8-1999

Synthesis of potent radiolabeled acetylcholinesterase inhibitors

George Albert Pacer

To the Graduate Council:

I am submitting herewith a thesis written by George Albert Pacer entitled "Synthesis of potent radiolabeled acetylcholinesterase inhibitors." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

George W. Kabalka, Major Professor

We have read this thesis and recommend its acceptance:

Richard Paqui

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by George Albert Pacer entitled "Synthesis of Potent Radiolabeled Acetylcholinesterase Inhibitors." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

George W. Kabalka, Major Professor

We have read this dissertation and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and Dean of
The Graduate School

Synthesis of Potent Radiolabeled Acetylcholinesterase Inhibitors

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville.

George Albert Pacer

August 1999

DEDICATION

To my parents, without whose love, help and support this work would not have been possible.

ACKNOWLEDGEMENTS

The author wishes to thanks Dr. George W. Kabalka for his guidance and support. A special thanks must go to all the graduate students (Kathy, Max, Rob and Shane) and post-graduate students, especially Dr. Murthy Akula, working in our laboratory for all their help and friendship, and to my parents for listening and advising through these years.

ABSTRACT

As positron emission tomography (PET) and single photon emission computed tomography (SPECT) become more available, appropriately labeled imaging agents will be needed to aid in the definitive diagnosis of neurological diseases. The goal of this research was the development of radiolabeled derivatives of acetylcholinesterase (AChE) inhibitors that could aid in the detection of Alzheimer's disease. The efficient syntheses of a fluorine-18 and an iodine-123 derivative of 1-(3-benzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine are described.

Table of Contents

List of Figures

List of Schemes

List of Tables

List of Abbreviations

Ach	Acetylcholine
AchE	Acetylcholinesterase
AD	Alzheimer's Disease
Å	Angstrom
AEC	Atomic Energy Commission
BBB	Blood-Brain-Barrier
d	Day
DAST	Diethylaminosulfur trifluoride
DMSO	Dimethylsulfoxide
EC	Electron Capture
EOS	End of Synthesis
FDA	Federal Drug Administration
FT-NMR	Fourier Transform Nuclear Magnetic Resonance
g	Gram
HPLC	High Performance Liquid Chomatography
h	Hour
HA	Hystamine
IC_{50}	Inhibitor concentration at 50% uptake of ligand
KeV	Kiloelectronvolt
LAH	Lithium Aluminum Hydride
MRI	Magnetic Resonance Imaging

MBq	Megabecquerel
MeV	Millionelectronvolt
MHz	Megahertz
μA	microamp
mL	milliliter
mmol	millimole
M	molar
nm	nanometer
nM	nanomolar
N	normal
NMR	Nuclear Magnetic Resonance
ppm	parts per million
β^+	positron
PET	Positron Emission Tomography
SPECT	Single Photon Emission Computed Tomography
THF	Tetrahydrofuran
TMS	Tetramethylsilane
TLC	Thin Layer Chromatography
TA	Tyramine
TME	Tyrosine methyl ester

CHAPTER 1
Radiolabeling

A. Introduction

Approximately 65 years ago, the production of artificial radioactive isotopes brought a new era to chemists and physicists, but little attention was given to possible medical applications.[1] However, within a short time, physiologists found that these new radioactive species to be powerful tools for studying metabolic processes. For example, in 1938 radioactive phosphorus was used as a substrate for the whole body x-irradiation in the treatment of certain blood discrasias.[2] At about the same time, the utility of radioactive iodine for thyroid function studies was demonstrated and within a short period of time it was used for the treatment of toxic goiter. By 1940 there was a considerable amount of literature dealing with medical uses of radioactive isotopes, but no one thought that they would be used as standard, diagnostic and therapeutic procedures. This was due to the fact that these isotopes were only obtainable from a cyclotron, and usually at a great cost before they could be used. In 1946 the discovery of the nuclear reactor (chain reacting pile) changed the situation and isotopes became available for non-military purposes. The Atomic Energy Commission (AEC) announced, in 1946, the availability of certain isotopes for medical use at a cost that was much lower that the cyclotron produced products. Shortly after that, more than 30 institutions received over 100 isotope shipments and physicians began using large quantities of isotopes.[3] Presently, thousands of institutions are authorized to make, obtain and experiment with radioactive species.

There are two main categories for the uses of isotopes in medicine for the benefit of humans: therapy and tracer studies. Therapy involves the use of the

radioactive emissions usually killing or deactivating a group of cells or certain tissues. Tracer studies, including medical imaging, constitute a much larger category. They use radioactivity to obtain information about the function of an organ or biological systems with minimal altering of its state or condition at the time of the experiment. Tracer studies generally include the synthesis of biologically active molecules followed by labeling with an appropriate isotope such that the molecules' location may be monitored external to the body with radiation detecting devices. The collected data, after processing by a computer, gives a detailed picture of the function of the system and insight into the metabolic biological process.

One compound of particular interest is that of acetylcholine. Acetylcholine (ACh) is the neurotransmitter that is responsible for carrying the messages throughout the brain. In a person that suffers from Alzheimer's Disease (AD) the amount of acetylcholine is reduced. To correct this, physicians have used many approaches. One was to inhibit the function of acetylcholineesterase (AChE) which is the enzyme that hydrolyzes acetylcholine and renders it useless to the brain.

The methods that physicians use to diagnose Alzheimer's Disease do not confirm the onset of the disease. The only definitive method for diagnosis is an autopsy. Through the use of a radiolabeled chemical, the disease can be detectable using tomography giving the treating physician a tool to provide the most comfortable environment for the patient.

B. Background

1. Radionuclides

There are two main types of radionuclides used in nuclear medicine imaging: gamma emitters and positron emitters. Those isotopes which emit gamma rays, either directly or via electron capture, are imaged using single-photon emission computed tomography (SPECT). Where as those isotopes that emit positrons, which annihilate electrons to generate two gamma rays, are imaged using positron emission tomography (PET). Some of the common isotopes used in SPECT and PET are presented in Table 1-1 along with their half-lives and decay modes.

Isotopes of carbon, nitrogen and oxygen such as ^{11}C, ^{13}N, and ^{15}O are positron emitters that are used in PET imaging along with other isotopes. But these have an advantage over other nuclides, such as fluorine-18, gallium-68 and bromine-75, in that they can be incorporated into a biologically active imaging agent without altering their chemical nature and thus the properties that make the molecule active. In relatively large molecules, the presence of elements other than C, N and O is usually not a problem but, in smaller molecules, the presence of a "foreign" label may render the molecule inactive. This is a concern in SPECT imaging where the most commonly used isotopes, iodine-123, bromine-82, thallium-201 and indium-111, are not normally a part of the original biologically active agent.

One of the advantages of using SPECT radioisotopes is that they generally have a longer half-life than those used in PET and they can either be produced by an on-site cyclotron or purchased commercially. The positron emitting isotopes must

Table 1-1. Properties of Some Important Isotopes		
Nuclide	Half-Life[a]	Decay Mode[b]
Carbon-11	0.34 h	β^+
Nitrogen-13	0.16 h	β^+
Oxygen-15	0.03 h	β^+
Fluorine-18	1.83 h	β^+
Bromine-75	1.62 h	β^+, EC
Bromine-76	16.2 h	β^+
Iodine-123	13.2 h	EC
Iodine-125	60.14 d	EC
[a]Times: d = day, h = hour; [b]Decays: positron = β^+, Electron Capture = EC.		

normally be produced on-site; they are usually so short-lived that commercial production is not feasible.

a. Radiofluorine

Hydrogen fluoride was discovered in 1771 by Scheele;[4] molecular fluorine itself was not prepared until 1886 by Moissan.[5] Organofluorine chemistry began to unfold with the work of Swarts[6] on the preparation of fluoride-promoted halogen-fluoride exchange reactions. The commercial utility of organofluorine compounds as refrigerants,[7] further accelerated the growth of the field by virtue of the economic incentives involved. Wartime requirements stimulated research on thermally stable

and chemically resistant materials, which led to a renewed interest in perfluorinated substances.

The half-life of ^{18}F is 110 minutes which gives the chemist ample time to incorporate the fluorine and purify the pharmaceutical before injection into the patient. A usual rule of thumb for synthesizing a radiolabeled compound is that chemical synthesis and purification should be accomplished within three half-lives. That would require that the introduction and purification of the sample would have to be done in less than five and one-half hours. Upon decay, two positrons are released with an energy of 510 KeV[8], which is easily detected by PET cameras.

One advantage of fluorine as a substituent in a biologically active molecule results from the pronounced electronic effects that may result on fluorination as well as on the fact that fluorine is not a sterically demanding substituent. With its small van der Waals radius (1.35 Å), fluorine closely resembles hydrogen (van der Waals radius 1.20 Å). The carbon-fluorine bond length, 1.39 Å, is comparable to that of the carbon-oxygen bond, 1.43 Å. The electronegativity of fluorine (4.0 vs. 3.5 for oxygen) can have pronounced effects on the electron distribution in the molecule affecting the acidity, basicity, neighboring groups, dipole moments within the molecule, and overall reactivity and stability. Fluorine can also function as a hydrogen bond acceptor because of its available electron density.[9, 10]

b. Radioiodine

There are three isotopes of iodine that are useful for medical research. These are iodine-123, iodine-125 and iodine-131. Of these iodine-123 is the most useful for imaging because it is not only a gamma emitter, it emits radiation at 159 KeV[4] which

makes it ideally suited for gamma detection cameras found in most nuclear medicine departments. Iodine-125 emits gammas and conversion electron with an energy of 35 KeV[4], which are too weak to be detected *in vivo*. Iodine-131 emits a gamma ray of 364 KeV[4] but it is also a beta emitter, which means that a larger dose of radiation is given to the patient. Iodine-125 and iodine-131 also have significantly longer half-lives (60 and 8 days, respectively, compared to 13 hours for iodine-123).[47] Iodine-131 is, however, one of the few isotopes that can be used for both imaging and for therapy.[47] The advantage of isotopes with short half-lives is based on the fact that the maximum specific activity theoretically attainable is inversely related to the half-life, therefore, a high activity can be attained for ^{123}I. Also, a short half-life reduces the radiation exposure to the patient. However, since the radionuclide needs to be administered to the patient within three half-lives of its production, a short half-life can result in significant synthetic and purification problems. For iodine-123, the nuclide must be delivered to the medical facility, incorporated in the imaging agent, purified and delivered to the patient within 39 hours.

Another consideration for radiolabeling with iodine is the potential effect of the iodine on the imaging agent. Iodine is a relatively large atom when compared to the hydrogen that it normally replaces. Fortunately, when a large molecule is used as the imaging agent, iodine does not usually sufficiently alter the activity of the agent so that it remains functional. Generally the stability of the carbon-iodine bond is also a potential concern. If iodine is attached to the imaging agent at an sp^3-hybridized center, the iodine can be cleaved from the molecule soon after administration to the patient and can collect in the thyroid. It is therefore necessary to attach the iodine to

an sp^2 carbon (a vinyl or aromatic center) in order to increase the *in vivo* stability whenever possible. While some biologically active agents contain sp^2 centers for attachments, others do not and must be modified to accommodate the iodine. This can add an additional foreign element to the molecule, which could possibly affect its activity. Again, this is most important in small molecules but can affect large molecules as well. Computer programs have been developed to aid in determining the changes in structure, electronic properties and lipophilicity caused by the addition of nuclides.[48] These structure-activity relationships must be considered when iodine is used as an imaging agent.

2. Radiolabeling Methods

A. Fluorine

a. Electrophilic

Electrophilic fluorination is the process by which fluorine is delivered to an electron rich reactant, such as an alkene, aromatic ring or carbanion, to form a carbon-fluorine covalent bond. The fluorination of a carbon double bond was utilized in early fluorine-18 labeling[90] and has remained an important reaction for over 10 years. Stereoselectivity in the addition of F_2 or acetylhypofluorite to alkenes can be very important in the synthesis of fluorine-18 labeled radiopharmaceuticals. The stereoselectivity of the addition of F_2 or acetylhypofluorite to tri-O-acetylglucal proves to be solvent dependent, an attribute that was not recognized until the early 1990's.[11, 12, 13, 14]

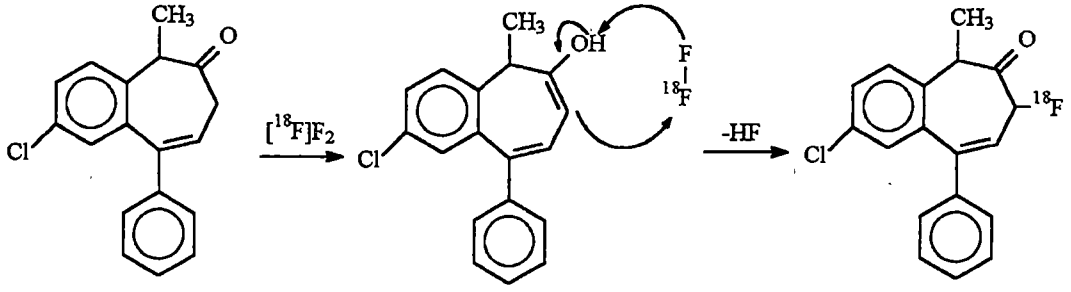


Figure 1-1. Electrophilic Attack of Fluorine

An interesting variant on the fluorination of alkenes is the reaction of $[^{18}F]F_2$ with diazepam, a 1,4-benzodiazepine, to yield the 3-fluoro derivative (Figure 1-1). The mechanism proposed is the electrophilic fluorination of the enol form of the amide, yielding, after fluorine attachment and the reformation of the carbonyl group, the α-fluoro carbonyl derivative. The success of this reaction (yields of 20 to 60%) has prompted further consideration of carbonyl groups of ketones and amides as "masked" alkene precursors for electrophilic fluorination involving fluorine-18 labeled reagents. Similarly, the reaction of N-[^{18}F]fluoropyridinium triflate with the methyl enol ether derived from cyclohexanone yielded the addition product 1-[^{18}F]fluoro-2-methoxy-3-cyclohexene[15], which might also be converted via ether cleavage to the α-[^{18}F]fluoro ketone.

As an alternative to simple addition of [^{18}F]fluorine gas to alkenes, vinyl [^{18}F]fluorides can also be prepared by the fluorination of vinylsilanes (Figure 1-2) as exemplified by the synthesis of 4-[F-18]fluoroantipyrine form 4-(trimethylsilyl)-antipyrine (18% yield EOS).[16]

In most applications of electrophilic fluorination of alkenes, the ultimate product is monofluorinated and arises from a subsequent hydrolysis or elimination (as in nucleic acid bases) step. In rare cases, the difluoro-adduct is intentionally

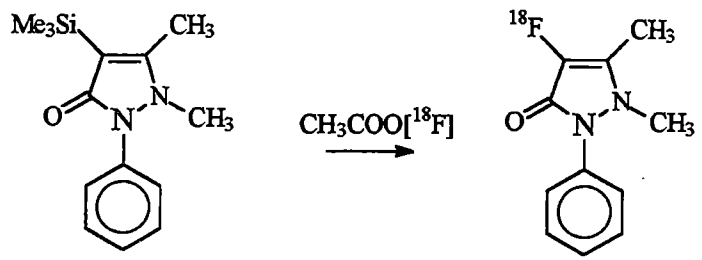


Figure 1-2. Electrophilic Attack by Acetyl hypo[^{18}F]fluorite

isolated, as in the synthesis of 2-deoxy-2-fluoroglucosyl fluoride.[17,18] In these instances, the maximum radiochemical yield is 100%.

