Paper E1

OPTIMAL YIELDS FOR THE Cu^{1+} ASSISTED RADIOHALOGENATION BY RESPECTING THE MECHANISTIC PARAMETERS

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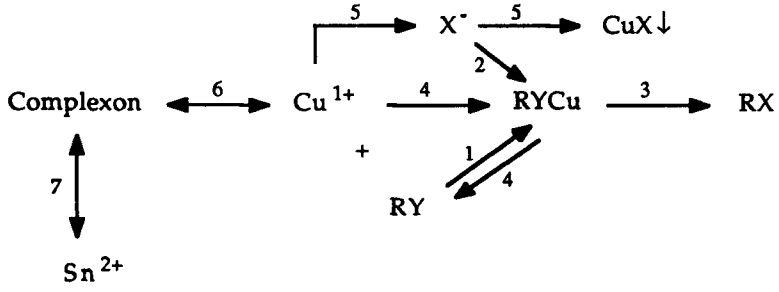
Radiohalogenation with the Cu^{1+} assisted nucleophilic exchange reaction in reducing conditions has been applied with success by us and other groups on a wide variety of compounds. In some cases lower labelling yields than expected were reported. This was often caused by transposition of the conditions described in one of our earlier publications without taking into account the appropriate parameters ruling the reaction under consideration. The Cu^{1+} method is based on a fundamental model including the reaction mechanism, the presence of reducing (Sn^{2+}, gentisic acid) and complexing (citric acid) agents, the absence of oxygen, the substrate (RY) to Cu^{1+} ratio and the Cu^{1+} to halogenide (X^-) ratio (related to solubility).
The overall labelling reaction can be represented by :



The Cu^{1+} reaction shows pseudo first order kinetics

$$\frac{-d[^*X^-]}{dt} = \frac{K_3\,[RY]_o\,[Cu^{1+}]_{o.f}}{K_1 + K_1\,[Cu^{1+}]_o + [RY]_o}\,[^*X^-]$$

The fundamental aspects concerning the type of the intermediate complex RYCu and reaction 1, leading to this complex suitable for nucleophilic attack 2 and yielding the labelled compound RX* (reaction 3), are discussed elsewhere (1). In practice : The introduction of unwanted halogens as a part of salts must be avoided. The RY/Cu^{1+} molar ratio must be kept between 5 and 10. Increasing the amount of Cu^{1+} is useless as reaction 4 causes the dissociation of the complex resulting in a steady state labelling yield. Only increasing the amount of RY results in a higher labelling yield.
As Cu^{1+}, just as Sn^{2+}, is partially complexed by citric acid (and acetic acid if present), for a fixed amount of complexing agents the amount of Cu^{1+}, the catalyst, can change with the amount of Sn^{2+}, due to the parallel reactions 6 and 7. The optimal pH for labelling is about 2.2. The labelling can proceed at higher pH (up to 3.5) if the amount of complexing agent is increased. Compounds with a thioketo function can partially precipitate as their Cu-complex.
Taking into account the considerations mentioned above must lead to a successful labelling.

(1) J. Mertens, M. Gysemans, New Trends in Radiopharmaceutical Synthesis. Quality Assurance and Regulatory Control, Ed.: A. M. Emran, Plenum Press, New York 1991.

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POLYMER-BOUND RADIOPHARMACEUTICALS: N-isopropyl-4-iodoamphetamine

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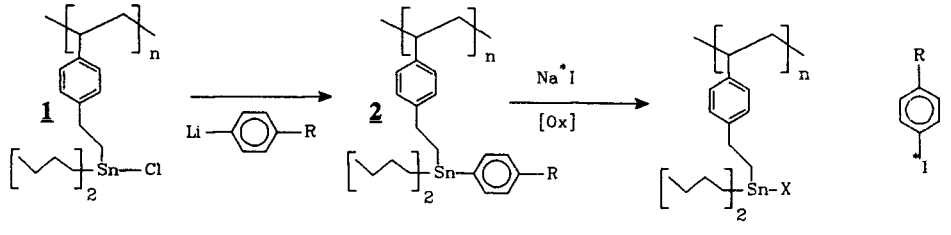
Organotin reagents are attractive precursors to radioiodinated pharmaceuticals since radioiodination often proceeds in good radiochemical yield under fairly mild conditions. Organotin precursors are also a source of *no-carrier-added* radiopharmaceuticals of iodine which continue to attract attention as radioactive probes for receptor systems in the human body.

Radiolabelling procedures at the *no-carrier-added* level carry with them the problem of separating the very small amount of radiopharmaceutical from the sideproducts and unreacted reagents which are usually present in large excess. These unwanted impurities include unreacted radioiodide in one or more of its oxidation states, the oxidizing agent or its reduced form and unreacted organotin precursor. The problem of the oxidizing agent can be solved by using 'Iodobeads' which are readily removed from the reaction mixture. There are procedures for removal of unreacted radioiodide. The one problem which remains is the separation of the unreacted organotin precursor from the structurally analogous radioiodopharmaceutical. This can result in a step involving HPLC or other chromatographic purification and isolation procedures. One approach to this problem is through a polymer-bound organotin precursor. In principle, unreacted precursor will remain bound to an insoluble polymer bead and the radiopharmaceutical will be released into solution.

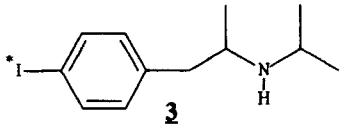
Although the use of polymer-bound reagents[1] in organic chemistry and biochemistry has enjoyed tremendous success for nearly half a century, the use of polymer-bound reagents in radiochemistry is relatively unknown. The organotin polymer **1*** has recently been reported[2] as a precursor to a sequestered organotin hydride reducing agent. We recognized that such a polymer, if treated with the appropriate organolithium or Grignard reagent, might produce a polymer-bound precursor suitable for use in a radiopharmaceutical "kit".



*The polymer is synthesized from purified commercial divinylbenzene which is an approximately equimolar mixture of the meta and the para isomers. The polymer will be represented as the para isomer throughout for simplicity.

Though simple in principle, few useful radiopharmaceuticals can be metallated. Our initial approach was to prepare protected precursors, metallate these precursors and then covalently attach them to the organotin polymer by substitution at the tin-chlorine bond. Problems with deprotection led us to reconsider the direct metallation of existing radiopharmaceuticals.

We were able to show that *p*-dibromobenzene could be mono-lithiated and coupled to the organotin polymer. The resulting polymer **2** (R=Br) was treated with $Na^{131}I$ and N-chlorosuccinimide in chloroform. After filtration of the polymer, 70% of the radioactivity was found in the filtrate. Reversed-phase HPLC analysis showed three radioactive peaks with 4-iodobromobenzene accounting for 67% of the total activity. The two other radiopeaks were attributed to iodobenzene and 4-iodophenol but not radioiodide. We believe that the monolithiation reaction may occur with some di-lithiation. If this is the case then iodobenzene resulting from the protonation of the dilithiated species or iodophenol arising from the oxidation of the dilithiated species may account for the presence of additional radioactive peaks.

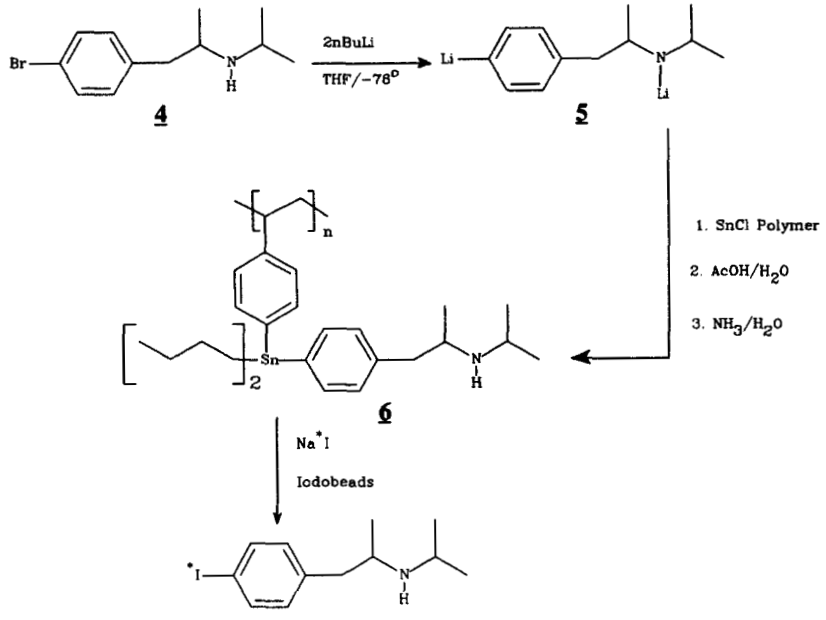


3

N-Isopropyl-4-iodoamphetamine[3] **3** seemed to be an appropriate chemical model for this approach although high specific activity is not critical for its use. We believed that treatment of the brominated analogue **4** with two equivalents of n-butyllithium would lead to the di-lithiated product **5**. Reaction of **5** with the SnCl polymer **1** was carried out yielding a new polymeric material which was characterized by XPS and combustion analysis indicating loss of chlorine and incorporation of nitrogen. This polymer **6** was also characterized chemically by its reaction with a solution of iodine in chloroform. Rapid decolorization and subsequent TLC analysis showed a single spot consistent with N-isopropyl-4-iodoamphetamine. HPLC analysis employing the system of Carlsen and Andresen[4] showed but one peak at the retention for **3** and verified the absence of N-isopropyl-4-bromoamphetamine.

Treatment of (64 mg, 0.15 meq) of the polymer **6** with excess iodine in chloroform followed by filtration and isolation of the chloroform-soluble residue yielded (11 mg, 0.036 mmol) of **3** which suggests, along with XPS and combustion results, that about 20% of the tin atoms are connected to N-isopropylamphetamine moieties. The residue yielded ^1H and ^{13}C NMR spectra consistent with N-isopropyl-4-iodoamphetamine.

The desired polymer **6** was also reacted with $Na^{131}I$ using "Iodobeads" as oxidant in acetonitrile. The radiochemical yield of the reaction was determined to be 44%.



We have therefore been able to demonstrate that a polymer-bound radiopharmaceutical can be used to produce a *no-carrier-added* iodinated analogue free from precursor impurities. Future work is directed towards optimizing "kit" conditions to produce radioiodinated *no-carrier-added* compounds to be used clinically.

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Sherrington D.C. and Hodge P. (eds.)- *Synthesis and Separations Using Functional Polymers*, John Wiley and Sons, London, **1988**

2. Gerigk U., Gerlach M., Neumann P., Veiler R. and Weintritt V.- *Synthesis*, 448 (1990)

3. Windrell H.S., Horst W.D., Braun L., Oldendorf W.H., Hattner R. and Parker H.; *J. Nucl. Med.* **21**: 947 (1980)

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Monoamine Storage Site Imaging Agent for SPECT: Iodinated Tetrabenazine.

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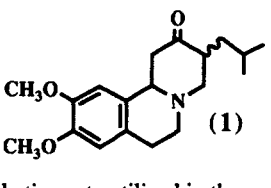
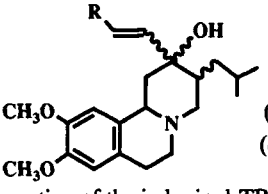
Monoamine neuronal systems, i.e., serotonergic, dopaminergic and adrenergic neurotransmitters, have been implicated in various neurological and psychiatric disorders. Different types of therapeutic agents aiming at these neuronal systems, as the pharmacological basis for treatment, are well known. Evaluation of the innervation of these neuronal systems is essential and important for understanding the pathophysiology, and for monitoring progress of patient treatment. Reserpine is a natural product which inhibits the amine uptake-storage mechanism of amine granules in the synapse. Tetrabenazine is an analog of reserpine which displays a similar biological profile. Both agents were proposed as antipsychotic agents in the 1950's. The depletion of catechol and serotonin in the brain by reserpine is long-lasting and the storage mechanism is irreversibly damaged. Tetrabenazine (TBZ, **1**) produces a similar effect; however, the drug effects of TBZ are of a shorter duration and do not induce irreversible damage on neurons[1]. Recently, [^{3}H]dihydro-TBZ has been used as a selective marker for the monoamine transport system in vitro[2]. The acetyl derivative of dihydro-TBZ has also been shown to retain high affinity for the transporter, suggesting that an iodovinyl group might be tolerated at this position[3]. Consequently, we report the synthesis and evaluation of [^{123}I]iodovinyl-TBZ (**4a** and **4b**) as a potential SPECT imaging agent for studying CNS monoamine uptake sites.



(1)



(4a, R = I)
(4b, R = ^{123}I)

The synthetic route utilized in the preparation of the iodovinyl-TBZ analog is shown in Scheme I. Ethynylation of tetrabenazine (TBZ; **1**) was accomplished by first treating trimethylsilylacetylene with n-butyllithium at -5 to 0 °C in THF, followed by dropwise addition of a solution of TBZ in THF. The trimethylsilyl group was removed using 5N NaOH. The ethynyl derivative (**2**) was then treated with tri-n-butyltin hydride in the presence of AIBN and the mixture was heated to 95 °C for 5 h. Treatment of the vinylstannane (**3**) intermediate dissolved in $CHCl_3$ with a 0.1 M solution of iodine in $CHCl_3$ afforded the cold iodinated compound (**4a**) in 27.5% overall yield. Elemental analyses and spectroscopic data for compounds **2**, **3**, and **4a** were consistent with the structures shown. The radiolabeled derivative (**4b**) was prepared by treating an ethanolic solution of the organotin intermediate with Na^{123}I and 1 N HCl in the presence of H_2O_2. After an HPLC purification the radiochemically pure compound displayed the same retention time as that of the authentic cold compound. Biodistribution of ^{123}I-**4b** in rats showed that the TBZ derivative can cross the intact blood brain barrier and localize in brain. However, the regional distribution ratio in the rat brain did not exhibit any selectivity. The lack of regional difference reflecting the distribution of monoamine neurons may be due to the fact that there are three optical centers for **4a**. Additional studies with the optically pure samples may be necessary to further characterize the proposed agent.

It is likely that determination of neuronal integrity may be important in differential diagnosis of CNS diseases. The proposed iodinated tetrabenazine will be useful for evaluation of the presynaptic neuronal function, especially the uptake and storage mechanism. The agent will not be specific for one type of neuronal system, but will be useful as a general indicator for serotonergic, dopaminergic and adrenergic systems. A monoamine storage site imaging agent for PET and SPECT may serve the need of a large population of patients receiving drug treatments targeting the monoamine neurons.

