a tin group, 4-chloro-2-fluoro-clorgyline(fluoroclorgyline) was selected for the following study. Radiofluorination was carried out with ^{18}F-acetylhypofluororite by fluorodestannylation reaction as outlined in Scheme I. The radiochemical yield of ^{18}F-fluoroclorgyline was 74% and the radiochemical purity was higher than 98% as measured by HPLC. In the biodistribution of ^{18}F-fluoroclorgyline, high brain uptake of the radioactivity was observed, followed unchanged radioactivity levels for more than 15min (Table 1). This retention profile of this radiolabel in the brain reflected the characteristic of the suicide inhibitor. Pretreatment with clorgyline induced a decrease of ^{18}F-fluoroclorgyline brain uptake to 79% at 1hr post-injection (Fig. 1). Thus, a higher in vivo specificity of fluoroclorgyline for MAO-A than that of clorgyline was achieved. Distribution studies at longer post-injection time of ^{18}F-fluoroclorgyline are now under consideration.

In conclusion, ^{18}F-fluoroclorgyline possesses good characteristics for imaging of MAO-A functional activity and distribution, and may serve as a potential PET radiopharmaceutical for MAO-A studies in the brain.

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Scheme I

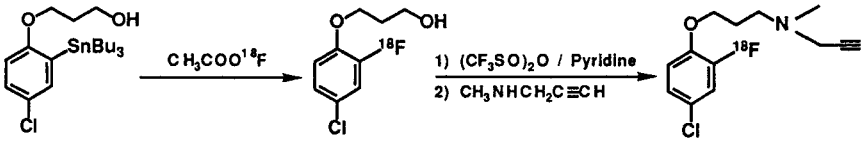
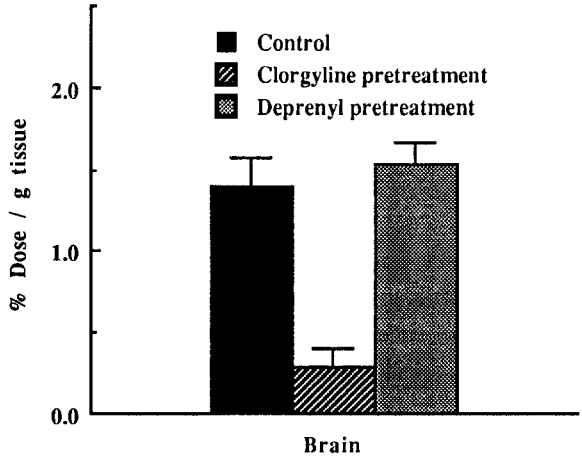


Table 1. Biodistribution of ^{18}F-Fluoroclorgyline in Normal Mice

	5min	15min	30min	60min
Blood	3.79±0.13	2.25±0.26	1.20±0.42	0.64±0.25
Liver	3.44±0.47	3.02±0.60	2.61±0.49	1.59±0.32
Kidney	10.05±1.78	11.15±2.55	11.99±3.70	5.52±1.45
Pancreas	3.81±0.26	3.16±0.64	2.29±0.32	1.81±0.16
Spleen	1.66±0.08	1.42±0.12	1.15±0.19	0.81±0.14
Bone	1.54±0.31	1.91±0.60	1.40±0.22	1.36±0.40
Lung	3.79±1.73	3.72±0.81	2.78±0.27	2.27±0.26
Heart	2.15±0.20	1.55±0.25	1.17±0.18	0.79±0.13
Brain	2.26±0.19	1.36±0.23	1.53±0.07	1.40±0.18

*Each value is mean±s.d. for 4 animals (% dose / g).

Fig. 1. Effect of Clorgyline and Deprenyl Pretreatment on Brain Uptake of ^{18}F-Fluoroclorgyline



RADIOIODINATED BENZAMIDE DERIVATIVE: A NEW SPECT RADIOPHARMACEUTICAL FOR MONOAMINE OXIDASE-B

Y. Ohmomo[1], M. Hirata[2], Y. Magata[2], C. Tanaka[1], H.F. Kung[3] and A. Yokoyama[2].
[1]*Osaka University of Pharmaceutical Sciences, Matsubara, Osaka, Japan,* [2]*Kyoto University, Kyoto, Japan, and* [3]*University of Pennsylvania, Philadelphia, PA, USA.*

We have synthesized and evaluated a novel series of iodinated analogues of benzamide for the development of new radiopharmaceuticals for mapping and functional studies of monoamine oxidase-B (MAO-B) in the living brain with single photon emission computed tomography (SPECT)[1]. Among those analogues, *N*-(2-aminoethyl)-2-chloro-4-iodobenzamide (Fig.1) (**1**) showed high inhibitory potency and selectivity toward MAO-B in *in vitro* studies. Strong and selective *in vivo* MAO-B inhibition by compound **1** was also confirmed. As shown in Fig. 2, there was an almost exclusive inhibition of MAO-B activity in the brain. On the contrary, MAO-A activity was only slightly affected up to the high dose.

We report here the synthesis and preliminary biological evaluation of the ^{125}I labeled compound **1**. No-carrier-added [^{125}I]-**1** was obtained by an iododestannylation reaction catalyzed by hydrogen peroxide followed by HPLC purification with a high radiochemical yield of 90-95% and final radiochemical purity of more than 99%. The *in vivo* tissue distribution of [^{125}I]-**1** was examined in mice. As summarized the results in Table I, the initial brain uptake of [^{125}I]-**1** was high, 1.30% dose/g at 5 min after injection, and then the brain radioactivity level increased gradually to the maximum level of 1.64% dose/g at 30 min after injection. The [^{125}I]-**1** exhibited desired prolonged retention in the brain (1.11% dose/g at 120 min after injection). However, the disappearance of the radioactivity from the blood was rapid, resulting in good brain to blood activity ratio of 2.19 and 2.41 at 60 and 120 min after administration, respectively. Then, further studies on the [^{125}I]-**1** specificity for MAO-B was carried out. Binding of the [^{125}I]-**1** to rat liver mitochondrial fraction was found to be saturable (Fig.3). Scatchard analysis of these data yielded the K_D of 0.22μM and the B_{max} of 11.3pmol/mg protein. Treatment with *l*-deprenyl caused 80% reduction of the [^{125}I]-**1** binding to mitochondrial fraction, while clorgyline treatment showed no significant effect. These competition data for [^{125}I]-**1** binding clarified the specificity of [^{125}I]-**1** toward MAO-B.

Gathered data on [^{125}I]-**1** indicated that ^{123}I labeled **1** would be a good candidate for advanced development as a radioligand that may be useful for functional MAO-B studies in the living human brain using SPECT.

1. Y. Ohmomo, *et al.*, *Chem. Pharm. Bull., in press.*

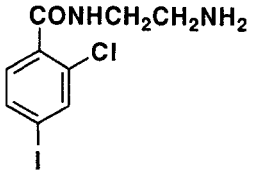


Fig. 1

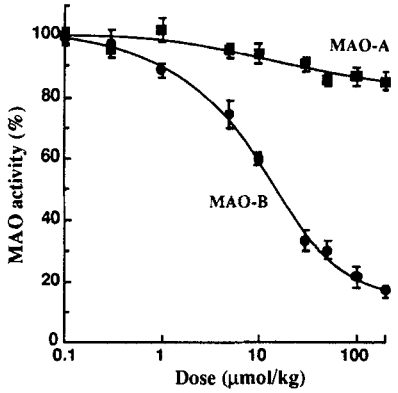


Fig. 2. Dose response curves for the inhibition of MAO-A and MAO-B activity in mouse brain *in vivo* by compound **1**. Data represent mean±s.d. of four animals as percent of control value.

Table I. Tissue Distribution of [^{125}I]-**1** in Mice *

	Time after injection				
Tissue	5 min	15 min	30 min	60 min	120 min
Blood	2.32 ± 0.21	1.59 ± 0.09	1.22 ± 0.11	0.69 ± 0.05	0.46 ± 0.04
Liver	11.11 ± 1.20	9.38 ± 0.69	6.78 ± 0.37	6.03 ± 0.51	4.91 ± 0.63
Kidney	30.59 ± 3.52	24.69 ± 2.84	14.41 ± 2.76	9.21 ± 4.92	3.48 ± 0.68
Heart	4.52 ± 0.38	2.55 ± 0.12	1.56 ± 0.14	0.98 ± 0.03	0.57 ± 0.05
Lung	13.11 ± 2.26	12.95 ± 2.29	8.40 ± 1.17	4.58 ± 0.83	1.95 ± 0.55
Pancreas	6.23 ± 0.52	4.95 ± 0.13	2.61 ± 0.30	1.69 ± 0.10	1.08 ± 0.09
Spleen	4.79 ± 0.21	4.07 ± 0.05	1.96 ± 0.23	1.08 ± 0.06	0.44 ± 0.04
Stomach	4.85 ± 0.56	5.65 ± 0.11	6.57 ± 0.36	2.36 ± 0.93	2.73 ± 0.57
Brain	1.30 ± 0.12	1.64 ± 0.09	1.64 ± 0.06	1.51 ± 0.09	1.11 ± 0.17
Brain/Blood	0.56	1.03	1.34	2.19	2.41

* Mean % injected dose ± s.d. per gram tissue of four animals.

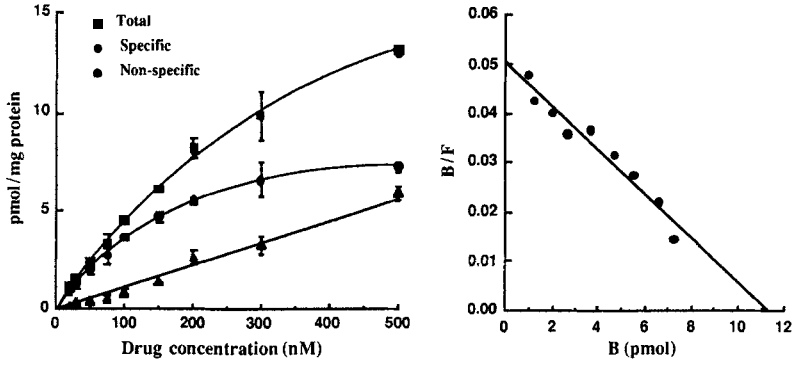


Fig. 3. Binding of [^{125}I]-**1** to rat liver mitochondrial fraction. Left panel: Binding curves of [^{125}I]-**1**. Non-specific binding was determined in the presence of 1μM *l*-deprenyl. Data represent mean±s.d. of triplicate determinations. Right panel: Scatchard plot of the specific binding.

[^{123}I]-Ro 43-0463, a potential SPET tracer for MAO-B imaging

BEER H.-F.; ROSSETTI I.; FREY L.D.; REMY N.; MAIER A.; SCHUBIGER P.A.
Paul Scherrer Institute, Radiopharmacy Division, CH-5232 Villigen PSI, Switzerland

The selective and reversible inhibitor of MAO-B, Ro 43-0463 [N-(2-Aminoethyl)-5-iodo-2-pyridinecarboxamide)], was labelled with ^{123}I to get a tool for measuring the MAO-B distribution in the human brain with SPET.

The labelling is performed with the well established halogen exchange reaction of the bromo-precursor (Ro 18-4950) using $CuSO_4$ (1)and ascorbic acid. The reaction conditions were optimized varying the parameters time (30 to 110 min), precursor concentration (1 to 3.5 mg) and temperature (125 to 175 °C). The corresponding results are shown in Fig.1. The purification of [^{123}I]-Ro 43-0463 is performed on HPLC (Lichrosorb RP-18, 5µm, 250x8) with 0.36 M H_3PO_4/EtOH 97/3 and 0.01 M $(NH_4)_2HPO_4$ (1.5 ml/min) as eluent. The labelling yield was found to range between 60 and 70% whereas the technical yield varied between 30 and 50%. The fraction with the product peak was neutralized with an equal volume of 0.45 M NaOH. The activity concentration ranged between 18.5 and 37 MBq/ml.

The sterilized radiopharmaceutical was used for biodistribution studies in femal Wistar rats (n=3) 10, 25, 40, 60, 180 and 900 min p.i. A major part of the radioactivity was found 60 min p.i. in the liver (4.1 %ID/g), kidneys (2.0 %ID/g), lung (1.7 %ID/g) and bladder (3.4 %ID/g). The brain/blood ratio of [^{123}I]-Ro 43-0463, which is a measure for the capability of the compound to penetrate the blood/brain barrier, varied between 1 and 1.2 within 1 h p.i. with the maximum 25 min p.i., shown in Fig. 2. These data are better than those published by Macwhorter et al. (2), who found 60 min p.i. a maximum of 0.53. This improvement can be explained with our labelling procedure which allows a nca production resulting in a higher specific activity of ca. 60 MBq/µg.

With the ^{125}I labelled compound, autoradiography with rat brain slices was performed which proved after displacement with Ro 18-4950, a high specific binding of Ro 43-0463.

Because of these promising results clinical trials are planed.

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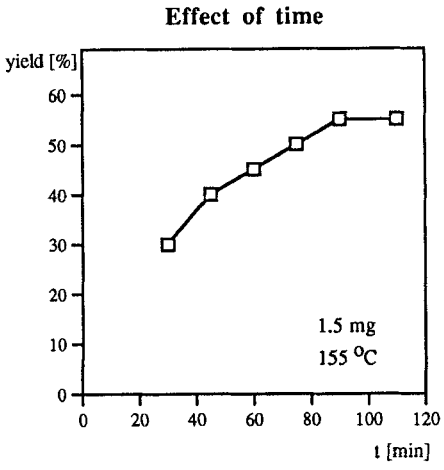
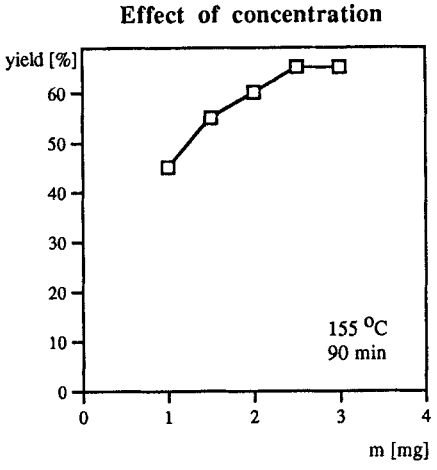




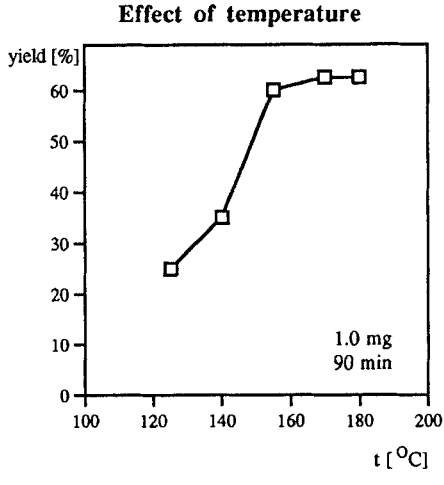


Fig. 1. Labelling of [^{123}I] RO 43-0463, yield depending on time, precursor concentration and temperature. Conditions: 34 - 740 MBq [^{123}I] NaI, 5 mg ascorbic acid, 5 µl 1% $CuSO_4 \cdot H_20$, 1ml 0.1M H_3PO_4

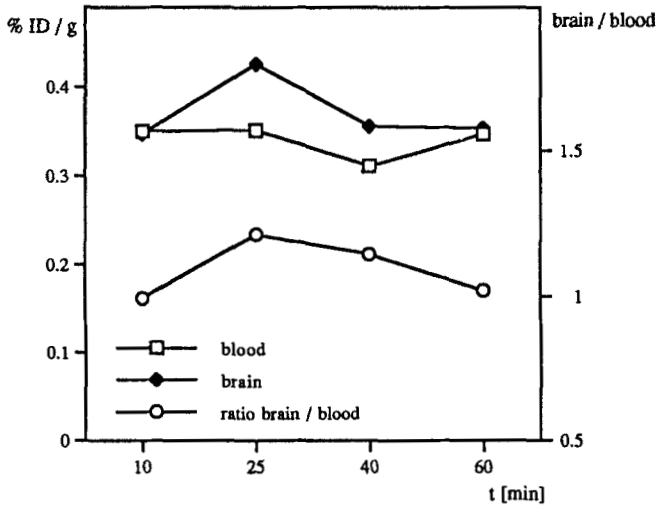


Fig. 2. Percent of injected dose of [^{123}I] RO 43-0463 per gram of tissue, mean value of three rats, the s.d. is ranging between 12 and 47 %.

[^{125}I]N-(2-PIPERIDINYLAMINOETHYL)4-IODOBENZAMIDE AND RELATED ANALOGS AS SIGMA RECEPTOR IMAGING AGENTS; HIGH AFFINITY BINDING TO HUMAN MALIGNANT MELANOMA AND RAT C6 GLIOMA CELL LINES.

JOHN, C.S.; BAUMGOLD, J.; VILNER, B.J.[1]; McAFEE, J.G.; BOWEN, W.D.[1] The George Washington University Medical Center, Washington, DC. and [1]Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD.

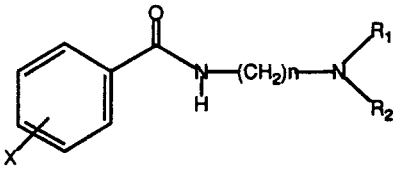
Sigma receptors were first thought to be a subtype of opiate receptor due to binding of (+/-)-N-allylnormetazocine and other related racemic benzomorphans. However, further pharmacological characterization has led to significant changes in the definition of sigma binding sites and distinction from other receptor systems such as phencyclidine, dopaminergic, and opiate receptors (1). Sigma sites bind most typical neuroleptic drugs with high to moderate affinity. Also, two subtypes, termed sigma-1 and sigma-2, have been identified which are are distinguishable by affinity for enantiomers of opiate benzomorphans and morphinans (1).

The interest in developing a highly potent and selective sigma receptor antagonist is due to its putative role in psychoses, movement disorders, neurodegeneration and various psychiatric disorders (2). The development of high specific activity sigma receptor imaging agents using SPECT and PET would enable external visualization of human sigma binding sites and the study of functional and physiological roles of sigma receptors. Recently, high densities of sigma sites have been demonstrated in several tumor-derived cell lines (3). These findings suggested that sigma receptors might be useful markers for the non-invasive visualization of tumors in-vivo. Towards these ends we have synthesized and characterized a series of halogenated benzamides of the general structures shown below and evaluated their biological activity in sigma assays.

The synthesis of halogen substituted benzamides was carried out by the condensation of an acyl chloride with the corresponding amine in the presence of triethylamine as an acid acceptor. The tributyltin precursors for the radioiodination were prepared by the reaction of bromobenzamides with palladium catalyzed stannylation reaction using bis(tributyltin). Alternatively, the halogenated benzamides containing a secondary amine as a terminal group were synthesized by the condensation of N-succinimidyl activated halobenzoic acid ester with a diamine.