Direct fluorination of aromatic rings is possible using [^{18}F]F_2, acetyl[^{18}F]hypofluorite, and [^{18}F]XeF_2 as electrophilic fluorinating reagents. Such fluorinations are not usually regioselective, due to the very reactive nature of these compunds.[19] For example, reaction of [^{18}F]F_2 and 3,4-dihydroxyphenylalanine (DOPA) in liquid hydrogen fluoride gave 2-, 5- and 6-[^{18}F]fluoroDOPA (35:5:59 ratio).[20] Reaction of electrophilic fluorination agents with a protected DOPA (e.g., methyl-*N*-acetyl-3-methoxy-4-acetoxyphenylalaninate) yields primarily the 2-, and 6-fluoro derivatives (1:1 mixture).[21] Isolation of the desired isomer (in this case, 6-fluoroDOPA) for both syntheses required careful chromatography (HPLC).

More recently, regioselectivity in electrophilic fluorination was shown by Chirakal *et al.* for the synthesis of 3-fluorotyrosine.[22] Reaction of tyrosine with F_2 in hydrogen fluoride solution gave exclusively 3-fluorotyrosine (46% yield), whereas the same reaction using the *O,N*-diacetylated derivative of L-tyrosine methyl ester

gave a mixture of 2- and 3-fluorotyrosines (yield 26%, 40:60 ratio) However, fluorination of O-acetyltyrosine (no protection of amine or acid groups) with [^{18}F]acetylhypofluorite gives a much better ratio of [^{18}F]fluorotyrosines (20% yield, 2-, 3- ratio 83:17). The characteristics of aromatic substitution and effects of reaction conditions (solvents, temperature, etc.) on fluorinations with fluorine-18 labeled electrophilic reagents have yet to be systematically defined.

Regioselective fluorodemetallation reactions have enjoyed more success. Here, regioselectivity is accomplished by prior functionalization of the aromatic ring, with fluorination occurring predominantly at that position. A number of metals (silicon[23, 24, 25, 26], tin[27, 28], germanium[23], mercury[29, 30, 31, 32]) have been utilized in such fluorination reactions, but a thorough comparison of all of these has not been published. A good comparison of aryl tin, aryl germanium, and aryl silicon derivatives has been made by Coenen and Moerlein.[23] In their work, various p-substituted aryl-metal substrates (CH_3O, CH_3, F, Br, CF_3, NO_2) were evaluated for radiolabeling using [^{18}F]F_2 and acetyl[^{18}F]F_2hypofluorite. Several important conclusions were drawn:

1) Tin appeared to be a better leaving group than silicon or germanium, yielding cleaner substitution products.
2) Temperatures from –78°C to 22°C could be utilized with little effect on yields.
3) Fluorodemetallation reactions could be conducted in perhalogenated methanes as solvents but proceeded in low or no yield in solvents with active hydrogens (e.g., CH_2Cl_2, DMSO, and acetonitrile).

4) For fluorodestannylation reactions with either F_2 or AcOF are satisfactory, but with less reactive silicon or germanium derivatives F_2 is the preferred reagent.
5) Strong electron withdrawing groups (NO_2, CF_3) diminish yields considerably.
6) Side products are few but include nonspecific ring fluorination and substitution of benzylic hydrogens.

b. Nucleophilic

The use of [^{18}F]fluoride ion as a synthetic precursor should, in principle, encompass a much smaller spectrum of reactions as there is one reagent (fluoride ion) to consider as compared to the multiple electrophilic fluorination reagents. For many years, the use of [^{18}F]fluoride ion as a synthetic precursor remained unexploited, with few successful fluorinations. More recently, [^{18}F]fluoride ion has emerged as a readily available, very valuable precursor for synthesis of fluorine-18 labeled radiopharmaceuticals, with numerous successful applications reported.

Aliphatic nucleophilic substitutions with [^{18}F]fluoride ion are, in concept, very simple S_N2 displacements of suitable leaving groups (e.g. halides, sulfonate esters). This particular reaction, however, has seen widespread variations in [^{18}F]fluoride source, as a necessity for added carrier fluoride, reaction conditions (solvents, catalysts, temperature), molecular structure and results. Comparison of all of the disparate literature reports of aliphatic nucleophilic fluorinations is difficult. At present, although there are several excellent syntheses using this reaction, a consensus has not been reached on the best method for nucleophilic [^{18}F]fluorinations with [^{18}F]fluoride ion.

Aliphatic nucleophilic substitutions are most easily classified according to the leaving groups. Many of the considerations of structures, solvents, etc., that are normally found with aliphatic nucleophilic substitutions are found in reactions with [^{18}F]fluoride ion. In particular, side products arising from elimination reactions (a problem using fluoride ion, which is an excellent base as well as a nucleophilic) and reaction of substrate (present in excess when doing no-carrier added fluorinations) with other nucleophiles present may make isolation of the desired [^{18}F]fluoroalkane difficult.

The use of fluoride ion in nucleophilic aromatic substitution reactions was reported initially in 1956 by Finger *et al.*[33] However, the use of [^{18}F]fluoride ion in aromatic nucleophilic substitutions was not seriously investigated until recently, when accounts of carrier-added and no-carrier added displacements of aryl halides and aryl nitro groups were reported.[34-40] The substitution reactions are proposed to proceed by the generally accepted mechanism for aromatic nucleophilic substitution. Fluorine and nitro are particularly good leaving groups in these reactions, but a large number of other leaving groups are possible (e.g., iodo, bromo).[41, 42] The use of cationic leaving groups, such as trimethylammonium[43], allows for reactions at lower temperatures and (potentially) a simple separation of aryl [^{18}F]fluorides from the charged precursors. The aromatic ring must be activated by the presence of one or more electron-withdrawing groups; in activated rings, the additional presence of a electron-donating substituent (e.g., methoxy) can be tolerated.[44, 45] In most cases, the activating groups are placed *ortho-* or *para-* to the leaving group, although successful

reactions have been achieved with one (*m*-dinitrobenzene)[40] or two (3,5-dinitrobenzonitrile)[46] activating groups placed *meta*- to the desired leaving group.

Nucleophilic aromatic substitution appears particularly well suited to the synthesis of carrier-added and no-carrier-added aryl [^{18}F]fluorides. The use of fluoride as the leaving group yields, necessarily, the carrier-added aryl [^{18}F]fluoride. The ^{18}F-for ^{19}F exchange has utility where high specific activity is not a requirement: if the ^{19}F-labeled molecule is available, the isotopic exchange reaction yields a product that, conceptually, would be very easy to purify. No-carrier-added aryl [^{18}F]fluorides are obtained with the use of other leaving groups, and this reaction provides the best available method for fluorine-18 labeling of aromatic rings. As there are a large number of variations possible (leaving groups, activating groups, ring substitution patterns), and intermediate products can be subsequently converted to more complex molecules, the number of possible fluorine-18 labeled compounds would appear almost endless. Indeed, the last few years have seen a proliferation of new [^{18}F]fluoroaromatic compounds.

Some other nucleophilic [^{18}F]fluorination methods include the diethylaminosulfur trifluoride (DAST) reactions. The reaction of carbonyl compounds or alcohols with DAST yields that corresponding *gem*-difluoro or monofluoroalkanes, respectively. The mechanism of DAST fluorination of alcohols is proposed to involve formation of the intermediate addition product, followed by S_N2 displacement with fluoride ion. Two approaches to use DAST and fluorine-18 have been described. It was initially reported that the preparation of fluorine-18 labeled DAST reacted with alcohols to give the expected [^{18}F]fluoroalkanes, with a

maximum yield of 33%.[88] Subsequently, the addition of soluble [^{18}F]fluoride ion to a normal reaction of alcohol and DAST (the DAST not being labeled) gave the[^{18}F]fluoroalkane (in this case, 3-deoxy-3-[^{18}F]fluoroglucose, figure1-3) in 90%

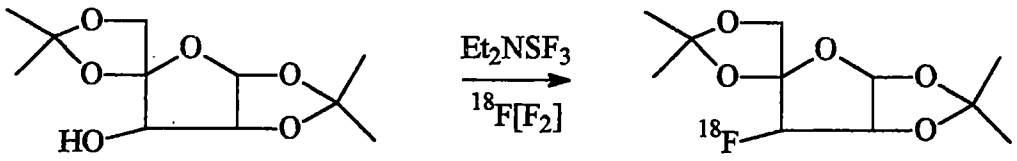


Figure 1-3. Fluorination using DAST

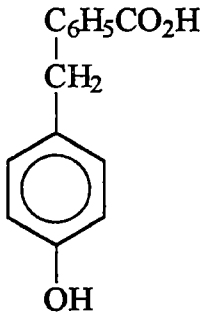
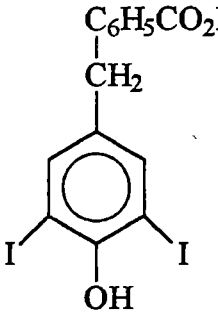
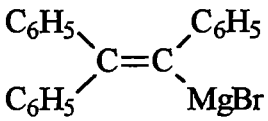
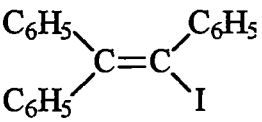
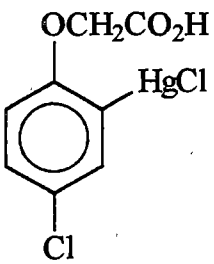
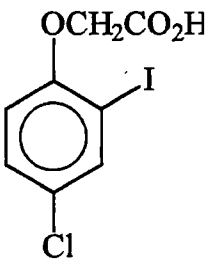
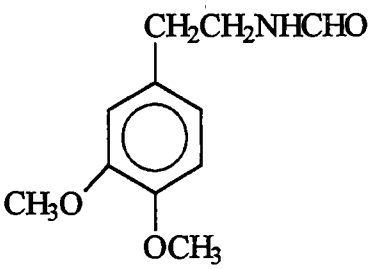
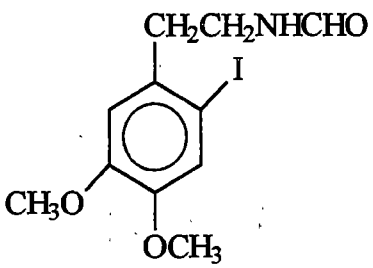
yield.[89] The yield, being greater than 33%, indicates that formation of fluorine-18 labeled DAST was not a prerequisite for reaction (and, indeed, did not occur *in situ* to an appreciable extent), but the fluorination proceeds in normal fashion with exchange of ^{19}F and ^{18}F within the free fluoride pool.

B. Iodine

a. Molecular iodine

One of the first classes of molecules to be radiolabeled were proteins and the first method of radioiodination utilized molecular iodine.[49] Since methods existed for iodinating phenols, these methods were adapted to protein radiolabeling which meant that a source of radioactive molecular iodine was needed. Several sources were developed in the late 1940's and early 1950's, all beginning with commercially available radioactive sodium iodide. The use of molecular iodine resulted in a maximum radiochemical yield of 50% and the volatility of the radiolabeling agent created potential safety problems. However, many proteins were successfully labeled in this manner. Table 1-2 contains representative examples of products synthesized using molecular iodine.

Table 1-2. Compounds Labeled by Reaction with Molecular Iodine

Starting Material	Product	Reference
		50
		51
		52
		53

b. Iodine monochloride

In 1958 McFarlane[54] published a procedure for radioiodination using iodine monochloride (ICl) which was first treated with sodium iodide to partially substitute radioactive iodine for unlabeled iodine in ICl. The mechanism for the reaction of ICl with protein was suggested to involve an electrophilic attack on the phenoxide ion followed by slow loss of a proton.[55] This reagent can be used to iodinate molecules which are activated towards electrophilic attack and which contain a double bond.

c. Chloramine-T

The most widely used radioiodination technique is the chloramine-T method which was developed by Hunter and Greenwood in 1962.[56] In water, chloramine-T forms HOCl which reacts with radioactive iodide ion to form an electrophilic species HOI.[57] This is thought to be the actual electrophile in the reaction.

In order to overcome the problem of removal of the chloramine-T, the oxidant, and its by-products from the reaction, a polymer-supported version of the oxidant, Iodobeads, has been developed which allows a simple filtration of the solid by-product of the oxidation.[58] This is now frequently used when time is an important factor as in the case of short-lived radionuclides.

d. Iodogen

Iodogen (Figure 1-4) is a molecule similar to chloramine-T and gives similar radiochemical yields but is somewhat less damaging to the molecule.[59] The phase transfer properties of the reagent result in less contact time between the product and the oxidizing agent.

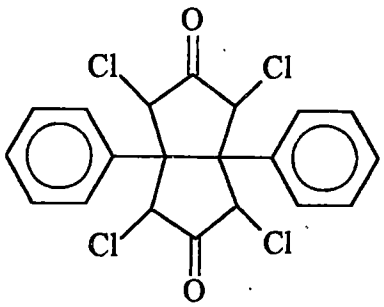


Figure 1-4. Iodogen

e. Electrolytic methods

Electrolysis can be used to produce molecular iodine from a radiolabeled sodium iodide. The sodium iodide is oxidized using an electrolytic cell and the product I_2 produced after the reaction with the imaging agent precursor can be reoxidized to molecular iodine so little of the labeled iodine is lost. This method has been used to iodinate estradiol,[60] producing 2-iodo, 4-iodo and the 2,4-diiodo derivatives in 20%, 23% and 4% yields, respectively.

f. Enzymatic methods

A milder alternative to the use of chemical oxidants is the use of enzyme peroxidases, which oxidize the radioiodide ion. The most commonly used enzyme is lactoperoxidase which, when mixed with a small amount of hydrogen peroxide, iodinates aromatic ring systems. If the direct use of hydrogen peroxide causes a problem, it can be generated *in situ* using a glucose/glucose oxidase system.[61] With this method the enzyme itself is iodinated and is usually removed using HPLC. Alternatively, a solid phase, coupled enzyme can be used and removed by gel filtration. Lactoperoxidase has been used to label phospholipids with the label being

attached to the fatty acid attached to C-2 of the glycerol.[62] Representative compounds labeled using this method are presented in Table 1-3.

g. Other direct methods

Several oxidizing systems have been developed to oxidize labeled sodium iodide to iodine and then reoxidize the by-product, iodide, formed by the reiodination reaction.

Table 1-3. Compounds Labeled by Enzymatic Radioiodination

Compounds
Phenol
Fluoresceinamine
Estradiol
Tyrosine
Uridine
Cytosine
Histidine
Phospholipids

h. Prosthetic groups

When a biologically active molecule to be radiolabeled has no site for iodination to occur, or the molecule is too sensitive to even mild oxidizing agents, a problem occurs. These kinds of problems can sometimes be overcome by the use of a prosthetic group. A prosthetic group contains an activated site such as an aromatic ring to make iodination easier and a connecting bridge to covalently attach the labeled group to the active molecule. This method is useful provided the prosthetic group does not significantly alter the biological activity of the imaging agent. This is often dependent on the size of the agent and the placement of the labeled group. In the attachment of the prosthetic group there are two choices. If the target molecule contains functional groups that are sensitive to oxidation, the prosthesis can be

radiolabeled then attached to the active molecule. In order to use this method, the attachment reaction must be rapid relative to the half-life of the radiolabel and the yield of the attachment reaction must be high so that the label is not lost. The second alternative is to attach the prosthesis to the active molecule and then perform the radiolabeling reaction. This increases incorporation of the radiolabel but the target molecule must be stable in the presence of the oxidizing agent.