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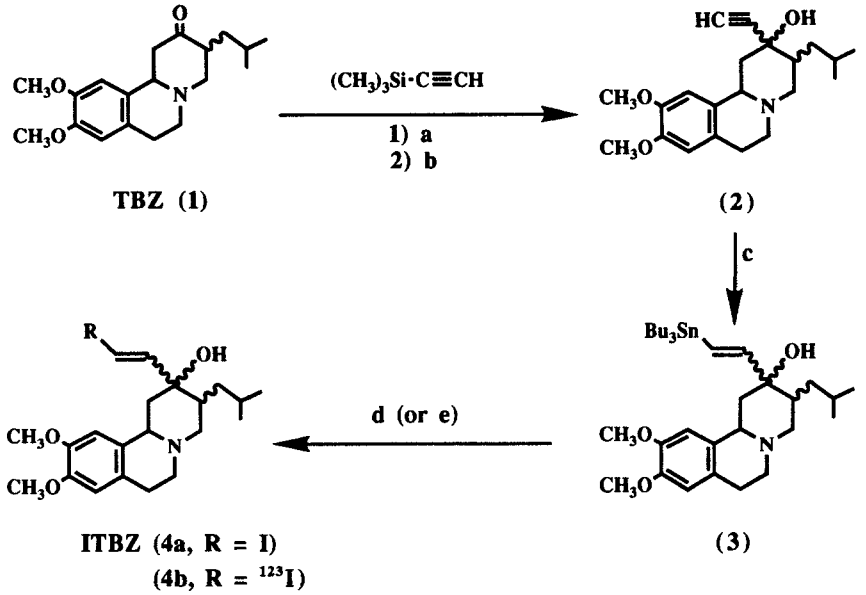
2. Henry JP, Sherman D; Biochem Pharmacol 38:2395-2404, 1989
3. Sherman D et al.; Mol Pharmacol 33:72, 1988

Acknowledgements: This work is supported by a grant from Nihon Medi-Physics Inc. Authors thank Catherine Cartwright for her editorial assistance.

Supporting Data: Canney et al

Scheme I. Synthesis of Iodovinyl-tetrabenazine (ITBZ)



a) n-butylLi, THF, 0° C; b) KOH, MeOH, 65° C; c) $HSnBu_3$, AIBN, Toluene, 95° C

d) I_2, $CHCL_3$, 25° C; e) $Na^{123}I$, 1N HCl/EtOH, H_2O_2

Table I
Biodistribution of ^{123}I-TBZ in rats

Organ[1]	2 min	30 min	60 min	120 min	240 min
Blood	5.99	1.84	2.46	2.16	1.82
Heart	1.33	0.34	0.35	0.18	0.18
Liver	11.1	9.75	8.57	6.71	4.57
Thyroid	0.06	0.03	0.05	0.08	0.15
Brain	0.92	0.36	0.28	0.16	0.13
Regional Uptake[2]					
Cerebellum	0.50	0.17	0.14	0.14	0.05
Striatum	0.54	0.21	0.12	0.12	0.06
Hippocampus	0.58	0.21	0.12	0.12	0.05
Cortex	0.55	0.20	0.15	0.15	0.05

1. %dose/organ; ave of 3 rats
2. %dose/g; ave of 3 rats

Paper E4

THE SYNTHESIS OF RADIOIODINATED VINYL IODIDES VIA ORGANOBORANE CHEMISTRY

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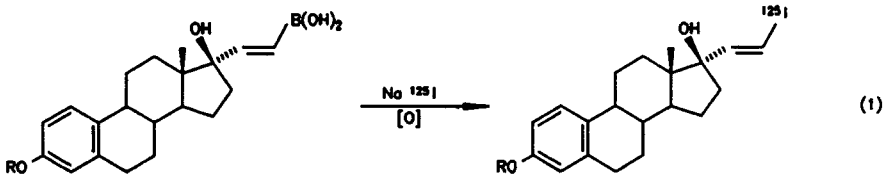
We reported the first synthesis of radioiodinated vinyl iodides over a decade ago (1,2). In that initial report, organoborane technology was utilized to prepare 17α-iodovinylestradiol derivatives (equation 1) due to the convenient properties of the stable, solid precursor boronic

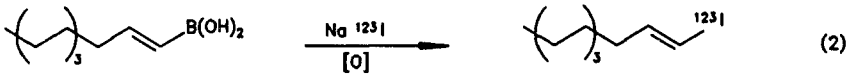


(1)

acids (2). During the ensuing years, other investigators utilized tin, mercury, silicon and boron to prepare radioiodinated iodovinyl derivatives of a wide variety of physiologically active agents (3). Each of the metals has advantages and disadvantages; the ultimate choice of methodology is often dependent on the ease of preparation of the metallated precursor. We prefer to use the boronic acid derivatives because they are, generally, air and water stable crystalline solids.

The most efficient method for radioiodinating vinylboronic acids involves the *in situ* oxidation of the radioiodide ion using mild oxidants (4). We wish to report the results of a systematic study of the iodination of a vinylboronic acid utilizing ten common oxidizing agents: hydrogen peroxide, sodium percarbonate, sodium perborate, N-chlorosuccinimide, chloramine - T, *meta*-chloroperbenzoic acid, *t*-butyl hypochlorite, sodium hypochlorite, iron(III) chloride, and potassium ferricyanide. 1-Decenylboronic acid (equation 2) was used in the study. The investigation revealed that chloramine-T is the most efficient oxidant for the radioiodination reactions under both carrier-added and no-carrier-added conditions. Chloramine-T oxidations

were then carried out in the presence of a variety of buffers, acids and bases; our results are summarized in Table 1.



(2)

TABLE 1. Preparation of (E)-1-[^{123}I]Iododecene Via Reaction of (E)-1-Decenylboronic Acid (DBA) with Na^{123}I and Chloramine-T (CAT).

	Ratio[a]			
Base	CAT/DBA	BASE/DBA	NaI/DBA	Yield (%)
-----	1.2	-----	nca[b]	46
H_3BO_3[c]	0.2	1.0	nca[b]	15
PO_4^{-3}[d]	0.2	1.0	nca[b]	54
NaOAc	0.2	1.0	nca[b]	15
NaOH	0.2	1.0	nca[b]	0
HCl[e]	0.2	1.0	nca[b]	20
-----	2.0	-----	1.0	85
PO_4^{-3}[d]	2.0	0.6	1.0	86
PO_4^{-3}[d]	1.0	2.4	1.0	25

[a]Molar equivalent ratio. [b]No-carrier-added. [c]Borate buffer, pH = 9.

[d]Phosphate buffer, pH = 7.

General Procedure: Radioiodination of 1-Decenylboronic Acid

1-Decenylboronic acid (10 micromoles, 0.10 mL of a 0.100 M solution in THF) was added to a vial containing a magnetic stirring bar and fitted with a septum cap. A charcoal filter constructed from a 2 mL syringe was inserted into the septum to prevent leakage of radioiodine. The vial was shielded from direct light and immersed in an ice-water bath. Bases (buffers, etc.) were injected as aqueous solutions prior to addition of the radiolabeled sodium iodide. The sodium [^{123}I]iodide (300 microCuries, no-carrier-added or contained in 10 micromoles of stable iodide) was then added in 0.10 mL of water. The appropriate oxidant (dissolved in 50% aqueous THF) was then added and the solution stirred for 10 minutes. [In carrier-added experiments the brown-orange color disappeared within 10 minutes.] The reaction was then quenched with 0.1 mL of a saturated sodium thiosulfate solution. Radiochemical purity was monitored by TLC on silica gel using 5% ethyl acetate in hexanes. Radio-TLC was performed using a Bioscan System 200 Imaging Scanner.

Research supported by The United States Department of Energy.

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RADIOIODINATED CLORGYLINE DERIVATIVE FOR MAPPING MAO-A ACTIVITY IN BRAIN WITH SPECT

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Clorgyline, a suicide inhibitor, radiolabeled with carbon-11 has been reported as positron ligand for functional studies of the monoamine oxidase type A (MAO-A) in the living brain [1]. Instead, our interest has been centered on iodinated radiopharmaceuticals and various clorgyline derivatives have been synthesized as MAO-A ligands for SPECT studies. Among these analogues, the 2-iodo-4-chloro-clorgyline (2-ICG) and 2,4-dichloro-6-iodo-clorgyline (6-ICG) (Fig.1) have shown high inhibitory potency and selectivity toward MAO-A, compared with clorgyline (IC_{50} 6.3×10^{-11} M, at 0.1 mM kynuramine). Neverthless preliminary in vivo study of 2-ICG has shown high non-specific binding in the brain at 1 hr post injection [2]. In the present work, an in vivo study of the 6-ICG specific binding with the MAO-A was considered and the determination of variables to distinguished it from non-specific binding was attempted.

No-carrier-added ^{125}I-6-ICG was obtained by an iododestannylation reaction catalyzed by hydrogen peroxide (radiochemical yield > 75-80 %, final radiochemical purity > 99 % as measured by HPLC) (Fig. 2). The precursor was prepared by the reactions outline of scheme I. Maximum brain uptake was registered at 5 min post injection; later, fast clearance was detected but after 1hr radioactivity remained unchanged for more than 6 hr, an advantageous characteristic to discreminate it from non-specific binding, Then, further work on 6-ICG specificity for MAO-A was carried out. Pretreatment with clorgyline and MAO-B inhibitor, *l*-deprenyl was performed. Only the pretreatment with clorgyline induced a decrease of the 6-ICG brain uptake down to 50, 78, and 95 % at 2, 6 and 24 hr post injection, respectively . This results indicated the specificity of 6-ICG for MAO-A in the brain. The specific binding offered the needed retention time for radioiodinated clorgyline derivative, 6-ICG to be used for in vivo mapping of MAO-A activity in the brain with SPECT, should the iodine-123 be selected.

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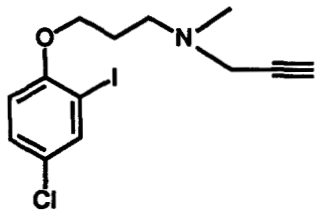
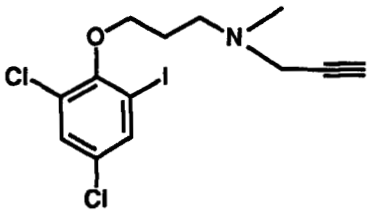
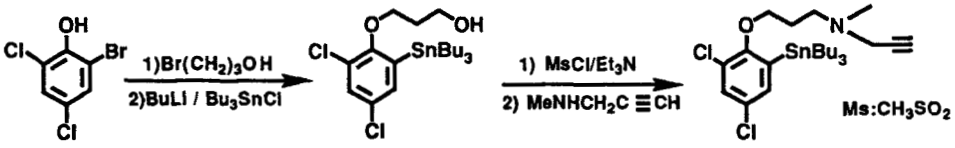


2-Iodoclorgyline
(2-ICG)



2,4-Dichloro-6-iodoclorgyline
(6-ICG)

Fig. 1. Chemical structure of iodonated clorgyline derivatives



Scheme I Synthesis of the precusor of 2,4-dichloro-6-iodo-clorgyline (6-ICG)

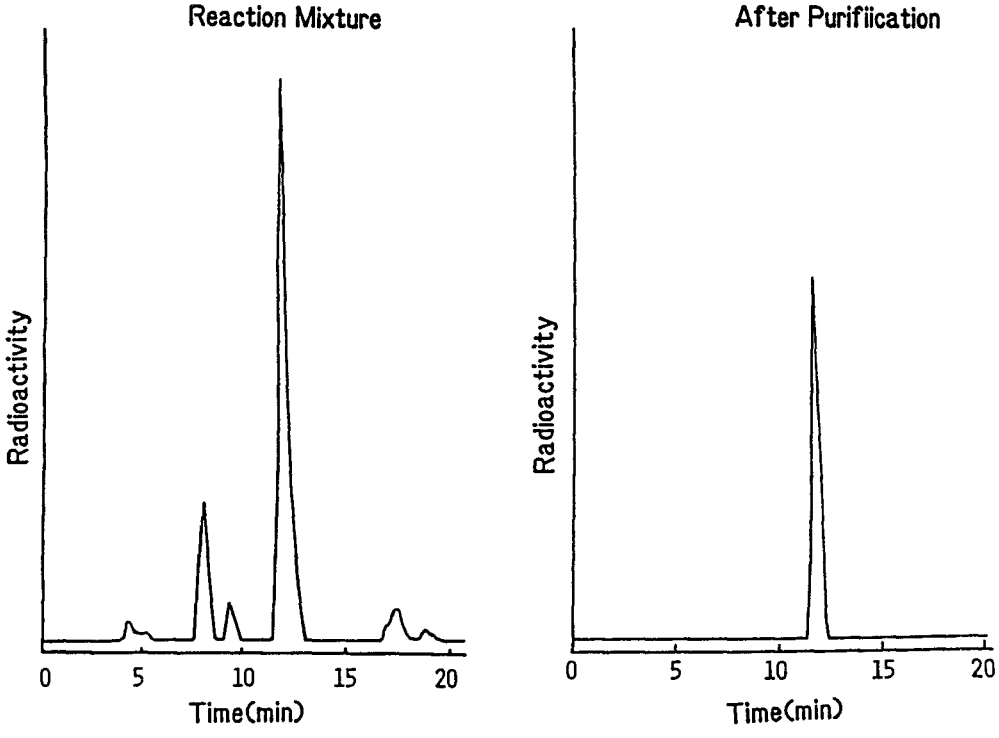


Fig.2 HPLC Profiles of ^{125}I-6-Iodoclorgyline(6-ICG)
Column : C-18 RP column (250 x 10mm)
Solvent : MeOH / $HCOONH_4$ = 9 / 1
Flow rate : 3ml / min

Use of aryltin substrates for high specific activity radiohalogenation of amine derivatives. Application to labeling of amphetamine, phentermine and benzamide derivative.

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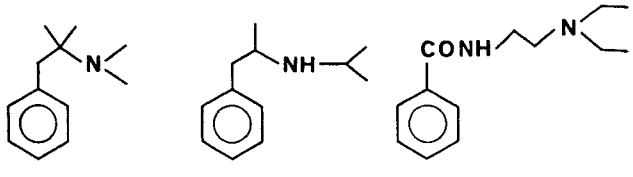
Despite its relative high price and low widespread distribution ^{123}I remains the isotope of choice for labeling substrates giving in vivo biochemical informations at molecular level. Mapping receptors or tracing biochemical pathways often requires the use of high specific radioiodinated compounds. This is generally achieved by a labeling step of substrate followed by an HPLC purification in order to separate iodinated radiopharmaceutical from native substrate and degradation products. This separation step, very efficient at laboratory level, is quite unsatisfactory when applied to production level where several hundred of millicuries of ^{123}I should be handled in hot cells.

We developed a labelling method based on organotin substrates which is able to provide high yields and relatively high specific activity without the use of an HPLC column separation[1,2].This method is based on the following properties displayed by the aryltin derivatives:

- strong reactivity toward iodination in mild conditions
- presence of a bulky structure (e.g. $SnBu_3$) allowing an easy separation on Sep Pak column.

We applied this method to the para radioiodination of three compounds:

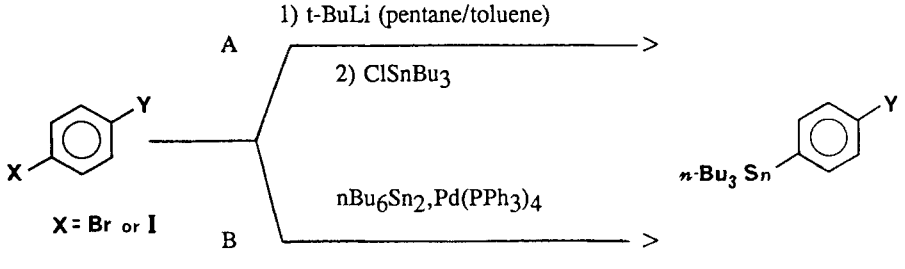
- N,N-dimethyl phentermine (**1**), N-isopropyl amphetamine (**2**),N-(2-diethylaminoethyl) benzamide (**3**)



(1) (2) (3)

Synthesis of p-aryltin substrates

Two routes were selected to perform the metallation reaction



The final isolated products were characterized by TLC, IR, ^{1}H NMR and MS analysis. The yields (without optimisation) were:

Product	A	B
1	52%	-
2	8%	20%
3	12%	40%

Radioiodination with ^{123}I

Labeling was carried out at room temperature with 40 nmoles of aryltin substrate and 185MBq (5mCi). The radiochemical purity was checked by TLC before and after separation on Sep Pak column.