N-(2-piperidinylaminoethyl)4-tributyltinbenzamide and N-(diethylaminoethyl)4-iodobenzamides were radioiodinated (I-125) using chloramine-T as oxidising agents to give high yields of [^{125}I](2-piperidinylaminoethyl)4-iodo

benzamide, [^{125}I]PAB and [^{125}I] N-(diethylaminoethyl)4-iodobenzamide, [^{125}I]DAB (4). The biodistribution of the radiolabeled [^{125}I]PAB and [^{125}I]DAB in normal mice showed that both the tracers were able to cross the intact blood-brain barrier. A high brain uptake (3.47 and 2.91 % ID/whole organ for [^{125}I]PAB and [^{125}I]DAB respectively) of the tracer was found at 2 min post i.v. injection. A slow washout of tracer from the brain at 15 min post injection (2.40 and 2.04 %ID/whole organ for IPAB and IDAB respectively) was observed. This high brain uptake in conjunction with the binding results (Table 1) demonstrate that IPAB and IDAB are highly potent sigma receptor imaging agents.



X=4-I; n=2; R_1=R_2=Et
X=4-I; n=2; R_1=R_2=-$(CH_2)_5$-
X=4-Br; n=2; R_1=R_2=Et
X=4-Br; R_1=R_2=-$(CH_2)_5$-
X=3-Br; n=2; R_1=R_2=Et
X=3-I; n=2; R_1=R_2=Et
X=3-Br; n=2; R_1=R_2=-$(CH_2)_5$-
X=3-I; n=2; R_1=R_2=-$(CH_2)_5$-
X=4-I; n=2; R_1=Et; R_2=H
X=4-Br; n=2; R_1=Et; R_2=H
X=4-I; n=2; R_1=Et; R_2=H
X=4-Br; n=3; R_1=R_2=Et
X=4-I; n=3; R_1=R_2=Et
X=4-I; n=2; R_1=R_2=-$(CH_2)_4$-
X=2-I; n=2; R_1=R_2=-$(CH_2)_5$-
X=4-I; n=2; R_1=R_2=$(CH_2)_2O(CH_2)_2$

The halogenated benzamides were evaluated for their activity at sigma receptors by competition in membranes of guinea pig brain, rat liver, and C6 glioma cells. Sigma-1 sites were labeled with sigma-1 selective ligand, [^{3}H](+)-pentazocine. Sigma-2 sites were labeled with subtype non-selective ligand [^{3}H]DTG in the presence of dextroallorphan to mask sigma-1 sites. The two compounds IPAB and IDAB bind with high affinity to sigma-1 sites, with IPAB being more potent (Table 1). Both compounds showed a marked selectivity for sigma-1 over sigma-2. However, IPAB had 10-fold higher affinity at sigma-2 compared to IDAB.

Recently we have shown that both [^{125}I]PAB and [^{125}I]DAB are taken up and retained in human malignant melanoma xenografts in nude mice model (4,5). In order to understand the mechanism of this uptake and retention we have studied the binding of these tracers in human malignant melanoma A2058 (melanotic) and A375 (amelanotic) cells. Both [^{125}I]PAB and [^{125}I]DAB bind to melanoma cells specifically with a high affinity. Ki for binding of IPAB

and IDAB to A2058 cells were found in a low nanomolar range. The saturation binding curve of [^{125}I]PAB and [^{125}I]DAB in amelanotic melanoma cells suggested that both tracers bind to the cell surface sigma receptors with very high affinity (Kd = 0.124 nM and 0.9 nM for IPAB and IDAB respectively) (Table 2). There was total inhibition of binding in the presence of 1.0 micromolar haloperidol and DTG (sigma binding ligands). No inhibition was found in the presence of raclopride or spiperone, or MABN (D-2 ligands), indicating that these compounds are devoid of dopamine D-2 activity. These results demonstrate that sigma receptors are present on human melanoma cells and C-6 glioma cells. Both IPAB and IDAB bind to these receptors with high affinity. It is therefore possible that both primary and metastatic melanoma could be imaged in humans using I-123 labeled benzamides.

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5) C.S. John; T. Saga; et al. J. Nuc. Med. 1992; 32, 889 (abstract).

Table 1. Sigma Binding Profiles of Selected Halogenated Benzamides.

	Sigma-1 (Ki, nM) [^{3}H](+)-Pentazocine		Sigma-2 (Ki, nM) [^{3}H]DTG+dextroallorphan	
	Guinea Pig Brain	C6 Glioma	Rat Liver	C6 Glioma
IDAB	11(+/-)3.93	6.86(+/-)1.95	2041(+/-)454	2900(+/-)488
IPAB	2.57(+/-)0.70	1.70(+/-)0.33	205(+/-)67	158(+/-)20

Table 2. Binding Profiles of Iodinated Benzamides in Melanoma Cells

	A2058 Human Melanoma Ki (nM)	A375 Human Amelanotic Melanoma Kd (nM)
IDAB	2.1	0.9
IPAB	6.0	0.124

A Radioiodinated Agent For Detecting Radicals In Vivo: Synthesis And Its Preliminary Evaluation.

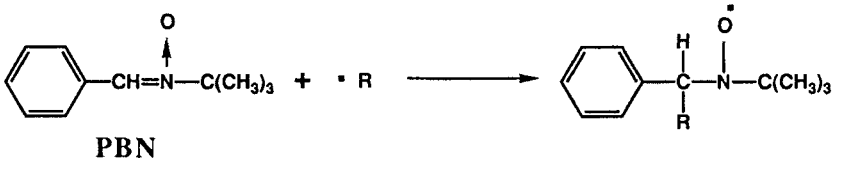
WADA, K.; FUJIBAYASHI, Y.; TAJIMA, N.; AND YOKOYAMA, A.
Department of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, 606, JAPAN.

In recent years, tissue damages caused by radicals, such as superoxide and hydroxyl radical, have been reported in various physiological and pathological states (1-3). Reaction of free radicals and its derived radicals with some nitrone or nitroso compounds yielding the radical adduct has been documented (4-5). One of those radical trapping agents, α-Phenyl-*N-tert*-butylnitrone (PBN), reacts with radical molecules as shown in Scheme I; conversely, PBN can be trapped in radical abundant area with the formation of PBN-radical adduct. Thus, PBN derivatives, α-p-Hydroxyphenyl-*N-tert*-butylnitrone (HPBN) and its iodinated derivative, α-p-Hydroxy-m-iodophenyl-*N-tert*-butylnitrone (HIPBN) were synthesized at first. The evaluation of their radical trapping properties was carried out by using the electron spin resonance (ESR) apparatus and in-vitro brain slices studies.

Synthesis of HPBN and non-radioactive HIPBN were carried out by dehydration of *N-tert*-butylhydroxylamine and p-Hydroxy-m-iodobenzaldehyde or p-Hydroxybenzaldehyde, respectively. Chemical purity was confirmed by elemental analysis and NMR. For the radiolabeling, HPBN was radioiodinated (I-125) using the chloramine T reaction with radiochemical yield of more than 90 %. The purification of radioiodinated I-125-HIPBN was carried out by HPLC (column; ODS 8 x 250mm, solvent; methanol : water = 1:1, flow; 1ml/min) (Scheme II). The radiochemical purity was more than 99 %.

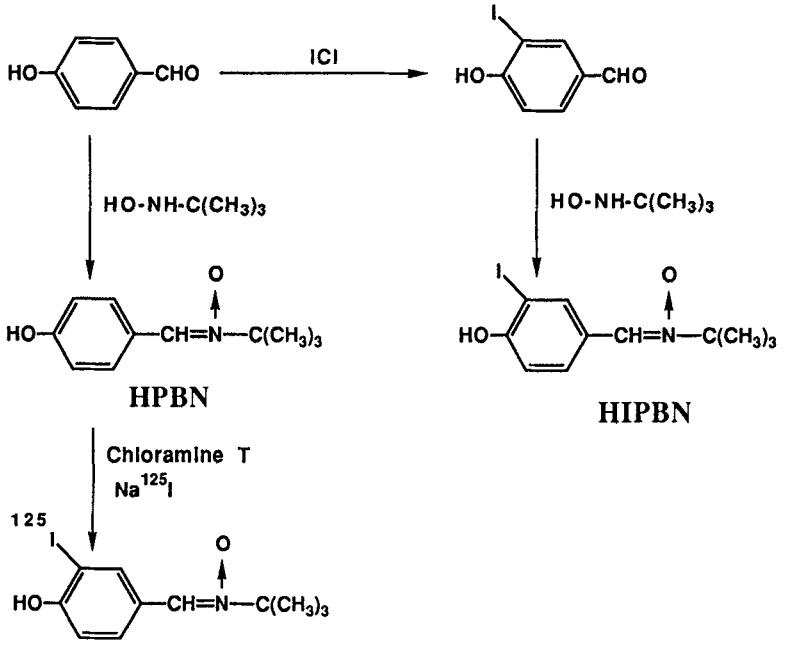
Radical trapping ability of the newly synthesized PBN derivative (HIPBN) was tested by ESR; the radical adduct formation of HIPBN and superoxide anion radical, produced by xanthine and xanthine oxidase, could be confirmed by its ESR spectrum as well as the parent compound, PBN. To investigate the I-125-HIPBN ability for detecting radical(s) in vivo, tissue binding study was carried out using rat brain cortex slices. The slices pre-exposed to the superoxide anion radical generated by xanthine and xanthine oxidase were incubated with I-125-HIPBN. After an incubation for 15 min, the tissue binding measurement indicated the presence of significant radioactivity bound to the exposed tissue compared with non-exposed control tissue. Also, significant tissue radioactivity increased with added amount of xanthine oxidase, indicating I-125-HIPBN potential as a celluar radical detecting agent.

In conclusion, the synthesized HIPBN preserved the PBN ability for the formation of radical adduct, and its radioiodinated I-125-HIPBN presented good potential for the detection of radical abundant area.



PBN

Scheme.I



I-125-HIPBN

Scheme.II

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Synthesis of 1-{1-[2-benzo(B)thiophenyl]cyclohexyl}, 3[^{123}I]iodomethyl piperidine ([^{123}I]BTCPCH$_2$I) for the *In Vivo* Study of the Dopamine Reuptake System

DONG, C.[1]; LOC'H, C.[1]; PONCHANT, M.[1]; BRUTESCO, C.[1]; KAMENKA, J-M.[2]; MAZIERE, B.[1]; 1: Service Hospitalier Frédéric Joliot, CEA, F-91406 Orsay, France, 2: INSERM U249, CNRS UPR8402, ENSCM, 8 rue de l'Ecole Normale, F-34053 Montpellier Cedex 1, France

1-{1-[2-benzo(B)thiophenyl]cyclohexyl}piperidine (BTCP), is a phencyclidine analogue which has a high affinity for the dopamine (DA) reuptake complex (1). The 3-[^{18}F]fluoromethyl derivative of BTCP has been synthesised and preliminary studies performed in baboons have shown that this radiotracer has the potential to be used to map the DA reuptake system by PET (2). For SPECT imaging purposes, we have prepared the radioiodinated derivative of BTCP, 3-[^{123}I]iodomethyl BTCP.

Triflic anhydride (92 μmol) in dry CH_2Cl_2 (1.6 mL) was added dropwise under nitrogen to a stirred solution of the alcohol ***1*** (30.4 μmol) in a refrigerated mixture of pyridine (92 μmol) and CH_2Cl_2 (2.8 mL) (-10°C). The cold bath was removed and the mixture was stirred for 40 min at room temperature. Then the reaction mixture was diluted to 10 mL and washed with water (20 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated, giving the triflate ***2*** (27 μmol, 89%) which was used directly without purification. For the radiolabelling, the triflate (1.0 mg in 1.0 mL acetone) and 370 MBq of [^{123}I] as NaI were let at room temperature for 40 min. The solvent was evaporated under vacuum and the residue, dissolved in 0.5 mL CH_2Cl_2, was poured onto a SEP-PAK silica cartridge. The column was eluted successively with 10 mL $CHCl_3$ to remove the by-products and with 3 mL of ethyl acetate/n-hexane (10/90, v/v) which provided 330 MBq of a crude radioactive product. 3-[^{123}I]iodomethyl BTCP ***3*** was purified first by straight-phase HPLC (Lichrosorb-Si 250x10 mm) with a mixture of EtOAc/cyclohexane/H_2O/TEA (1/99/0.002/0.005, v/v/v/v) at a flow rate of 1.5 mL min^{-1} (Rt = 21 min) and then by reversed-phase HPLC (μBondapak C18, 300x3.9 mm) with a mixture of EtOH /H_2O /TEA (70/30/0.005, v/v/v) at a flow rate of 1.3 mL min^{-1} (Rt = 28 min). The chemical and radiochemical purities of 3-[^{123}I]iodomethyl BTCP were >98% and >99% respectively. The total radiochemical yield was >85 % and the specific activity of the radiotracer was >74 GBq/μmol.

In vivo biodistribution studies in rats showed that 3-[^{123}I]iodomethyl BTCP crossed easily the blood brain barrier (0.1% ID/g 10 min post injection). However, due to the high lipophilicity of the radiotracer (Log P_{oct} > 4) the uptake was essentially non specific and could not be displaced by a load of cold BTCP.

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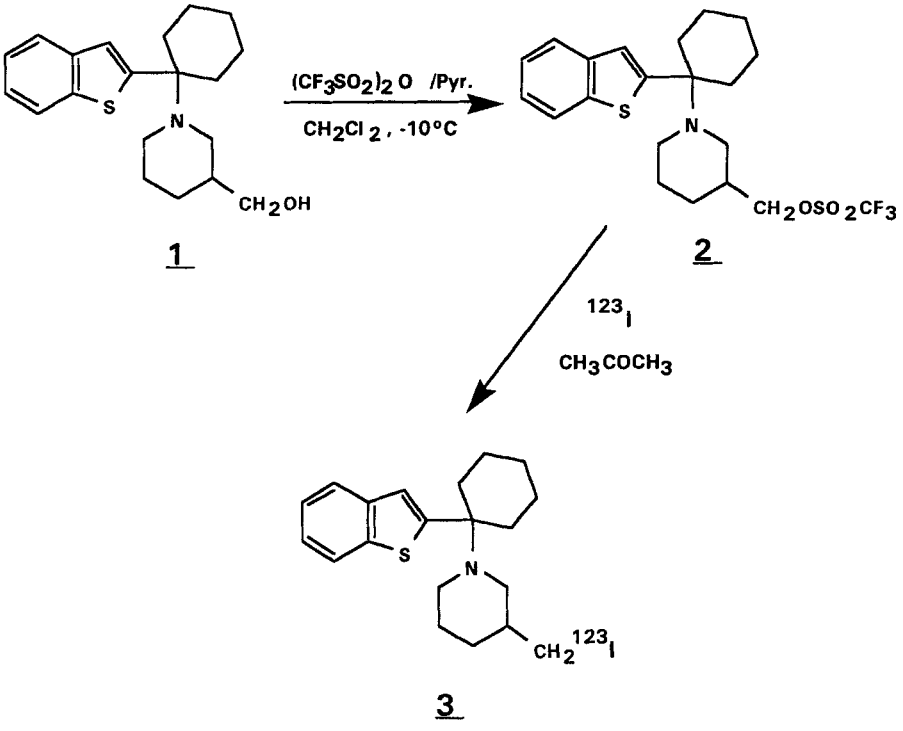
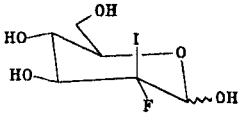
REACTION SCHEME



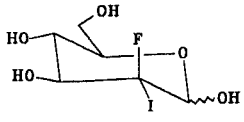
2-Fluoro-2-Iodo-Mannose and -Glucose as Potential SPECT Imaging Agents. [1]J.D. McCarter, [2]M.J. Adam and [1]S.G. Withers, [1]Department of Chemistry, University of British Columbia and [2]TRIUMF, Vancouver, B.C., Canada.

There have been many recent attempts to develop a ^{123}I labelled glucose analog of 2-fluoro-2-deoxy-glucose (FDG) to study various diseases of the brain and heart (1). Most of these compounds have little or no uptake in heart or brain or are poor substrates for hexokinase. This is not surprising given the major structural differences between most of these compounds and glucose. Several years ago it was shown that 2-iodo-glucose (2IDG) is unstable and cannot be isolated (2). Because of this we have attempted to synthesize an analog that is structurally similar to (2IDG) without being unstable.

Cold 2-fluoro-2-iodo-mannose and -glucose were both prepared from 2-fluoro-glucal. 2-Fluoro-2-iodo-mannose (2-FIM) was prepared by iodination of 2-fluoro-glucal with KI oxidized with hydrogen peroxide. 2-Fluoro-2-iodo-glucose (2-FIG) was prepared by iodohydration of acetylated 2-fluoro-glucal followed by deprotection and chromatography. Both of these compounds are stable *in-vitro* and were isolated and their structures determined by nmr.



2-FIM



2-FIG

Labelling with ^{123}I was attempted only on the mannose derivative since the radiolabelling procedure was the same as the synthesis of the cold compound. Briefly, 1 mg of 2-fluoro-glucal was dissolved in pH3 citrate buffer, ^{123}I-NaI was added followed by hydrogen peroxide (30%). The mixture was allowed to stir for 60 minutes and the reaction analyzed by tlc and compared to an authentic standard of the iodinated product. The radiochemical yield was 60%.

Yeast hexokinase studies were carried out in-vitro on both of these compounds and no phosphorylation was detected. Further studies in animals are underway.

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HIGH-LEVEL PRODUCTION AND USE OF CARBON-11: A STRATEGY FOR CONTAINMENT OF STACK EMISSIONS.

M.R. Kilbourn, D. Norton, J. Moskwa, T. Mangner, J. Viola, T. O'Donnell, D. Palmieri, M. Driscoll, and D.E. Kuhl
The University of Michigan, Ann Arbor, MI, 48109, U.S.A.

Clinical research protocols at our institution, in combination with radiochemical research projects, require production of several curies of carbon-11 per each working day. Early in 1990, we became aware of problems with releases of carbon-11, nitrogen-13, oxygen-14 and oxygen-15 labeled volatile materials through our facility exhaust stacks. To eliminate the environmental health, public relations, and regulatory questions regarding such emissions, we instituted a program to reduce and eventually eliminate releases of short-lived radioactive gases from the stacks serving our radiochemistry hot cells. After considerable discussion, we finally settled on a system to collect, compress, and store the carbon-11 gases until sufficiently decayed to allow safe release to the environment. This systems was designed, constructed and implemented in the first half of 1992, and has been in constant daily use for the past year. The characteristics of this system are described here.