Bolton and Hunter reported, in 1973, *N*-succinimidyl 3-(4-hydroxyphenyl) propanoate a molecule developed as a prosthetic group.[63] The prosthesis is first radiolabeled using chloramine-T and then attached to a protein. A similar reagent is methyl 4-hydroxybenzimidate hydrochloride[64], which has the advantage of retaining the positive charge on the protein after attachment.

i. Isotope exchange

Isotope exchange is perhaps the easiest method for radiolabeling many small organic molecules. The compound to be radiolabeled is iodinated with stable iodine-127 and then stirred in a solvent with radioactive sodium iodide so that iodine exchange occurs. The solvent must be able to dissolve the organic molecule as well as the inorganic iodide. Obviously, compounds of low specific activity are obtained but this may be acceptable in some cases. This method avoids the dangers of an oxidizing agent but wastes the majority of the label.

j. Isotope exchange in melt

Since many compounds exchange very slowly even in heated solvent, another method was developed for exchange to take place: melt exchange. This simple technique involves heating the organic compound to be labeled to its melting point

then mixing it with the inorganic labeled iodide. The compound must be stable at its melting point and it must be able to dissolve the inorganic iodide. This method had been used to radioiodinate *m*-iodohippuric acid[66] and 2,3,5-triiodobenzoic acid[67] in low radiochemical yields.

If the organic compound to be labeled does not dissolve the inorganic iodide, the exchange reaction can be done in an acetamide melt.[68] Acetamide melts at 82 °C and is stable up to 200 °C. Compounds labeled by this method include 2,3,5-triiodobenzoic acid,[68] 3,5-diacetamido-2,4,6-triiodobenzoic acid,[69] and 1,2,3-trihydroxypropane-1,3-dipalmitate 2-(3-iodobenzoate).[70] The radiochemical yields for these compounds were reported as modest to good.

The third type of melt labeling is carried out below the melting point of the substrate. The compound to be labeled is heated in a mixture of ammonium sulfate and radioactive sodium iodide at 120 – 160° for one to four hours.[71] The exchange is promoted by the gradual decomposition of the ammonium sulfate producing ammonia and sulfuric acid. Sulfuric acid can be substituted for ammonium sulfate to promote the exchange. Compounds iodinated by this method include 2,3,4-triiodobenzoic acid, a series of iodobenzylguanidines and other iodo aromatic compunds.[72]

k. Exchange for bromide

The advantage of using exchange for bromide instead of exchange for iodide is very high specific activities can theoretically be obtained if the bromide compound can be separated from the radioiodinated product. The time required for this separation must be considered. 16-α-Iodoestradiol has been labeled in this manner

but the presence of a small amount of 16β-bromoestradiol in the 16α-bromoestradiol produced 16β-iodoestradiol, which could not be easily removed.[73]

l. Catalyzed exchange

In order to improve yields and reduce reaction times, catalysts have been developed for the exchange reactions. The alkyl halide exchanges can be catalyzed by polymer-supported phosphonates[74] and dicyclohexyl-18-crown-6.[75] Copper (I) salts have also been shown to catalyze the exchange in iodoaromatics.[76] Silica gel has been shown to catalyze the exchange of iodide for hydrogen in the formation of 4-iodoantipyridine.[77]

m. Iododediazonization

The standard iododediazonization procedure is well known and involves the treatment of an aromatic diazonium salt with an inorganic iodide to produce the corresponding aromatic iodide. In order to use this procedure, the appropriate aniline must be available and other functional groups in the molecule must be stable to or protected from the nitrous acid or alkyl nitrite used to form, the diazonium salt. A variation of the standard procedure is the Gatterman reaction which used copper-bronze to catalyze the reaction.[78] Recently, the Wallach[79] reaction has gained popularity as a variation of the iododediazonization reaction which uses a secondary amine to trap the diazotized amine by formation of a triazene. The triazenes are stable, isolable solids that can be reacted with inorganic halides in acid to give aromatic halides. This reaction has been shown to be useful for radioiodination.[80]

n. Iododemetallation

The iododeboronation method is an excellent way to incorporate radioiodine into a molecule at a specific site with retention of configuration. Initially, these reactions were carried out with trialkylborane and molecular iodine giving the iodoalkyl product (figure 1-5) but they require a strong base. This method was improved by using iodine monochloride that requires no strong base and by starting with the appropriate vinyl or aromatic borane derivative so as to produces the more stable iodoalkenyl or iodoaromatic compound.[81]

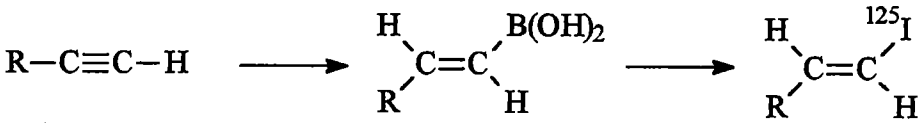


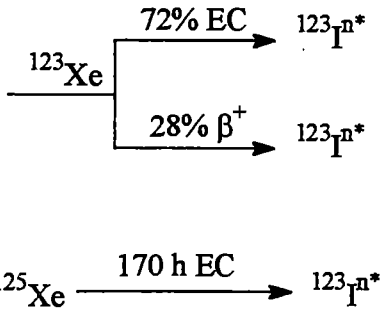
Figure 1-5. Iododemetallation

The carbon-silicon bond is somewhat similar to the carbon-tin bond and has been utilized in reactions with molecular halogens. Aromatic trimethylsilanes can be treated with labeled sodium iodide and *tert*-butyl hypochlorite in acetic acid to give good yields of the radioiodinated product.[82]

Thallium (III) trifluoroacetate will electrophilically attack aromatics to give an arythallium bis(trifluoroacetate) which can be treated with an inorganic iodide to produce aromatic iodide.[83] The labeled resides in the *para* position of the aromatic ring except where strong electron-withdrawing groups are present where it is in the meta position and where benzoic acid is used it is *ortho*.[83] An aromatic derivative of cellulose[84] and two phenyl-fatty acid analogues have been successfully radioiodinated using this method.[85]

o. Recoil labeling

The radioactive decay of Xe isotopes produce highly positive iodine species called recoil atoms. This result of the electron capture decay and loss of Auger electrons makes possible the use of Xe for radioiodination as shown in Scheme 1-1. Compounds labeled by this method include serum albumin which, when exposed to ^{123}Xe for 6-12 hours at liquid nitrogen temperatures, bound 80% of the iodine produced.[86] When insulin was treated with radioxenon using KIO_3 as a catalyst, [^{123}I]monoiodoinsulin was produced in 50% yield and [I]monoiodoinsulin was



EC = electron capture

Scheme 1-1. Production of Iodine Isotopes

produced in 80% yield.[87] Also, deoxyuridine and L-tyrosine were labeled in this way to give quantitative yields.[87]

C. Research goal

As SPECT and PET instruments become more available, imaging agents that can be used to detect certain brain abnormalities are needed to aid in the definitive diagnosis of neurological diseases. The goal of this research was to develop new agents that could aid in the detection of Alzheimer's disease by detecting the

inhibition of acetylcholinesterase (AChE) which is the enzyme that is responsible for the breakdown of acetylcholine, a neurotransmitter.

CHAPTER 2
Mapping Acetylcholine

A. Introduction

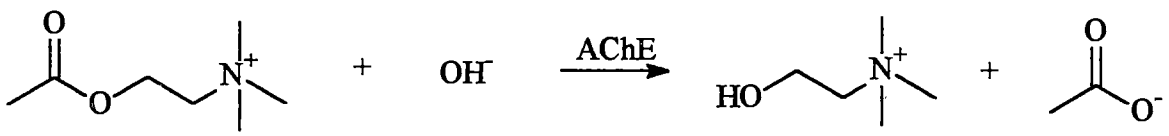
Mapping biological functions is a challenge that has faced scientists over the years. One of the most difficult goals has been to elucidate the role of ACh in neurology with emphasis on location, role, and *in vivo* concentration. It has been proposed that the concentration of acetylcholine can be correlated with the onset of Alzheimer's Disease.[114] If an acetylcholinesterase inhibitor, a chemical responsible for the disruption of the breakdown of acetylcholine, could be produced which had an indicator attached that could be detected externally, it might be possible to measure the *in vivo* concentration of acetylcholine.

B. Background

Alzheimer's Disease (AD) is a progressive degenerative disease in which brain cells die and are not replaced. This results in a person with AD having impaired memory and behavior. AD has been termed one of the most common dementing illnesses. It is estimated that approximately 4 million Americans are afflicted with AD. It is the fourth leading cause of death among adults and it is estimated that, by the year 2050, 14 million will have the disease. Of those afflicted, ten percent are over 65 and over half of those are over the age of 85. AD strikes men and women of all races and socioeconomic groups. The current diagnosis is by default. There is no single or simple AD test that can be performed that can yield a definitive answer. The only definitive diagnosis is an autopsy. The current process used to determine if a person has AD involves the following: a detailed medical history is acquired; a

complete physical exam is performed; a series of neurological exams are collected over a period of time. This information is used to rule out any other potential cause of the symptoms. Although AD is diagnosed by default, current diagnoses are up to 90 percent accurate.

It is difficult to externally image the functions of the living brain. Traditional medical imaging methods, such as magnetic resonance imaging (MRI), can be used only to identify the presence of acetylcholine. This does not help physicians because what needs to be determined is the concentration of acetylcholine present and how fast it is being hydrolyzed. Scheme 2-1 outlines the process by which acetylcholine is hydrolyzed to choline. With the advent of PET and SPECT scanners, the concen-



Acetylcholine Choline Acetate

Scheme 2-1. Hydrolysis of Acetylcholine

tration of acetylcholine present and how fast it is being hydrolyzed can now be obtained. These imaging methods allow clinical diagnosis and prognosis for dementia and disease mechanisms as well as facilitate the development of therapeutic drugs. There have been several attempts to synthesize a radiolabeled agent that would target acetylcholine but it has been found difficult to incorporate the radiolabel without affecting the biological function of the AChE inhibitor. Usually, the radiolabeling is carried out in the final step in the synthesis due to the relatively short half-life of the most useful radioactive elements. To be successful, one must design a

compound that has high selectivity for acetylcholine while maintaining its ability to cross the blood brain barrier.

The design and development of radiolabeled physiologically active compounds have become very sophisticated. Generally, there is a biochemical and physiological basis for the compound's design, as well as an understanding of how it might target a particular site. It is also important that the radiolabeled portion of the molecule not adversely affect the targeting, solubility, transport, permeability and incorporation. Also, there must not be a significant increase in the compound's toxicity. The problem with compound development is that it is difficult to predict the effect of the radiolabel on these parameters.

The measurement of AChE has been carried out postmortem. This method has demonstrated a cholinergic deficit in AD and has produced the data that led to the first neurochemically specific and efficacious therapy for AD, but even this drug has limitations. There have been three general approaches to the study of cholinergic systems in patients: 1) presynaptic terminals have been studied with derivatives of vesamicol designed to label the vesicular ACh transporter;[91, 92] 2) receptor systems have been studied with a number of ligands for nicotinic and muscarinic receptor subtypes;[93] and 3) AChE activity has been evaluated by labeling known AChE inhibitors.[94, 95] The study of AChE activity has several distinct advantages. First, the development of tracers for AChE activity has the biochemical advantage that these ligands can be designed as substrates for AChE that are hydrolyzed by the enzyme and then "trapped" in tissue where their distribution can be mapped by PET and SPECT. Second, because AChE is a good marker for both cholinergic and

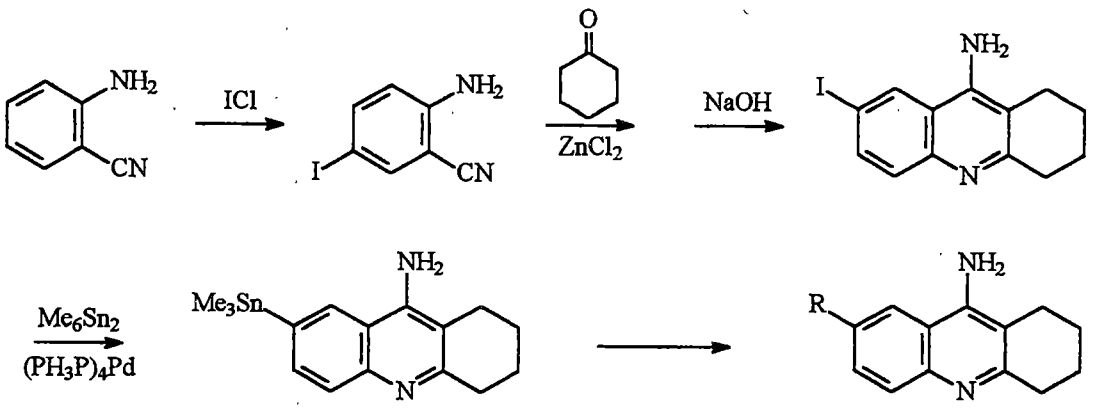
cholinoceptive neurons in the brain, it provides an index of cholinergic function that is more widespread than that seen in presynaptic terminals or receptor subgroups. Finally, AChE activity is itself the target of therapeutic interventions in AD, so that AChE measurements could have direct clinical relevance.

C. Types of Acetylcholinesterase Inhibitors

1. Aminoacridine

The first successful AChE inhibitor to be reported was 9-Amino-1,2,3,4-tetrahydroacridine, more commonly known as Tacrine®. Tacrine® was the first compound approved by the Federal Drug Administration (FDA) for the use in treatment of AD. Tacrine® has the advantage of being reversibly bound to AChE through lipophilic and hydrogen-bonding interactions. It also crosses the blood-brain barrier (BBB) in its uncharged, neutral form and yet interacts with AChE in its charged form.[96] It has been suggested that Tacrine® binds to the anionic site of AChE at the ring nitrogen, with the 9-amino group oriented away from the esteratic site. However, aminoacridines suffer from dose-limiting hepatoxic effects[97-100] which are believed to be structure related.[101] Fortunately, the minimal doses administered to the patients in radioisotope studies, would preclude side effects.

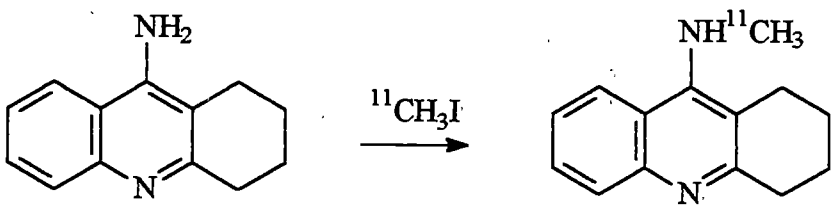
There have been few attempts at incorporating a radiolabel into the structure of Tacrine®. Kabalka *et al.* have devised methods to synthesize both a fluorine-18[102] and an iodine-123[103] derivative of Tacrine® as shown in Scheme 2-2. The syntheses yielded pure compounds. Tavitan *et al.*[104] also synthesized a carbon-11 derivative of Tacrine®, shown in Scheme 2-3.



R = F-18, reagents used are $[^{18}F]F_2$ in $AcOH/CHCl_3$

R = I-123, reagents used are $Na^{123}I$ in $CH_3CO_3H/MeOH$.