Substrate	% p-^{123}I derivative	Specific activity without separation	Specific activity after Sep Pak	Specific activity by isotopic exchange method
1	80%	200 mCi/mg	4 Ci/mg	5 mCi/mg
2	50%	125 mCi/mg	2,5 Ci/mg	0,7 mCi/mg
3	50%	125 mCi/mg	2,5 Ci/mg	0,5 mCi/mg

This method gives acceptable yields of aryltin substrates, good yields of ^{123}I derivatives and high specific activity without tedious separations. It may be used when great amount of radioactivity has to be handled in hot cell facilities.

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Paper E7

SYNTHESIS OF [I-125]P-IODOPHENYLTRIPHENYLPHOSPHONIUM NITRATE AS A TRACER FOR MALIGNANT CENTRAL NERVOUS SYSTEM.

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Photodynamic therapy (PDT) has drawn recent attention because of its potential treating solid tumors. This therapy takes advantage of the properties of certain cationic lipophilic compounds: 1) Being accumulated selectively in significant amounts. 2) Being retained for prolonged periods of time by neoplastic lesions.

There has also been significant interest in enhancing the selectivity of tumor targeting through the exploitation of functional or biochemical differences between normal and malignant cells.

It is known that tetraphenylphosphonium chloride demonstrates the greater uptake and retention by adenocarcinoma[1] and especially by some malignant central nervous system tumors.[2,3] Our ongoing interest in developing new potential agent of PDT for malignant central nervous system led us to synthesize ^{125}I-p-iodophenyltriphenylphosphonium nitrate **8** as a model compound for a series of cationic lipophilic agents.

Our initial attempt (Scheme 1) revolved on preparing a precursor compound **2** from diiodobenzene for iodine-iodine exchange with I-125 by a well established procedure. However the formation of many other unidentified by products and the difficult separation in making compound **2** made that scheme impractical.

We devised a new synthetic route (Scheme 2) which involves regiospecific introduction of I-125 at the para position to the phosphonium ion. The conversion of p-iodoaniline to the triazene **5** was done by a one pot reaction. This triazene was reacted with triphenylphosphine and a catalytic amount of palladium(II) acetate to give p-piperidinylazophenyltriphenylphosphonium iodide, which in turn, was labeled with I-125. The radiochemical yield was 70% after purification on a short silica gel column using acetone as eluant. The synthesis of the cold p-iodophenyltriphenylphosphonium nitrate was achieved by the same methods shown in Scheme 2.

The selectivity and retention of this radioiodinated prototype tracer was compared to its tritiated analog. This lipophilic cationic compound is model molecule for a series of potential diagnostic and radiotherapeutic agents.

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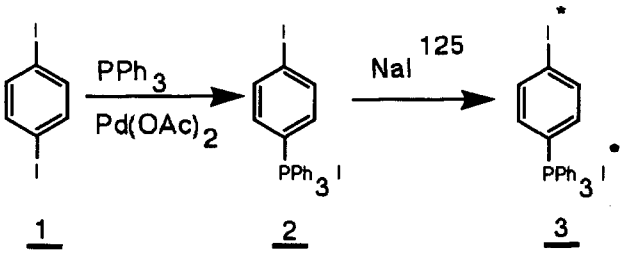
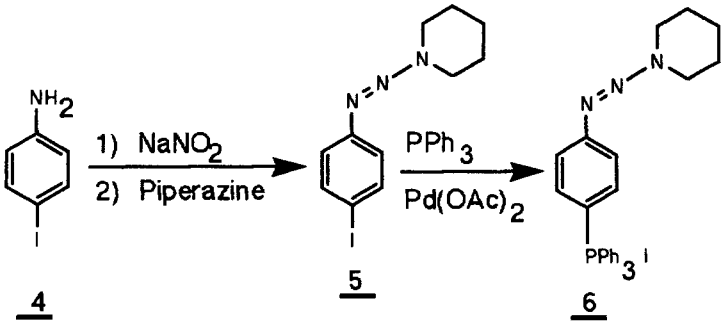
Scheme 1

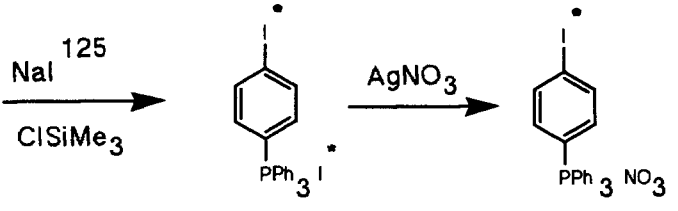


1 2 3

Scheme 2



4 5 6



7 8

ACYLATION REAGENTS FOR THE RADIOHALOGENATION OF PEPTIDES.

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Substantially reduced loss of label *in vivo* and improved tumor accumulation have been observed when antibodies have been labeled using *N*-succinimidyl 3-iodobenzoate (SIB) (1) or *N*-succinimidyl 5-iodo-3-pyridinecarboxylate (SIPC) (2). While SIB and SIPC are equally useful for labeling antibodies, SIPC should be the superior reagent for use with smaller peptides because of the lower lipophilicity of its corresponding acid (octanol-water partition coefficient: 0.05, 5-iodonicotinic acid; 0.48, 3-iodobenzoic acid).

Initial studies were performed using phenylalanine ethyl ester (Phe-Et). Phe-Et conjugates of both SIPC and SIB were synthesized in 70-85% yield and their structures confirmed by NMR and elemental analyses. HPLC was performed using a silica column eluted with EtOAc:Hexane:AcOH (30:70:2) at a flow rate of 1 ml/min. As shown in Table 1, retention times suggest a lower lipophilicity for the SIPC (9.6 min) versus the SIB (5.6 min) conjugate of Phe-Et.

As a model peptide, the 8-amino acid somatostatin analog octreotide was used. Radiolabeled octreotide is of considerable interest because of recent reports demonstrating its utility for imaging tumors such as small-cell lung cancer that have elevated somatostatin receptor populations (3); however, ^{123}I-labeled Tyr-3-octeotide appears to be readily dehalogenated *in vivo* (4). The octreotide used in these studies was obtained from Sandoz as the acetate and used at its original concentration (0.5 mg/ml).

Starting with the corresonding *N*-succinimidyl trialkylstannyl ester precursor, [^{131}I]SIPC and [^{131}I]SIB were synthesized using *N*-chlorosuccinimide and *tert*-butylhydroperoxide, respectively, as described (1, 2). The labeled esters were reacted with 100 μl of octeotide in pH 8.5 borate buffer for 30 min. With SIPC, yields for the ^{131}I-labeled octreotide conjugate were 30-35% at 25°C and 45% at 37°C. Lower yields were obtained with SIB (18-20% at 25°C and 25% at 37°C). The labeled octrotide conjugates were purified using a reverse phase column eluted with methanol:154mM NaCl (65:35) at 1 ml/min and HPLC retention data indicate that the SIPC conjugate of octreotide is considerably less lipophilic that the SIB conjugate (Table 2).

The tissue distribution of the [^{131}I]SIPC-octreotide conjugate was studied in normal mice. Similar to reported data for conventionally labeled Tyr-3-octreotide (4), at 1 hr, liver (25%) and intestine uptake (68%) were high and decreased rapidly by 24 hr. However, thyroid uptake at 24 hr was only 0.2% of the injected dose compared to 7% for Tyr-3-octreotide, suggesting a greater retention of label *in vivo* with [^{131}I]SIPC-octreotide conjugate. The 5-[^{211}At]astatonicotinic acid conjugate of octreotide has also been synthesized and its tissue distribution in normal mice was similar to its ^{131}I-labeled analog.

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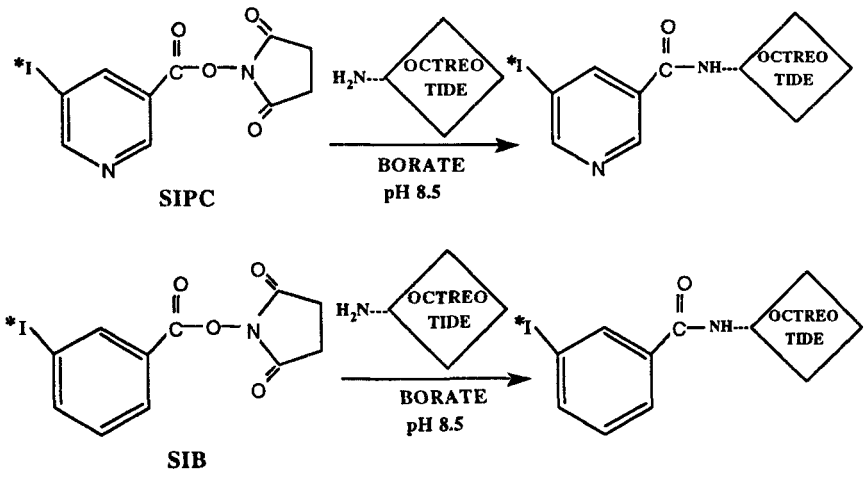

FIGURE 1: FACILE HALOGENATION OF OCTREOTIDE USING SIPC AND SIB

Table 1: HPLC Retention Time on Silica Column

Compound	Retention Time (min)
Phe-SIB	5.6
Phe-SIPC	9.6
SIB	10.2
SIPC	15.1

Table 2: HPLC Retention Time on Reverse Phase Column

Compound	Retention Time (min)
Octreotide	7.2
Octreotide-SIPC	14.4
Octreotide-SIB	23.0

Paper E9

Radioiodinated Corticotrophin-Releasing Factor (CRF): A potential radiopharmaceuical for CRF-receptor imaging.

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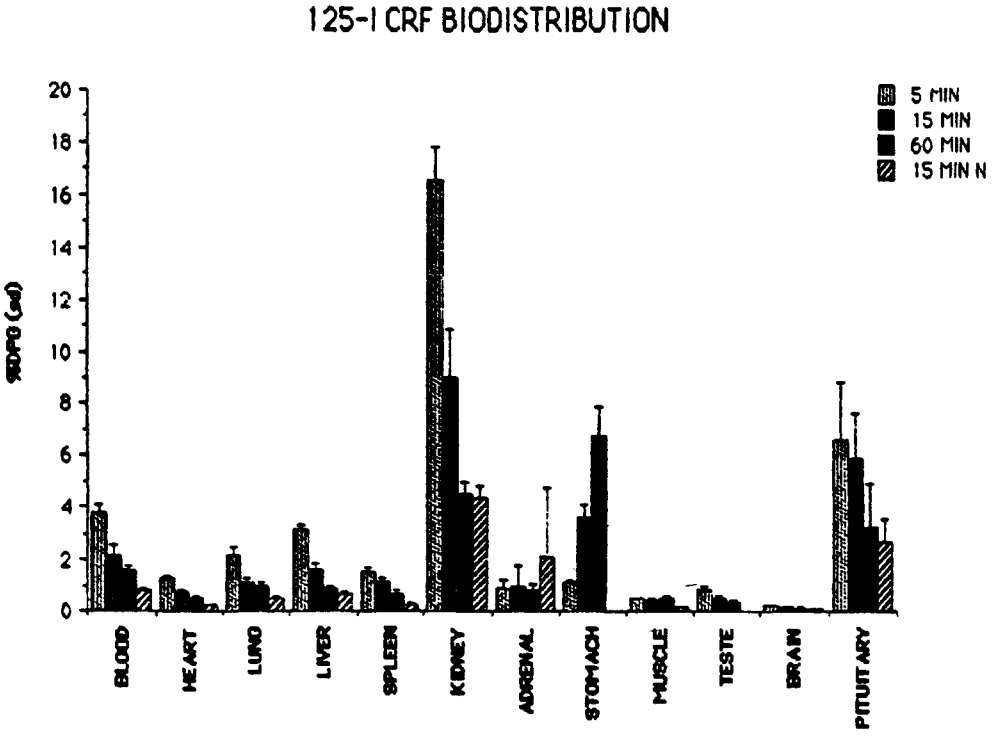
CRF is a an essential element in the integrated neuroendocrine control of the hypothalamic-pituitary-adrenal(HPA) axis. Receptors to CRF exist in the anterior pituitary and adrenal medulla as well as in a variety of neuronal and extra-neuronal tumors. The aim of this study was to develop a labelled CRF analog and evaluate its biological activity in in-vivo models. Synthetic ovine Tyr-CRF was used for all labeling experiments due to its longer plasma half-life relative to human/rat CRF and the presence of the amino-terminal tyrosine group. CRF was reconstituted in 5 mMolar HCl to 1mg/ml. Radioiodination was performed using I-125 and chloramine-T. The effect of Chloramine-T:CRF molar ratio on labelling yield was studied in pH 7.5 phosphate buffer at a fixed incubation time of 1 min. The optimal reaction conditions were 20 uL 0.25M phosphate buffer pH 7.5, 10 uL ovine-Tyr-CRF(10ug), 10 uL (1 mCi) I-125-NaI, and 10 uL Chloramine-T vortexed for 1 min. Separation of the unreacted I-125 from the labeled peptide was accomplished using a C18-reverse phase sep-pak column. The reaction mixture was loaded in NH_4OAc then eluted with H_2O, acetic acid (0.5N) and ethanol, respectively. The iodinated peptide was recovered in the ethanol fraction (yield ca. 45%), brought to dryness under N_2 and redissolved in a minimum amount of 5 mM HCl, diluted with buffered saline and sterile filtered. Radiochemical purity (RCP) was determined using C18-reverse phase TLC plates using Methanol:phosphate(pH 3.2), (1:1). The Rfs for free iodide and I-125-CRF in this system were 0.8 and 0.1, respectively. RCP was routinely >95%. The biological activity of I-125-CRF was determined in a series of biodistribution studies in rats. Since stress induces release of CRF from the hypothalamus it was important to isolate the pituitary from endogenous CRF release. This was done pharmacologically by chlorpromazine-morphine-pentobarbital blockade of the HPA axis stress response in one group of animals and by physically removing the HPA axis by transplanting the pituitary to the renal capsule in another. The control groups were normal rats which were sacrificed by either pentobarbital or ether overdoses. The localization (as %dose/gram) of I-125-CRF was (except for kidney) highest in the pituitary as compared to other tissues and reached a peak concentration at 5 min post injection. The %dose/gram in transplanted pituitaries were 6.55 (+/- 2.3), 5.86 (+/- 1.7) and 3.19 (+/- 1.7) at 5, 15 and 60 mins, respectively, compared with 2.63 (+/- 0.9) in normal pituitary at 15 min. Relative pituitary uptake (expressed as ratio of %d/g) in pharmacologically blocked rats sacrificed with pentobarbital overdose compared with pentobarbital and ether sacrificed rats was 1.44 and 2.23 times greater, respectively. Our data suggest radioiodinated CRF may be a useful tracer for the study of CRF receptors.