Requirements. We wished to utilize a pro-active radioactive gas containment system which would automatically engage for every carbon-11 synthesis. Control of the system would not be accessible by the radiochemistry or cyclotron personnel, and all system parameters could be monitored but not altered. The location of our cylotron facility within the close-packed confines of the Medical Center, and the lack of available space in any other building or outside our building, required that all components of the system be accomodated within our existing facility.

Design. The containment system has three basic components. First, the hot cells have been largely sealed (limited entry points), the normal exhaust ducts fitted with high-efficiency gate valves, and the cells equipped with a supplementary, low-volume (50 ft3/min) exhaust system. This supplementary system feeds into the second part of the system, a pair of high pressure, hermetically sealed inert gas compressors. Finally, the compressors fill a tank farm of 28 K-bottles (4 sets of 7 bottles) located along one wall of our cyclotron vault.

Operation. The entire system is controlled by a programmable logic controller (PLC) system, custom designed for our application, and which is monitored in real time on a Macintosh IIci personal computer. The containment system control is also directly connected to the computer controlling our cyclotron targetry, preventing delivery of carbon-11 to the radiochemistry laboratory without the containment system in full operation. At end of beam, the normal exhaust mode is cut off, the supplementary exhaust system engaged, the system compressors are brought up to full speed, and a five minute required waiting period imposed: if the system is fully functional, the carbon-11 is then delivered into the hot cell for the beginiing of the radiochemical synthesis. For 45 minutes, the hot cell is under the containment cycle, and all effluent is collected, compressed, and stored in one of four tank farms: after 45 minutes the system resets and is available for another synthesis.

Performance. This system has been in operation for a year with minimal difficulties. The exhaust stacks are constantly monitored for radioactivity, and have shown effectively complete containment of radioactive gas effluents. The forty minute turn-around time has not impeded either chemical research or clinical production of PET radiopharmaceuticals.

PRODUCTION 0F [^{18}F]F WITH PROTONS ON NEON: IS IT ^{20}Ne(p, p2n)^{18}Ne-->^{18}F or ^{20}Ne (p,2pn)^{18}F? A REEXAMINATION.

REDDY, G.N.; BEER, H.F.; and SCHUBIGER, P.A.
Division of Radiopharmacy, Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland.

Introduction: [^{18}F]F-Labelled radiopharmaceuticals have been recognized to play a major role for the development of positron emission tomography (PET) as an *in vivo* modality in biomedical research. The half-life (109.6 min) of [^{18}F]F provides an ideal time frame for PET investigations. However, it is an elusive isotope to deal with, both for the target chemist and radiochemist.[1] [^{18}F]F is generated currently in two main forms either as fluoride ion or as fluorine gas. The most commonly employed two nuclear processes are: ^{20}Ne(d,α)^{18}F and ^{18}O(p,n)^{18}F. The latter process is predominantly used for the production of NCA [^{18}F]fluoride and the former for the production of carrier-added [^{18}F]F_2. Generation of [^{18}F]F_2 with protons on [^{18}O]O_2 has been implemented, but this method has its disadvantages.[2,3] Thus, PET centers that have proton-only-cyclotrons are handicapped for the production of [^{18}F]F_2. However, with medium energy protons (35-40 MeV), it could be generated by ^{20}Ne(p,x)^{18}F reaction. This process is not much studied and is being used only at TRIUMF, UBC PET Center.[4] At our Institute, two injectors of proton energy 72 Mev are in use for isotope production and hence we examined this nuclear process in detail.

Materials and Methods: The target body and windows were made of pure nickel. The cylindrically shaped target is 1.8 cm in diameter and 18 cm long. The outer window has a thickness of 0.3 mm and the inner one 0.5 mm with 2.2 mm circulating water. The volume of the target is 64.0 mL. Two different incident proton energies of 35 and 39 MeV were employed. One shoot and two shoot protocols as described by R.J. Nickles et al. were implemented.[3] Extracted activity was trapped either in KI solution or NaOAc cartridge and KI solution.

Results and Discussion: Two modes of production of [^{18}F]F are possible from neon: (a) ^{20}Ne(p, p2n)^{18}Ne-->^{18}F and/or (b) ^{20}Ne (p,2pn)^{18}F. The excitation functions for these two reactions are given in Figure 1. The excitation functions reach maxima at the proton range of 45-55 MeV for ^{18}Ne and 35-45 MeV for ^{18}F production. However the total cross-section values are somewhat similar for the proton range of 25-40 MeV employed for this study. The cross-section values were obtained with ALICE 82, a computer-based evaporation code. Theoretical [^{18}F]F yields from these nuclear reactions were calculated to be approximately 5 mCi / μAh for (p,p2n) and 4 mCi / μAh for (p,2pn) process for the proton range 28-35 MeV and 10 mCi / μAh for (p,p2n) and 5 mCi / μAh for (p,2pn) process for the proton range 28-39 MeV. Experimentally extracted total values for a two-step protocol are 3-4 mCi / μAh for the proton range 28-35 MeV and 5-6 mCi / μAh for the proton range 28-39 MeV. These values represent 30-45% extraction of activity from the target. If the experimental total production values (slightly higher than predicted by code ALICE) were used the extraction values would be lower.[5] However, these amounts allow us to produce routinely carrier-added 6-FDOPA at our Institute.

In a two-step production protocol, first the target is filled with pure neon and bombarded for a desired period and is emptied. It is then filled with desired amount of (carrier) fluorine gas and bombarded again to facilitate exchange between F_2 and [^{18}F]F. It is assumed that in the first bombardment [^{18}F]F is generated and deposited onto the inside

walls of the target. **However contrary to previous observations[4], we observe that considerable amount of activity is extracted when pure neon gas alone is bombarded with protons and emptied from the target. When neon gas was emptied directly into a water tank, a violent reaction takes place with the evolution of white dense fumes. The identity of this reactive species is not established. We speculate that it is a nascent form of [^{18}F]F that is formed as ^{18}Ne passes through water column. However, it is questionable whether one can visibly observe reaction of nanomolar quantity of [^{18}F]F with water. The fumes deposit radioactivity which is that of [^{18}F]F and if the gas is directly trapped from the target, it exhibits initially a high activity which then decays quickly.** If short bombardment periods were employed and the target gas emptied immediately, the above phenomenon is very noticeable. However, if the target gas is emptied after being kept in the target for 5 to 10 min or is emptied through sodalime and charcoal, only a little amount of radioactive gas is observed. We believe that [^{18}F]F activity is not directly generated but indirectly from positron decay of [^{18}Ne]Ne. It is also possible that both processes are taking place at the same time. However the contribution of each process to the total activity is not clear. Studies are underway to characterize the components of the target gas mixture.

Conclusion: We have reason to believe that [^{18}F]F is indirectly produced by positron decay of [^{18}Ne]Ne($T_{1/2}$= 1.68 sec) produced by ^{20}Ne(p,p2n)^{18}F nuclear process.

Acknowledgment: We thank Dr R.J. Nickles for his help in starting this work and for his helpful discussions.

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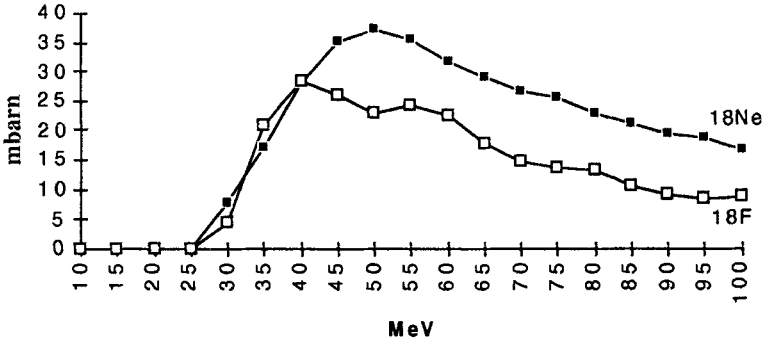
Figure 1: Excitation Functions for 18Ne and 18F



High Pressure Liquid 18F-Fluoride Target Systems - Effect of HPLC Water Quality on Radiochemical Yields

GEE*, A.D. and GILLINGS N., The Clinical PET Centre, UMDS, University of London, St Thomas' Hospital, Lambeth Palace Rd, London SE1 7EH, UK.

The use of a high pressure target which offers yields of ^{18}F-fluoride at least twice those available using a conventional low-pressure target has recently been reported[1,2]. This paper describes our experience gained over the last 12 months in utilising this target in the routine production of ^{18}F-FDG. It is reported that the quality of HPLC water used to purge the ^{18}F-fluoride from target has a significant effect on the degree of ^{18}F-fluoride available for incorporation in nucleophilic labelling reactions.

SYSTEM DESCRIPTION (see Fig. 1)

The target and delivery lines are continually kept in contact with natural water which can be fed into the system using an HPLC pump. At the start of a production run, 440 ul ^{18}O-water (>95% enrichment) is loaded into the target (R69 and R70 closed, R 71 open, HPLC off) displacing natural water to waste. The target system is pressurised to 3 MPa with natural water (HPLC pump on, R 69, R70 and R71 closed). After a typical bombardment of 40 uA for 60 min., the system is vented to atmosphere by rapid cycling of R 69 (or R70). ^{18}F-Fluoride is transferred to one of the Chemistry Processing Control Units (CPCU's) by purging the fluoride bolus through the delivery lines with water supplied from the HPLC pump. Displaced natural water is piped to waste. Upon arrival of the ^{18}F-fluoride, a valve is switched to deliver the radioactivity to the CPCU. A volume of 110 ul delivered to the CPCU contains *ca.* 98% of the total radioactivity purged from the target.

FDG SYNTHESIS

FDG was synthesised using a Siemens/CTI Chemical Processing Control Unit (CPCU) by a standard procedure.[3]

In initial investigations (25/3-26/5), target purging with natural water was achieved using Fresenius water for injection BP producing decay corrected radiochemical yields of FDG in the range 10 - 40%

To enhance sterility precautions, subsequent syntheses (26/5 - 21/8) used Baxter sterile water for injection as the source of HPLC water. Reference to Table 1 clearly shows a marked decrease in FDG yields during this period (range 5-25%, average 10%).

From 25/8 to 1/12/92 a return to the use of Fresenius water for injection BP clearly shows a marked increase in FDG yields (range 16-40%, average 33%)

It was reasoned that the presence of dissolved metal ions and/or fluoride in the HPLC water could cause contamination of the ^{18}F-fluoride bolus and exert an influence on the FDG yields.

Since 8/12/92, high purity 'water for inorganic analysis' (Fluka) has been used to purge the fluoride bolus resulting in dramatically increased FDG yields (range 40-59%, average 48%). Since using the Fluka water, typical syntheses now produce 8-12 GBq FDG measured at end of synthesis.

The sources of water mentioned above are presently being analysed for content of anions, cations, organics etc. These results will be presented at the symposium.

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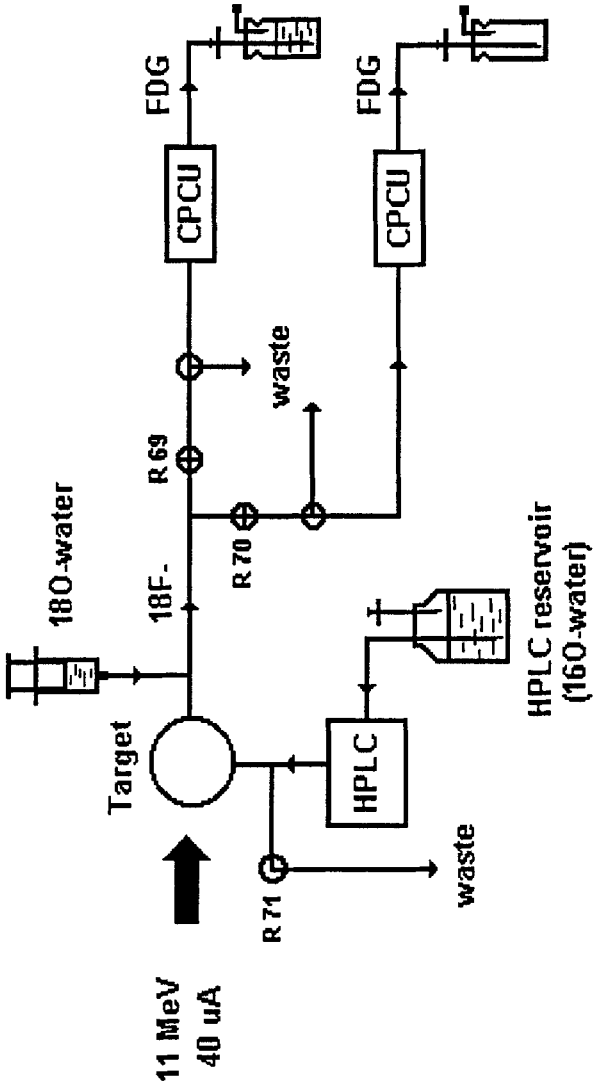
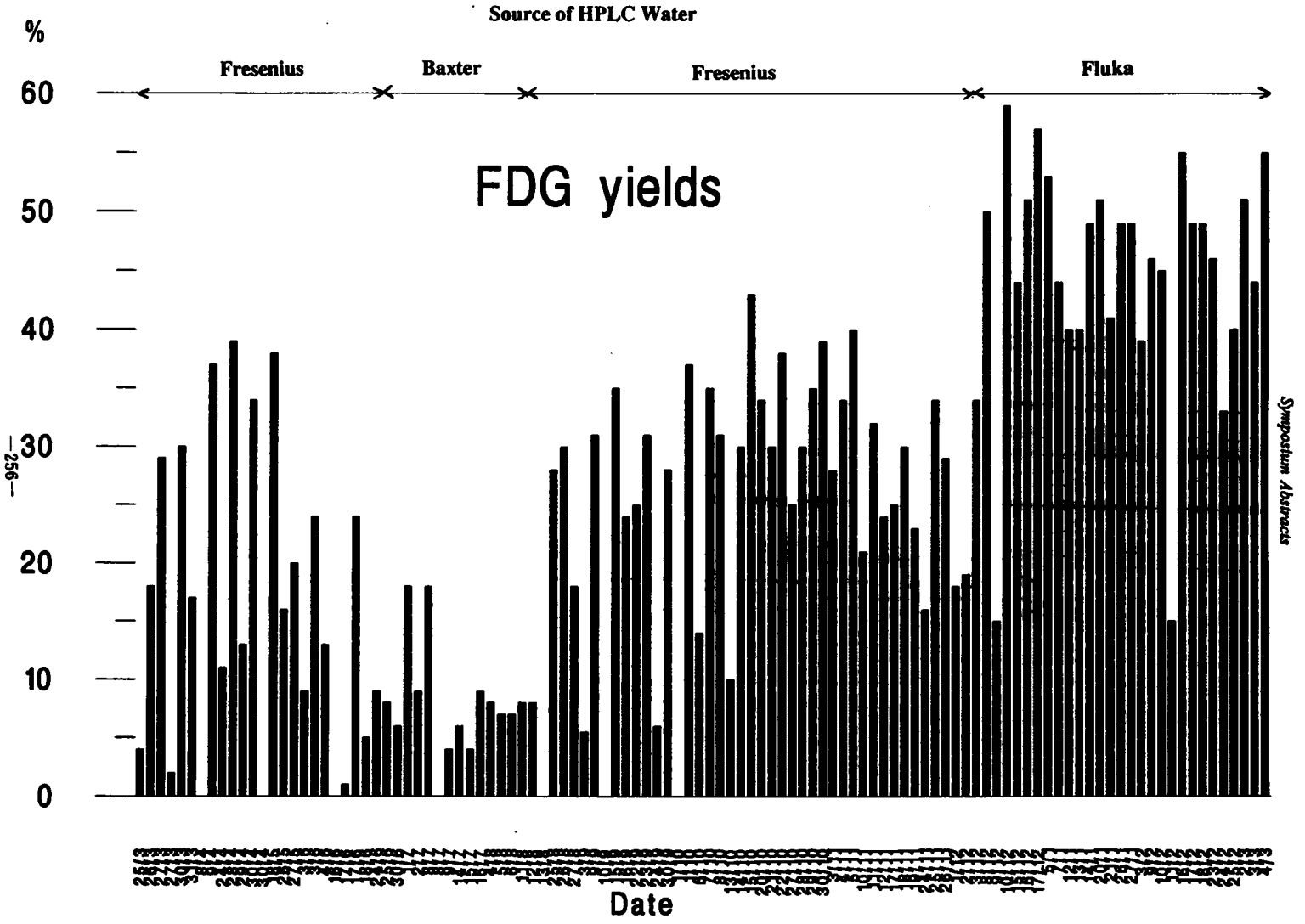


Fig 1. High Pressure 18F-Fluoride Target Scheme


Source of HPLC Water
%
Fresenius
Baxter
Fresenius
Fluka
60
50
40
30
20
10
0
FDG yields
Date

The Yield of I-124 from Different Target Materials in the $^{124}Te(d,2n)^{124}I$ Reaction and an Improved Recovery Method for Te-124.

Mahmoud L. Firouzbakht, David J. Schlyer and Alfred P. Wolf, Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973. USA.

Iodine-124 is a positron-emitting isotope with a longer half-life (4.2 days) which has been used in preliminary studies for PET (1,2). Monoclonal antibodies labelled with this isotope offer promise in using PET to quantitate the biodistribution of a monoclonal. The biodistribution information can then be used to plan therapy using the monoclonal labelled with I-131.

The most practical way to make the I-124 on a medium energy cyclotron is from the $^{124}Te(d,2n)^{124}I$ reaction on enriched Te-124 (3). The $^{125}Te(d,3n)^{124}I$ reaction has been used but has been found to be less desirable due to the lower yields. The extremely high cost of the enriched Te-124 demands that nearly all the target material be recovered. In this light we have undertaken a study of yield and recovery of Te-124 based on the widely used chemistry for production of I-123 from the $^{124}Te(p,2n)^{123}I$ reaction and the recovery of the Te-124 (4).

The target used in this study was a flat plate target as shown in Figure 1. The target material is pressed into the groove in the aluminum plate with 20,000 psi. The tellurium was either mixed with 10% aluminum powder to aid heat conduction or pressed in with no additives.

We have made a direct comparison of the yields of I-124 from a pure tellurium powder target, a tellurium powder target with added aluminum and a tellurium oxide pellet target (all natural abundance). The results of this study are given in Table 1. The relative I-124 radioactivity was determined by measuring the area under the 602.7 keV γ-ray peak. The aluminum blank is the target holder. In this case the target material was left in the holder and the entire assembly counted. In the case of no blank, the target material was removed from the aluminum plate and counted separately. All targets were counted 1 day after irradiation.