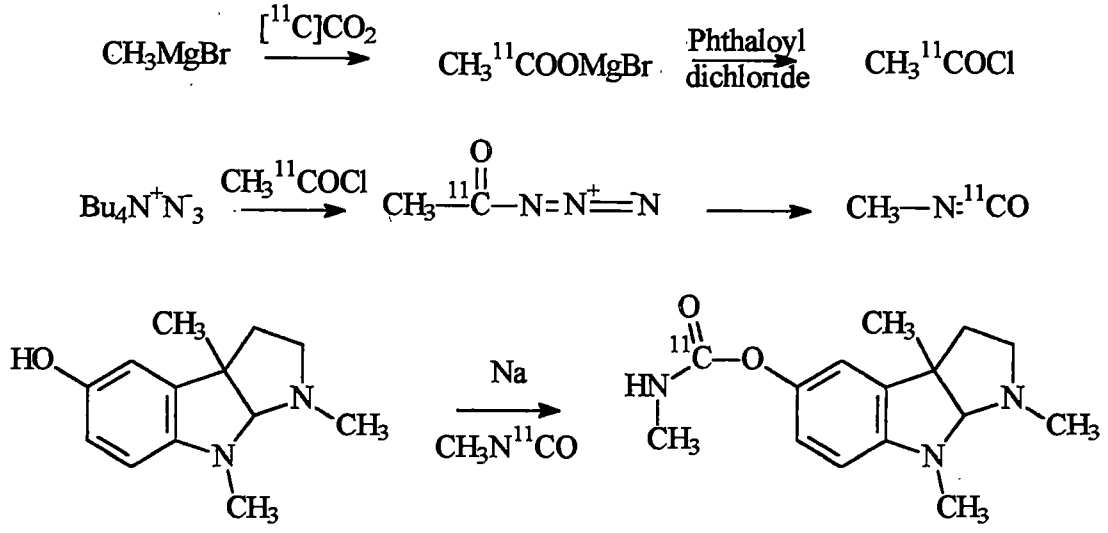
Scheme 2-2. Synthesis of ^{18}F and ^{123}I Labeled Tacrine®



Scheme 2-3. Synthesis of ^{11}C Labeled Tacrine®

2. Carbamate Inhibitor

Carbamyl agents, such as physostigmine, form a carbamyl enzyme more stable than the acetyl enzyme by reacting with the active site of serine. Because the carbamyl enzyme is unable to hydrolyze ACh, carbamyl agents are used in therapy as potent inhibitors of ACh hydrolysis. The anti-AChE property of physostigmine, an alkaloid extracted from calabar bean, had been known for years. Studies revealed that the distribution of [^{11}C]physostigmine and AChE activity were superimposable when studies were done on rat brains.[105] When primates brains were studied, similar results were found with the regional distribution of radioactivity paralleling that of AChE activity.[106] Bonnot-Lours, *et al.*[107] devised a synthesis, shown in Scheme 2-4, of [^{11}C]physostigmine which produced the radiolabeled product in a yield of 11-18%.

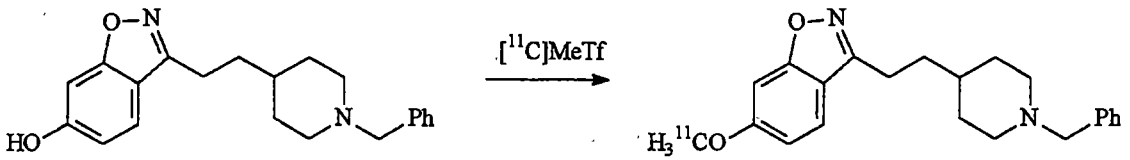


Scheme 2-4. Synthesis of ^{11}C Labeled Physostigmine

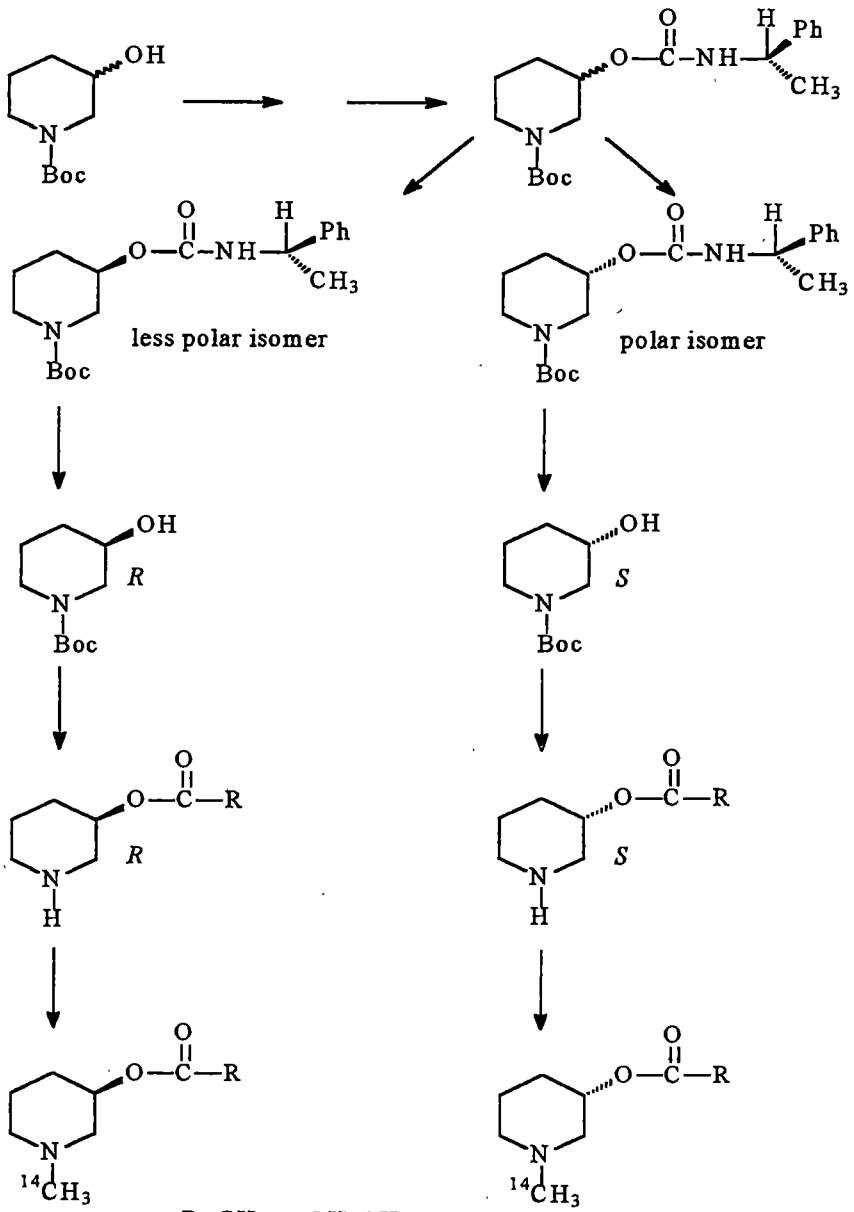
3. Substituted Piperidyls

This class of AChE inhibitor appears to overcome the unfavorable side effects and poor pharmacokinetics associated with the previously discussed AChE inhibitors.[108] In addition, the *N*-benzylpiperidine derivatives display good selectivity *in vitro* for acetyl- as compared to butyrylcholinesterase.[109-111] The benzoyl-containing functionality and the *N*-benzylpiperidine moiety are believed to be the key features for both binding to and inhibition of AChE.[111] Furthermore, a strategy was envisioned for preparing novel prototypes which would be devoid of chiral centers that might be susceptible to racemization.

There have been many different piperidyl-based AChE inhibitors developed utilizing a variety of radiotacers. Irie, *et al.*[112] have carried out research on PET analogues of AChE inhibitors. The first prepared were the [^{14}C/^{11}C]methyl-3- and 4-piperidyl esters. They have shown that these agents are able to cross the BBB and are hydrolyzed mainly by AChE and thus trapped in the brain. Three esters were studied initially: acetate MP4A, propionate MP4P, isobutyrate MA4IB, all being 4-substituted piperidyl derivatives that eliminated problems due to enantiomers. Irie, *et al.*[113] later studied the 3-substituted piperidyl derivative whose syntheses are shown in Scheme 2-6. They found that there was a significant difference in activity between the *R* and *S* enantiomers. Their findings revealed that the *R* was the favored enantiomer, with rate of hydrolysis ratios of *R/S* = 3.3 in MP3A and 8.1 in MP3P. Brown-Proctor, *et al.*[114] synthesized a C-11 derivative of benzisoxazole, shown in Scheme 2-5, in greater that 97 % radiochemical purity; however, it was unable to differentiate AChE distributions in the brain region.



Scheme 2-5. Synthesis of ^{11}C Labeled Benzisoxizole



R=CH_3 or CH_3CH_2

Scheme 2-6. Synthesis of ^{14}C Labeled MP3A and MP3P

D. Conclusion

With the increasing number of people diagnosed with AD every year, there is a need for a definitive, non-invasive *in vivo* method for AD detection. It has been shown that *N*-benzylpiperidines demonstrate promise for the early detection of AD. However, the synthesis of a 4- substituted *N*-benzylpiperidine that is equivalent to or more effective than [^{11}C]physostigmine has yet to be developed. The focus of this research is the synthesis of a 4-substituted *N*-benzylpiperidine imaging agent that could be utilized in the diagnosis of AD. The synthesis and the conversion to the radiolabeled derivative of a potent AChE inhibitor will be outlined in the remainder of this thesis.

CHAPTER 3

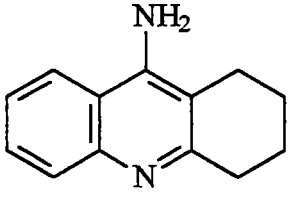
Synthesis of Radiolabeled 1-Benzyl-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine

A. Introduction

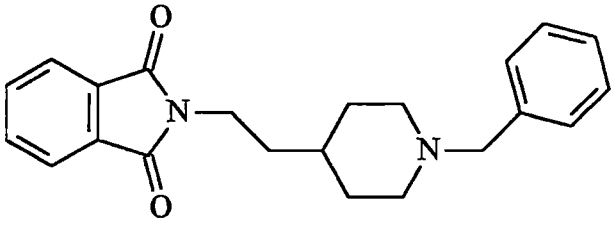
Para-substituted *N*-benzylpiperidine derivatives are promising AChE inhibitors. However, the development of a radiolabeled derivative that maps ACh has not been achieved. The focus of this research is the development of a radiolabeled acetylcholinesterase inhibitor. The synthesis of a fluorine-18 and an iodine-123 derivative of potent acetylcholinesterase inhibitors will be discussed in the remainder of the thesis.

B. 9-Amino-1,2,3,4-tetrahydroacridine

It was recognized that 9-amino-1,2,3,4-tetrahydroacridine (Tacrine®) enhanced the memory of patients with AD. This led to studies regarding the uptake and distribution utilizing PET. Tacrine®, Figure 3-1, was synthesized by various routes, one of which is illustrated in Scheme 3-1.[115]

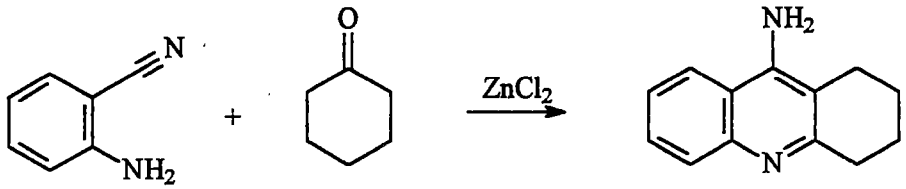


9-Amino-1,2,3,4-tetrahydroacridine



1-Benzyl-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine

Figure 3-1. Examples of Some Potent AChE Inhibitors



Scheme 3-1. Synthesis of Tacrine®

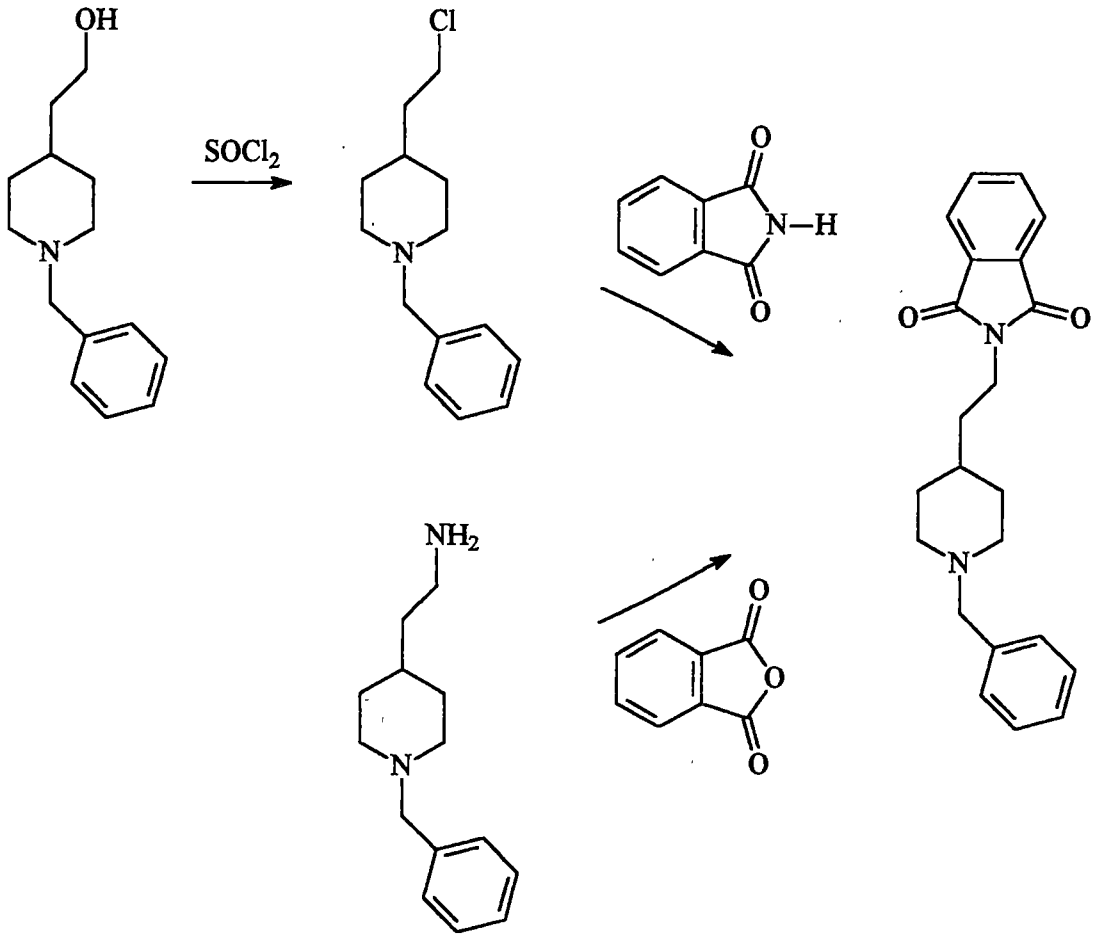
C. 1-Benzyl-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine

1-Benzyl-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine, Figure 3-1, was first synthesized by Sugimoto *et al.*[116] in 1992 via the route presented in Scheme 3-2. During inhibition studies, it was discovered that 1-benzyl-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine had a higher selectivity for acetyl- over butyrylcholine and exhibited an IC_{50} value of 30 nM.[117]

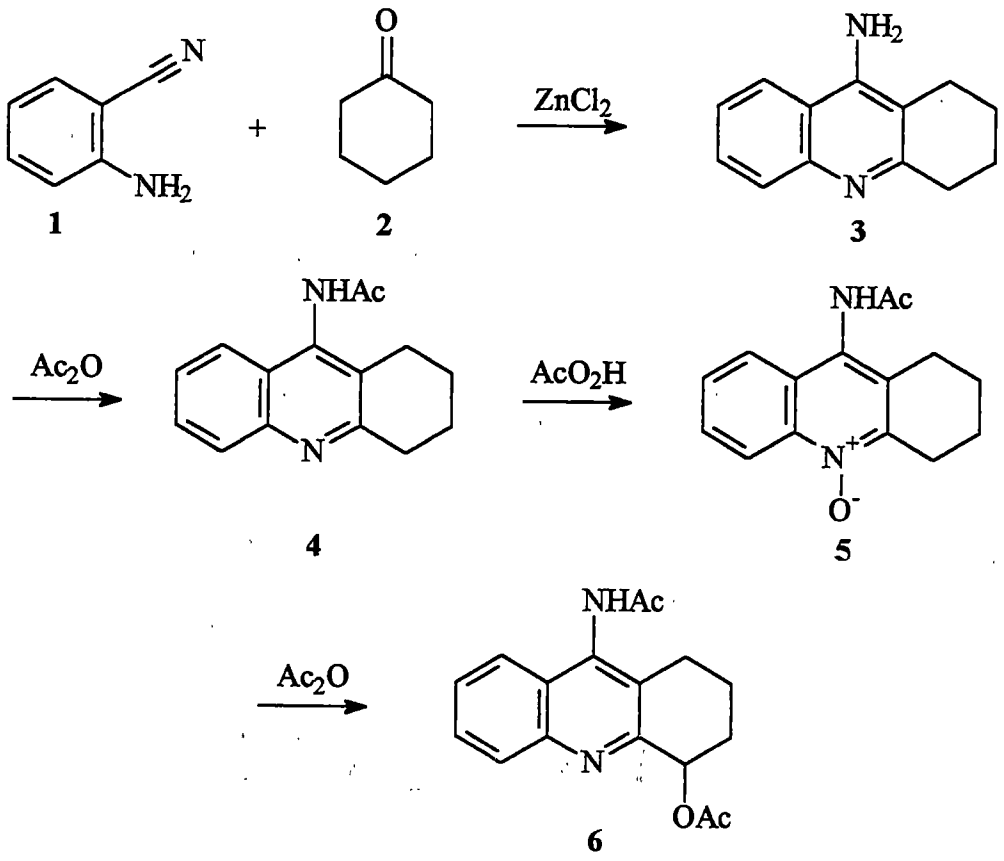
D. Synthesis of 9-Amino-4-fluoro-1,2,3-trihydroacridine