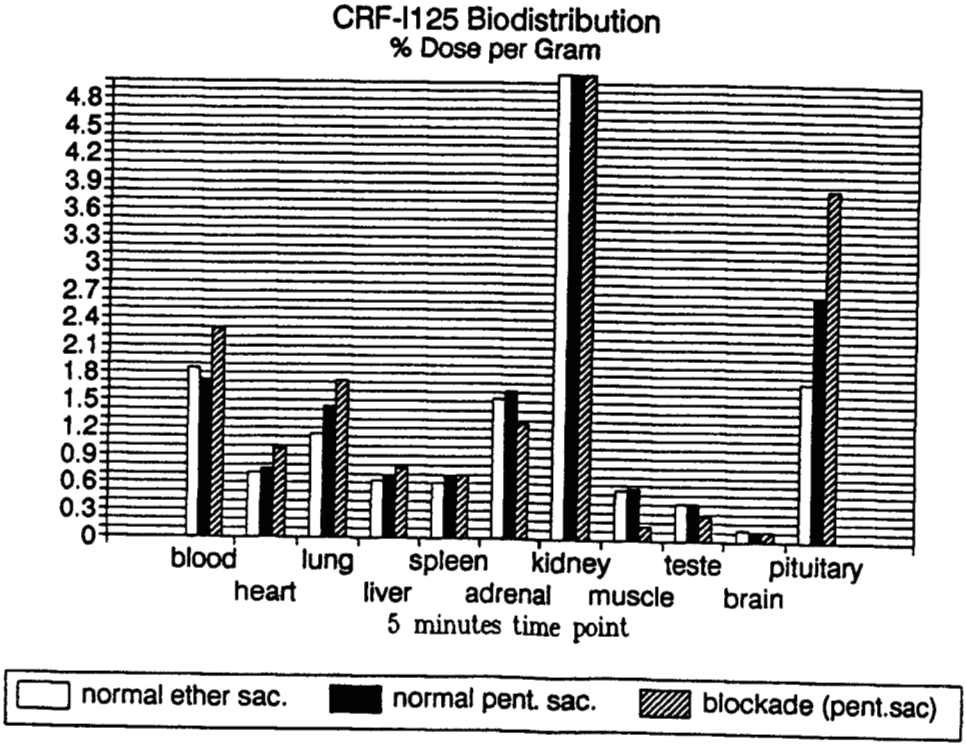
supporting data:
Radioiodinated Corticotrophin-Releasing Factor (CRF): A potential radiopharmaceuical for CRF-receptor imaging.
JW Babich, et al

BIODISTRIBUTION OF I-125-OVINE-TYR-CRF IN PITUITARY TRANSPLANTED RATS AT 5 MIN, 15 MIN AND 60 MIN POST INJECTION COMPARED WITH NORMAL RATS AT 15 MIN POST INJECTION (15N)



supporting data:
Radioiodinated Corticotrophin-Releasing Factor (CRF): A potential radiopharmaceuical for CRF-receptor imaging.
JW Babich, et al

BIODISTRIBUTION OF I-125-OVINE-TYR-CRF IN CHLORPROMAZINE-MORPHINE-PENTOBARBITAL BLOCKED RATS AT 5 MIN POST INJECTION COMPARED WITH UNBLOCKED RATS SACRIFICED WITH ETHER OR PENTOBARBITAL OVERDOSE.



Paper E10

Synthesis of halogenated analogues of 5-(4-chlorophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo(2,1-a) isoindole or mazindol as new dopamine uptake carrier ligands.

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* Vub-Cyclotron, Vrije Universiteit Brüssel, Laarbeeklaan 103, 1090 Brüssels, BELGIUM

For exploration of the dopamine transport system by SPECT, we synthesized halogenated derivatives of mazindol. Iodomazindol (1) and bromomazindol (2) were obtained by a procedure according to Houlihan (1) (scheme 1) with a high yield. Radioiodinated derivative has been obtained according to Mertens (2) by iodide for bromide nucleophilic exchange (scheme 2). The radioligand (3) was obtained with a yield of 50%, it's a high yield regarding the desactivated ring. (3) was completly removed from the precursor (2) by HPLC procedure : RP 18 column, CH_3OH/1% TEA in CH_3COONH_4 (40/60). So (3) was obtained with no carrier added (specific activity : 74 TBq / mmole).

The affinity of these ligands for the dopamine transporter has been determinated in vitro using rat striatal membrane preparation in competition with [^{3}H]-GBR 12935.

IC_{50} obtained were respectively :

- Mazindol : 100 nM
- Bromomazindol (2) : 100 nM
- Iodomazindol (1) : 250 nM

In conclusion, the affinity of new halogenated derivatives for the dopamine transporter was in the same magnitude than the affinity of mazindol. In vivo experiments in animal are currently performed.

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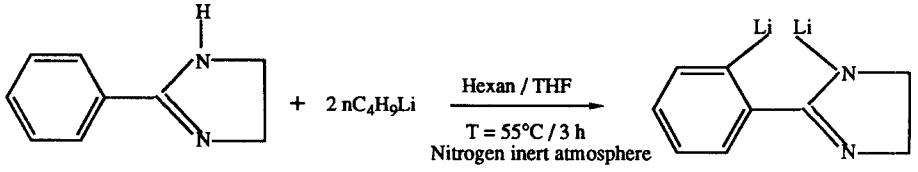
Acknowledgements :

This work was supported by the Région Centre France and by the Franco (INSERM) - VLAAMSE GEWEST projet.

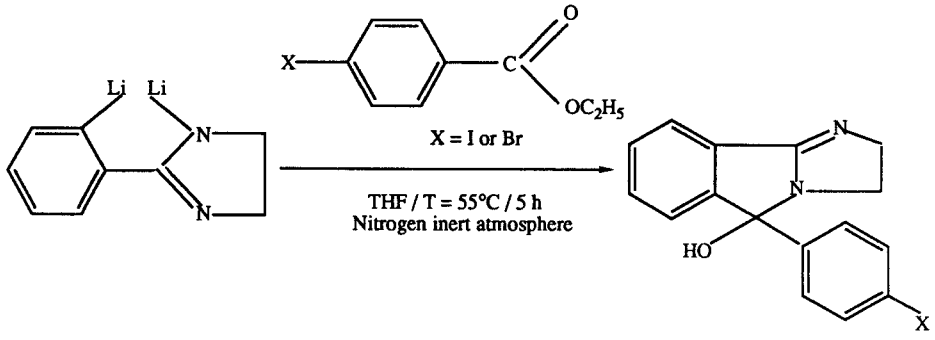
Scheme 1 :

Synthesis of :

- 5-[4-iodophenyl]-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]isoindole (1)
- 5-[4-bromophenyl]-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]isoindole (2)



2-Phenylimidazoline

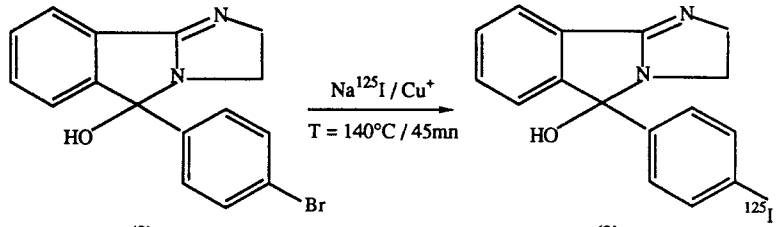


(1) : X = I

(2) : X = Br

Scheme 2 :

Radio synthesis of 5-[4-iodophenyl]-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]isoindole (3)



(2) (3)

Paper E11

S-ADENOSYL-*L*-HOMOCYSTEINE HYDROLASE AS A POTENTIAL TARGET FOR DIAGNOSIS WITH RADIOHALOGENATED NUCLEOSIDES

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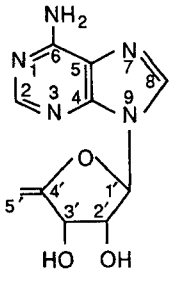
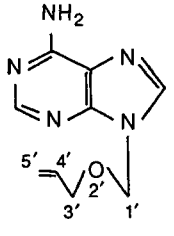
Nucleosides are enzymatically incorporated into nucleic acids, DNA and RNA, which form the basis of mammalian tumor and virus genetic systems and play a critical role in protein and cell-membrane biosynthesis. Of significant importance, however, is the study of enzymes which control nucleotide metabolism. There are several enzymes and at least 85 enzymatic reactions that contribute to the *de novo* biosynthesis of nucleic acids.

The enzyme, *S*-Adenosyl-*L*-homocysteine (SAH) hydrolase, catalyses the reversible hydrolysis of SAH to adenosine and homocysteine through a mechanism involving the oxidation of the 3'-hydroxyl group of the nucleoside substrates with concomitant reduction of enzyme bound NAD^+ to NADH (2). Adenine-based unsaturated sugar nucleoside, 4',5'-didehydro-5'-deoxyadenosine (adenosin-4'-ene) is an intermediary metabolite of SAH and a substrate for NAD⇄NADH redox system. Similar to adenosin-4'-ene (adenosinene), the 5'-fluorinated analogues of adenosinene are also enzymatically oxidized to the corresponding electrophilic substrates which covalently bind to a specific nucleophilic site on SAH hydrolase and render the enzyme inactive (3). The enzyme inactivation is supported by measuring (by ^{19}F NMR) the release of 1 mole of fluoride ion per mole of enzyme inactivated (3).



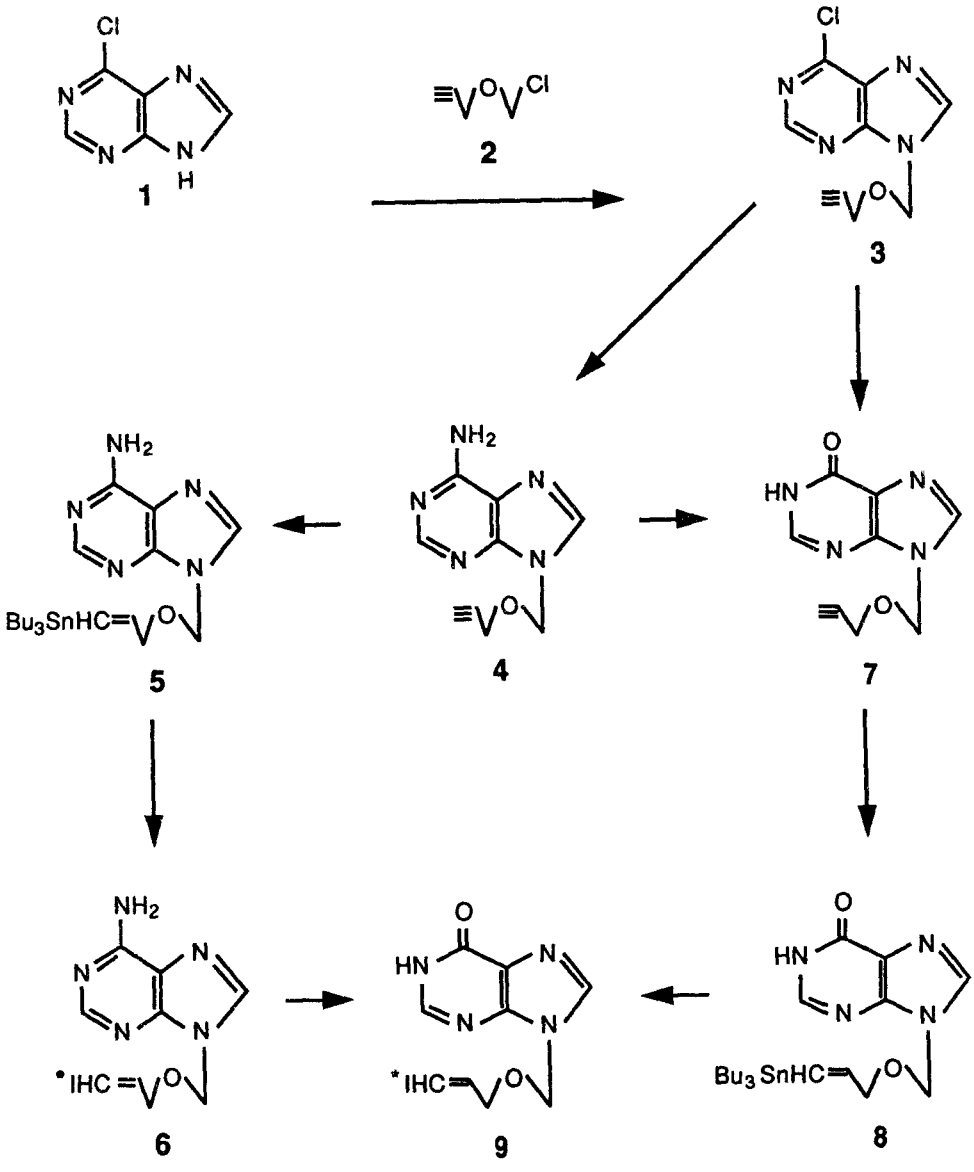
ADENOSINENE



HOMOACYCLOADENOSINENE

Substitution of 5'-halogen in adenosinene analogues with ^{18}F or ^{123}I, ^{125}I, or ^{131}I, and subsequent *in vivo* quantitation by PET or SPECT, may provide a unique and useful means to study SAH hydrolase in intact tissue. The localized differences in enzyme activities in specific tissues may have biochemical significance related to the metabolic abnormality of various tissues including tumor.

In this report we describe the synthesis and biological screening data of a vinylic acyclonucleoside analogue of adenosinene, homoacycloadenosinene, radiolabeled with ^{125}I. The 9-propargyl-oxymethyladenine (**4**) and 9-propargyloxymethylhypoxanthine (**7**) analogues (Scheme I) have been


Cl
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NH2
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O
HN
7
Bu3SnHC
5
*IHC
6
9
Bu3SnHC
8

prepared and converted to the corresponding 5'-tributylstannyl intermediates (5 and 8), respectively, which gave 9-(Z-5-iodo-5-propenyloxymethyl)adenine (iodohomoacycloadenosinene, 6) and 9-(Z-5-iodo-5-propenyloxymethyl)hypoxanthine (9) after iododestannylation. The radioiodination of 5 and 8 provided [^{125}I]-6 and [^{125}I]-9, respectively.

Table 1. Mean Biodistribution and Elimination Data for [^{125}I]Iodohomoacycloadenosinene (^{125}I-6) in Tumor-Bearing Female Balb-C Mice Following Intravenous Administration.[a,b]

Time After Injection	Tumor	Blood	Kidney	Liver	Lung	Thyroid[c]	Urine[d]
1 h	1.50	3.15	3.68	3.40	3.41	1.29	
4 h	0.60	1.33	1.00	1.34	1.30	3.61	
24 h	0.06	0.34	0.20	0.33	0.35	3.49	94.36

[a]Four animals per group were used. [b]ID = 0.28 μCi/1 mg in 0.1 ml saline. [c]Values as % ID/thyroid. [d]Values as % ID/animal.

Biodistribution results (%ID/gm) of [^{125}I-6) in Balb-C mice indicate initial uptake in tumor and other organs with subsequent washout (24 h) of the radioactivity from all the organs except thyroid (Table 1). Approximately 94% of the radioactivity was excreted in the urine (24 h). The TLC analysis (solvent: ethyl acetate, isopropanol, water; 7:1:2; V/V; top layer) of urine by UV and radioscanning indicated the absence of any detectable amounts of either [^{125}I]-6 (control 6: R_f 0.71) or the corresponding deaminase metabolite [^{125}I]-9 (control 9: R_f 0.60). Three major spots on TLC were detected: one radioactive spot (R_f 0.87) was attributed to an iodide. The other two spots (R_f 0.19 and R_f 0.15), visible under UV light were non-radioactive.