The commonly used methods of separating the I-124 (5,6,7) and recovery of the enriched tellurium were explored and a new method was developed which gives better recovery of tellurium-124. The sample was dissolved in 10 mL of 7M H_2SO_4 and then 1.5 mL of 30 % H_2O_2 was added in small portions at which point a clear solution was obtained. Twenty-five mL of distilled water was added to the cooled mixture and 25 mL was distilled off. This fraction contained the iodine-124. Then 10 mL of 50% hypophosphorus acid was added to the cooled solution to precipitate the tellurium. The solution was filtered and the filtrate washed and dried. This method gives a recovery of 99.0 ± 0.5% recovery of the tellurium.

This work was carried out at Brookhaven National Laboratory under contract DE-AC02-76CH00016 with the U.S. Department of Energy, supported by its Office of Health and Environmental Research and grant No. NINDS, NS 15380.

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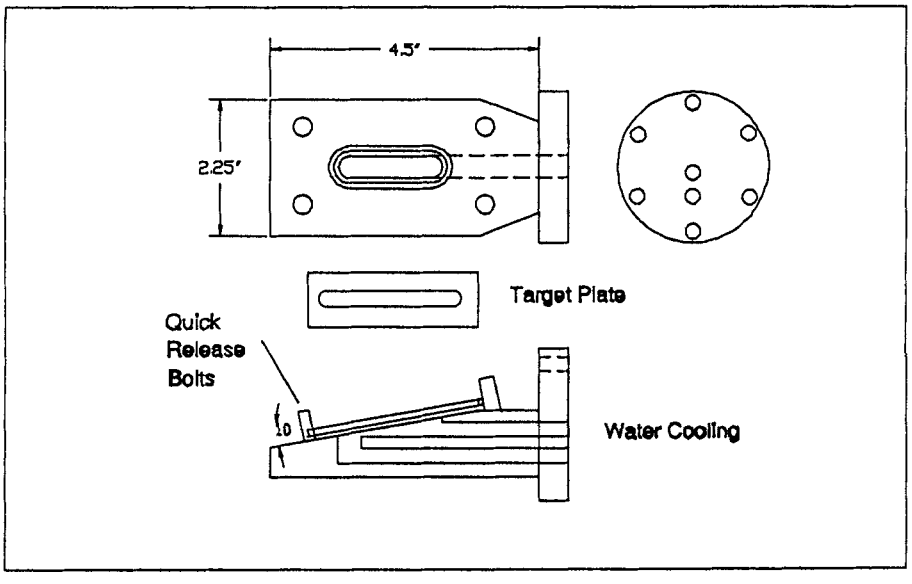
Table 1

Comparison of Target Materials
Percentage of I-124 Activity

Te,Al/Te with Al blank	Te,Al/Te without Al blank	TeO2/Te with Al blank	TeO2/Te without Al blank	TeO2/Te,Al with Al blank	TeO2/Te,Al without Al blank
84.5±1.0	86.8±1.9	71.7±2.8	67.7±3.6	81.0±5.2	79.2±4.6



Production and Processing of Bromine-75 from the $^{75}As(^{3}He,3n)^{75}Br$ Reaction.

David J. Schlyer, Steven Tischer, and Mahmoud Firouzbakht
Department of Chemistry, Brookhaven National Laboratory Upton, NY 11973 and King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.

Bromine-75 is an isotope which is slowly increasing in use in the field of Positron Emission Tomography (1,2,3). The half-life of 98 minutes is nearly ideal for receptor binding studies and the carbon-bromine bond is significantly stronger than the carbon-iodine bond which makes the denaturation of organic molecules less facile.

Production of bromine-75 can be carried out with several reactions. The one which does not rely on an enriched isotope is the $^{75}As(^{3}He,3n)^{75}Br$ nuclear reaction. Since natural arsenic is 100% As-75, the radionuclidic impurities are greatly reduced. The major radionuclidic impurity is bromine-76 with a 16.2 hour half-life. By using a thin electroplated target, it was possible to obtain bromine-75 with a yield of 8 mCi/μA-hr and with a 2.0% Br-76 impurity at an energy of 38 MeV.

The arsenic was deposited on a copper plate from a solution which consisted of 10 grams of As_2O_3 and 7 grams of KOH dissolved in 30 mL of water. The arsenic was applied at a voltage of 2.8 volts over the period of about 1 hour.

The arsenic target was disassembled and the target plate placed in a teflon dissolving dish. The target material was dissolved off the target plate with 5 mL of 1M NaOH and 1-2 mL of 30% H_2O_2. Dissolution took about 10 minutes. After destruction of the excess peroxide, this solution was passed through a cation exchange resin (H^+ form) and rinsed with 15 mL of water. The effluent from the column was then passed through a anion exchange resin (Cl^- form) and rinsed with about 5 mL of water. The arsenic was removed from the column with 7 mL of .05 M HCl. The bromine was removed with 10 mL of normal saline. The processing time is about 50 minutes. The apparatus used is shown in Figure 1.

Quality assurance test on the bromine-75 are given in Table 1. Since the arsenic target material is not enriched, no attempt was made to recover it. The total amount of arsenic used in the target was 65 mg.

This work was carried out at Brookhaven National Laboratory under contract DE-AC02-76CH00016 with the U.S. Department of Energy, supported by its Office of Health and Environmental Research and grant No. NINDS, NS 15380.

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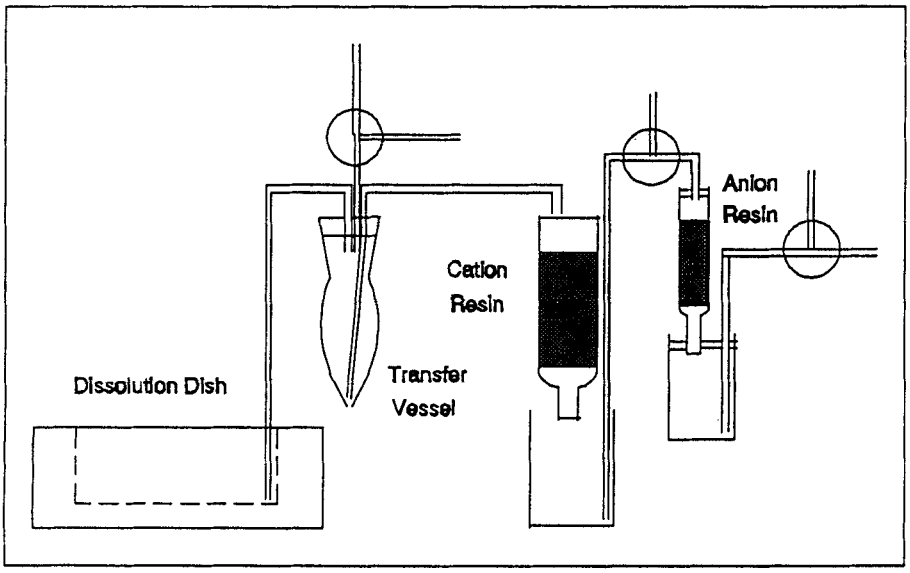
Table 1
Quality Assurance tests on Bromine-75

Radionuclidic Purity	2.0% ^{76}Br at EOB
Chemical Purity	< 5.0 µg As/ml
Radiochemical Purity	> 95% Br^-
pH	5-7 (normal saline)



The Sr-82 Cyclotron Production For Medical Application. Method. Experimental Results.

VERESHAGIN,YU.I.; ZAGRYADSKIY,V.A.; PRUSAKOV,V.N. Russian Scientific Centre "Kurchatov Institute",123182 Moscow,Russia.

Rubidium-82 is one of the perspective positron emission radionuclides,which can be used at the positron tomography method. The short half-life of the ^{82}Rb (75s) allow significantly to reduce radiation dose to patients and hospital personnel. Rubidium-82 is obtained from a generator system through its 25-day half-life parent ^{82}Sr. This report is devoted to problem of the ^{82}Sr cyclotron production for the ^{82}Sr-^{82}Rb generator. The ^{82}Sr was received into RbCl(99% ^{85}Rb) target with help of the ^{85}Rb(p,4n) reaction, using 70 MeV protons of inner cyclotron bunch at the current up to 75 μA. Chemical processing included chromotographic isolation of Sr with help of ion exchange column. The target construction, experimental method and results are presented in this report.

High Yield Production of Pure ^{89}Zr; a Positron Emitter for Labelling of Proteins

MEIJS[1] W.E., HERSCHEID[1] J.D.M., HAISMA[2] H.J., van LEUFFEN[3] P.J., MOOY[3] R. and PINEDO[2] H.M. [1]RadioNuclideCentre(RNC), Free University; [2]Department of Oncology, Free University Hospital; [3]B.V. Cyclotron, Amsterdam, The Netherlands.

The physical characteristics of ^{89}Zr suggest the use of this positron emitter for labelling proteins. Due to its relatively long half-life ($t_{1/2}$ = 78.4 h), it can be used as positron label for quantifying the biodistribution of antibodies. It decays for 23% by positron emission and for 77% by electron capture to ^{89}Y (stable). The total particle release per desintegration of approximately 100 keV even opens the possibility for therapeutic applications.
Zr has been found to form very stable complexes with the chelating agent desferal (Df); this compound might be a promising chelator for labelling the antibodies with Zr[1].

The aim of our study was to produce very pure ^{89}Zr in amounts sufficient for patient-studies. ^{89}Zr can be produced by a (p,n) as well as a (d,2n) reaction on ^{89}Y (100 % natural abundancy)[2,3]. Increasement of the ^{89}Zr yield by raising the beam current necessitates a good heat transfer. This was obtained by sputtering the ^{89}Y on a copper target holder and cooling the target holder with water. Two hours of bombardment, using the internal radiation facility of a Philips AVF cyclotron with 14 MeV protons at 97 μA, gave 220 mCi Zr-89. Due to the small angle of the beam with the target (1-3 degrees), an Y-sputtering layer of only 25 μm (about 300 μmol Y) was sufficient for efficient production.
After bombardment, the Y-target was dissolved in 2 ml of 1 M HCl; at this HCl concentration the Y is dissolved, while the copper of the target holder hardly is. Subsequently 1 ml of H_2O_2 was added to oxidize the Zr to the IV-state, followed by addition of concentrated HCl to obtain a final HCl concentration of 3 M. The amount of long living radionuclidic impurities was low ($6*10^{-3}$%, table 1).

TABLE 1 Radionuclidic impurities of the ^{89}Zr-solution before purification on a hydroxamate column

isotope	half live (days)	production	% impurity*
Zr-88	83.4	^{89}Y(p,2n)	$2.5 * 10^{-6}$
Y-88	106.6	^{89}Y(p,pn) and daughter ^{88}Zr	$6.5 * 10^{-6}$
Zn-65	244	^{65}Cu(p,n)	$2.5 * 10^{-3}$
V-48	16	^{48}Ti(p,n)	$2.7 * 10^{-3}$
Co-56	79	^{56}Fe(p,n)	$0.65 * 10^{-3}$

* Percentage of radionuclidic impurity present in the ^{89}Zr-solution at EOB.

With PIXE-experiments we found that 75 nmol Fe was present as an impurity in the Y-target (± 300 μmol). This explains the formation of ^{56}Co (by a (p,n)reaction on ^{56}Fe). Because of the very high affinity of desferal (Df) for Fe, the presence of such a relatively large amount of Fe seriously competes with or even prohibits the formation of ^{89}Zr-Df.
Zr was separated from the large amounts of Y and Fe using a hydroxamate column[4]. Hydroxamate groups form complexes with several metal ions, but Zr is the only metal ion thus far known, which is able to form complexes with the hydroxamate groups at high HCl concentrations[5]. Optimal separation conditions were studied using ^{59}Fe, ^{88}Y and ^{88}Zr* as tracers. With 3 M HCl all ^{59}Fe and ^{88}Y was eluted (fig. 1), while most of the Zr was retained on the column. More than 95% of the Zr was then eluted with 2 ml 1 M oxalic acid. The excess of oxalic acid was removed by sublimation under vacuum.

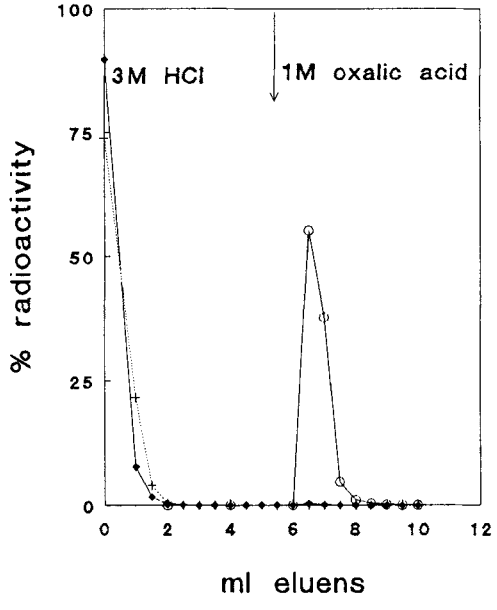


FIGURE 1 Separation of ^{88}Zr (—o—) from ^{59}Fe (...+...) and ^{88}Y(— ◆ —) using a hydroxamate colum eluted with 6 ml 3 M HCl and subsequently with 6 ml 1 M oxalic acid.

*Zr-88, a longer living isotope of Zr ($t_{1/2}$ = 83.4 days), can be used for *in vitro* and animal experiments. Zr-88 was produced by a (p,2n)reaction on Y-89 in a yield of 9 mCi after 1 hour of irradiation with 27 MeV protons (130 μA).

The capability of Zr in forming complexes with Df might be reduced by two factors. Firstly the presence of the competing chelating agent oxalic acid and secondly the formation of Zr-hydroxy products, which are easily formed in aqueous solutions.
However, the total process gives Zr, which is still capable of forming Df-complexes, even at very low Df-concentrations.

In conclusion, high amounts of Zr-89 were produced, with high radionuclidic purity. The Zr can be separated simply and efficiently from Y and Fe. After removing the oxalic acid the Zr is still able to form complexes with the chelating agent desferal.

TABLE 2 Chelation of Zr by desferal

Df conc.	% ^{88}Zr-Df mean ± sd (n=4)
10 mM	98.1 ± 0.7
1 mM	98.3 ± 1.0
100 μM	96.9 ± 1.9
10 μM	95.8 ± 3.7
1 μM	59.6 ± 30

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High Purity Production of the Positron Emitting Technetium Isotope ^{94m}Tc

RÖSCH, F.; NOVGORODOV*, A.F.; QAIM, S.M.; STÖCKLIN, G.

Institut für Nuklearchemie, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany
* Laboratory for Nuclear Problems, JINR Dubna, Russia

The positron emitting technetium isotope ^{94m}Tc ($T_{1/2}$ = 52 min, β^+ = 70 %, $E_{\beta^+,max}$ = 2.47 MeV) is a promising radionuclide for special PET-studies. As a substitute of the important SPECT isotope ^{99m}Tc it allows to obtain data on the quantitation of uptake kinetics and the mechanisms of [^{99m}Tc]radiopharmaceuticals and consequently, to better estimate their diagnostic potential. First experiments on the production of ^{94m}Tc were done by irradiating natural molybdenum with 13.5 MeV protons or 13 MeV deuterons [1] as well as with 11 MeV protons [2-4]. Later studies involved a radiochemical separation of ^{94m}Tc from molybdenum oxide followed by first nuclear medical investigations [2-4]. Recently, a comparison of [^{94m}Tc]MIBI with [^{99m}Tc]MIBI and $^{13}NH_4^+$ was published [5]. The ^{94m}Tc used in these studies was relatively impure. Furthermore, the basic nuclear data for the possible production routes are unknown. In this work we to determined the nuclear data relevant to the production of ^{94m}Tc via the $^{94}Mo(p,n)$-reaction on highly enriched target isotope and measured the experimental thick target yields as well as the level of isotopic impurities. Additionally, a radiochemical separation for a fast isolation of ^{94m}Tc in high yields from $^{94}MoO_3$ including recovery of the target material was developed.

Using a suspension of metallic ^{94}Mo powder (isotopic enrichment 93.9 %) in acetone and collodium, samples of 10 mm diameter and 3-6 mg/cm^2 thickness were prepared by a sedimentation method on 25 μm thick Cu backing foils of 13 mm diameter. The samples were homogeneous, chemically stable and could be handled well. To avoid contamination, the surface of each sample was covered with a 10 μm thick Al foil, having the same diameter as the Cu backing foil. The sandwiched samples were stacked together and irradiated with 19 MeV protons at beam currents of about 100 nA for 15 min at the Jülich compact cyclotron CV28. The activities of the radioactive products were determined via Ge-detector γ-ray spectroscopy. Excitation functions were obtained for the reactions $^{94}Mo(p,n)^{94m,94g}Tc$ and $^{94}Mo(p,2n)^{93m,93g}Tc$ from threshold to 19 MeV. They are shown in Fig. 1. The reaction of interest, i.e. $^{94}Mo(p,n)^{94m}Tc$, shows a maximum of 480 mb at about 12 MeV. Considering both nuclear reaction yield and radionuclidic purity of ^{94m}Tc, the optimum proton energy range for the production was found to be E_p = 13 → 7 MeV. The expected thick target yield is 54 mCi (2 GBq) / μAh. The isomeric cross section ratio $\sigma_{(^{94m}Tc)}$ / $\sigma_{(^{94m+g}Tc)}$ shows its highest value of 0.9 at low proton energies. It decreases with increasing proton energy and reaches a level of about 0.5 at 19 MeV. Over the optimum proton energy range the level of ^{94g}Tc at EOB after an one hour irradiation amounts to 5.5 %.

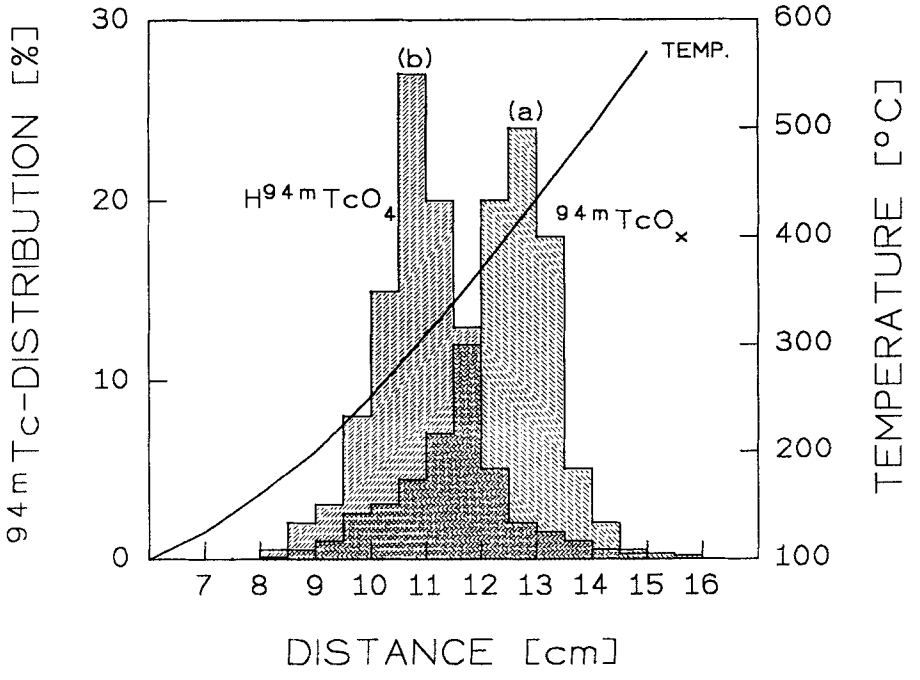

Fig. 2 Thermal adsorption of "carrier-free" technetium on quartz in dry (a) and moist air (b) vs. temperature distribution.