The first acetylcholinesterase inhibitor to be reported was 9-amino-4-fluoro-1,2,3,-trihydroacridine. An aminoacridine was chosen because its activity in the body was well known. The synthesis utilized is shown in Scheme 3-3. The starting material was anthranilonitrile, **1**. The first step involved ring fusion with cyclohexanone, **2**. This was accomplished by using an inorganic catalyst and the formation of a cake-like precipitate indicated that the desired product formed. The addition of aqueous sodium hydroxide freed the cyclized aminoacridine, **3**.[115]

The amino group was then protected to prohibit further reactions at this position. The aminoacridine **3** was refluxed in a solution of acetic anhydride containing a small amount of a base to deprotonate the amine group. The amine nitrogen nucleophilically attacks one of the carbonyls of acetic anhydride displacing



Scheme 3-2. Synthesis of 1-Benzyl-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine



Scheme 3-3. First Synthetic Route to a Substituted Tacrine®

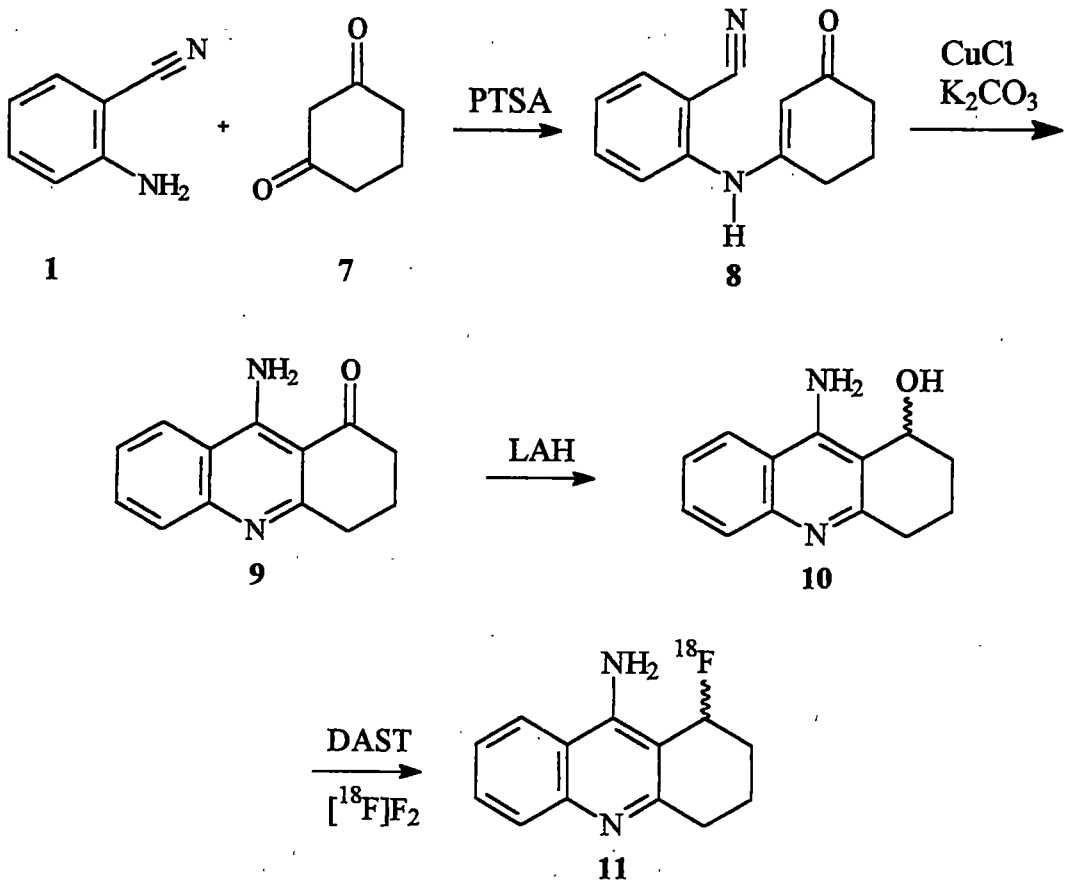
an acetate ion. The quaternary amine was then deprotonated by pyridine to yield *N*-acetyl Tacrine®, **4**.

N-Acetyl Tacrine® was then allowed to react with peracetic acid to generate the *N*-oxide, **5**. The *N*-oxide bond is polar with the nitrogen carrying a formal positive charge and the oxygen a negative charge.

The *N*-oxide **5** was rearranged in the next step. Compound **5** was allowed to react with acetic anhydride resulting in 9-(*N*-acetyl)-amino-1,2,3-trihydro-4-acetoxy-acridine, **6**. The oxygen attacks one of the carbonyls of acetic anhydride forming *N*-acetoxy compound *in situ*. The released acetate ion then deprotonates the α-methylene. This proton is acidic because it is in an allylic position with respect to the positively charged nitrogen. The project was terminated because compound **6** could not be isolated in a sufficiently high yield. According to Boekelheide and Linn,[118] the desired rearrangement should be possible.

E. Synthesis of 9-Amino-1-fluoro-2,3,4-trihydroacridine

While designing the synthetic route for the first target compound, an alternate scheme was developed for preparing a related compound, **11**, shown in scheme 3-4. The first step involved joining the two rings. Anthranilonitrile, **1**, was allowed to react with cyclohexanedione, **7**, utilizing a Dean-Stark apparatus to separate water. The amine group of **1** nucleophillically attacks one of the protonated carbonyl groups of **7** displacing water and regenerating the acid catalyst, yielding *N*-(3-oxocylohexen-1yl)-2-aminobenzonitrile, **8**.[119]



1 7 8 9 10 11

Scheme 3-4. 2nd Synthetic Route to a Fluorinated Tacrine®

With compound **8** in hand, construction of the center ring was then performed by refluxing **8** in the presence of a mild base and cuprous chloride. The cyclization did not occur in the absence of cupric chloride according to Basato *et al.*[120] Apparently, the key step involves the coordination of the nitrile to the metal which leads to an increase in the electrophilicity of the CN carbon atom. This enhances attack by the metal-co-ordinated ketone and yields the expected 9-amino-3,4-dihydroacridin-1(2*H*)-one, **9**.[119]

Lithium aluminum hydride was used to reduce **9** to the desired alcohol. Lithium aluminum hydride transfers a hydride to the carbonyl group resulting in the formation of 9-amino-1,2,3,4-tetrahydroacridin-1(2*H*)-ol, **10**, being formed in high yield.[119]

The final step involved the fluorination of **10**. Diethylaminosulfur trifluoride, DAST, had been used successfully to fluorinate compounds with unprotected amine groups.[121] DAST can also be used to incorporate F-18 into a molecule if $[^{18}F]F_2$ is bubbled into a solution containing DAST. When DAST is added, it deprotonates the hydroxyl group causing the oxygen to attack the sulfur displacing a fluoride ion. This fluoride ion can then attack the carbon-oxygen bond displacing the oxidized DAST molecule. However, if $[^{18}F]F_2$ is in solution, the attacking fluoride can be fluoride-18 which yields the desired labeled product 9-amino-1-[^{18}F]fluoro-2,3,4-trihydroacridine, **11**.[122]

In our hands, the fluorination was not quantitative using DAST. The desired product was obtained but in very low yields. Since the 1 position is allylic, using DAST as the fluorinating agent apparently led to the formation of polymers.[123]

F. Synthesis of 1-(3-Trimethylstannylbenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]-piperidine

We then investigated an alternative potent AChE inhibitor in the *N*-benzylpiperidine family, specifically 1-benzyl-4-[2-(*N*-phthalimid-1-yl)ethyl]-piperidine, **12**, Figure 3-2. When compared to Tacrine®, compound **12** is a more potent AChE inhibitor, with an IC_{50} value of 30 nM compared to 80 nM for Tacrine®.[124] The incorporation of the radiolabel into compound **12** appeared to be

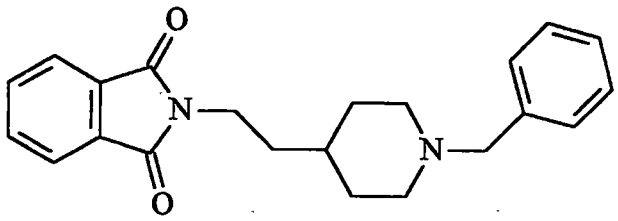
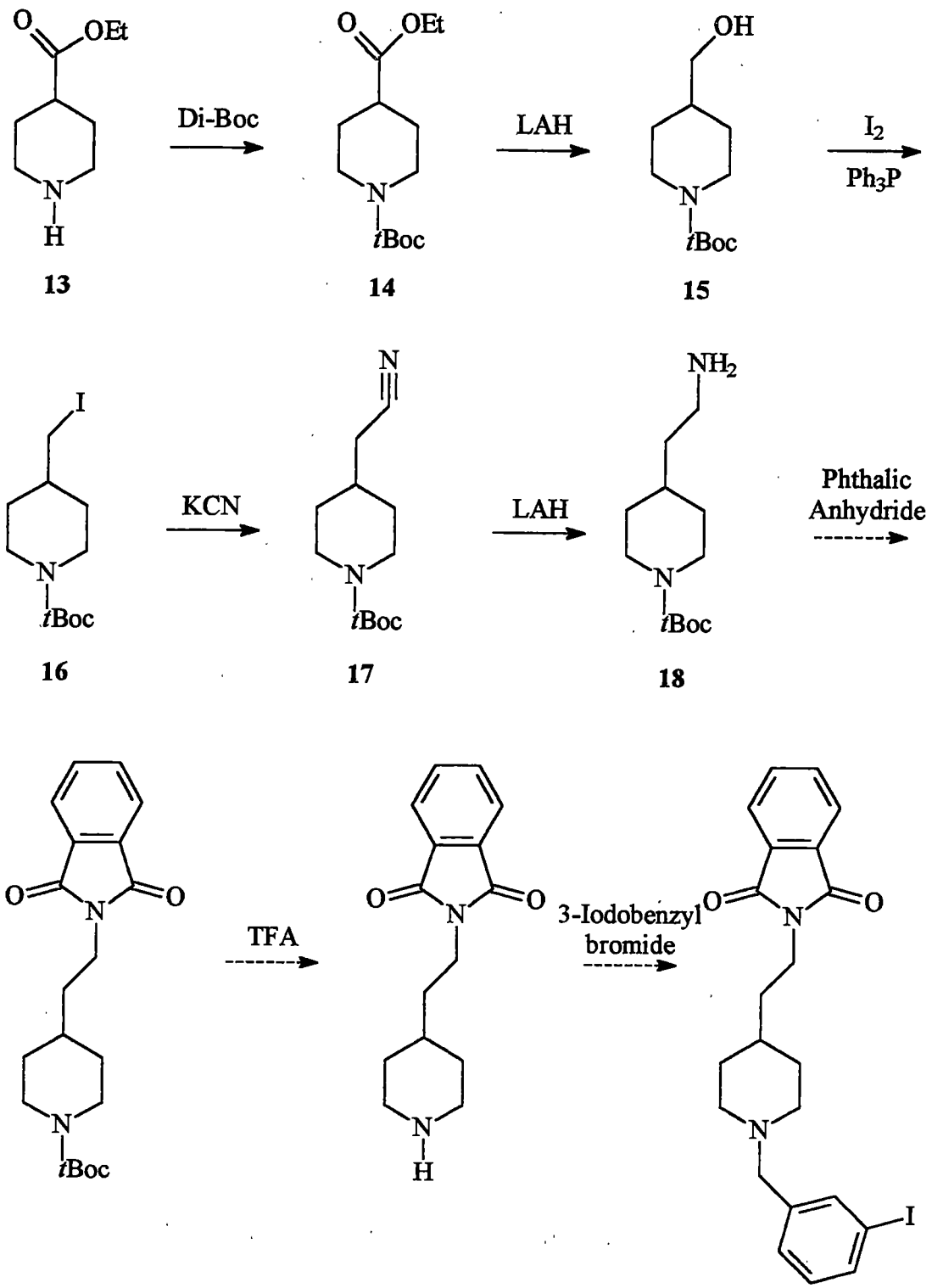


Figure 3-2. 1-Benzyl-4-[2-(*N*-phthalimid-1-yl)ethyl]-piperidine

straightforward. It was felt that a tin precursor could be radiohalogenated and the desired radiolabeled product prepared. The synthesis of the tin precursor and its conversion to both a fluorine-18 and an iodine-123 derivative of **12** will be described in the remainder of this chapter.