Our studies with radiolabeled iodohomoacycloadenosinene indicate that this compound is readily metabolized *in vivo* to a non-radioactive (deiodinated) compound and support dehalogenation, potentially by a mechanism similar to that involving the attack of enzyme hydrolase nucleophile on fluorinated nucleoside substrates (3). These results point out the need for a systematic study of halogenated unsaturated adenine nucleosides for potential evaluation of enzymes of nucleotide metabolism in intact cell and tissue systems.

Research supported by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. The authors wish to thank C. R. Lambert for technical assistance.

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Paper E12

DESIGN OF ^{123}I PRODUCTION TECHNOLOGY ON IAE CYCLOTRON

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123182, USSR

The use of the proton-induced reactions on ^{124}Xe is one of the most effective methods for the production of ^{123}I. The technology has been realized in IAE cyclotron laboratory too[1].

Experimental data on $^{124}Xe(p,x)$ reactions cross-sections have been not published. Therefore, this cross-sections were measured for the proton energy range of 15-32 MeV[2]. To continue this work we have measured[3] the cross-sections of $^{124}Xe(d,3n)^{123}Cs$ and $^{124}Xe(d,p2n)^{123}Xe$ reactions for energy range of 20-29 MeV and compared two methods of ^{123}I production: (p,x) and (d,x). Radionuclide impurity data is also very important. ^{126}Xe, which ^{125}I is formed from, is the main impurity in ^{124}Xe. $^{126}(p,2n)^{125}Cs$ and $^{126}Xe(p,pn)^{125}Xe$ reactions cross-sections were measured in the proton energy range of 13.0-35.5 MeV, and the ^{125}I impurity was calculated for different irradiation times and irradiated gas decay as in the target, as in the decay vessel.

Since 1988 ^{123}I is routinely produced. For 4-hour irradiations with 20 μA 30 MeV proton beam ^{123}I yield is about 10 μCi/μAh, while calculated proton energy losses being 11.8 MeV (without taking into account gas heating). According to excitation functions of $^{124}Xe(p,x)$ reactions, the ^{123}I yield should be 18.6 μCi/μAh. Considering losses due to beam scattering (10 %) and due to gas heating (20 %) the yield should be 13.4 μCi/μAh. The discrepancy of experimental and calculated yields can be explained by ^{123}I carryover from the internal walls of the decay vessel in Xe stream during gas pumping to the storage vessel. Experiments showed, that the shape of the decay vessel, its material and the temperature of its walls during the pumping strongly influence the value of ^{123}I yield.

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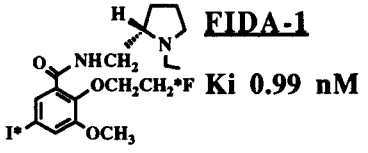
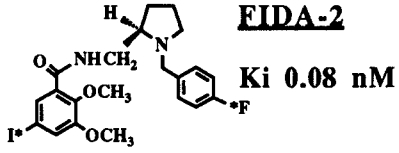
Paper E13

Fluorinated and Iodinated Dopamine Agents (FIDA): D2 Imaging Agents for PET and SPECT. Chumpradit, S.; Kung*, M.P. and Kung, H.F. Department of Radiology and Psychiatry*, University of Pennsylvania, Philadelphia, PA 19104.

In developing new dopamine receptor selective radiopharmaceuticals for routine clinical use, several major criteria must be met: The agents have to demonstrate desired in vitro and vivo properties indicating the agent is receptor selective (high basal ganglia/cerebellum ratio); has easy radiochemical synthesis, exhibits in vivo binding kinetics and metabolic properties suitable for quantitative modeling, shows acceptable radiation dosimetry; and uses a simple and relatively short imaging procedure (data acquisition time in minutes). Many D-2 imaging agents for SPECT and PET imaging have been reported[1-7]. In order to maximize the effort and cost in drug development, we investigated an unique series of dual-labeling D1 and D2 postsynaptic imaging agents, which can take advantage of the best of both modalities--PET and SPECT. In general, it is well recognized that PET has higher resolution, higher sensitivity and better quantitation capability. However, the SPECT has the advantage of being more readily available, cheaper to perform, and without the need of an on-site cyclotron and therefore, technically less demanding. Current status in developing receptor specific ligands is such that the data obtained for PET can not be easily transferred to SPECT and vice versa. The incompatibility is due to the fact that different agents are used for each modality; even though in many cases they are close analogs, but since they are not the same molecule, therefore, pharmacokinetic and metabolic differences prevent their cross comparison. To bridge the gap and to enhance the potential of a single radiopharmaceutical for clinical application, a series of novel dual-labeling D-2 dopamine agents, which can be labeled with either ^{18}F and ^{123}I for both PET and SPECT, were prepared and characterized.





Preparation of related fluorinated benzamide derivatives for PET have been reported previously[8-10]. The desired compounds FIDA-1 and FIDA-2 are prepared by schemes I and II. The corresponding precursors for radioactive iodination were prepared. The cold compounds displayed high affinity to the D2 receptor using rat striatal membrane preparation (Ki = 0.99 and 0.08 nM with ^{125}I-IBF, which showed a Kd value of 0.07 nM). In vivo biodistribution study in rats also exhibited high selective concentration in the striatal area of the brain. The target to background ratio (striatal/cerebellar ratio) reached 29.3 and 13.1 at one hr post injection for FIDA-1 and FIDA-2, respectively. Preliminary data suggest that this series of compounds are potentially useful D2 dopamine receptor imaging agents for PET and SPECT. By using the same molecule for two imaging modalities, one can expect the same pharmacological profile, same toxicology and same pharmacokinetics (to the extent that the biodistribution of the ^{123}I and ^{18}F labeled parent compound is the same). However, the similarities and differences of the biodistribution of metabolite(s) of different ^{123}I and ^{18}F moieties will have to be investigated and validated.

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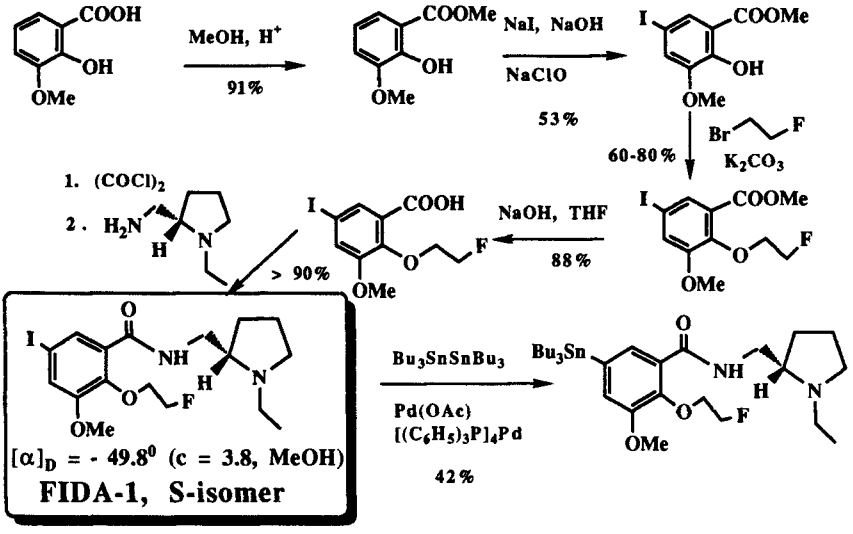
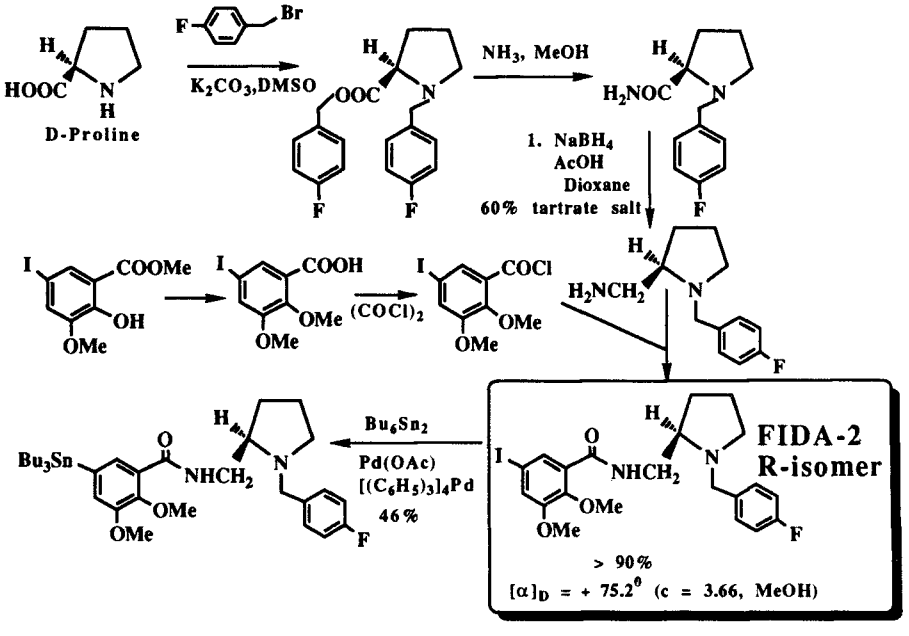
Supporting Data: Chumpradit et al

Scheme I. Synthesis of FIDA-1



Scheme II. Synthesis of FIDA-2



Paper E14

SYNTHESIS AND TARGET TISSUE UPTAKE OF THE 7α-METHYL AND 11β-METHOXY DERIVATIVES OF THE 20Z AND 20E ISOMERS OF 17α-[^{125}I]IODOVINYL-2- AND -4-FLUOROESTRADIOLS.

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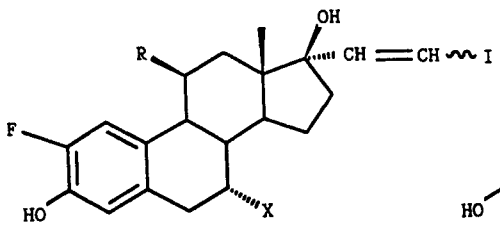
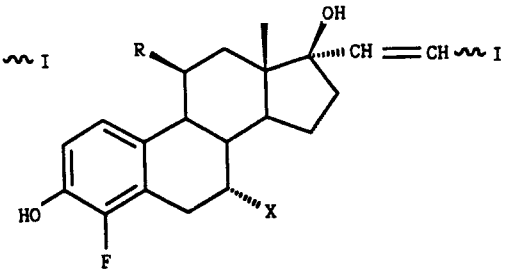
The nature and site of substituents onto estradiol are critical factors in the design of an improved estrogen receptor (ER) binding radiopharmaceutical for imaging healthy and malignant endocrine tissues. In the case of radioiodination, the bulky and electro-negative halogen must be inserted at a stable position on the steroid molecule while exerting minimal interference with the exquisite interaction between the hormone and the ER. Among the radioiodinated estrogens proposed for nuclear imaging, the 20E and 20Z isomers of 17α-iodovinylestradiol exhibit good *in vivo* stability. Added 7α-methyl or 17β-methoxy substituents further enhances binding to the ER resulting in improved localization in ER-rich target tissues (1-3). Addition of 2- and 4-fluoro substituents onto estradiol also augments *in vivo* stability of the parent molecule while preserving good binding affinity for the ER (4). On such accounts we evaluated the effect on ER binding affinity, *in vivo* stability and target tissue uptake, of added 2- or 4-fluoro substituents onto the isomeric iodovinylestradiol derivatives.

Commercially available 2- and 4-fluoroestradiols were converted to the corresponding 17α-ethinyl derivatives. The analogues 11β-methoxy and 7α-methyl derivatives were prepared *via* direct A-ring functionalization with an electrophilic fluorination agent, N-fluoropyridinium triflate. The fluorination of 11β-methoxy- and 7α-methylestrone gave 2- and 4-fluoro derivatives which were subsequently converted to the 17α-ethinyl derivatives, and purified by HPLC. We have previously reported that stereospecific formation of the 20E and 20Z tri-n-butyltin derivatives can be controlled by the choice of catalyst, the polarity of the solvent, and the reaction temperature (1). The selectivity of the method was further improved by the use of either bis(triphenylphosphine)palladium(II) chloride, palladium(II) acetate or triethylborane as a catalyst. Reacting the acetylenic estrogen derivatives with tributyltin hydride in the presence of a palladium catalyst for a period of 10-15 min, at room temperature, gave exclusively the Z isomer. Changing the catalyst for triethylborane while maintaining the other reaction conditions the same,

mainly gave the 20Z isomer. The isomeric tin intermediates were purified by HPLC and reacted with iodine in chloroform to yield the corresponding (17α,20E)- and (17α,20Z)-iodovinylestradiols in 70-90% yield. The corresponding isomeric products **1a-c** and **2a-c** were characterized by ^{1}H NMR and mass spectral analysis. The analogues ^{125}I-derivatives were obtained by treatment of the corresponding tin intermediates with [^{125}I]NaI in the presence of H_2O_2.



1



2

a: R = X = H

b: R = OCH_3; X = H

c: R = H; X = CH_3

Relative binding affinities (RBA) of the estradiol derivatives for ER were estimated *via* a competitive binding assay. The 4-fluoro derivatives exhibited higher RBA values than the corresponding 2-fluoro derivatives, while the 20Z isomers exhibited higher RBA values than the 20E isomers, i.e. **1a**(20E) = 6 *versus* **1a**(20Z) = 53; **2a**(20E) = 55 *versus* **2b**(20Z) = 66; estradiol = 100. Tissue distribution and uterus uptake of [^{125}I]**1a-c** and [^{125}I]**2a-c** was studied in immature female Long Evans rats. All 4-fluoro derivatives (**2**) were better uterus localizers than the corresponding 2-fluoro derivatives (**1**). Except for **2b**(20E) and **2c**(20E), the 20Z isomers exhibited more persistent uterus retention. This most likely reflects the higher instability of the 20Z isomers, which was also evident from higher thyroid uptake. Among these isomeric estrogens the 4-fluoro-11β-methoxy(17α,20E)iodovinylestradiol (**2b**) showed the best uterus localization properties and the highest uterus to blood/nontarget tissue ratios.

Supported by a grant from the Medical Research Council of Canada.

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Paper E15

Radiopharmaceutical development of ^{123}I-3-iodotyrosine(A14)-insulin, a site-specific labeled ligand for in-vivo insulin-receptor scintigraphy.

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In patients with type II diabetes insulin action is impaired, probably caused by defects of insulin-receptors located mainly in the liver (1). For the study of these receptors in-vivo, an insulin ligand labeled with ^{123}I specifically at the A-chain Nr. 14 tyrosine residue (A14) was required (2).

Since insulin contains 4 tyrosine residues at positions 14 and 19 in the A-chain, 16 and 26 in the B-chain, radioiodination will yield a mixture of iodine-isomers requiring HPLC-separation. On the basis of reversed phase peptide HPLC (3) we developed a high resolution separation aiming at low MeCN concentration and replacement of TFA by a physiologic buffer to preserve receptor-binding capability, as well as isocratic elution for fast preparative application. The final system consisted of a Hypersil BDS C18 5 µm, 4 x 250 mm column eluted with a mixture of 0.2M NH_4OAc, 0.01M NaH_2PO_4/H_3PO_4(pH3):MeCN:i-Propanol 70:20:10 % v/v at 1 ml/min and separating the A19-, B26-, B16- and A14-ITyr-insulin isomers in that sequence (Fig. 1).