In summary, the separation is completed about 30 min after EOB, guaranteeing ≈ 90 % radiochemical yield (uncorrected ≈ 60 %) and > 99 % radiochemical purity of ^{94m}Tc-pertechnetate. This compound is the starting chemical form for labelling reactions. The enriched target material is well localized within the quartz tube and exists exclusively as $^{94}MoO_3$, thus offering the possibility of its cyclic use in the production of ^{94m}Tc. The batch yield of ^{94m}Tc utilizing one hour irradiations at 3 μA and 300 mg $^{94}MoO_3$ (226 mg/cm^2) is in the order of 70 mCi (2.6 GBq) at EOB.

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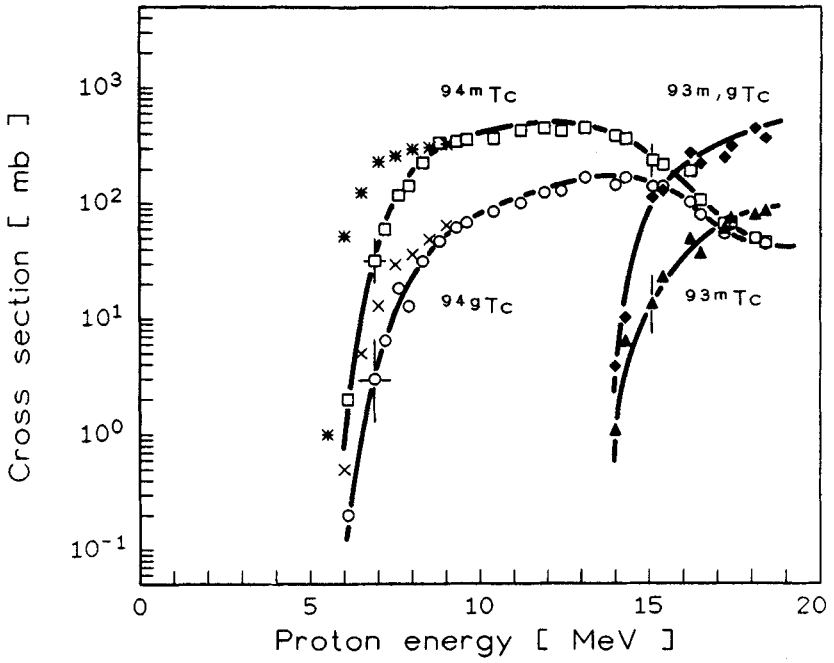

Fig. 1: Excitation functions of ^{94}Mo(p,xn)-processes leading to the formation of ^{94m}Tc, ^{94g}Tc, ^{93m}Tc, ^{93m,g}Tc. The values for ^{93m,g}Tc describe the cummulative formation cross section for the 2.75 h ^{93g}Tc. Typical error bars in different energy regions are given. Some literature data are also included [6](✳,✕).

As the production of ^{94m}Tc in high yields and with high radionuclidic purity demands the use of highly enriched ^{94}Mo as target material, an efficient radiochemical separation of the carrier-free ^{94m}Tc from macroamounts of $^{94}MoO_3$ (100-400 mg), including the recovery of $^{94}MoO_3$ is mandatory. Applying a thermochromatographic technique involving the high-temperature (800 °C) volatilization of ^{94m}Tc in a wet air stream (15 ml/min), > 99 % of the radiotechnetium is separated from the simultaneously sublimated $^{94}MoO_3$ on quartz surfaces according to a temperature gradient. The basic thermochromatographic properties of carrier-free technetium species, investigated in dry or wet air stream, are illustrated in Fig. 2.

The ^{94m}Tc fraction is desorbed almost completely using diluted alkaline solution. No traces of molybdenum could be detected in this solution. Quality control on the composition and purity of carrier-free ^{94m}Tc compounds was performed using thin layer chromatography on silica gel plates and acetone as the solvent. For aqueous solutions at pH 7-9, ^{94m}Tc-pertechnetate is analysed at r_f = 0.95; the other ^{94m}Tc species appears at r_f of 0-0.1. A final purification is recommended to obtain high radiochemical purity since the solution contains 5-10 % of a radiotechnetium species other than $^{94m}TcO_4^-$. This purification was done by column chromatography on alumina.

Development of Alternative Technologies for a Gel-Type Chromatographic ^{99m}Tc Generator.

LE VAN SO*., LAMBRECHT, R.M#.,Nuclear Research Institute, Dalat, Vietnam*, Biomedicine and Health Program, ANSTO, PMB 1, NSW 2233, AUSTRALIA#.

There is continuing concern worldwide about the need for multiple sources of the ^{99}Mo generator, and the large investment required to introduce the use of ^{235}U (n, f) ^{99}Mo derived production at a new facility. This is sufficient justification required for research on the development [2,3] of technologies for a gel-type chromatographic ^{99m}Tc generator based on the ^{98}Mo(n,γ)^{99}Mo nuclear reaction.

The synthesis of Titanium-Molybdate (abbrev. TiMo) generator packing gel pre-formed for ^{98}Mo(n,γ)^{99}Mo neutron activation of TiMo; and Zirconium-Molybdate (abbrev. ZrMo) gel columns containing (n,γ) produced ^{99}Mo for preparation of chromatographic ^{99m}Tc generators was studied. The chemistry of polymerisation, and amorphous or crystalline TiMo and ZrMo formation of the molybdate polyanion was investigated for the interpretation of the ^{99m}Tc-elution performance from the gel generators. Preparatory conditions of TiMo and ZrMo were found to be very critical. The complexity in the preparation of TiMo and ZrMo is attributed to the large capability of polymerisation of molybdate ions, their weakly acidic properties, and the formation of polymeric hydroxl complexes of Ti^{+4} and Zr^{+4} ions in the aqueous solution. The preparation of TiMo and ZrMo requires a process of high standardisation to give a product of consistent quality. Optimum gel composition characteristics were: Mo content 345.6 ± 1.3mg/g gel; Mo/Ti molar ratio of 1.29 ± 0.03; and crystal water content of 6.21 ± 0.2%. X-ray diffraction patterns of the MoTi gel were similar between batches.

Fig. 1. Depicts the potentiometric tritation curve of TiMo gel. Fig. 2. Illustrates the effect of drying temperature of the TiMo gel and the resultant $^{99m}TcO_4$ yield.

Behaviours, such as target temperature, irradiation stability, and neutron self-shielding, of the TiMo gel under reactor irradiation conditions that effect the performance of the generator and the quality of the ^{99m}Tc pertechnetate eluate were measured.

The irradiation stability of TiMo gel in HIFAR (Australia) and in Dalat reactor (Vietnam) at neutron flux lower than 5.10^{13} n/cm^2 .sec was certified. The specifications of TiMo and ZiMo gel columns of high ^{99m}Tc elution performance, and chromatographic ^{99m}Tc generator options yielding ^{99m}Tc fulfilling the requirements for nuclear medicine applications with several conventional kits will be illustratively described. The eluents used in the generator options are saline, redistilled water, or even organic solvent such as acetone, MEK. Typically a 250 mCi generator which was produced from a 100 hr irradiation (5 g of MoTi gel column weight) grade >80% radiochemical yield of $99mTcO_4$ with a 10 mL elution with saline. Table 1. Summarises representative performance tests. Fifty gel generators this type were tested in different hospitals in Vietnam, and gave satisfactory results. The gel generator concept is particularly relevant to hospitals in developing countries that do not have access to fission produced ^{99}Mo generators. Also a satisfactory gel-type generator based on (nγ) ^{99}Mo should be economical to produce.

Elution Performance of TiMo Gel Column Labelled with 200 mCi ^{99}Mo

Batch No.	Tested columns	Separation yield % (*)	Molybdenum content of Eluate µg/m (**)	Lowest Separation yield (%)	Radio-chemical purity (% TcO_4^-)
1	10	81 ± 5	< 5	78	> 99.8
3	10	80 ± 6	<5	75	> 99.8
5	10	82 ± 5	<5	79	> 99.8

* (10 radioactive equilibrium-elution cycles for each generator were investigated).
** (After passing the clean-up column of 1 g alumina or zirconia).

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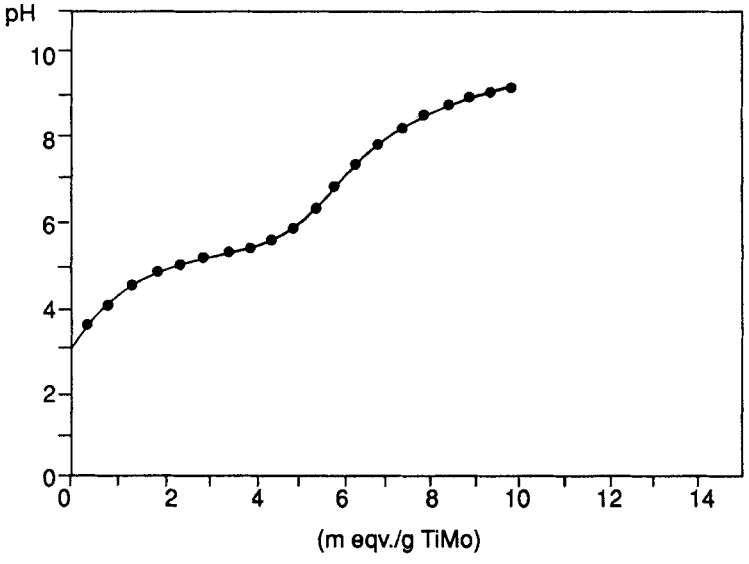

Fig 1: Potentiometric titration curve of TiMo gel.

(Batches No. :1—5)

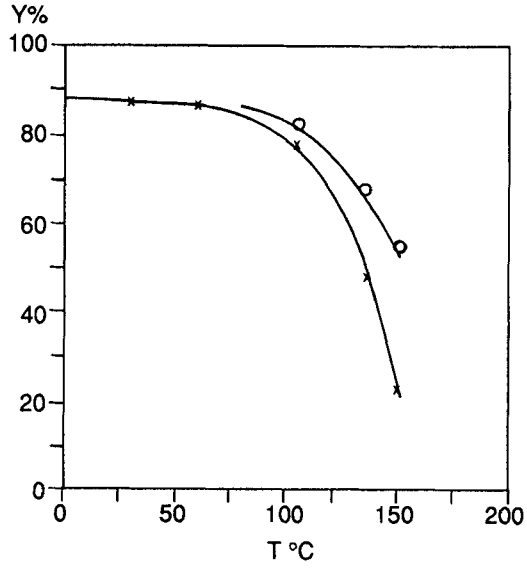

Fig. 2: Effect of the drying temperature on the Tc—99m elution yield of TiMo gel (Y%).

—o—o— Sample heated in tightly closed glass ampoule
—x—x— Sample heated in open air.

Carrier-free ^{166}Ho from ^{166}Dy/^{166}Ho Biomedical Generator System

MIRZADEH, S.[1,2]; HETHERINGTON, E.[2]; KNAPP, F.F. JR.[1]; LAMBRECHT, R.M.[2],
(1) Nuclear Medicine Program, Oak Ridge National Laboratory (ORNL), Oak Ridge, TN, 37831, USA and (2) Biomedicine and Health Program, Australian Nuclear Science and Technology Organisation (ANSTO), Lucas Heights, 2234 Australia.

Holmium-166 (^{166}Ho) is a potential candidate for various therapeutic applications (1-2) due to its attractive properties which include emission of high-energy β^- particles (E_{av}=666 MeV), an appropriate half-life ($t_{1/2}$=26.4 h) and decay to stable daughter. In addition, ^{166}Ho has chemical characteristics suitable for protein labelling through bifunctional chelates. Holmium-166 also emits a low intensity and low energy γ-ray (80 keV, 6%) suitable for imaging. Due to the absence of high energy γ-rays in its decay, ^{166}Ho may be used for outpatient therapy without significant external radiation to other individuals.

Although ^{166}Ho, with a moderate specific activity, can be produced by a simple neutron capture reaction, interestingly, its radionuclidic parent (81.5-h ^{166}Dy) can serve as a source of high specific activity ^{166}Ho. In certain applications, such as protein labelling, the use of a high specific activity radioisotope is essential. In addition, generator produced ^{166}Ho is free from 1200-y ^{166m}Ho. This isotope is unavoidably coproduced with ^{166}Ho by the ^{165}Ho[n,γ] reaction (3).

Dysprosium-166 is produced by double neutron capture on ^{164}Dy in a nuclear reactor as shown schematically in Figure 1. In this presentation we report the production yield of ^{166}Dy from two reactors and the preliminary data for the separation of carrier-free ^{166}Ho from μg quantities of Dy. As shown in Table 1, the experimental yields of ^{166}Dy were 2.2 and 3.5 Ci/mg of ^{164}Dy for 1 and 8 days of irradiation in the hydraulic tube of the ORNL HFIR. The relative reduction of the yield for the 8-day irradiation is due to the very large effective decay constant ($\lambda+\phi_n\sigma$) of the short-lived intermediate nuclei, ^{165}Dy, which results in substantial target depletion. The low yield from the ANSTO HIFAR simply reflects the 40 fold reduction in the neutron flux.

The preliminary results from the separation of carrier-free ^{166}Ho from neutron-irradiated Dy targets are shown in the Figure 2. This separation scheme is based on reverse phase ion exchange chromatography in which Ho and Dy are partitioned between the cation exchange resin and the mobile phase containing the weakly complexing ligand α-hydroxyisobutyric acid (α-HIBA) in pH 4.3-4.6. The applicability of this technique for large scale production is currently under investigation.

Acknowledgements: Research supported inpart by (1) OHER US-DOE, Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc., and (2) S. Mirzadeh visiting scientist at ANSTO supported by DITAC.

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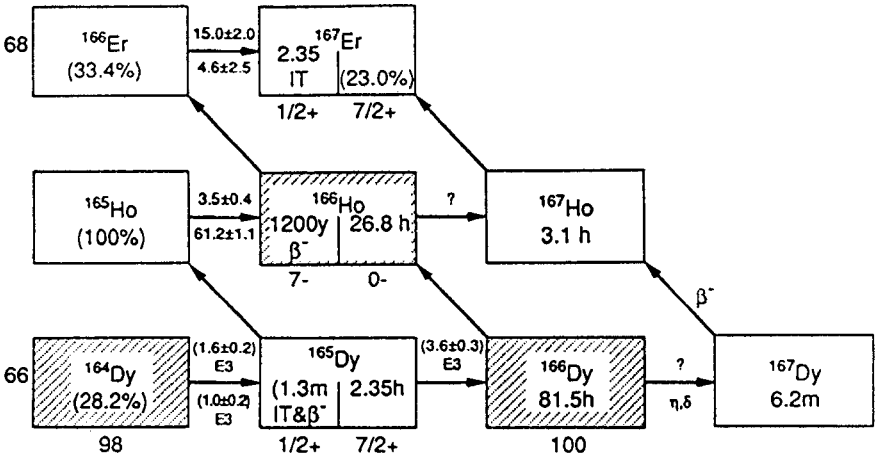

Figure 1. Production of ^{166}Dy in a Nuclear Reactor

Table 1. Reactor Production of ^{166}Dy[a]

Reactor/ Irradiation Position	Neutron Flux ($n.s^{-1}.cm^{-2}$)	T_{irr} (d)	Yield (mCi/mg of ^{164}Dy)
HFIR-HT[b]	2.0×10^{15}	8	3500
		1	2200
HIFAR-NT[c]	5×10^{13}	0.5	2

[a]Targets were highly enriched $^{164}Dy_2O_3$
[b]HFIR-HT: ORNL High Flux Isotope Reactor, Hydraulic Tube
[c]HIFAR-PT: ANSTO High Flux Australian Reactor, Pneumatic Tube

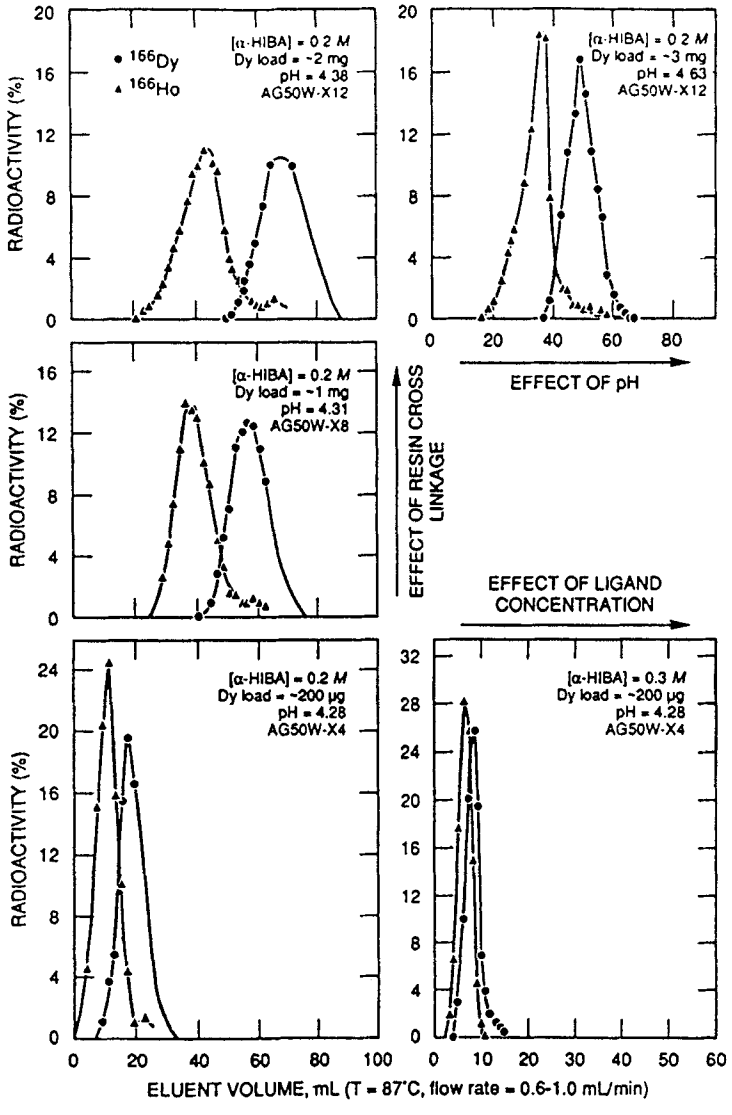

Figure 2. Separation of Carrier-free ^{166}Ho from Neutron Irradiated Dy Target by Reverse Phase Ion Exchange Chromatography

^{166}Dysprosium-^{166}Holmium *In Vivo* Generator.