The first attempt to synthesize compound **12** was long and laborious as shown in Scheme 3-5. Ethyl isonipicotate, **13**, was first protected using di-*t*-butoxy dicarbonate. The electron pair on the nitrogen attacked one of the carbonyls in di-Boc displacing the remaining Boc group. The nitrogen was deprotonated by base resulting in 1-(*t*-Boc)ethyl isonipecotate, **14**.[125]



Scheme 3-5. Original Synthetic Route

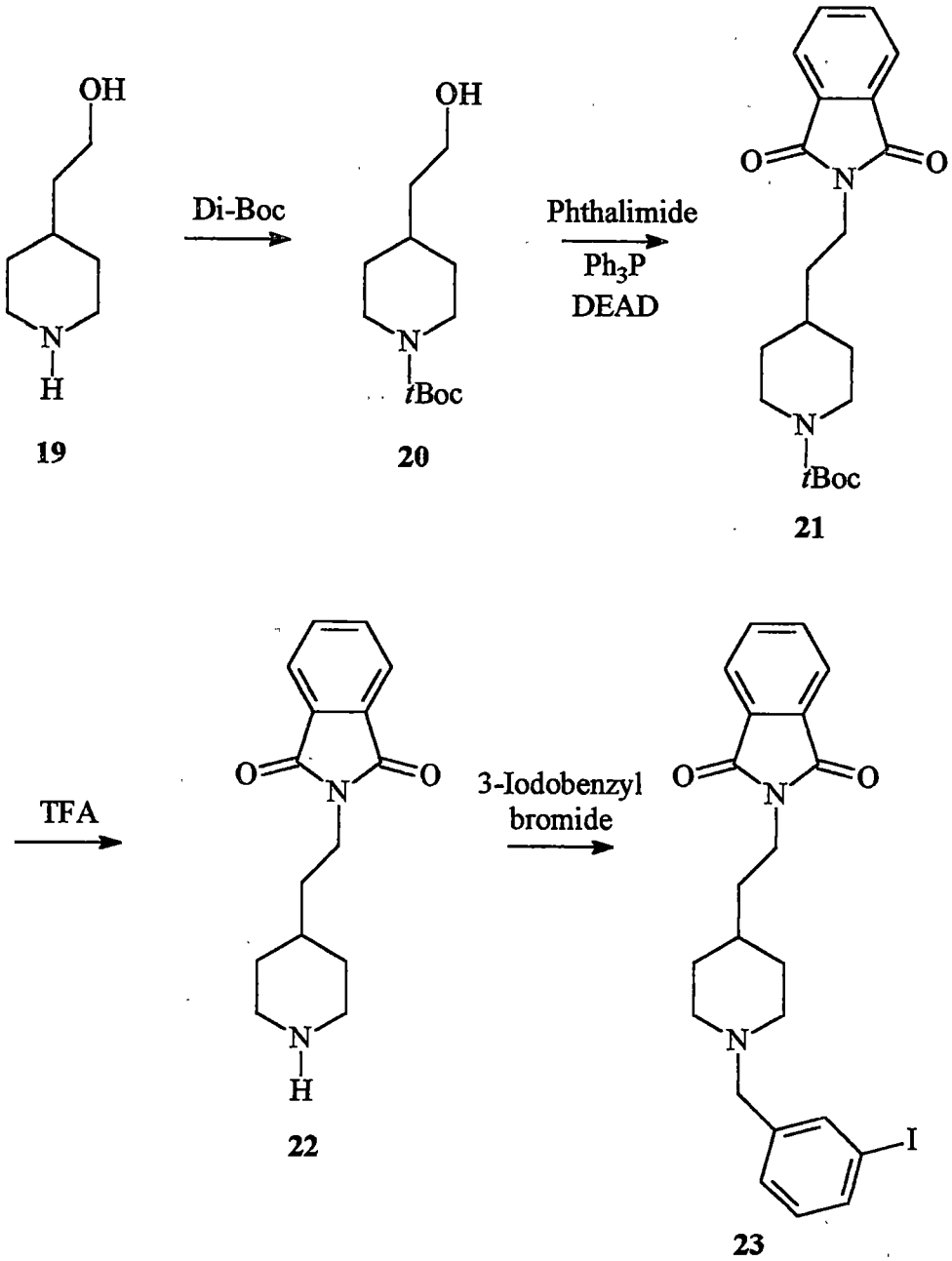
Compound **14** was then reduced by lithium aluminum hydride to yield the alcohol 1-(*t*-Boc)-4-(hydroxymethyl)piperidine, **15**, which was isolated in good yield.[125]

With **15** in hand, the hydroxyl group was replaced with iodine. A solution of **15** was reacted with triphenylphosphine, a base, and molecular iodine. The oxygen attacked the phosphorus generating a tetravalent phosphorus. The oxygen was then deprotonated by the base. Iodide ion attacked the carbon-oxygen bond via a S_N2 mechanism displacing the pentavalent phosphorous in triphenylphosphine oxide to form 4-iodomethyl-(*N*-*t*-Boc)piperidine, **16** in moderate yield.[125]

Intermediate **16** was then reacted with potassium cyanide to displace the iodide and generate the desired product, **17**. The cyanide ion nucleophilically replaced iodide via a S_N2 mechanism.

Intermediate **17** was then reduced to 4-(2-aminoethyl)-(*N*-*t*-Boc)-piperidine, **18**, using lithium aluminum hydride. The remaining proposed steps are shown in Scheme 3-5. The synthesis was terminated because a shorter route was developed which reduced the first six steps to two, with much greater efficiency. The new route is presented in Scheme 3-6.

4-Piperidineethanol, **19**, was allowed to react with di-*t*-butoxy dicarbonate to give 1-*t*-butoxycarbonyl 4-piperidineethanol, **20**.[126] **20** was then subjected to a Mitsonubu reaction wherein the hydroxyl group was replaced by a phthalimidyl group by allowing **20** to react with triphenylphosphine, diethyl azidodicarboxylate, and DEAD to yield 1-*tert*-butoxycarbonyl-4-[2-(*N*-phthalimido)ethyl]piperidine, **21**. A lone pair of electrons on the phosphorous attacks one of the nitrogens of DEAD



Scheme 3-6. 2nd Synthetic Route to the Iodo Intermediate

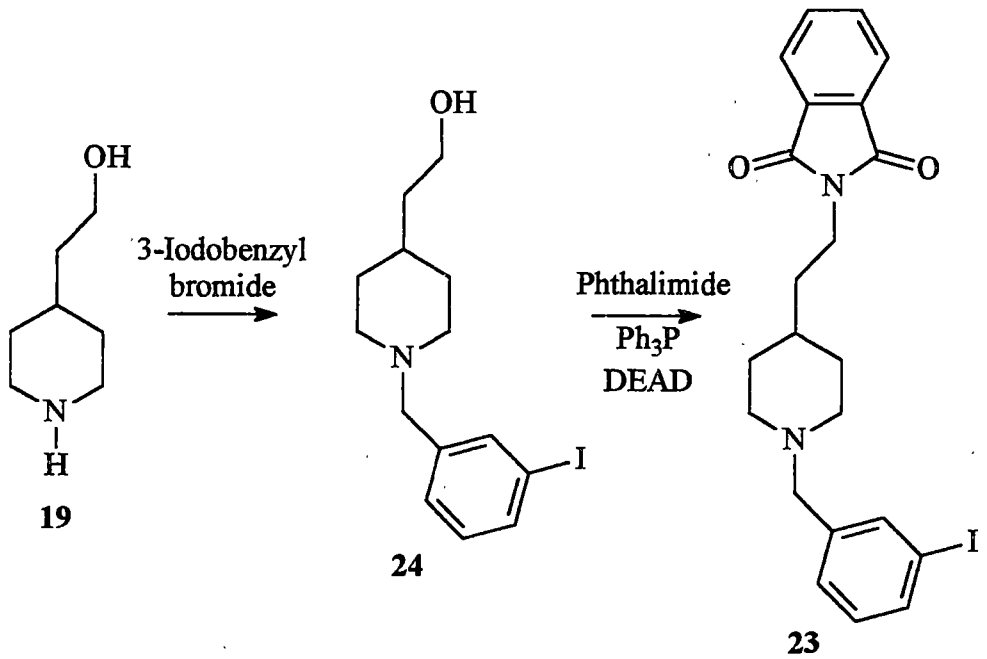
forming a quaternary phosphonium salt. The proton of the phthalimide protonates the other nitrogen of DEAD forming an alkoxyphophonium salt. S_N2 displacement of the salt yields diethyl hydrazinedicarboxylate and a quaternary phosphonium salt. The salt yields triphenylphosphine oxide, resulting from oxidized triphenylphosphine and **21**.[126] **21** was then deprotected using trifluoroacetic acid to yield 4-[2-(*N*-phthalimido)ethyl]piperidine, **22**.[132]

Compound **22** was then iodobenzylated using 3-iodobenzyl bromide to give 1-(3-iodobenzyl)-4-[2-(*N*-phthalimido)ethyl]-piperidine, **23**. The lone pair on the nitrogen attacks the benzyl carbon displacing the bromine via a S_N2 reaction.

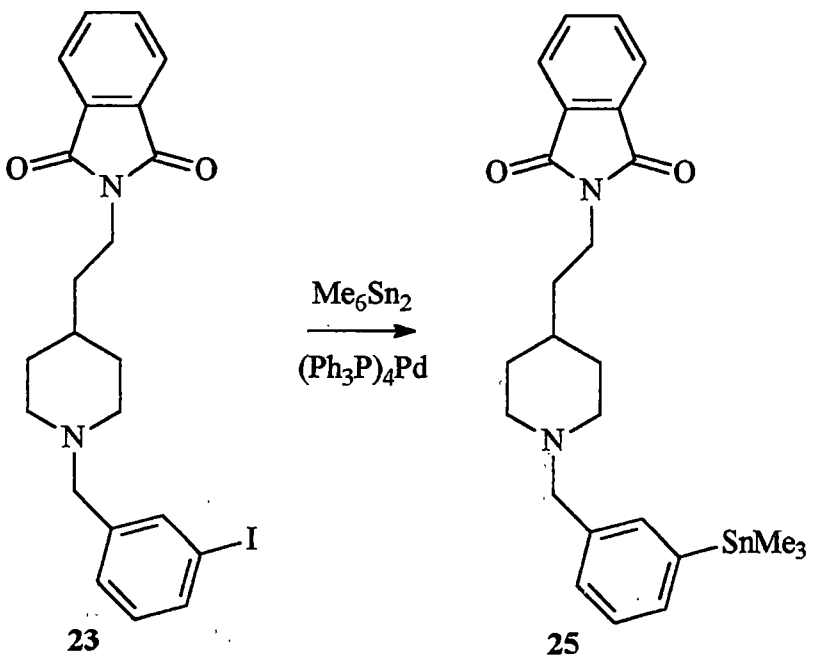
Another route to **23** was discovered and is shown in Scheme 3-7. 4-Piperidineethanol, **19**, was first iodobenzylated using 3-iodobenzyl bromide yielding 1-(3-iodobenzyl)-4-piperidineethanol, **24**. **24** was then subjected to a Mitsonubu reaction with phthalimide to yield 1-(3-iodobenzyl)-4-[2-(N-phthalimido)ethyl]-piperidine, **23**.

Once the iodo intermediate was prepared, its conversion to a trimethyltin derivative was straightforward. **23** reacted with hexamethylditin and tetrakis-(triphenylphosphine)palladium to yield 1-(3-trimethylstannyl)-4-[2-(*N*-phthalimido)-ethyl]piperidine, **25**, Scheme 3-8. The iodine was electrophilically replaced with trimethyltin utilizing the tetrakis(triphenylphosphine)palladium as the catalyst. The end of the reaction was indicated by precipitation of the palladium dust.[126]

With the trimethylstannyl derivative in hand, the formation of the fluorine-18 and iodine-123 derivatives was now feasible.



Scheme 3-7. Final Synthetic Route to the Iodo Intermediate



Scheme 3-8. Synthesis of Stannyl Precursor

G. Synthesis of 1-(3-[^{123}I]Iodobenzyl)-4-[2-(*N*-phthalimido)ethyl]piperidine

The iodine-123 derivative was first prepared using a carrier-added iodination sequence. **25** was dissolved in anhydrous methanol, to this was added a methanolic solutions of sodium iodide containing sodium [^{123}I]iodide (14 MBq). Finally a methanolic solution of 0.3% peracetic acid was added and the mixture allowed to stir. A drop of saturated sodium thiosulfate was added to remove excess iodine. This process yielded 1-(3-[^{123}I]iodobenzyl)-4-[2-(*N*-phthalimido)ethyl]piperidine, **26**. The peracetic acid was added to oxidize the iodide to iodine. The presence of the iodine was indicated by a brown-orange color. It has been shown that trimethyltin can be electrophilically replaced by iodine. The by-product is the trimethyltin iodide.

No-carrier-added **26** was also synthesized. **25** was dissolved in anhydrous methanol, to this was added a methanolic solution of no-carrier-added sodium [^{123}I]iodide (11 MBq). A methanolic solution of 0.3 % peracetic acid was then added and the reaction allowed to stir. A drop of saturated sodium thiosulfate was added to remove excess iodine. This process yielded no-carrier-added 1-(3-[^{123}I]iodobenzyl)-4-[2-(*N*-phthalimido)ethyl]piperidine, **26**.

H. Synthesis of 1-(3-[^{18}F]Fluorobenzyl)-4-[2-(*N*-phthalimido)ethyl]piperidine

To synthesize the fluorine-18 labeled product, **25** was dissolved in anhydrous chloroform. [^{18}F]Acetyl hypofluorite (952 MBq) was bubbled through the solution by passing [^{18}F]F_2 through a plug of freshly fused sodium acetate. The reaction mixture was stirred for 15 minutes. This process yielded 1-(3-[^{18}F]fluorobenzyl)-4-[2-(*N*-phthalimido)ethyl]piperidine, **27**. The fluorine from acetyl hypofluorite

electrophilically replaces the trimethyltin yielding the desired product. Acetyl hypofluorite was used because it is more selective than fluorine gas.

I. Conclusion

Although *N*-benzylpiperidines have shown limited success in mapping ACh, the usefulness of AChE inhibitors to help map ACh has been proven. With the prevalence of AD, the need to develop a definitive diagnosis of AD is needed. Derivatives of *N*-benzylpiperidines, such as the ones described here, will require further examination to determine their efficacy in mapping AChE.

Experimental

General

All glassware, syringes and syringe needles were oven-dried for 24 hours prior to use. Reactions were magnetically stirred and monitored by TLC. Products were purified by flash chromatography. NMR spectra were recorded on a Bruker AC 250 FT-NMR or a Varian Mercury 300 FT-NMR spectrometer. The ^{1}H NMR spectra were referenced to tetramethylsilane (TMS). The ^{13}C NMR spectra were referenced to $CDCl_3$ (with the center of the triplet at 77.0 ppm) or CD_3OD (with the center of the pentet at 49.0 ppm). ^{19}F NMR spectra were referenced to $CFCl_3$ (centered at 0.0 ppm). Thin layer chromatography was performed using Analtech Silica Gel 60 Å plates (250 μm thickness) with a fluorescent indicator (254 nm). Radio-TLC was performed using Analtech silica gel G PF-254 aluminum plates (250 μm) which were analyzed using a Bioscan System 200 Imaging Scanner controlled by an IBM Personal Computer. Column chromatographic separations were accomplished using ICN Silitech 60Å (230-400 mesh) silica gel. All reagents were analytical grade and were used without further purification. Atlantic Microlabs Inc., Norcross Georgia, performed elemental analyses. The commercially available chemicals and solvents used and their manufacturers are listed in table A-1.

Production of $[^{18}F]F_2$

The cyclotron is a particle accelerator used for the production of radioisotopes such as fluorine-18. A cyclotron consists of a pair of hollow, semicircular metal electrodes (called "dees" because of their shape), positioned between the poles of a large electromagnet. The dees are separated from one another by a narrow gap. An

ion source (typically an electrical arc device in a gas) near the center of the dees is used to generate charged particles. During operation, particles are generated in bursts, of 20 μA, by the ion source. A filament located in the ion source assembly creates a negative charge on the hydrogen ions through the addition of two electrons to the hydrogen. As the negative ions enter the vacuum tank, they gain energy due to a high frequency alternating electric field induced on the dees, 27 MHz. As the negative ions flow from the ion source, they are exposed to this electric field as well as a strong magnetic field generated by two magnet poles, one above and one below the vacuum tank. Because these are charged particles in a magnetic field, the negative ions move in a circular path. When the negative ions reach the edge of the dee and enter the gap, the RF oscillator changes the polarities on the dees. The negative ions are repelled as they exit the previously positive but now negatively charged dee. Each time the particles cross the gap they gain energy, so the orbital radius continuously increases and the particles follow an outwardly spiraling path. The particles are "pushed" from the first dee and drift along a circular path until they are attracted or "pulled" by the second dee which has become positively charged. The result is a stream of negative ions containing 11.2 MeV of energy, which are accelerated, in a circular path spiraling outward. The stream of negative ions is directed towards a thin carbon foil that strips each H^- ion of its two electrons. When the negative ions lose both electrons, they become H^+ ions or protons. The stripping foil is positioned partway into the beam so that a portion of the beam is extracted. The remainder of the particles continue in a circular path completing an additional orbit. In preparation for bombardment, oxygen-18 gas is loaded into a target

chamber. The proton beam from the cyclotron enters the target chamber and by means of a nuclear reaction, the oxygen-18 is changed into fluorine-18. The chamber is pressurized with argon and then delivered to the hot cell in preparation for synthesis of acetyl hypo[^{18}F]fluorite.