In order to achieve mono-iodination and to preserve receptor-binding insulin must not be exposed to an excess of electrophilic iodine and oxidising agent. Therefore radioidination by enzymatic methods and by a solid-phase oxidant was studied.

A. Solid phase enzymatic system of glucose oxidase (GO) and lactoperoxidase (LPO) immobilized on hydrophilic spheres: on addition of glucose GO generates a trace amount of H_2O_2 which, catalyzed by LPO, oxidises ^{123}I-NaI to electrophilic iodine (4). Using this system and varying insulin-, iodide-concentration and pH we obtained HPLC-isolated yields (Y) of 12 - 37 % A14 (Table 1). A particular problem of this system was that the spheres had to be separated from the solution before HPLC and retained significant proportions of radioactivity.

B. Enzymatic iodination in homogeneous solution using very low concentrations of H_2O_2 and 5 µg dissolved LPO (5). Insuline-, iodide- and H_2O_2-concentration, pH and reaction time were studied as parameters. Best results were achieved with 0.2 mM insulin, 0.01 mM iodide (trace amount of carrier added to ^{123}I-NaI) and 0.012 mM H_2O_2 in citrate-buffer pH 5.6. Upon prep. HPLC of ~ 100 µl reaction mixture A14 was isolated in 53 ± 5 (n = 13) % Y (Table 2).

C. Finally the solid-phase oxidant Iodogen, useful for radioiodination of sensitive proteins, was employed. Using common Iodogen coating technique, labeling could not be made reproducible. However by reaction with 1 µg Iodogen in suspension for only 1 min, followed by prep. HPLC the highest Y of 70±3(n=5) % A14 was obtained (Table 3) (Fig. 1). The isolated A14-peak, protected by addition of 1 mg HSA, was vacuum evaporated, formulated in PBS/0,5 % HSA and sterile membrane filtered, overall product-Y ~ 60 %. The good Y and reliability of method C enabled clinical application of ^{123}I-ITyr(A14)insulin.

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Table 1: Solid-phase enzymatic ^{123}I-labeling of insulin[1)]

insulin nmol / 100 µl	NaI nmol / 100 µl	pH	react. time min	HPLC % ^{123}I in reaction mixture*) inorg.I	ITyrA19	ITyrA14
1	0.02	7.2	30	65	7	12
2	0.02	7.2	30	47	8	26
5	0.02	7.2	30	36	8	29
10	0.5	7.2	30	43	5	30
10	0.5	5.6	30	29	9	37

*) mean of 2-3 experiments
1) 50 µl Enzymobead-suspension/100 µl reaction mixture

Table 2: Enzymatic ^{123}I-labeling of insulin using 5 µg dissolved LPO[1)]

insulin nmol / 100 µl	NaI nmol / 100 µl	H_2O_2 nmol / 100 µl	pH	HPLC % injected ^{123}I*) inorg.I	ITyrA19	ITyrA14
2	0.5	0.6	5.6	30	11	32
10	0.5	0.6	5.6	23	10	39
20	0.5	0.6	5.6	20	8	46
20	0.1	0.12	5.6	35	11	39
20	1.0	1.2	5.6	14±6	12±2	53±5 (n=13)
10	0.5	0.6	7.0	10	51	25
10	0.5	0.6	4.6	40	10	30
20	1.0	1.2	5.6[2)]	16	11	51

*) mean of 2-3 experiments
1) reaction time 15 min
2) reaction time 30 min

Table 3: ^{123}I-labeling of insulin using Iodogen in suspension at pH 7.4[1)]

insulin nmol / 100 µl	NaI nmol / 100 µl	Iodogen nmol / 100 µl	react. time min	HPLC % injected ^{123}I*) inorg.I	ITyrA19	ITyrA14
20	1	2.3	15	6	12	57
20	0.25	0.57	1	14	16	53
20	0.5	1.15	1	6	11	68
20	1	2.3	1	4.5±1.3	13±4	70±3(n=5)

*) mean of 2-3 experiments
1) 0.5 mg Iodogen in 0.1 ml acetone mixed with 4.9 ml PBS, 2.5, 5 or 10 µl of mixture used per 100 µl reaction volume.

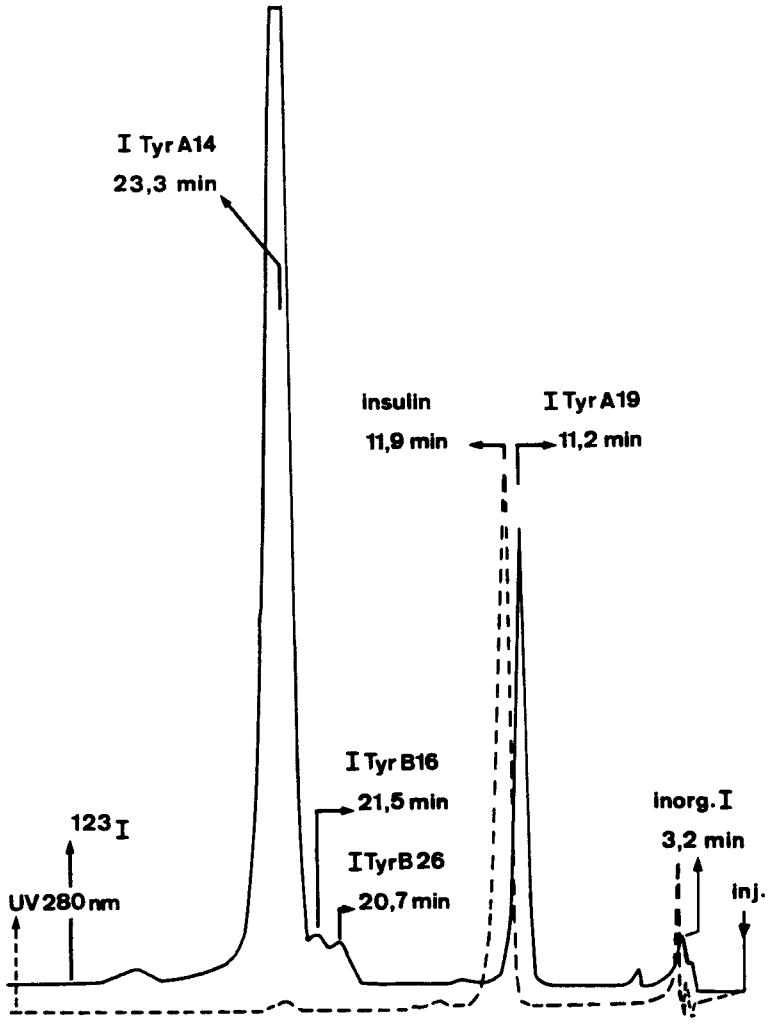

Fig. 1. Preparative HPLC of insulin ^{123}I-iodination with Iodogen-suspension. Column: Hypersil BDS C18 5 µm, 4 x 250 mm. Eluent: 0.2M NH_4OAc, 0.01M NaH_2PO_4/H_3PO_4 (pH3):MeCN:i-Propanol 70:20:10 % v/v at 1 ml/min. Detectors in series: 1st = Scintillation det. (^{123}I), 2nd = UV 280 nm.

Paper E16

RADIO-EMBOLISMIC THERAPY AGENT FOR LIVER CANCER---A HIGH YIELD RADIOIODINATION OF IOPHENDYLATE

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Radioiophendylate has been clinically applied in the redioembolismic therapy of liver cancer. Clinical applications demonstrated that radioiophendylate can be injected into the liver via artery intubation and selectively concentrated in liver tumours. It embolises in the blood capillary and blocks the blood supply, thus controlling the propagation of the tumour cell. Embolism and radiation therapy were able to control the growth of hepatocellular carcinoma and reduce the size of the tumour. The Liver Cancer Institute of Shanghai Medical University(1) had applied I-125(I-131)-iophendylate to the treatment of liver cancer using a comprehensive therapy of radioiophendylate and followed by surgical excision.

The chemical name for iophendylate is Ethyl-10-(p-iodophenyl) undecylate. In our search for a simple radioiodinated method with a high labelled yield, we employed a nucleophilic isotopic exchange, based on the use of Cu(I) in an acidic medium in the presence of an excess of reducing agent (method by J. Mertens(2)). We studied the labelling conditions of this method and the factors influencing the labelling yield.

Labelling procedure: NaI-125(NaI-131) 10mCi were added to a nitrogen-filled closed penicilline vial containing iophendylate 100mg, ascorbic acid 16mg, gentisic acid 8mg, copper sulfate saturation solution 0.010ml, acetic acid(glacial) 0.15ml, and ethanol(anhydrous) 0.85ml. The vial was heated in a boiling water bath for 60 min., with agitation. After completion of the reaction, radioiophendylate was washed with water, the solvent removed and the labelling compound dried by vacuum. The purity was verified by chromatography using an ITLC Silica Gel plate solvent system consisting of toluene: chloroform(1:1 v/v). Chromatograms were scanned and sectioned into 0.5cm strips and counted for radioactivity using LKB-1282 Compu Gamma, labelling product Rf=0.75, free iodine Rf=0.

Solution influence on labelling yield: Labelling yield of reaction in solvents of different acetic acid/ethanol ratio. The percentage of glacial acetic acid used was 0%, 5%, 10%, 20%, 30%, 40% respectively in ethanol. The results are shown in figure 2. It was found that the labelling efficiency(>90%) was the highest in a mixture containing 15% acetic acid and 85% ethanol.

Reaction temperature to labelling yield influence: The temperature of the reaction was varied from 80^0C-120^0C, while keeping the other reaction conditions constant. The results are summarized in figure 3. The labelling yield was found to be greater than 90% when the temperature was 100^0C. However, the labelling yield decreased when the temperature was increased above 100^0C.

Reaction times to labelling yield influence: The relationship between reaction time and the labelling yield is shown in figure 4. It was found that the labelling yield increased with increasing reaction time. The labelling yields were constant(>90%) when the reaction time was longer than 60 min..

Influence of different amounts of saturated $CuSO_4$: When using $CuSO_4$ in an acidic

medium using an excess of reducing agent, the $CuSO_4$ is reduced into Cu(I) by ascorbic acid and gentisic acid. The influence of copper sulfate on the labelling yield is shown in figure 5. The results show that an optimum labelling yield results when 0.010ml of copper sulfate solution is used.

In summary: The optimal radioiodination of iophendylate conditions are: acidic acid/ethanol=15%, temp. 100^0C, time 60 min. and $CuSO_4$ 0.010ml for labelling 10mCi iophendylate. Using the above conditions, the labelling yield is 93.26%(n=13 Max=95.71% Min=89.32%).

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Fig. 2 Labelling yields of reaction in solvent of different acetic acid/ethanol ratio. The percentage of ice acetic acid: 0%, 5%, 10%, 20%, 30%, 40%,

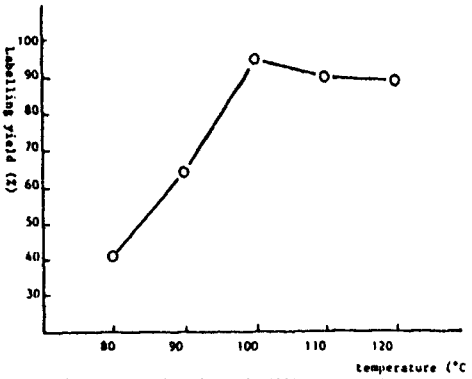

Fig. 3 Labelling yields in different reaction temperature: 80°C, 90°C, 100°C, 110°C, 120°C.

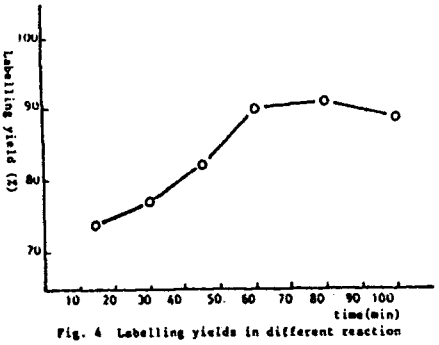

Fig. 4 Labelling yields in different reaction time: 15min, 45min, 60min, 80min, 100min.

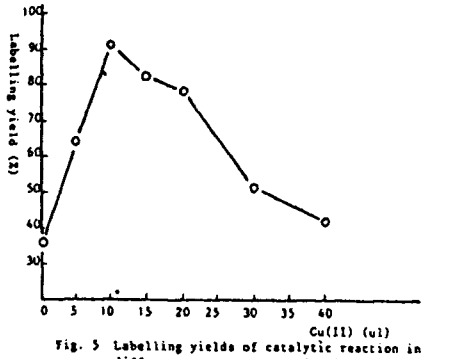

Fig. 5 Labelling yields of catalytic reaction in different amount saturated $CuSO_4$ solution: 0, 5ul, 10ul, 15ul, 20ul, 25ul, 30ul, 35ul, 40u

Paper E17

EXCITATION FUNCTIONS OF DEUTERON-INDUCED NUCLEAR REACTIONS ON ^{124}Xe: PRODUCTION OF ^{123}I

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Both a cross sections of $^{124}Xe(d,2n)^{123}Cs$, $^{124}Xe(d,p2n)^{123}Xe$ reactions and ^{123}I differential yields over the deuterons energy range from the thresholds to 29 MeV were measured using enriched ^{124}Xe (99,98 %). The methods of a measurement of deuteron beam intensity and activities of forming radionuclides and also the shape of irradiated capsules with xenon and the target system design were described in the article by Kurenkov et al.(1989) on proton irradiation of xenon. The results are given in the table. ^{123}I yield data received on natural xenon by Tarkanyi et al. (1989) exceed our values.

Cross sections of deuteron-induced reactions on ^{124}Xe and differential ^{123}I yields

Energy, MeV	Cross section, mbarn		Diff. I-yield,
	$^{124}Xe(d,3n)$	$^{124}Xe(d,p2n)$	μCi/(μAh·MeV)
20.2	63±9	-	-
20.9	69±10	87±18	147±13
21.9	151±15	75±12	228±33
23.0	163±16	154±22	292±40
24.2	267±22	185±22	399±47
25.2	304±24	238±26	500±50
25.7	298±23	132±15	413±49
27.0	410±28	287±27	620±62
27.8	468±31	288±27	667±63
29.2	453±30	444±39	719±68
29.2	612±80	-	-

References:

Kurenkov N.V., Malinin A.B., Sebyakin A.A., Venikov N.I. (1989). Excitation functions of proton-induced nuclear reactions on ^{124}Xe: production of ^{123}I. J. Radianal. Nucl. Chem., Letters. 135 (1), 39-50.

Tarkanyi F., Kovacs Z., Qaim S.M., Stöcklin G. (1989). Differential production yields of ^{123}I. Radiochim. Acta, 47, 25-27.

Paper E18

Alternate Syntheses of (*S*)-*N*-[(1-Ethyl-2-Pyrrolidinyl)Methyl]-3-[^{125}I]Iodo-5,6-Dimethoxysalicylamide (Ioxipride) and (*S*)-*N*-[(1-Ethyl-2-Pyrrolidinyl)Methyl]-5-[^{125}I]Iodo-2,3-Dimethoxybenzamide (Epidepride): High Affinity and Selective Dopamine D-2 Receptor Radioligands.