MIRZADEH, S.[1,2]; DI BARTOLO, N[1]; SMITH, S. V.[1]; LAMBRECHT, R.M.[1] (1) Biomedicine and Health Program, Australian Nuclear Science and Technology Organisation, Lucas Heights, 2234 Australia and (2) Nuclear Medicine Program, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, USA.

Recently, there has been an increasing interest in ^{166}Ho ($t_{1/2}$=26.8 h, 100% ß$^-$, $E_ß^{av}$=666 KeV) for various therapeutic applications (1-2). A novel approach to deliver this isotope to tissue is via the *in-vivo* decay of its 81.5-h parent, ^{166}Dy (100% ß$^-$, $E_ß^{av}$=130 MeV) - **an *in-vivo* generator system** (3). It is hoped the flexibility afforded by using this parent-daughter system may result in the reduction of radiation dose to sensitive non-target tissues which until now has limited the efficacy of the radiotherapy.

In this scenario, the ^{166}Dy-radiopharmaceutical prepared from pure ^{166}Dy is attached to a tumour specific antibody. During the *in vivo* localisation of the radiolabelled antibody, the resultant dose to non-targeted tissues is reduced because of sub-equilibrium amounts of ^{166}Ho. Once the ^{166}Dy labelled radiopharmaceutical has localised in the target tissue the therapeutic dose can be generated by the decay of its ^{166}Ho daughter.

A critical question for the *in vivo* ^{166}Dy/^{166}Ho generator system is whether translocation of the daughter nucleus occurs following the uptake of the parent at the target site. In an effort to address this question the biodistribution of ^{166}Dy-DTPA was performed, and the bone uptake was carefully analysed for both ^{166}Dy and ^{166}Ho in a gamma-spectrometer employing a Ge(Li) detector. The choice of Dy-DTPA was based on a previous report (4) that the integrity of the ^{166}Ho-DTPA complex is preserved following its formation via ^{166}Dy-DTPA ß$^-$ decay.

The Dy-166 was produced in the ANSTO HIFAR nuclear reactor by double neutron capture of highly enriched ^{164}Dy. After irradiation, the Dy_2O_3 target was dissolved in a minimum amount of 9M HCl, evaporated to dryness and the residue dissolved in 0.01M HNO_3 (1ml). The resultant solution contained 0.05M of dysprosium and ~5 mCi/ml of ^{166}Dy.

The Dy complex of DTPA was prepared *in situ* by mixing DTPA (16 µmol) with a slight molar excess of the ^{166}Dy solution, described above. The pH of the solution was adjusted to pH ~8 using sodium hydroxide and then heated at 50^{0}C for 5 min. The reaction mixture was made slightly acidic (pH 5-6) by the addition of HNO_3 (0.01M) and the free metal salts and/or metal hydroxide species were removed by passing the mixture through a Chelex column (5x10 mm), using 0.9% NaCl (~2 ml) as eluent. The purified sample was diluted with 0.9% NaCl, filtered through 0.22 µm filter and 100 µl aliquot of the resultant solution was injected intravenously into balb/c mice. The animals were sacrificed at varying time intervals, dissected, and the organs counted in a Packard gamma counter. The results of the biodistribution data show that the ^{166}Dy DTPA complex, as expected, has high renal clearance (see Fig. 1).

The uptake of both parent and daughter isotopes in bone was determined using a Ge(Li) detector and found to be negligible. The clearance of the radioisotopes from the bone appeared to be related to blood clearance as illustrated in Fig. 2. The ratio of ^{166}Dy and ^{166}Ho is plotted in Fig. 3 for 3 and 30 minute time points. The results indicate that ^{166}Ho appeared to be in equilibrium with its parent isotope indicating that there was no increase in ^{166}Ho uptake in bone relative to that of ^{166}Dy (Fig. 3).

Acknowledgements: Research supported inpart by (1) S. Mirzadeh visiting scientist at Ansto supported by DITAC and (2) OHER US-DOE, Contract DE-AC05-84OR21400 with Martin Marrieta Energy Systems, Inc.

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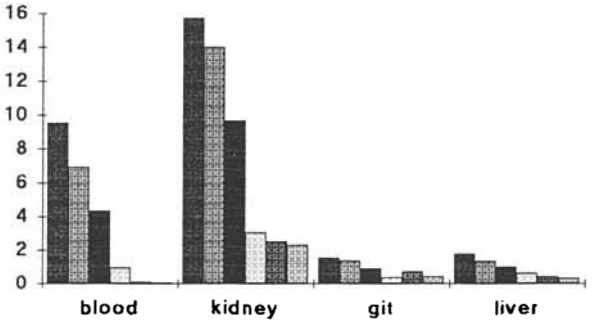

Figure 1. Biodistribution of ^{166}Dy-DTPA.

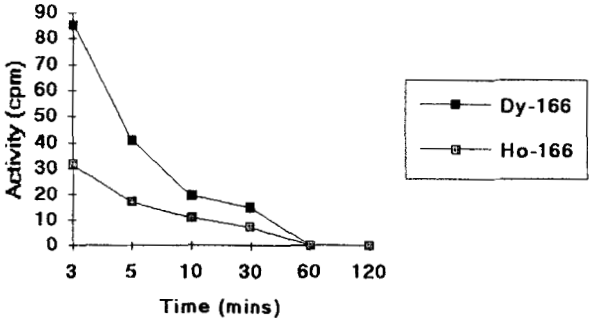

Figure 2. Activity in Bone as a Function of Time

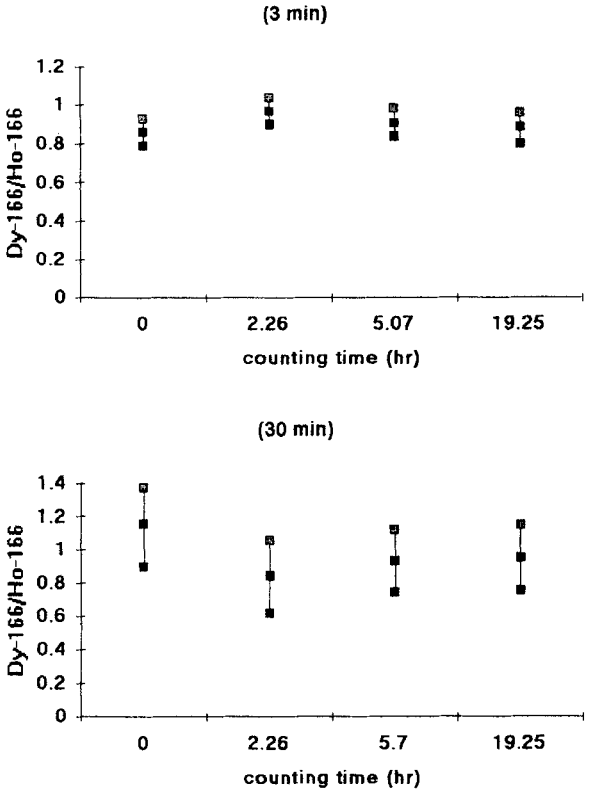

Figure 3. Ratio of ^{166}Dy to ^{166}Ho in Bone as a Function of Counting Time.

Pre-Clinical Evaluation of $^{188}W/^{188}Re$ Biomedical Generator. KAMIOKI, H.[1*]; MIRZADEH, S.[1#]; LAMBRECHT, R.M. Biomedicine and Health Program, Australian Nuclear Science and Technology Organisation, Lucas Heights, 2234 Australia.

In order to assess the performance of clinical scale generators, a 13.5 mCi generator has been prepared and evaluated for ^{188}Re yield and elution profile, and ^{188}W breakthrough using various reagents. The generator is alumina-based and eluted on a bi-weekly schedule. The results for a five-week evaluation period are presented. To address the problem of low specific volume (1), the normally used saline solution (as eluent) was replaced by reagents which could be easily destroyed by gentle evaporation from acidic solution (e.g. NH_4Cl and NH_4NO_3).

Tungsten-188 with a specific activity of ~5 mCi/mg and activity concentration of ~13.5 mCi/ml in 0.1 M NaOH was purchased from ORNL. The generator column (13x21 mm) was fabricated with a coarse glass frit support at the bottom and held 750 mg of alumina (80-120 mesh, pre-equilibrated with 0.01 M HNO_3). One ml of the ^{188}W stock solution (~3 mg) was titrated with 1 M HNO_3 until pH 2-3 was obtained. The mixture was loaded on the column and ^{188}Re was typically eluted with 4x10 ml portions of various eluents. At the end of each elution, the generator was equilibrated with 10 ml of the next eluent and then was purged dry. This was to reduce the effects of radiolysis which has been shown to result in erratic yield. The 10-ml eluents were collected directly in 10-ml injection vials and the activity of ^{188}Re was measured without further dilution with a calibrated ionisation chamber. After about 2 weeks of decay to insure the equilibrium, the samples were recounted at the surface of a Ge(Li) detector and the breakthrough of ^{188}W was evaluated from the intensity of the 155 keV γ-ray of ^{188}Re.

The yield and elution profile of ^{188}Re for various reagents are shown in Fig. 1 and 2, respectively. Within 9 elutions, the yield of ^{188}Re decreased from 80% to 70%. As shown in Figure 2, replacement of Na^+ ion with H^+ or NH_4^+ ions in the eluent had no observable effect on the ^{188}Re elution behaviour. Consistent with previously reported results obtained from a 1 mCi generator (2), the optimum concentration of cations for sharp elution of Re (in the 1st 10 ml of eluent) is about 0.05 M. Concentrations below this optimal value resulted in an increase in retention time as well as an increase in the FWHM of elution curve (2,3). No effect was observed due to replacement of the Cl^- counter ions with NO_3^- ions (Re in nitrate solutions could be further purified and concentrated in an anion exchange column) (2).

The breakthrough of ^{188}W is shown in Fig. 3. As seen, the breakthrough per elution (40 ml of eluent) had actually decreased, from $1.2x10^{-5}$ to $1.1x10^{-6}$ %, in the first 5 elutions.

Efforts remain underway to examine the proposed "alumina/anion-exchange" tandem generator system (2) for the preparation of highly purified ^{188}Re.

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1. Visiting Scientist, permanent address: *Japan Atomic Energy Research Institute, Ibaraki-Ken, 311-13 JAPAN. #Oak Ridge National Laboratory, Oak Ridge, TN 37831-6229, USA

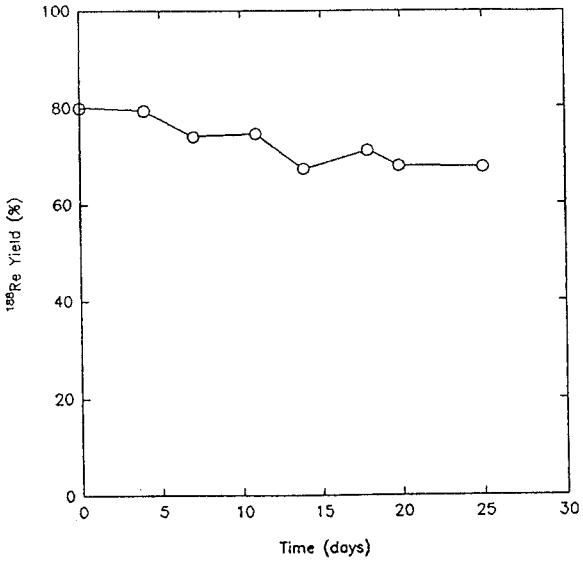

Figure 1. Elution Yield of Carrier-free ^{188}Re as a Function of Time (see Fig 3 for the corresponding elution profiles and types of reagents).

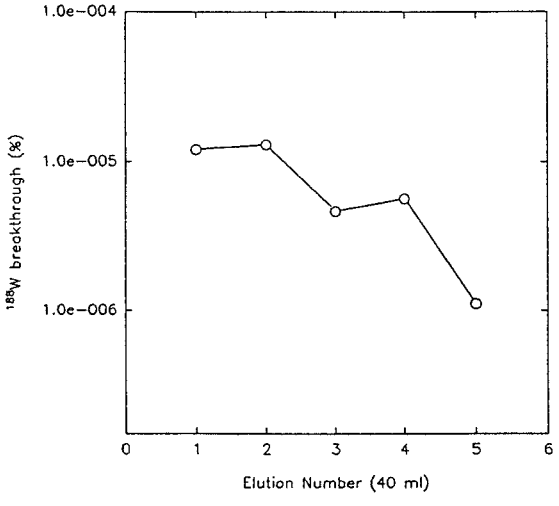

Figure 3. Breakthrough of ^{188}W

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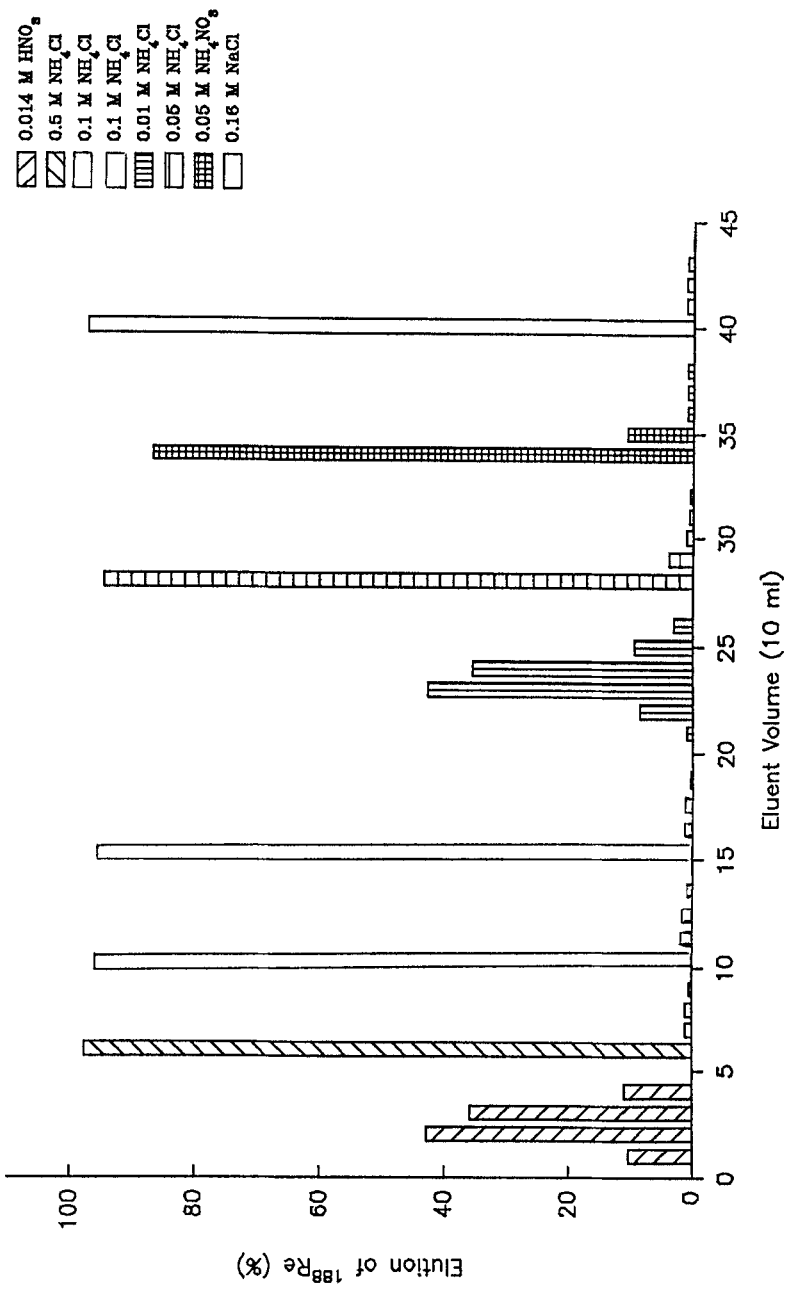

Figure 2. The Elution Profiles of ^{188}Re for Various Reagents

DEVELOPMENT OF ^{68}Ge/^{68}Ga GENERATOR IN INSTITUTE OF BIOPHYSICS.

M.D.Kozlova, A.B.Malinin. G.E.Kodina and A.S.Sevastyanova.

Radiopharmaceutical Dept., Institute of Biophysics, Ministry of Public Health, Moscow 123182, Russia.

Levin and Sokolov (1) developed ^{68}Ge/^{68}Ga generator on the base of chromatographic alumina in our laboratory in the end of the 60-s. ^{68}Ge sorbtion was produced from 0.005 M EDTA solution having pH 8 on 0.6 g of alumina at the static conditions, which then was transferred into a glass column having the diameter of 7 mm (with lower protective layer of alumina). The yield changed in the range of 47 % (in two days after the generator preparing) to 24 % (in 180 days), when washing out ^{68}Ga with 2 ml of 0.005 M EDTA solution having pH 8. In this case ^{68}Ge breakthrough in eluate increased for the same period in the range of 0.02 to 0.35 % respectively.

Later (in the beginning of the 80-s) we have developed another type of the generator, providing the ^{68}Ga washing out in ionic state with 0.1 M solution of hydrochloric acid. As a sorbent it was used the modified titanium oxide on which ^{68}Ge sorbtion is produced from 0.001-0.05 M solutions of hydrochloric acid, having yield of more than 95 % (^{68}Ge sorbtion is $\geq$ 98 % in the case if the acid concentration is in the range of 0.01-0.03 M).

The generators, based on this sorbent, have high stability and reproducibility of the main parameters during a very long period of time (fig.1 and fig.2): when let passing 3-4 ml of eluent ^{68}Ga yield changes usually in the range of 60 to 35 % for 2 years of work (the total volume of eluent is more than 1 litre). ^{68}Ge breakthrough is not more than $5 \cdot 10^{-3}$ % for the period pointed above,and the impurities of chemical elements in eluate (Ti, Fe, Si and B) are not more than 2 μg/ml, but it is necessary to say, that Ti impurity is not more than 0.5 μg/ml.