9-Amino-1,2,3,4-tetrahydroacridine (3).[130] To a solution of anthranilonitrile, **1**, (2.47 g, 20.9 mmol) in cyclohexanone, **2**, (8.00 mL, 76.9 mmol) was added zinc chloride (3.10 g, 22.8 mmol). The mixture was heated to 130 – 140 °C for 30 minutes. A colored complex precipitated from the solution. The solid was isolated by filtration and washed with ethyl acetate. The solid was then suspended in water and basified with 6 N sodium hydroxide to obtain a white solid. Drying under vacuum yields the required compound (2.92 g, 71 %), mp 183 °C. ^{1}H-NMR (CD_3OD/TMS) δ ppm: 1.84 (*s*, 4H), 2.46 (*s*, 2H), 2.83 (*s*, 2H), 7.43 (*d*, 1H, J = 2 Hz), 7.56 (*t*, 1H, J = 4 Hz), 7.68 (*t*, 1H, J = 2 Hz), 8.12 (*d*, 1H, J = 8 Hz). ^{13}C-NMR ppm: 30.4, 34.6, 35.5, 112.9, 119.2, 120.7, 123.7, 127.6, 128.4, 147.2, 157.1, 163.4.

9-(*N*-Acetyl)-amino-1,2,3,4-tetrahydroacridine (4).[128] To a solution of **3** (4.30 g, 21.7 mmol) in acetic anhydride (5.00 mL, 52.9 mmol) was added pyridine (18.0 mL, 22.3 mmol). The mixture was heated to 100 °C, for 30 minutes and allowed to cool to room temperature, and then poured in to 20.0 mL of ice cold water. Saturated aqueous sodium carbonate was then added until basic. The product was collected by filtration and the solid was washed with water and dried under vacuum to yield the desired product (4.88 g, 88 %), mp 249 °C, dec. ^{1}H-NMR (CD_3OD/TMS) δ ppm: 1.84 (*s*, 4H), 2.10 (*s*, 3H), 2.46 (*s*, 2H), 2.83 (*s*, 2H), 7.43 (*d*, 1H, J = 2 Hz), 7.56 (*t*, 1H, J = 4 Hz), 7.68 (*t*, 1H, J = 2 Hz), 8.10 (*s*, 1H, exchanges with D_2O), 8.12 (*d*,

17.6, 30.4, 34.6, 35.5, 112.9, 119.2, 120.7, 123.7, 127.6, 128.4, 147.2, 157.1, 163.4, 168.2.

9-(*N*-Acetyl)-amino-1,2,3,4-tetrahydro-(*N*-oxo)-acridine (5). To a solution of **4** (1.03 g, 4.27 mmol) in 40 mL of chloroform was added 32% peracetic acid (1.00 mL, 14.9 mmol). The reaction mixture was allowed to stir for 45 minutes and then poured into 5 mL of ice cold water. The organic layer was separated, dried over magnesium sulfate and the volume reduced under vacuum to yield the desired product (0.432 g, 41 %) as a colorless oil. ^{1}H-NMR (CD_3OD/TMS) δ ppm: 1.84 (*s*, 4H), 2.10 (*s*, 3H), 2.46 (*s*, 2H), 2.83 (*s*, 2H), 7.43 (*d*, 1H, J = 2 Hz), 7.56 (*t*, 1H, J = 4 Hz), 7.68 (*t*, 1H, J = 2 Hz), 8.10 (*s*, 1H), 8.73 (*d*, 1H, J = 8 Hz). ^{13}C-NMR ppm: 17.6, 30.4, 34.6, 35.5, 112.9, 119.2, 120.7, 123.7, 127.6, 128.4, 147.2, 157.1, 163.4, 168.2. IR ν (N-O) 1315 cm^{-1}. Anal. Calcd for ($C_{15}H_{16}N_2O_2$) C 70.29, H 6.29, N 10.93. Found C 70.01, H 6.27, N 10.96.

9-(*N*-Acetyl)-amino-1,2,3-trihydro-4-acetoxy-acridine (6). To a solution of **5** (0.0966 g, 0.377 mmol) in chloroform (10 mL) was added acetic anhydride (0.710 mL, 7.55 mmol). The mixture is refluxed for 30 minutes and allowed to cool to room temperature. Mixture was washed with water (3 x 3 mL) and collected the organic layer. Drying over magnesium sulfate and concentration under vacuum yields the desired product (0.0231 g, 21 %) as a colorless oil. ^{1}H-NMR (CD_3OD/TMS) δ ppm: 1.84 (*s*, 2H), 2.10 (*s*, 6H), 2.19 (*s*, 2H), 2.83 (*s*, 2H), 4.70 (*m*, 1H), 7.43 (*d*, 1H, J = 2 Hz), 7.56 (*t*, 1H, J = 4 Hz), 7.68 (*t*, 1H, J = 2 Hz), 8.10 (*s*, 1H, exchanges with D_2O), 8.12 (*d*, 1H, J = 8 Hz). ^{13}C-NMR ppm: 17.6, 28.6, 30.4, 38.9, 78.2, 112.9, 119.2,

120.7, 123.7, 127.6, 128.4, 147.2, 157.1, 163.4, 168.2, 171.0. Anal. Calcd for ($C_{17}H_{18}N_2O_3$) C 68.44, H 6.08, N 9.39. Found C 69.64, H 6.09, N 9.37.

***N*-(3-Oxocylohexen-1-yl)-2-aminobenzonitrile (8).**[96] A suspension of anthranilonitrile, **1**, (25.5 g, 211 mmol) in 130 mL of toluene containing 1,3-cyclohexanedione, **7**, (26.2 g, 233 mmol) and *p*-toluenesulfonic acid monohydrate (2.67 g, 1.50 mmol) was refluxed for 2 h, and the water was collected in a Dean-Stark water separator. The reaction mixture was then chilled to 0 °C and the product was filtered off and washed with cold toluene. The crude product obtained in this manner was slurried with 130 mL of water and stirred for 1h, after which it was filtered off and dried under reduced pressure to give 40.3 g (90 %) of **8**, mp 189 °C. ^{1}H NMR δ ppm: 2.10 (quintet, 2H, J = 7 Hz), 2.40 (*t*, 2H, J = 7 Hz), 2.60 (*t*, 2H, J = 7 Hz), 5.59 (*s*, 1H), 7.0 (*b s*, 1H), 7.25 (*t*, 1H, J = 8 Hz), 7.60 (*m*, 3H). ^{13}C-NMR δ ppm: 17.4, 39.2, 42.5, 96.3, 98.7, 113.0, 116.5, 117.6, 132.8, 133.6, 147.0, 161.7, 197.6.

9-Amino-3,4-dihydroacridin-1(2*H*)-one (9).[96] In 125 mL of THF was suspended **8** (5.05 g, 23.8 mmol), anhydrous K_2CO_3 (1.06 g, 7.67 mmol), and CuCl (0.190 g, 1.92 mmol). The reaction mixture was refluxed for 5 h and then the hot mixture was filtered into 100 mL of hexane. Precipitate **9** was filtered off, washed with water, and dried to give 2.78 g (55 %), mp 238 °C. ^{1}H NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.18 (quintet, 2H, J = 7 Hz), 2.74 (*t*, 2H, J = 7 Hz), 3.10 (*t*, 2H, J = 7Hz), 7.40 (*d t*, 1H, J = 8 Hz, J = 1 Hz), 7.70 (*d t*, 1H, J = 8 Hz, J = 1 Hz), 7.6 - 7.8 (*b s*, 1H, exchanges with D_2O), 7.80 (*dd*, 1H, J = 8 Hz, J = 1 Hz), 8.20 (*dd*, 1H, J = 8 Hz, J = 1 Hz). ^{13}C-NMR ppm: 21.4, 35.3, 42.5, 112.9, 114.8, 121.2, 124.6, 128.5, 131.0, 148.2, 155.4, 166.9, 197.6.

9-Amino-1,2,3,4-tetrahydroacridin-1(2*H*)-ol (10).[96] Ketone **9** (2.52 g, 11.9 mmol) was suspended in 50 mL of dry THF and a solution of LAH in THF (12.5 mL of 1.00 M, 12.5 mmol) was added dropwise over 15 minutes. The reaction was allowed to stir for 2 h and then quenched by the careful addition of saturated aqueous sodium thiosulfate. The free base **10** was filtered off. Purification using silica gel chromatography and acetone as the eluent yielded 1.28 g (50 %) of the desired product. MP 172 °C. ^{1}H NMR ($CDCl_3$/TMS) δ ppm: 2.7 – 3.1 (*m*, 4H), 2.8 – 3.0 (*m*, 2H), 4.90 (*s*, 1H), 5.35 (*br s*, 1H, exchanges with D_2O), 7.64 (*t*, 1H, J = 8 Hz), 7.80 (*d*, 1H, J = 8Hz), 7.92 (*t*, 1H, J = 8Hz), 8.52 (*d*, 1H, J = 8 Hz), 8.6 (*br s*, 2H, exchanges with D_2O). ^{13}C-NMR δ ppm: 28.4, 35.8, 42.2, 69.7, 112.9, 119.2, 120.7, 123.7, 127.6, 128.4, 147.2, 157.1, 163.4.

9-Amino-1-fluoro-2,3,4-trihydroacridine (11). DAST (0.250 g, 1.50 mmol) was dissolved in methylene chloride (3 mL) and pyridine (0.300 mL), and the solution was cooled to 0 °C under nitrogen. **10** (0.300 g, 1.40 mmol) was slowly added and the solution warmed to room temperature. The solvent was removed under vacuum and the product chromatographed on silica gel. The desired compound could not be isolated.

1-*tert*-Butoxycarbonyl ethyl isonipecotate (14).[125] A solution of ethyl isonipecotate, **13**, (5.03 g, 31.9 mmol) and triethylamine (4.60 mL, 32.8 mmol) in 1:1 dioxane-H_2O (300 mL) was cooled to 0 °C. After 15 minutes, di-*t*-butyl dicarbonate (8.93 g, 40.8 mmol) was added and the resulting mixture was allowed to warm to room temperature overnight. The mixture was extracted with ethyl acetate (4 x 20 mL) and the combined organic layer was washed sequentially with 1 N HCl, H_2O,

and brine, dried ($MgSO_4$), filtered, and concentrated to give a light orange oil. Kugelrhor distillation (0.05 Torr, 80 – 90 °C) yielded the desired product (8.10 g, 95 %) as a colorless oil. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.23 (*t*, 3H, J = 7.2 Hz), 1.43 (*s*, 9H), 1.52 – 1.66 (*m*, 2H), 1.81 – 1.86 (*m*, 2H), 2.40 (*tt*, 1H, J = 11.0 Hz, J = 3.9 Hz), 2.80 (*br t*, 2H, J = 11.6 Hz), 3.97 – 4.05 (*m*, 2H), 4.11 (*q*, 2H, J = 7.2 Hz). ^{13}C-NMR ppm: 13.6, 24.8, 28.7, 38.1, 44.9, 59.8, 70.9, 155.7, 176.0.

1-*tert*-Butoxycarbonyl-4-(hydroxymethyl)piperidine (15).[125] Lithium aluminum hydride (1.20 g, 31.8 mmol) was added to a cold solution (0 °C) of **14** (7.22 g, 28.9 mmol) in THF (250 mL). After 30 minutes, the ice bath was removed and the reaction mixture was allowed to stir overnight at room temperature. Saturated sodium sulfate was added carefully until evolution of gas subsided. After stirring for 1 h, the mixture was filtered through a Celite pad and the filtrate was concentrated. Recrystallization (Et_2O/hexanes) yielded the desired product (6.59 g, 92 %) as a white solid, mp 80 °C. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.15 (*ddd*, 2H, J = 23.2 Hz, J = 12.0, J = 4.3 Hz), 1.47 (*s*, 9H), 1.60 – 1.73 (*m*, 3H), 2.70 (*br t*, 2H, J = 12.0 Hz), 3.49 (*d*, 2H, J = 6.4 Hz), 4.04 - 4.26 (*m*, 2H). ^{13}C-NMR δ ppm: 25.7, 28.7, 34.0, 47.5, 70.9, 71.1, 155.7.

1-*tert*-Butoxycarbonyl-4-(Iodomethyl)piperidine (16).[125] Triphenyl-phosphine (9.19 g, 35.2 mmol) was added to a mixture of iodine (8.70 g, 34.2 mmol) in benzene (300 mL). After 5 minutes, pyridine (5.55 mL, 67.8 mmol) was added followed by addition of alcohol **15** (5.55 g, 25.7 mmol). The resulting mixture was heated to reflux for 1.5 h. The cooled reaction mixture was filtered, and the filtrate washed sequentially with saturated $Na_2S_2O_3$ and brine, dried ($MgSO_4$), filtered, and

washed sequentially with saturated $Na_2S_2O_3$ and brine; it was then dried (anhydrous $MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (1:4 v/v EtOAc-hexanes) yielded 7.60 g (91 %) of **16** as a clear oil which solidified upon standing, mp 59 °C. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.11 (*ddd*, 2H, J = 24.7 Hz, J = 12.7 Hz, J = 4.3 Hz), 1.43 (*s*, 9H), 1.52 – 1.64 (*m*, 1H), 1.80 (*br d*, 2H, J = 12.9 Hz), 2.66 (*br t*, 2H, J = 13.1 Hz), 3.08 (*d*, 2H, J = 6.5 Hz), 4.09 (*br d*, 2H, J = 13.1 Hz). ^{13}C-NMR δ ppm: 14.9, 28.7, 30.4, 34.8, 46.3, 70.9, 155.7

1-*tert*-Butoxycarbonyl-4-(cyanomethyl)piperidine (17). To a solution of DMSO (20 mL) containing NaCN (0.199 g, 4.06 mmol) was added **16** (1.31 g, 4.00 mmol). The mixture was heated to 80 °C for 2 h. The cooled solution was diluted with 20 mL of water and extracted into ether (3 x 3 mL). The combined organic layer was washed sequentially with water and brine; it was then dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to yield the desired product (0.722 g, 81 %) as a colorless oil. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.11 (*ddd*, 2H, J = 24.7 Hz, J = 12.7 Hz, J = 4.3 Hz), 1.43 (*s*, 9H), 1.52 – 1.64 (*m*, 1H), 1.80 (*br d*, 2H, J = 12.9 Hz), 2.03 (*br t*, 2H, J = 13.1 Hz), 3.08 (*d*, 2H, J = 6.5 Hz), 4.09 (*br d*, 2H, J = 13.1 Hz). ^{13}C-NMR δ ppm: 26.3, 26.4, 28.6, 28.7, 46.7, 70.9, 117.7, 155.7. Anal. Calcd for ($C_{12}H_{20}N_2O_2$) C 64.29, H 8.99, N 12.49. Found C 64.16, H 9.00, N 12.52.

4-(2-Aminoethyl)-1-*tert*-butoxycarbonylpiperidine (18).[129] To a solution of **17** (0.113 g, 0.510 mmol) in ether (5 mL) was slowly added LAH (0.0250 g, 0.660 mmol). After the mixture was allowed to stir for 2 h, the excess LAH was destroyed by the careful addition of aqueous saturated sodium sulfate until a white precipitate appeared. Purification using silica gel chromatography (4:1 v/v ethyl

acetate:hexanes) yielded the desired product (0.0810 g, 71 %) as a colorless oil. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.11 (*ddd*, 2H, J = 24.7 Hz, J = 12.7 Hz, J = 4.3 Hz), 1.43 (*s*, 9H), 1.52 – 1.64 (*m*, 1H), 1.80 (*br d*, 2H, J = 12.9 Hz), 2.59 (*br t*, 2H, J = 13.1 Hz), 2.72 (*s*, 2H, exchanges with D_2O), 3.08 (*d*, 2H, J = 6.5 Hz), 4.09 (*br d*, 2H, J = 13.1 Hz). ^{13}C-NMR δ ppm: 28.2, 28.7, 29.4, 40.1, 42.5 47.5, 70.9, 155.7.