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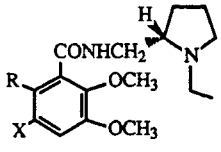
Several radioiodinated ligands (I-125 and I-123) have been developed for purposes of assaying dopamine D-2 receptors, both *in vitro* as well as *in vivo*. Several of these agents labeled with iodine-123 have been used in *in vivo* experiments, both in primates and humans using single photon emission computed tomography (SPECT). Substituted benzamides have attracted particular attention due to their selective, high affinity and reversible nature of binding at the D-2 receptor site. Within this class of compounds, [I-123]IBZM has been extensively studied as a potential imaging agent. More recently, several new high affinity compounds have been synthesized which have the potential of being successfully developed as SPECT radiotracers. Some of these are [I-123]IBF, [I-123]epidepride, [I-123]NCQ 298 (also known as ioxipride). An additional advantage of these high affinity agents is their capability to provide information about extrastriatal dopamine receptors. We have investigated alternate syntheses and radiosyntheses of (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-3-[^{125}I]iodo-5,6-dimethoxysalicylamide (ioxipride) and (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-[^{125}I]iodo-2,3-dimethoxybenzamide (epidepride) both of which have been recently reported to be selective, high affinity antagonists for the dopamine D-2 receptor and are therefore suitable candidates for use in SPECT imaging.

Figure-1



1. R= OH, X = I Ioxipride
2. R= H, X = I Epidepride
3. R= OH, X= ^{125}I
4. R= H, X= ^{125}I

Synthesis of (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-3-iodo-5,6-dimethoxysalicylamide (ioxipride) was accomplished in three steps from 2,3-dimethoxybenzoic acid in 20% yield. Reported synthetic methods of 5,6-dimethoxysalicylic acids relied on tedious efforts required to demethylate the 2,6-dimethoxy substituted benzoic acids (or benzamides), followed by separation and purification of the reaction mixture in order to obtain the desired product. Bengston and Hogberg recently reported selective hydroxylation of 2,3-dimethoxybenzamides by first generating the anion regiospecifically followed by oxidation of the anion. We have used one-electron oxidations using cupric salts of 2,3-dimethoxybenzoic acid in order to obtain 5,6-dimethoxysalicylic acid. The pure dimethoxysalicylic acid was then reacted with diethylaminosulfur trifluoride to obtain the corresponding acid fluoride which was then treated with (*S*)-2-(aminomethyl)-1-ethylpyrrolidine to provide (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5,6-dimethoxysalicylamide. Radioiodination of (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-5,6-dimethoxysalicylamide with sodium [I-125]iodide and chloramine-T provided [I-125]ioxipride in 70-80 % radiochemical yield with specific activities of 2000 Ci/mmol after RP-HPLC purification.

Synthesis of (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide (epidepride) was accomplished in six steps from 3-methoxysalicylic acid (see supporting data). 3-Methoxysalicylic acid was brominated to provide regiospecifically the 5-brominated product. Refluxing with dimethylsulfate provided the methyl ester of 5-bromo-2,3-dimethoxybenzoic acid. This was saponified to provide the corresponding benzoic acid which was then converted either to the acid chloride or acid fluoride. The acyl fluoride/chloride was reacted with (*S*)-2-(aminomethyl)-1-ethylpyrrolidine to provide (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-5-bromo-2,3-dimethoxy-benzamide. Reaction of the bromo derivative with bis(tri-n-butyltin) in the presence of catalytic

amounts of tetrakis(triphenylphosphine)palladium(0) provided (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-5-(tri-n-butyltin)-2,3-dimethoxybenzamide. Radioiodination of (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-tri-n-butyltin-2,3-dimethoxybenzamide with sodium [I-125]iodide and chloramine-T provided [I-125]epidepride in 80-90 % radiochemical yield with specific activities of 2000 Ci/mmol.

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Supporting Data

Figure-2: Synthesis of Ioxipride

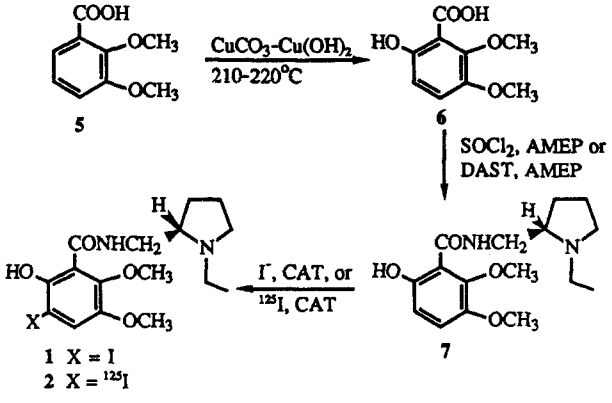


Figure-3: Semi-preparative HPLC purification of [I-125]ioxipride using acetonitrile-2 mM H_3PO_4, (5% acetonitrile to 70% acetonitrile in 22 minutes, at a flow rate of 3 mL/min. Ultraviolet peat at 12.7 min was the starting material. The radioactive peak at 4.90 min was unreacted iodide-125 and at 16.4 min was [I-125]Ioxipride.

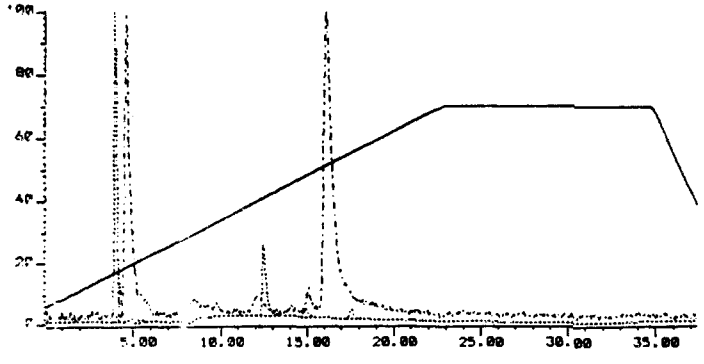


Figure-4: Synthesis of Epidepride

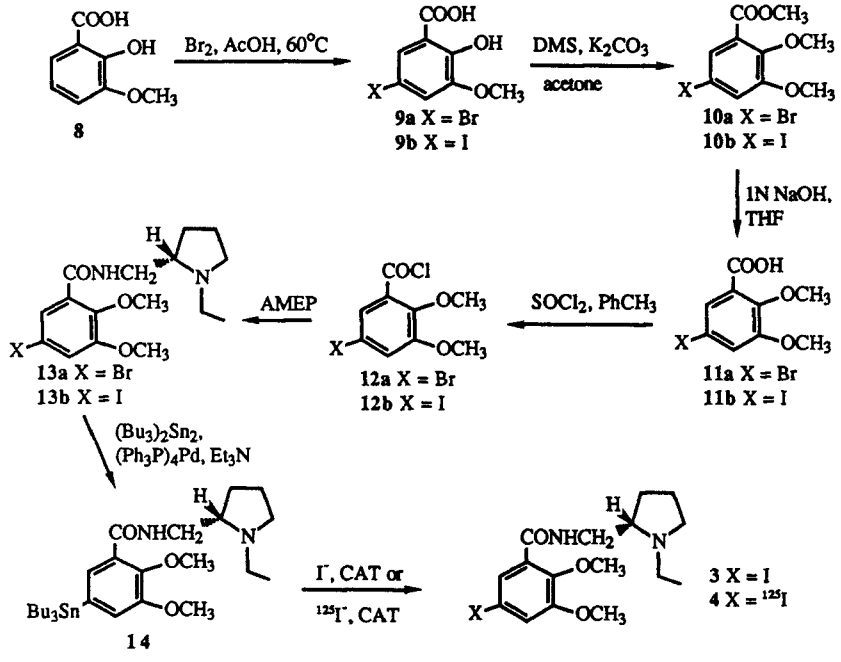
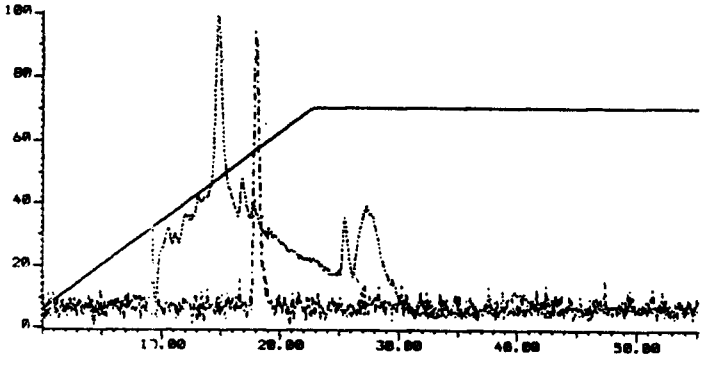


Figure-5: Semi-preparative HPLC purification of [I-125]epidepride using acetonitrile-2 mM H_3PO_4, (5% acetonitrile to 70% acetonitrile in 22 minutes, at a flow rate of 3 mL/min. Ultraviolet peat at 25.3 min was the starting material. The radioactive peak at 18.1 min was [I-125]epidepride.



Paper E19

SYNTHESIS OF IODINE-123 LABELED 3-N-ALKYL-5-IODO-2-THIENYL-BUTYROPHENONES FOR SPECT ANALYSIS OF DOPAMINE RECEPTORS

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A variety of neuropsychiatric diseases have been associated with abnormalities in dopaminergic synaptic transmission. Several iodine-123 (1-5) analogs of spiperone, a potent neuroleptic drug with high affinity for dopamine (D2) receptors, have been synthesized as potential radiotracers for imaging and quantifying dopaminergic receptor sites in the brain using single photon emission tomography (SPECT) techniques. Among the iodinated analogs of spiperone evaluated 2'iodospiroperidol (2'-ISP) showed the highest dopamine receptor affinity and appears to be the best candidate for SPECT imaging. Radioiodination of 2'-ISP is carried out via iodine exchange or the Balz-Schiemann reaction. Unfortunately, these methods are not readily adaptable to a "no carried added" scale for the preparation of very high specific activity products which is crucial for receptor imaging. In order to provide a iodine-123 labeled high affinity dopamine receptor radioligand which can be readily labeled by "no carrier added" iododemetallation methods we report the development of the synthesis of iodine-123 labeled 3-N-alkyl-5-iodo-2-thienylspiroperidol analogues where N=methyl (19), ethyl (20), propyl (21) and butyl (22).

Compounds 19-22 were prepared by the synthetic approach described in Figure 1. Compounds 15-18 were radioiodinated with 'no-carrier added" $Na^{123}I$ and chloramine-T, in EtOH and pH=7.0 phosphate buffer (Table 1 and 2). The dopamine, and serotonin receptor binding affinities and striatum to cerebellum ratios of compounds 19-22 will be presented.

Table 1. Radiochemical Yields for ^{123}I-Iodination of Tin Substrates

Substrate	Radiochemical Yield (%)
15	90%
17	66%
18	75%

2mg of substrate (3 µmole) in 1 mL 100% ETOH, 0.1 mL 0.06 M phosphate buffer (pH=7.0), 0.1 mL 0.02 M chloramine T at 25 C. TLC Al_2O_3 CH_2Cl_2:MeOH 75:1 Rf=0.9.

Table 2. Radiochemical Yield for Removal of Ketal

Product	Radiochemical Yield (%)
19	64%

1 mL 0.4 M HCl in MeOH at 50 C 5 min. TLC Al_2O_3 CH_2Cl_2:MeOH 75:1 Rf=0.71.

Research supported by the Office of Health and Environmental Research, U.S. Department of Energy.

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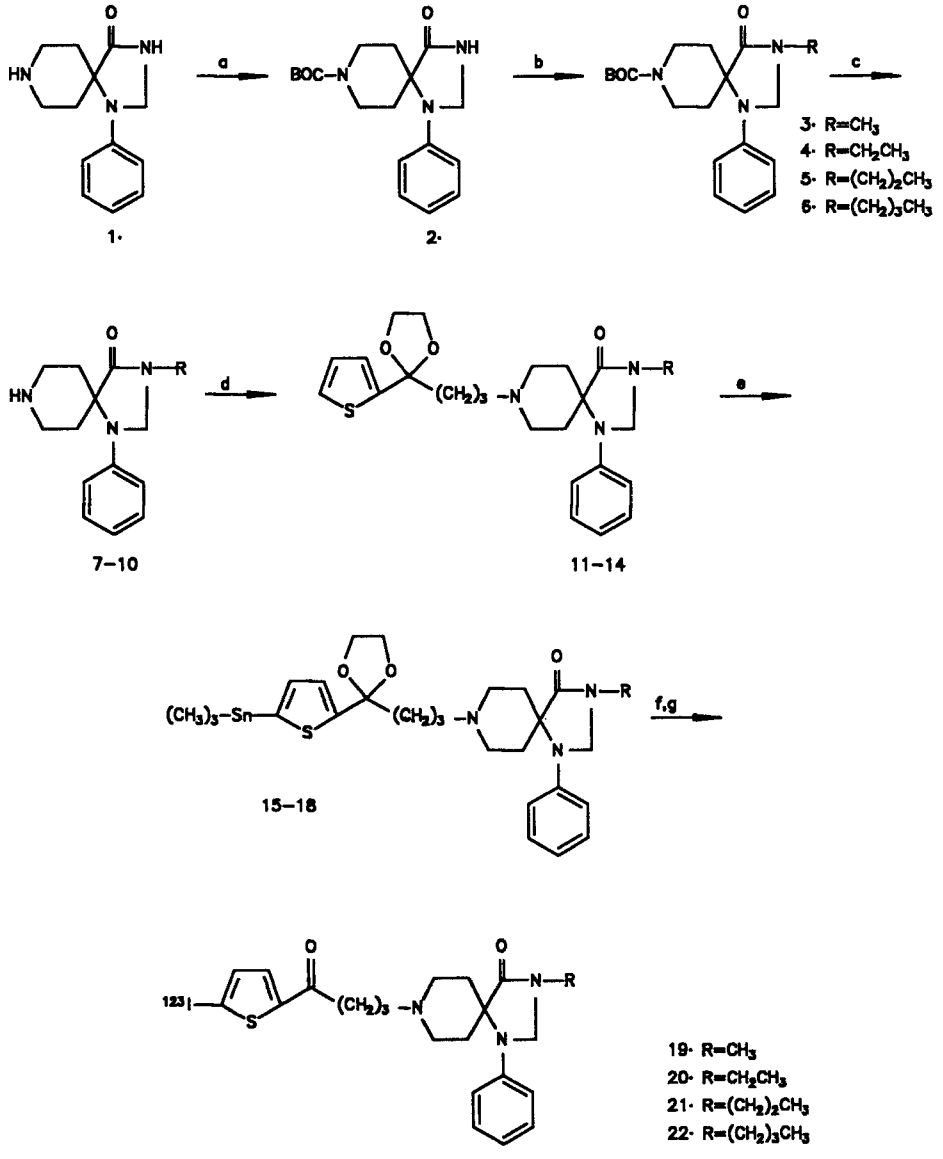

Reagents: a = BOC–ON/N–Et_3,CH_2Cl_2; b = CH_3I,CH_3CH_2I,$CH_3(CH_2)_2I$,$CH_3(CH_2)_3I$/NaH,THF;

c = 4M HCl/Dioxane; d =  /K_2CO_3,KI,DMF; e = n–BuLi,Et_2O/$(CH_3)_3SnCl$;

f = Chloramine–T,EtOH,Na^{123}I,Na_2PO_4 PH=7; g = 40:1 CH_3OH:HCl

Figure 1

Paper E20

20E - and 20Z-17α - iodovinylestradiol and 20E- and 20Z- 17α – iodovinyl – 11 β methoxyestradiol : Synthesis and biological properties comparison.