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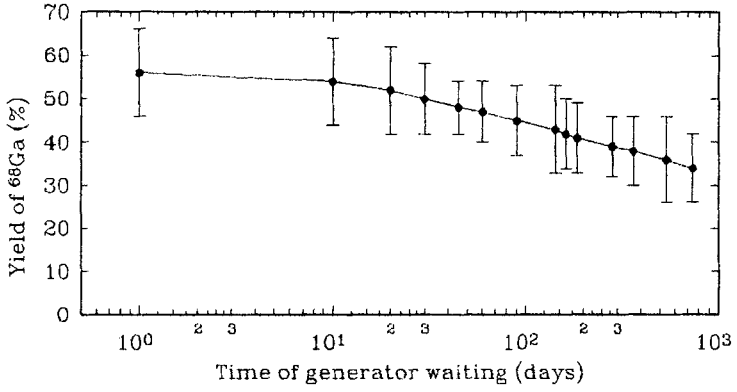

Fig.1 Influence of duration of generator exploration on ^{68}Ga yield. Weighted mean for 5 generators.

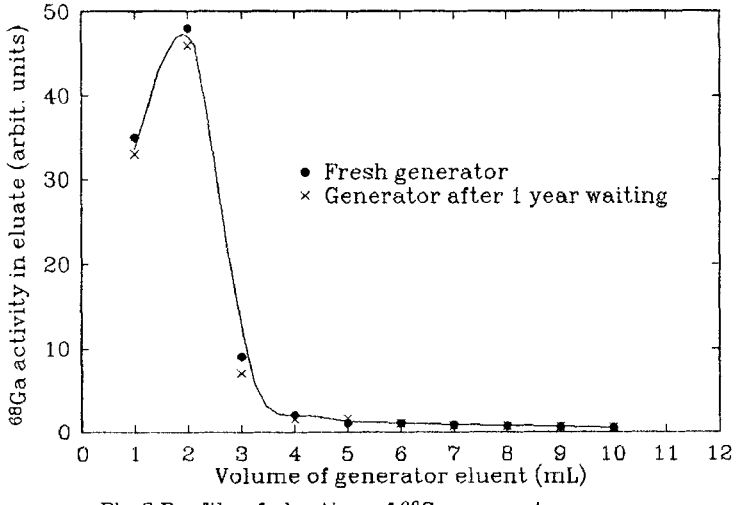

Fig.2 Profile of eluation of ^{68}Ga generators

Synthesis of C-11 Labeled Ceramide by Ketene Method

Ryou Fujii, Yoshio Imahori*, Satoshi Ueda*, Kazuo Wakita, Yoshio Ohmori*, Noburo Higashi, Takahiro Kanatsuna

*Nishijin Hospital, Kyoto, Japan; Department of Neurosurgery, Kyoto Prefectural University of Medicine, Kyoto, Japan**.

It is reported that the sphingolipids has various biological functions in vivo. The expression of various gangliosides on the cell surface has been correlated with cell transformation and tumor progression, and gangliosides are thought to participate in various cell regulatory functions(1,2,3). Moreover, the recent studies demonstrated that the sphingolipid turnover affects cellular responses. From those above, we tried to visualize the sphingolipid metabolism in vivo. Ceramide is an essential form of sphingolipid, so we synthesized C-11 labeled ceramide by *KETENE METHOD* (4-9). We performed preliminary animal experiment for the tracer distribution in the rat and obtained the brain autoradiograms.

Synthesis procedure of C-11 labeled ceramide is shown in Figure 1. The C-11 labeled ceramide was synthesized by *KETENE METHOD* (4-9). The C-11 dioxide was produced via $^{13}N(p,\alpha)^{11}C$ reaction using a compact cyclotron (BC1710, The Japan Steel Works LTD., Japan). The C-11 ethylketene was produced using Ketene Automated Synthesis System (ARIS-C8, The Japan Steel Works LTD., Japan). The sphingosine, as a precursor for synthesis of C-11 labeled ceramide, was dissolved in CH_2Cl_2 (1ml) containing with DMAP (0.5 μmol), and C-11 ethylketene was bubbled into a reaction vessel. After the reaction CH_2Cl_2 was evaporated and 0.5ml of solvent (hexane/isopropyl alcohol; 8/2) was added into the reaction vessel, mixed using Voltex Mixer. The products in the solvent were passed through the SEPPAK silica, and sphingosine and C-11 labeled impurity were removed with 5ml of same solvent. The solvent was evaporated and dissolved in 0.5ml of CH_2Cl_2. C-11 labeled ceramide was obtained by HPLC separation.

The biodistribution of C-11 labeled ceramide was studied by the following procedure. Four male Wistar rats (each weighing 160g) were used. C-11 labeled ceramide was dissolved in 0.5ml of saline containing 0.1% bovine serum albumin and 0.5% DMSO, and was injected into the tail vain. Thirty minutes after injection, the rats were decapitated. The cerebral cortex, cerebellum, pancreas, spleen, heart, lung, kidney, liver, testes, blood were rapidly removed and those tissue weight and radioactivity were measured. Each uptake value was expressed as %dose/g. The data obtained from quadruplicate experiments were presented as average ± s.d.

The in vivo autoradigraphy was performed by the following procedure. C-11 labeled ceramide dissolved in saline, as described above, was injected into the tail vein. After 30 min, the rat was decapitated and the brain was rapidly removed. The frozen brain was fixed on a bed and was sliced at 40μm thickness by cryotome. The sample was contacted to imaging plate and autoradiography was reconstructed with a BASS 2000 system.

The HPLC profiles of unlabeled ceramide as an authentic standard and C-11 labeled ceramide are shown in Figure 2. The synthesis was finished within 15 minutes from starting of ketene reaction. The yield of C-11 labeled ceramide was 41.7% at the end of C-11 ketene reaction, and the purity was more than 97%. The biodistribution of C-11 labeled ceramide in rats is shown in Table 1. The liver and kidney demonstrated high uptake. The typical autoradiography of rat brain is shown in Figure 3. In the rat brain, these autoradiograms indicated high accumulation in the cortex, thalamus and caudato-putamen, in contrast to the hippocampus and amygdala.

In this article,we synthesized C-11 labeled ceramide for the first time and investigated the sphingolipids metabolism. Sphingolipids have various biological functions. Especially we gave attention to the sphingolipid turnover because of the correlation to the cellular response. Sphingosine has a primary alcohol and amino-residue. The characteristic of ketene reaction is higher reactivity to the amino-residue than the hydroxyl group, so C-11 labeled ethylketene attacked to the amino-residue of the precursor in this reaction. Almost of the reaction was occurred to the amino-residue and 5~8% was reacted to the hydroxyl-residue in the total radioactivities of the ketene adducts. C-11 labeled ethylketene was transferred with diluted HCl gas, so we examined whether the acylation efficiency to the amino-residue may be predominant to the hydroxyl-residue under the basic condition. We employed pyridine as the basic solvent. However, no effect of the reactivity to the amino-residue was demonstrated between pyridine and dichloromethane.

In actually, sphingosine is a little contaminated with ceramide. The elution time of unlabeled ceramide and C-11 labeled ceramide were different on HPLC because of the difference of each acyl carbon chain. Then C-11 labeled ceramide was obtained by separative HPLC with high specific activity (≥5Ci/μmol).

The sphingolipids image was obtained in the rat brain in vivo. The C-11 labeled ceramide was accumlated in the cortex, caudato-putamen and thalamus. Furthermore we examined the metabolism of C-11 labeled ceramide in rat brain by the method of modified Folch's method (TLC data is not shown). As a result of TLC analysis, some spots were appeared on the TLC plate (not confirmed). C-11 labeled ceramide, intravenously injected, may be metabolized into various intermediates via the catabolic pathway and anabolic pathway. It suggests that C-11 labeled ceramide is a probe of sphingolipid metabolism.

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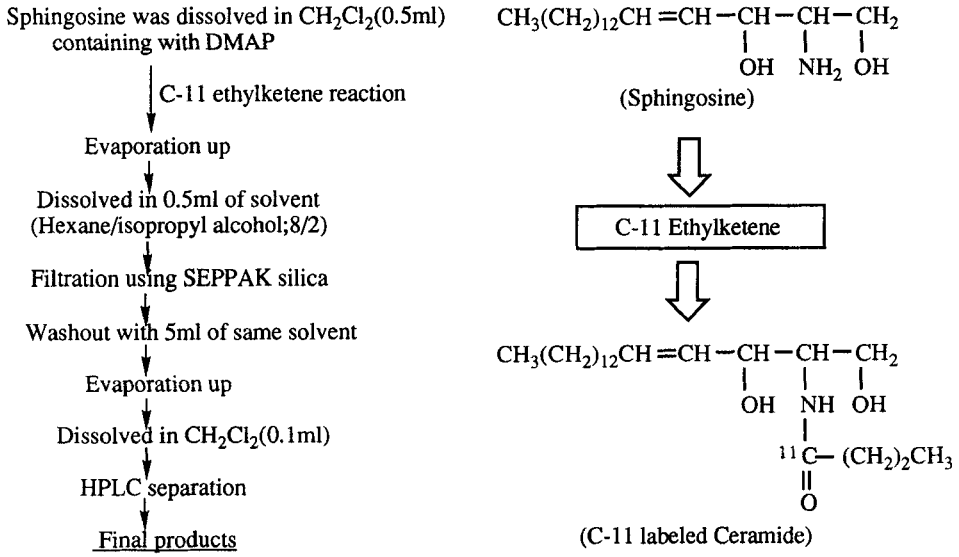

Figure 1 Synthesis procedure of C-11 labeled Ceramide and structures

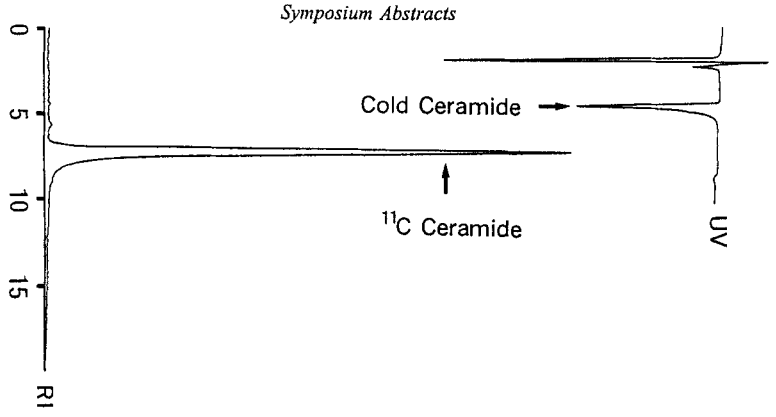

Figure 2 HPLC analytical data of C-11 Ceramide and cold Ceramide

HPLC Conditions Column : Zorbax SIL(Ø4.5mm X 250mm)
Eluent : Hexane/Ether/isopropyl alcohol(10/2/1)
Flow rate : 1.8ml/minute

Table 1 Biodistribution of C-11 Ceramide
(30minutes after IV injection)

	Optimal distribution means ± s.d.	Blood ratio
Cortex	0.2021 ± 0.0049	0.6930
Cerebellum	0.2089 ± 0.0175	0.7165
Lung	0.8181 ± 0.0906	2.8054
Heart	0.6116 ± 0.0407	2.0975
Pancreas	0.6831 ± 0.0688	2.3426
Spleen	0.6254 ± 0.0155	2.1447
Kidney	2.1746 ± 0.6013	7.4576
Liver	2.8036 ± 0.1182	9.6144
Testes	0.1396 ± 0.0096	0.4787
Blood	0.2916 ± 0.0230	1.0000

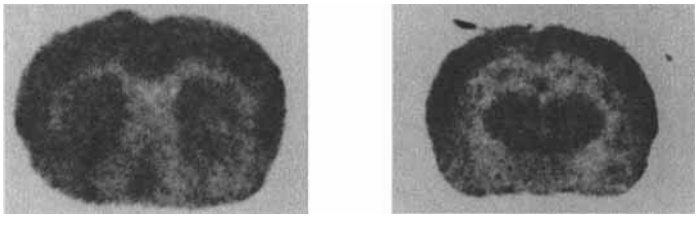

Figure 3 The rat brain autoradiograms of C-11 labeled Ceramide

Synthesis of [^{11}C]Rotenone and Preliminary Biodistribution Studies in Mice and Monkey. CHARALAMBOUS, A., MANGNER, T.J., FREY, K.A., GREENAMYRE, T.J.*; and KILBOURN, M.R. Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI 48109, and *Departments of Neurology, Neurobiology and Anatomy, and Pharmacology, University of Rochester Medical Center, Rochester, New York 14642.

In normal physiological state the mitochondria electron transport chain (ETC) promotes, through electron movement, the synthesis of ATP which then fuels the ion pumps that restore the ionic cellular gradient after cell depolarization. The ETC consists of five complexes (I-V). Recent studies suggest that the pathogenesis of several neurodegenerative diseases is related to defects in the functions of the ETC complexes[1,2]. For example, in Parkinson's and Huntington's diseases the activity of complex I is reduced in the substantia nigra and striatum, respectively[1,2], and in Alzheimer's disease decreased activity of complex V has been reported[1]. Most recently, neuroleptic-induced toxicities have been related to interactions of such drugs with complex I of the ETC[3]. As *in vivo* imaging of complex I would provide a new approach to the study of neurodegenerative diseases, we have undertaken the synthesis of radioligands for specific binding sites on complex I.

Rotenone (**1**) has long been established as a potent inhibitor of complex I activity[4,5]. Recently, a tritiated rotenone derivative has been used autoradiographically to localize rotenone binding sites in rat brain. These experiments revealed relatively uniform brain distribution with slightly increased binding in the cerebellum molecular layer and in the hippocampal dentate gyrus[6]. Thus, appropriately labeled rotenone could be a potential PET radioligand tracer for studying losses of complex I activity in neurodegenerative diseases. Here, we report the synthesis of [^{11}C]rotenone (Scheme 1) and the preliminary results obtained from biodistribution studies in mice and monkey.

Commercially available rotenone (**1**) was demethylated selectively at position 2 using boron tribromide[7]. This reaction also involves a concommitant ring opening and re-closure of the E-ring, producing loss of stereochemistry at the 5'-position. Since desmethylrotenone (**2**) decomposed when using traditional [^{11}C]methylating conditions (base, [^{11}C]methyl iodide or [^{11}C]methyltriflate), the ketone function was protected as the methyl enol ether by treatment of **2** with trimethyl orthoformate. The desmethylrotenone methyl enol ether (**3**) (1 mg) was then methylated in dry dimethylformamide (200 µL) using *n*-tetrabutylammonium hydroxide (methanol solution, 1 equiv.) and [^{11}C]methyl iodide for 5 min at room temperature. Hydrolysis of the enol ether to the ketone was accomplished using a trifluoroacetic acid/water solution (500:300 µL) for 2 min at 60° C. Injectable [^{11}C]rotenone **4** was obtained after normal phase HPLC purification, evaporation of the organic solvent and formulation (10% ethanol in saline). The yields of [^{11}C]rotenone were 7-10% (corrected for decay), in an overall synthesis time of <50 min, and with radiochemical purities >95% and specific activities >3500 Ci/mmol (EOB).

Biodistribution studies in female CD-1 mice showed that [^{11}C]rotenone has good blood brain barrier permeability (>6.2% of the injected dose in brain at two minutes post-injection) and little or no regional binding selectivity in the brain structures examined (consistent with the results obtained autoradiographically). Additionally, monkey PET imaging and mouse studies demonstrated clearance of the radiotracer with a $T_{1/2}$ of

approximately 26 and 16 min, respectively. Further studies in mice and primates are in progress to evaluate the specificity of [^{11}C]rotenone binding and its potential as an imaging agent for PET.

Aknowledgements. This work was supported by grants from the National Institutes of Health (MH47611, NS 15655) and Department of Energy (DE-FG02-88ER60639).

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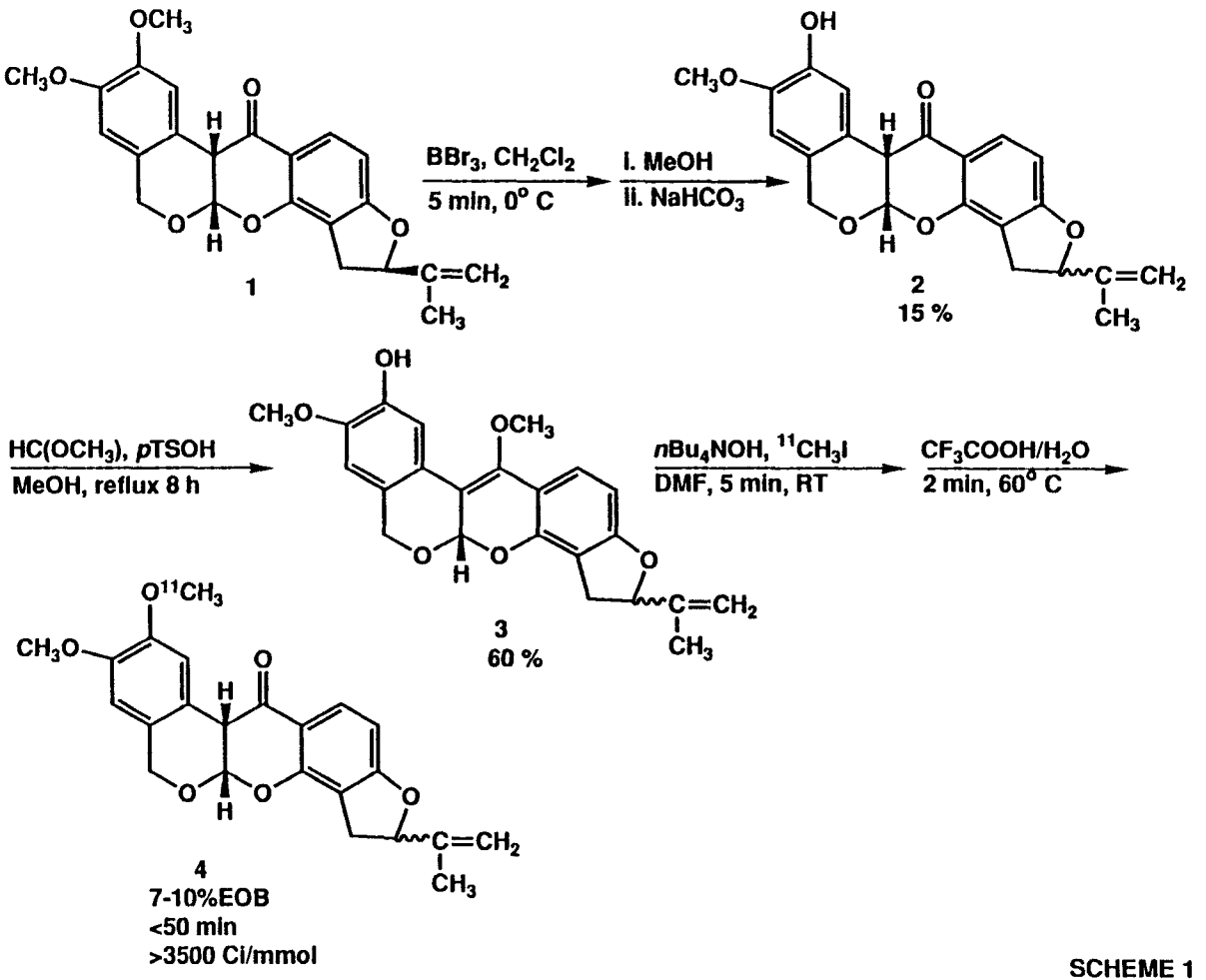
SYNTHESIS OF [^{11}C]ROTENONE



SCHEME 1

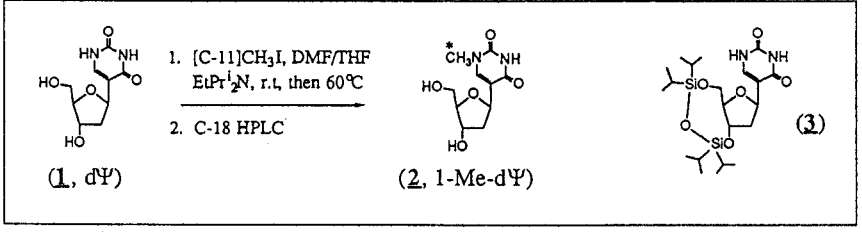
Radiosynthesis of 2'-deoxy-1-[C-11]methyl-pseudouridine, an isostere of labeled thymidine, as an agent for the measurement of DNA synthesis *in vivo*.