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We present the synthesis of 20E- and 20Z-17α- iodovinylestradiol (IVE2) and 20E- and 20Z-17α- iodovinyl-11β methoxyestradiol (MIVE2), in order to compare their biological properties.

In 1988, Ali and al. presented a synthetic way to enhance the formation of 20E- IVE2 or 20Z- IVE2, according to the solvent used. We extend this route to the methoxy derivatives. Both labeled and unlabeled iodide compounds were synthesized from their respective tributyl-tin precursors. These latter came from the action of the tributyl-tin hybride on the ethynyl derivative in the adequat solvent (scheme 1). The four precursors and each corresponding iodide compound were purified by HPLC and their structures determined by NMR.

Biological behaviors on the trans isomers (20E-IVE2 and 20E- MIVE2) were compared in-vitro and in-vivo. The dissociation constant of the two derivatives were determined in-vitro using cytosol from immature sheep. The scatchard analysis of our data gives the following values : Kd(20E-IVE2) = 11.10^{-10} M and Kd (20E-MIVE2) = 16.10^{-10}M. We conclued that in-vitro, the two trans derivatives present about the same affinity for estrogen receptor.

The in-vivo distribution realized in 21 day mice (n= 10) shows that 20E-[^{125}I]-MIVE2 presents a best affinity for the target tissue. Indeed, we obtained the following uterus/blood ratio : 42 for the 20E-[^{125}I]-MIVE2 and 13 for 20E-[^{125}I]-IVE2. This result can be explained by the lower fixation of the 20E-[^{125}I]-MIVE2 on the plasma proteins due to the presence of the methoxy (Raynaud et al., 1973). Coinjection of unlabeled estradiol shows that binding of the two components is specific.

The best affinity of the methoxy derivative leads us to compare the in-vivo affinity of 20E-[^{125}I]-MIVE2 and 20Z-[^{125}I]-MIVE2, the trans derivative usterus/blood ratio is 16 times higher than the cis one.

In conclusion, 20E-[^{125}I]-MIVE2 shows a good affinity for estrogen receptor and these results are in agreement with other reports (Hanson et al., 1991). In-vivo distribution in immature mice demonstrates the high 20E-[^{125}I]-MIVE2 affinity for uterus. These encouraging results indicate that 20E-[^{123}I]-MIVE2 could be useful for estrogen receptor exploration.

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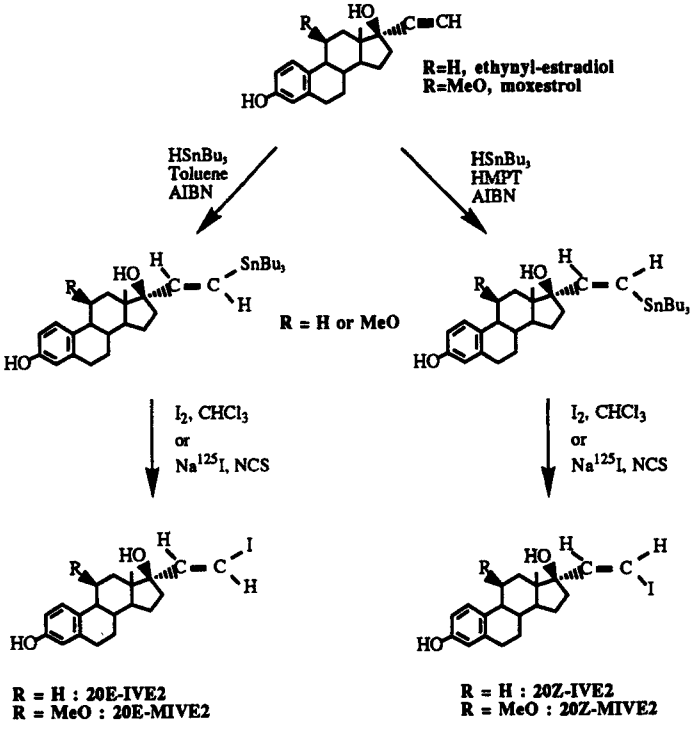
Aknowledgements :We thank Cis Biointernational compagny and the "Association pour la Recherche contre le Cancer " for their financial supports.



Scheme 1 : Synthesis

LABELLING OF METYRAPONE DERIVATIVES WITH RADIOIODINE - IN VITRO AND IN VIVO STUDIES

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2-Bromo-phenyl-metyrapone showed higher 11ß-hydroxylase inhibition activity when compared with a number of metyrapone derivatives (1,2). Metyrapone, metyrapol and phenyl-metyrapone showed almost identical binding. 4'-bromo-metyrapone and metyrapone-mono-N-oxide are weaker binders. Derivatives of phenyl-metyrapone substituted in 2-position showed the highest binding affinity.

When exchanging bromine for iodine in the 2-position a potential tracer for SPECT is obtained. NCA labelling is achieved by Cu^{+1} assisted non-isotopic exchange in reducing conditions during 60 minutes (3). The parameters affecting labelling were studied using increasing amounts of precursor (0.1 - 1 mg), reaction time (up to 1 h), and temperature (60 - 100°C). The importance of Cu^{+1} - catalyst for the exchange reaction was demonstrated. Without the addition of Cu^{+2}- ion, no labelling is obtained. Because of the activated 2-position, 100°C is sufficient for the exchange of bromine with more than 95% incorporation of ^{123}I.

[^{123}I]-2-iodophenyl-metyrapone was used for in-vitro binding studies and for the biodistribution in rats. The apparent K_D - value (14 nM) indicated a higher binding affinity to adrenocortical mitochondria than observed with the original metyrapone tracer. When injected, adrenal uptake was 1.7% of the intravenously injected activity.

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Paper E22

A Solid Xenon Ice Target for the Production of I-123

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The use of the $^{124}Xe(p,2n)^{123}Cs \rightarrow ^{123}Xe \rightarrow ^{123}I$ nuclear reaction sequence has resulted in the ability of centers with medium energy cyclotrons to produce very high purity iodine-123. The use of this very high purity iodine results in a lower patient dose in Nuclear Medicine studies and makes it possible to study receptor systems in vivo using iodine labeled radiopharmaceuticals. One of the problems with the use of xenon-124 as a target material is the very high cost of the enriched isotope. This cost makes the possibility of a foil failure resulting in the loss of the target gas a very serious prospect. Recently a solid water ice target has been described for the production of fluorine-18 (1). We have adapted this technology in the design of a xenon ice target for the production of I-123.

The target consists of a copper cone which is immersed in liquid nitrogen (see Figure 1). The liquid nitrogen cooling bath keeps the xenon in the form of a solid during irradiation. The heat transfer through the solid is sufficient that the xenon ice does not melt during irradiation. The angle of the cone is 15° to the perpendicular which allows the use of a relatively small quantity of the gas to form the solid layer (about 0.4 liters of gas at STP). The second advantage is that if there is a catastrophic failure of the foil, the gas will not be lost since it is still at liquid nitrogen temperature. After irradiation, the liquid nitrogen is evaporated and the xenon gas transferred to a storage vessel for decay to I-123.

Preliminary results with a prototype target demonstrate that I-123 can be produced with this target at levels similar to those obtained with the gas target (2). More extensive yield measurements with a production type design are currently under way. The primary advantages of this target are the low inventory of enriched xenon required for the target and the reduction of the risk of loss due to catastrophic failure of the foil. **Research carried out under contract DE-AC02-76CH00016 with the USDOE/OHER and also by the NIH Grant NS-15380.**

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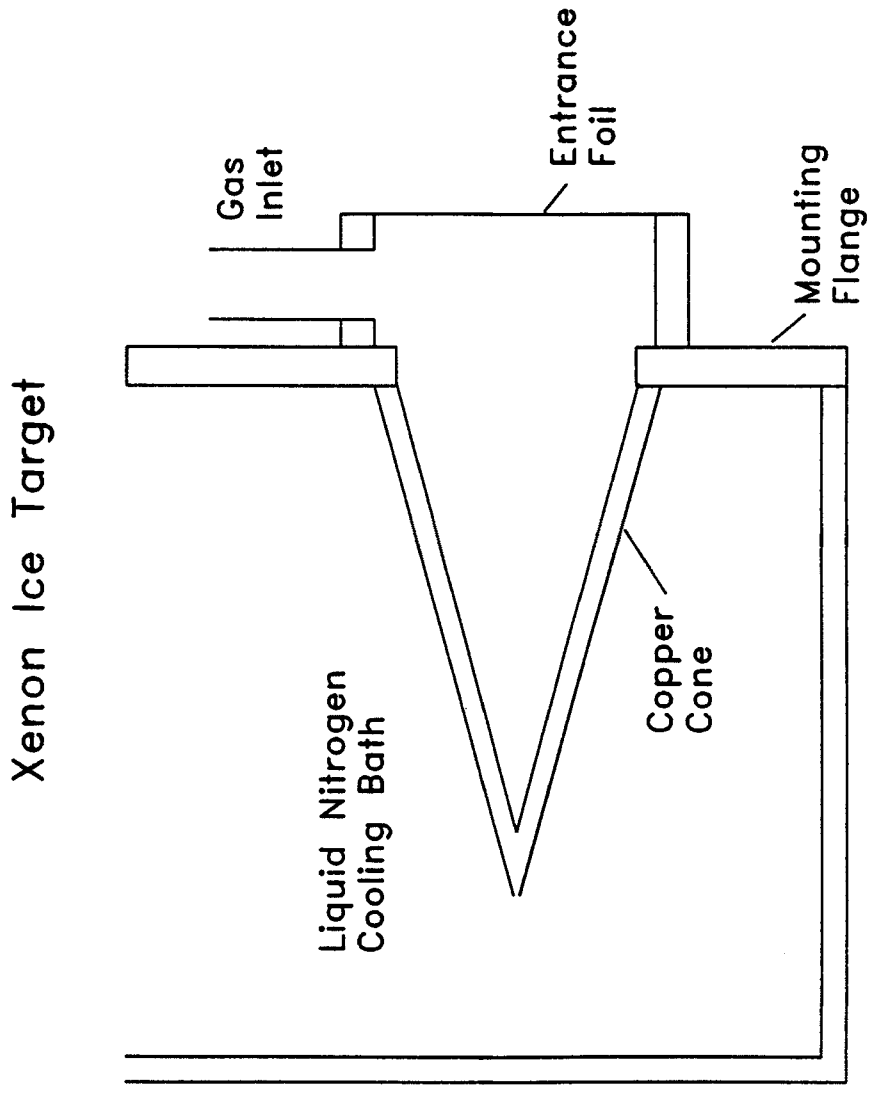
Xenon Ice Target
Gas Inlet
Entrance Foil
Mounting Flange
Copper Cone
Liquid Nitrogen Cooling Bath

Paper E23

DESIGN, LIPOPHILICITY AND SAR STUDY OF METYRAPONE ANALOGUES POTENTIAL IN VIVO SPECT AND PET TRACERS.

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Hays et al (1) have shown that replacement of the A-ring pyridyl group by a phenyl in Metyrapone enchances 11-β-hydroxylase inhibiting activity as compared to the original compound. Substitution of a halogen or methoxy on the propiophenone group will increase the lipophilicity. The increase of lipophilicity caused by substitution of ^{18}F,^{75}Br, $^{11}CH_3O$ (PET) and ^{123}I (SPECT) is estimated from k'values of Reversed Phase HPLC and SAA calculations (solvent accessible area in $Å^2$) as presented in the next table.
– Phenyl Metyrapone = 2 (3-pyridinyl) propiophenone.

Phenyl Metyrap. Analogue	k'	SAA	Analogue	k'	SAA
Phenyl Met.	4.71	448	3-Br-Phenyl	-	484
2-F-Phenyl	4.81	452	3-I-Phenyl	-	495
2-Br-Phenyl	7.18	473	4-I-Phenyl	-	495
2-I-Phenyl	8.02	481	2-OH-Phenyl	4.80	453
2-Br-4-F-Phenyl	-	479	2-CH_3O-Phenyl	5.88	467
2-I-4-F-Phenyl	-	487			

For the in 2 position H, F, Br, I subtituted analogues a linear relationship is observed obeying SAA = 400 + 10,2 k' with R^2 = 0,999. The increase in lipophilicity is the lowest for substitution in position 2 due to lipophilic compensation by the keto function. In vivo stability, i.e. deactivation of the phenyl for enzymatic hydroxylation and deiodination, is enchanced by substitution or additional substitution of F on the 4 position. This only increases the lipophilicity with 6 SAA units. 4-^{18}F-Phenyl Metyrapone can be obtained by ^{18}F for NO_2 exchange on the NO_2 analogue in which the NO_2 function is highly activated.
As compared to Phenyl Metyrapone, 2-I, 2-Br and 2-CH_3O Phenyl Metyrapone show higher binding affinity to the bovine adrenal mitochondrial P 450 enzyme system. For 2-Radioiodo and 2-Br-Phenyl Metyrapone an apparant K_D value of $9.5\,10^{-9}$M is observed. Moreover a Hill coefficient of 1.02 points to a one site - one compound interaction. The 2-^{123}I-Phenyl Metyrapone and 2-$^{11}CH_3O$-Phenyl Metyrapone analogues seem to be potential in vivo tracers for SPECT and PET respectively.

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RADIO SYNTHESIS OF (I-125) OF N-IODOALLYL NOR GBR12935

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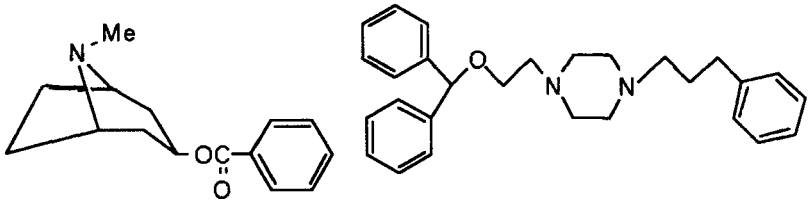
As part of our program to develop new probes for the dopaminegic system we have focused upon the selective inhibitors of dopamine uptake cocaine (I) and GBR 12935 (II). Work related to the labeled cocaine



I II

analogs CFT, and CIT has recently provided valuable data concerning the dopamine uptake system (1-4).

The analysis of the DA pathway using GBR 12935 has been hampered by the lack of derivatives which can be readily labeled with radiohalogens or carbon-11 (5). Our approach to solving this problem involves replacing the N-phenylpropyl group with an iodoallyl moiety, a substitution which retains most of the steric properties of the parent molecule and which can be rapidly labeled.

The synthesis of the target compound was acheived via the procedure depicted in Scheme I. Using the liteature methods we prepared the NOR GBR 12935(6) which was then alkylated with propargylbromide. Hydrostannation gave the trans N-tributylstannylallyl Nor GBR 12935 in good yield.

The intermediate could be converted to either its cold iodo product with NIS (80%) or to the (I-125)-N-iodoallyl nor GBR 12935 with I-125 sodium iodide and H_2O_2-acetic acid (80%). The radiolabeled compound was readily purified on a Sep-pak using methanol for elution.

Acknowledgement: This work has been supported in part by a Biomedical Research Support Grant 507RR05830.

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Scheme 1