GRIERSON* JR, SHIELDS AF, and COURTER JH. Department of Radiology, School of Medicine, University of Washington, Seattle, WA 19195.

Pseudonucleosides are C-linked rather than N-linked nucleosides and isosteres of their natural counterparts. Because of this they are expected to be readily accepted as substitutes in the processes of nucleoside incorporation into DNA. However, by virtue of their C-linked base and sugar moiety, they are not readily de-glycosylated *in vivo*. If labeled with [C-11] for PET imaging, these pseudonucleosides could effectively act as probes for the measurement of DNA synthesis and yet be devoid of the problems associated with the rapid degradation of natural nucleosides in the blood and liver. For example, the major problem with using 2-[C-11]thymidine is that 60-75% of labeled carbon is found as labeled bicarbonate in plasma within 10 minutes of injection (Shields et al., 1990).

Labeling 2'-deoxy-1-methyl-pseudouridine (Figure 1, 1-[C-11]methyl-dΨ, 2) has several advantages over labeling thymidine. While thymidine may be labeled using [C-11]cyanide by a labor intensive multi-step process, 1-[C-11]methyl-dΨ can be labeled directly from unprotected dΨ (2'-deoxy-pseudouridine, 1) in one-pot with n.c.a. [C-11]methyl iodide, followed by C-18 HPLC purification. Our labeling experiments show significant yields (5-8% decay corrected yield from [C-11]MeI) of purified 1-[C-11]methyl-dΨ can be obtained from a non-position specific labeling reaction at 60°C in dry DMF (150 μL) containing N,N-diisopropylethylamine (2.5 equiv.). A radio-chromatogram for this reaction is shown in Figure 2. The reaction is complete within 10 min after the tertiary amine addition. We find that adapting the literature method of methylating per-silylated dΨ (Chu et al., 1977) with methyl iodide in THF to afford the desired 1-N-methylated product is unproductive with n.c.a. [C-11]CH_3I at room temperature or at reflux.

Figure 1: Labeling chemistry for 1-[C-11]methyl-dΨ



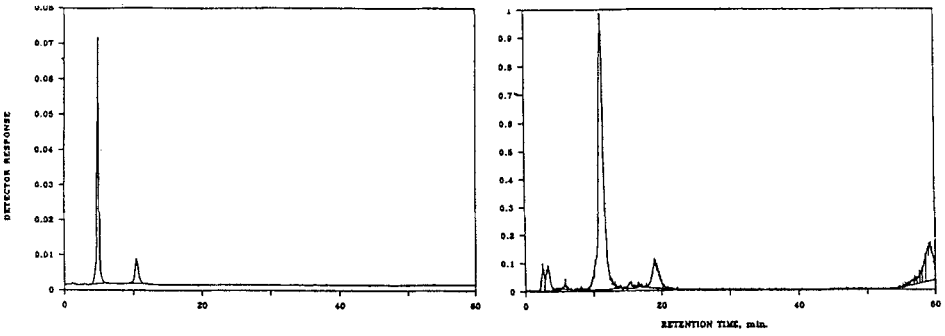
It is important to avoid using bases dissolved in protic media in these reactions. For example, the use of either n-Bu4NOH (50 μL, 1M in MeOH) or NH3/MeOH(1 mL, 2M) leads to rapid production of 1-[C-11]methyl-dΨ and a closely related substance (1:1 by HPLC). Presumably, this competitive undesired product formation may result from a well known facile β– to α–nucleoside isomerization of the labeling substrate prior to its reaction with labeled methyl iodide.

The presence of DMF is a complicating factor for large scale production of 1-[C-11]methyl-dΨ. While it is useful for solubilizing the labeling substrate for reaction, even small volumes (>100 μL) of DMF are incompatible with the semi-prep HPLC purification process. This fact limits the volume of the reaction mixture that

can be injected onto the HPLC column. (The chromatographic separation shown in Fig. 2 was achieved with a 3000:1 dilution of a reaction aliquot prior to HPLC analysis.) To overcome the difficulty of using DMF we have also considered the reaction of 3', 5'-silyl-protected-dΨ (3) which allows solubilization of the labeling substrate in THF /CH_3CN that can be easily evaporated after reaction. The labeled product would then be rapidly (< 5 min) deprotected at r.t. with soluble fluoride ion. Unfortunately, the labeling efficiency with this alternative substrate was poor.

We are encouraged by these results and are continuing to pursue this methyl labeling reaction of 1-methyl-dΨ using [C-11]methyl iodide.

Figure 2. HPLC purification of 1-[C-11]methyl-dΨ



Left: UV detection (254 nm); first peak= dΨ, second peak= co-injected authentic 1-methyl-dΨ. **Right**: Gamma detection (NaI/Tl); 1-[C-11]methyl-dΨ at ~ 10 min, longer retained material not shown. **HPLC conditions**: Phenomenex Ultracarb 5 ODS-30, 25 cm x 4.6 mm (id) column, 2.5% CH_3CN-water, 1.5 ml/min.

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[18F]-6-Fluoro-9-alkylpurines: A Potential Radiotracer for Measuring Brain Glutathione-S-Transferase by PET.

FUKUSHI,K. and IRIE, T. Division of Clinical Research, National Institute of Radiological Sciences, Chiba, Japan

Foreign, lipophilic compounds with an electrophilic center can conjugate with reduced glutathione (GSH) either spontaneously or by catalysis of glutathione-S-transferase (GST). We have previously observed (ref. 1) that [18F]-6-fluoro-9-benzylpurine ([18F]-FBP) was readily taken up by the brain, converted into [18F]-fluoride and thus the radioactivity was retained in the brain. However, the precise mechanisms of defluorination remained unknown. In this study, we have synthesized 6-fluoro-9-methylpurine (FMP) with a double label, [18F]-6-fluoro-9-methylpurine ([18F]-FMP) and 6-fluoro-9-[14C]-methylpurine ([14C]-FMP), to investigate the relevance of GSH/GST systems to defluorination of [18F]-6-fluoropurine derivatives in the brain.

[18F]-FMP was prepared by the reaction of trimethyl-9-methylpurin-6-ylammonium chloride (5 mg) and NCA [18F]-fluoride/K2CO3 in DMF (1 mL) containing 18-crown-6 (100 μmol) for 10 min at 65°C and purified by column chromatography (silica gel, elution with a mixture of ethylacetate:ethanol=9:1). Radiochemical yield was 87% and purity was >99%. [14C]-FMP was obtained by the reaction of 6-fluoropurine and [14C]-iodomethane in acetone/K2CO3 with stirring at room temperature for 1 h, followed by purification by preparative TLC (silica gel, ethylacetate:ethanol:ammonia=90:10:0.5). Radiochemical yield was 44% and purity was 92.5%.

Mice (C3H strain, male) were used for biodistribution study. Time-activity curves of [18F] radioactivity from [18F]-FBP and [18F]-FMP in brain and blood are shown in Fig.1, indicating that elimination of [18F] radioactivity from the brain is slow. Similarity in T1/2 (ca. 1h) of [18F]-clearance from the brain between [18F]-FBP and [18F]-FMP suggests that [18F]-FMP is also converted into [18F]-fluoride in the brain. Higher uptake of [18F]-FBP compared to [18F]-FMP may be due to higher lipophilicity and/or defluorination rate of the former.

To examine whether defluorination of [18F]-6-fluoropurines in the brain is due to GSH/GST, the effects of 2mM GSH (physiologic concentration in brain) and brain tissue homogenates on the reaction rates, [18F]-fluoride formation rate for [18F]-FBP and GSH-conjugate formation rate for [14C]-FMP, were measured (Table). In brain homogenates supplemented with 2mM GSH, rapid conversion of fluoropurines into metabolites occurred (Tissue+GSH in Table). When the tissues were depleted of GSH by standing in 2% diethylmaleate 4h, the rates were much slower (Tissue only in Table), and the spontaneous rates were also slow (GSH only in Table). GST exists in substantial quantities in liver and the rate of GSH-conjugation of [14C]-FMP was much higher in the liver than in the brain. Furthermore, incubation of FMP with GSH and purified GST (equine liver) afforded GSH-conjugate. These results indicate that defluorination of [18F]-FBP and FMP in the brain is due to GST-catalyzed conjugation with GSH.

In expecting that fluoropurines with a stable radiolabel would have better retention in brain and other tissues, both [18F]-FMP and [14C]-FMP were simultaneously injected in mice and their biodistribusions were compared (Fig.2). In spite of longer retention of [18F] in the brain, neither brain nor liver showed retention of [14C]-radioactivity. Recently, it is reported (ref. 2) that 6-chloropurine is transformed into GSH-conjugate in liver in vivo, and that the conjugate is rapidly eliminated from the liver into blood. Our results suggest that there are some efflux-transport mechanisms that carry GSH-conjugate of the purine or its further metabolites out of the brain (Fig. 3).

Fig. 1.
Time-Activity Curves of [18F]-Radioactivity from [18F]-FBP and [18F]-FMP in Mice

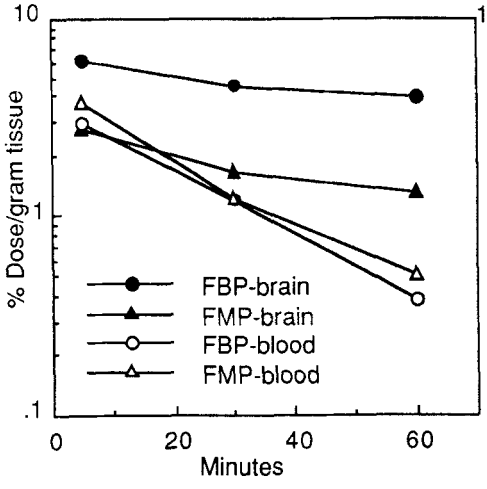


Fig. 2.
Comparison of Tissue Kinetic Curves between [18F]-FMP and [14C]-FMP

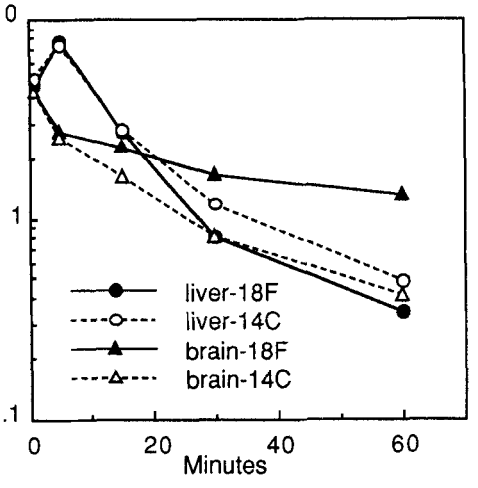
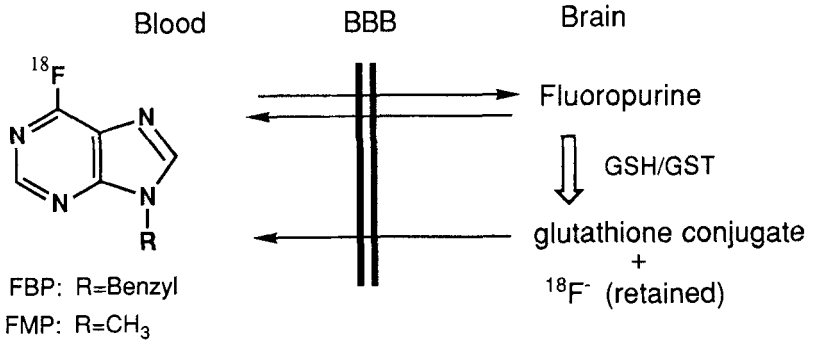


Table
Reaction Rates of [18F]-6-Fluoro-9-Alkylpurines with GSH and Tissue Homogenates

		Rate constant (fraction/min/g tissue/mL)		
		Tissue + GSH	Tissue only	GSH only
[18F]-FBP	Brain (rat)	0.320	0.060	0.019
[14C]-FMP	Brain (mouse)	0.340	0.001	0.005
[14C]-FMP	Liver (mouse)	3.380	0.004	0.005

Fig. 3 The Retention Mechanism of [18F]-6-Fluoro-9-Alkylpurines in the Brain



FBP: R=Benzyl
FMP: R=CH_3

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Effect of Radionuclide on Membrane-transport and Intracellular Hybridization of c-myc Probe in Cultured Murine Leukemic Cells. M.K. DEWANJEE, A.K. GHAFOURIPOUR, L. WILLEM, A. GANJU-KRISHAN, M. KAPADVANJWALA, A.N. SERAFINI, G.N. SFAKIANAKIS. University of Miami, School of Medicine, Miami, FL USA.

The c-myc oncogene amplified in leukemia and solid tumors was used as a model target for comparative evaluation of radionuclide label on membrane-transport and intracellular hybridization in P388 leukemic cells (1-3). The 16-mer oligonucleotide (ON) sequence specific for c-myc oncogene, was synthesized, aminolinked [sense (SN) and antisense (AS) phosphodiester (O) and monothioester (S)] and coupled to DTPA-isothiocyanate and aliquots (10μg) were lyophilized. Conjugated oligonucleotide was labeled with In-111, Ga-67, Sm-153, Tc-99m and I-125 (100-500 μCi) and free radionuclide was separated by gel-filtration. The P388 log phase cells were grown in roller bottles in media (RPMI 1640) supplemented with 10% fetal bovine serum, 24 hours prior to experiments and used at a concentration of (1.1-1.9)10^6/ml. For plateau phase cultures, these cells are diluted 6-fold and cultured for 48 hours in serum supplemented media followed by 24 hour incubation in serum free media (RPMI and antibiotics) and used at a density of (1.5-2.0)X10^6 cells/ml.

The preferential cellular uptake of the same c-myc antisense probe (16-mer) labeled with different radionuclides is in the following order: In-111 (41%) > Tc-99m (32%) > Ga-67 (25%) > Sm-153 (17%) > I-125 (7%). The parameters of stability of labeled probe, specific activity of radionuclides, ionic charge of radionuclide, length of aminohexyl spacer linkage, length and nucleotide sequence of probe, donor atoms of the conjugated probe (O, S, N, P), type of chelating agent or halogenating activated phenyl ring and stereoisomers (S) and secondary structure of probe (GC%), may affect both the cell-uptake, intracellular hybridization and probe retention. Labeled cells were lysed and radioactivity bound to membrane, total RNAs, mRNA, protein and DNA were separated by CsCl density gradient centrifugation. The ribosomal RNA was separated from mRNA with polyadenylate tail, by oligo-deoxythymidylate conjugated cellulose column (Boehringer-Mannheim Inc., Germany). More than 70-80% of the antisense probe was bound to mRNA. The probe may work as a catalyst for continuous degradation of probe-mRNA complex by RNaseH. The ß-radiation from I-125 and Sm-153 radionuclides degrades the phosphorothioate derivatives of oligonucleotides, faster than phosphodiester. The antisense oligonucleotide provides a specific and efficient vehicle for delivering γ- and ß-emitting radionuclides to cancer cells that may be beneficial for both diagnosis and therapy in cancer patients.

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Figure 1. Effect of radionuclide on membrane-transport and intracellular hybridization of c-myc probe in cultured murine leukemic cells (P388); the same sequence at similar specific activity was used at all incubation studies.

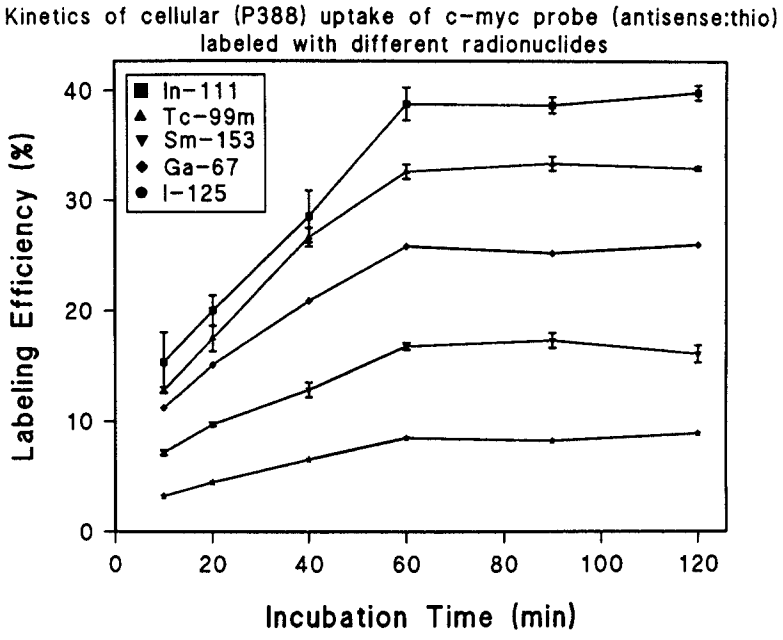

Figure 2. Kinetics of intracellular hybridization of mRNA of c-myc oncogene with Sm-153 labeled antisense deoxyoligonucleotide probes in leukemic cells (P388). The kinetics of AS probe-mRNA complex formation was studied from samples taken at 10, 20, 60, 90 and 120 minutes of incubation by HPLC with a TSK-300 column eluted by phosphate-buffered saline.