1-*tert*-Butoxycarbonyl-4-piperidineethanol (20).[126] Di-*tert*-butyl dicarbonate (2.19 g, 10.0 moL) was added to a vigorously stirred biphasic mixture of 4-piperidineethanol, **19** (1.08 g, 8.36 mmol) in 5% $NaHCO_3$:$CHCl_3$ (10 mL, 1:1 v/v). The mixture was refluxed for 16 h, cooled and extracted into CH_2Cl_2 (3 x 40 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was distilled (127 °C/0.8 torr) to give 3.39 g (94%) of a colorless oil. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.08 (*m*, 2H), 1.40 (*s*, 9H), 1.48 (*m*, 3H), 1.61 (*br d*, 2H, J = 12.32 Hz), 2.21 (*br s*, 1H), 2.63 (*br t*, 2H, J = 12.69 Hz), 3.62 (*br t*, 2H, J = 6.05 Hz), 4.02 (*m*, 2H). ^{13}C-NMR δ ppm: 27.1, 28.7, 29.7, 41.3, 47.5, 60.8, 70.9, 155.7.

1-*tert*-Butoxycarbonyl-4-[2-(N-phthalimido)ethyl]piperidine (21).[126] Di-ethyl azodicarboxylate (0.600 mL, 3.81 mmol) was added to a solution of N-*tert*-butoxycarbonylpiperidineethanol (0.999 g, 4.36 mmol), phthalimide (0.901 g, 6.12 mmol) and triphenylphosphine (1.21 g, 4.61 mmol) in the THF (12 mL). The solution was stirred for 3 days and subsequently concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (10 mL) and the solution treated with hexanes until it turned cloudy. The precipitated Ph_3PO was removed by filtration, and the filtrate concentrated under reduced pressure. The residue was passed through SiO_2,

eluting with 4:1 hexanes:acetone. The eluent was subsequently concentrated under reduced pressure to give 1.27 g (81%) of a colorless oil that solidified on standing, mp 113 – 115 °C. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.04 (*dt*, 2H, J = 3.5 Hz, J' = 11.5 Hz), 1.25 (*m*, 1H), 1.38 (*s*, 9H), 1.55 (*m*, 2H), 1.68 (*br d*, 2H, J = 12.6 Hz), 2.60 (*br t*, 2H, J = 12.0 Hz), 3.65 (*t*, 2H, J = 7.29 Hz), 4.01 (*br d*, 2H, J = 12.8), 7.65 (*m*, 2H), 7.75 (*m*, 2H). ^{13}C-NMR δ ppm: 28.2, 28.7, 29.4, 37.1, 38.6, 47.5, 70.9, 126.8, 129.3, 133.0, 155.7, 165.9.

4-[2-(*N*-phthalimido)ethyl]piperidine (22).[132] Trifluoracetic acid (1.00 mL, 12.9 mmol) was added to a solution of **21** (0.611 g, 1.71 mmol) in methylene chloride (9 mL). The solution was refluxed for 3 h, cooled and subsequently poured into water (10 mL). The resulting solution was adjusted to pH 11 with 10% NaOH and extracted with CH_2Cl_2 (3 x 8 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was chromatographed on SiO_2 eluting with MeOH:Et_3N (100:1). Concentration of the desired fractions yielded 0.308 g (70%) of a colorless oil. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.20 (*m*, 2H), 1.65 (*m*, 2H), 1.68 (*br d*, 2H, J = 11 Hz), 1.84 (*s*, 1H, exchanges with D_2O), 2.65 (*br t*, 2H, J = 13 Hz), 3.72 (*t*, 2H, J = 7.2 Hz), 4.08 (*br d*, 2H, J = 10.9 Hz), 7.65 (*m*, 2H), 7.82 (*m*, 2H). ^{13}C-NMR δ ppm: 28.5, 32.2, 37.1, 38.6, 47.7, 126.8, 129.3, 133.0, 165.9.

1-(3-Iodobenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine (23). 3-Iodobenzyl bromide (0.165 g, 0.560 mmol) was added to a solution of **22** (0.143 g, 0.550 mmol) in methylene chloride (10 mL). To this was added triethylamine (0.700 mL, 0.720 mmol), and the mixture was refluxed overnight. The cooled solution was

washed with water (3 x 4 mL), dried over anhydrous magnesium sulfate, filtered and then concentrated. The crude product was purified on SiO_2 using acetone:hexanes (1:1 v/v) as the eluent. The corresponding fractions were collected and concentrated to give 0.216 g (83 %) as a white solid, mp 83 – 84 °C. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.29 (*m*, 3H), 1.61 (*q*, 2H, J = 5 Hz), 1.75 (*d*, 2H, J = 9 Hz), 1.92 (*t*, 2H, J = 11 Hz), 2.83 (*d*, 2H, J = 9 Hz), 3.39 (*s*, 2H), 3.71 (*t*, 2H, J = 7 Hz), 7.02 (*t*, 1H, J = 7 Hz), 7.26 (*d*, 1H, J = 8 Hz), 7.53 (*d*, 1H, J = 3 Hz), 7.66 (*s*, 1H), 7.69 (*m*, 2H), 7.82 (*m*, 2H). ^{13}C-NMR δ ppm: 28.5, 32.0, 37.1, 38.6, 52.0, 62.5, 97.0, 126.8, 127.9, 129.3, 129.8, 133.0, 135.9, 137.9, 165.9. Anal. Calcd for ($C_{22}H_{23}N_2O_2I$) C 55.29, H 4.94, N 5.82. Found C 55.71, H 4.89, N 5.91.

1-(3-Iodobenzyl)-4-piperidineethanol (24). To a solution of **19** (1.00 g, 7.74 mmol) in methylene chloride (15 mL) was added 3-iodobenzyl bromide (2.41 g, 8.11 mmol) and triethylamine (1.50 mL, 10.7 mmol). Mixture was refluxed overnight, upon cooling the reaction mixture was washed with water, brine, dried (anhydrous $MgSO_4$), and concentrated. Purification using silica gel chromatography (1:1 v/v acetone:hexanes) yielded the desired product (2.21 g, 83 %) as a colorless oil. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.27 (*m*, 3H), 1.50 (*t*, 2H, J = 6 Hz), 1.66 (*d*, 2H, J = 15 Hz), 1.82 (*br s*, 1H, exchanges with D_2O), 1.93 (*t*, 2H, J = 10 Hz), 2.83, (*d*, 2H, J = 12 Hz), 3.41 (*s*, 2H), 3.67 (*t*, 2H, J = 6 Hz), 7.03 (*t*, 1H, J = 8 Hz), 7.27 (*t*, 1H, J = 4 Hz), 7.57 (*d*, 1H, J = 8 Hz), 7.68 (*s*, 1H). ^{13}C-NMR δ ppm: 27.4, 32.3, 41.3, 52.0, 60.8, 62.5, 97.0, 127.9, 129.8, 135.6, 137.9. Anal. Calcd for ($C_{14}H_{20}NOI$) C 48.68, H 5.87, N 3.99. Found C 48.71, H 5.84, N 4.06.

1-(3-Iodobenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine (23). To a solution of **24** (0.818, 2.40 mmol) in THF (25 mL) was added triphenylphosphine (0.654 g, 2.45 mmol), phthalimide (0.360 g, 2.45 mmol), and finally DEAD (0.440 mL, 2.45 mmol). The mixture was allowed to stir for 3 days and then concentrated under vacuum. The residue was taken up in methylene chloride (5 mL) and triphenylphosphine oxide was then precipitated by the addition of hexanes. Filtration removed the precipitate and the filtrate was concentrated under reduced pressure and chromatographed on silica gel to yield the desired product 0.921 g (82 %). NMR was consistent with sample described earlier.

1-(3-Trimethylstannylbenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine (25). To a solution of **23** (0.350 g, 0.740 mmol) in 1,4-dioxane (12 mL) was added hexamethylditin (0.610 g, 1.86 mmol) and tetrakis(triphenylphosphine)palladium (0.0590 g, 0.051 mmol). The solution was refluxed for 6.5 hours. The cooled solution was filtered and the filtrate was collected and concentrated under reduced pressure. The residue was purified on silica gel using ethyl acetate:petroleum ether (1:2 v/v) as the eluent to give **25**, 0.302 g (80 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 0.28 (*t*, 9H, J = 26 Hz), 1.29 (*m*, 3H), 1.61 (*q*, 2H, J = 5 Hz), 1.75 (*d*, 2H, J = 9 Hz), 1.92 (*t*, 2H, J = 11 Hz), 2.83 (*d*, 2H, J = 9 Hz), 3.39 (*s*, 2H), 3.71 (*t*, 2H, J = 7 Hz), 7.23 (*m*, 3H), 7.39 (*s*, 1H), 7.69 (*m*, 2H), 7.82 (*m*, 2H). ^{13}C-NMR δ ppm: 2.4, 28.5, 32.0, 37.1, 38.6, 52.0, 63.4, 126.8, 128.0, 128.7, 129.3, 133.0, 134.4, 136.1, 136.4, 141.6, 165.9. Anal. Calcd for ($C_{25}H_{32}N_2O_2Sn$) C 58.74, H 6.31, N 5.48. Found C 58.68, H 6.31, N 5.49.

1-(3-Fluorobenzyl)-4-piperidineethanol (26). To a solution of **19** (0.401 g, 3.10 mmol) in methylene chloride (5 mL) was added 3-fluorobenzyl bromide (0.385 mL, 3.14 mmol) and triethylamine (0.500 mL, 3.59 mmol). The mixture was refluxed overnight; upon cooling the reaction mixture was washed sequentially with water and brine, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure. Purification using silica gel chromatography (1:1 v/v acetone:hexanes) yielded the desired product (0.581 g, 79 %) as a colorless oil. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.27 (*m*, 2H), 1.30 (*m*, 1H), 1.50 (*t*, 2H, J = 7 Hz), 1.65 (*br d*, 2H, J = 3 Hz), 1.95 (*m*, 2H), 2.18 (*br s*, 1H, exchanges with D_2O), 2.83, (*d*, 2H, J = 12 Hz), 3.46 (*s*, 2H), 3.67 (*t*, 2H, J = 6 Hz), 6.93 (m, 1H), 7.05 (m, 2H), 7.25 (m, 1H). ^{13}C-NMR δ ppm: 32.3, 39.37, 53.8, 60.3, 62.8, 113.6 (*d*, J = 21 Hz), 115.7 (*d*, J = 21 Hz), 124.5 (*d*, J = 3 Hz), 129.3 (*d*, J = 8Hz), 141.1 (*d*, J = 7 Hz), 162.5 (*d*, J = 245 Hz). ^{19}F-NMR δ ppm: -114.2. Anal. Calcd for ($C_{14}H_{20}NOF$) C 70.86, H 8.49, N 5.90. Found C 70.83, H 8.50, N 5.91.

1-(3-Fluorobenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine (27). To a solution of **26** (0.403, 1.70 mmol) in THF (15 mL) was added triphenylphosphine (0.459 g, 1.75 mmol), phthalimide (0.257 g, 1.75 mmol), and finally DEAD (0.275 mL, 1.75 mmol). The mixture was allowed to stir for 3 days then concentrated under reduced pressure. The residue was taken up in methylene chloride (3 mL) and triphenylphosphine oxide was then precipitated by the addition of hexanes. Filtration removed the precipitate and the filtrate was concentrated and chromatographed on silica gel to yield the desired product 0.516 g (83 %) as a colorless oil. ^{1}H-NMR ($CDCl_3$/TMS) δ ppm: 1.29 (*m*, 3H), 1.61 (*q*, 2H, J = 5 Hz), 1.75 (*d*, 2H, J = 9 Hz),

1.92 (*t*, 2H, J = 11 Hz), 2.83 (*d*, 2H, J = 9 Hz), 3.39 (*s*, 2H), 3.71 (*t*, 2H, J = 7 Hz), 6.91 (*m*, 1H), 7.05 (m, 2H), 7.26 (m, 1H), 7.70 (*m*, 2H), 7.83 (*m*, 2H). ^{13}C-NMR δ ppm: 32.1, 33.4, 35.1, 35.6, 53.7, 62.8, 113.6 (*d*, J = 21 Hz), 115.6 (*d*, J = 21 Hz), 123.0, 124.4, 129.3 (*d*, J = 8 Hz), 132.4 (*d*, J = 8 Hz), 163.2 (*d*, J = 245 Hz), 168.2. ^{19}F-NMR δ ppm: -114.2. Anal. Calcd for ($C_{22}H_{23}N_2O_2F$) C 72.11, H 6.33, N 7.64. Found C 72.09, H 6.34, N 7.66.

Synthesis of Carrier-Added 1-(3-[^{123}I]Iodobenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine (28). To a solution of **25** (14.3 mg) in anhydrous methanol (200 μL) was added a solution of carrier-added sodium [^{123}I]iodide (40 μL, $6.7x10^{-2}$ M, 14 MBq). Then 0.3% methanolic peracetic acid (1.1 mL) was added. The reaction vial was closed and allowed to stir for 10 minutes. The vial was then opened and 1 drop of saturated aqueous sodium thiosulfate was added. Contents were carefully removed and loaded onto an alumina Sep-Pak cartridge for purification using 1:9 methanol:chloroform by volume. Radiochemical purity was determined by radio-TLC to be >96 % and the decay corrected radiochemical yield was 47 %.

Synthesis of No-Carrier-Added 1-(3-[^{123}I]Iodobenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine (28). To an anhydrous methanolic solution of **25** (100 μL of $2.2x10^{-2}$ M) was added no-carrier-added sodium [^{123}I]iodide (11 MBq) in sodium hydroxide. Then 0.3% methanolic peracetic acid (100 μL) was added and the reaction vial was closed and allowed to stir for 10 minutes. The reaction vial was opened and 1 drop of saturated aqueous sodium thiosulfate was added. Contents were carefully removed and loaded onto an alumina Sep-Pak cartridge for purification

using 1:9 methanol:chloroform by volume. Radiochemical purity was determined to be >96 % and the decay corrected radiochemical yield was 48 %.

Synthesis of Carrier-Added 1-(3-[^{18}F]Fluorobenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine (29).[131] To a solution of **25** (56 mg, 0.11 mmol) in anhydrous chloroform (5 mL) was added acetyl hypo[^{18}F]fluorite (952 MBq) and the reaction was stirred for 20 minutes. The reaction vial was opened and the contents were carefully removed and loaded onto an alumina Sep-Pak cartridge for purification using 1:9 methanol:chloroform by volume. Radiochemical purity was determined to be >94 % and the radiochemical yield was 38 %.

REFERENCES

1. Chiewitz, O.; Hevesy, G. *Nature* (London) **1935**, *13*, 754.

2. Chargaff, E. *J. Am. Chem. Soc.* **1938**, *60*, 1700.

3. *Principles of Nuclear Medicine*, Henry N. Wagner (ed.), W. B. Sanders Publishing Company, 1969, 7.

4. Hudlicky, M. *Chemistry of Organic Fluorine Compounds*, 2nd ed., Ellis Horwood, Chichester, 1976.

5. Moissan, H. *Compte Rendu* **1886**, *102*, 1543.

6. Swarts, F. *Bull. Acad. Roy. Belg.* **1892**, *24*, 474.

7. Midgley, T.; Henne, A. L.; *Ind. Eng. Chem.* **1930**, *22*, 542.

8. Phelps, M.; Mazziotta, J.; Schelbert, H. *Positron Emission Tomography and Autoradiography*, Raven Press, New York, 1986.

9. Baker, A. W.; Shulgin, A. T. *Nature (London)* **1965**, *206*, 712.

10. Doddrell, S.; Wenkert, E.; Demarco, P. V. *J. Mol. Spectrosc.* **1969**, *32*, 162.

11. Bida, G. T.; Satyamurthy, N.; Barrio, J. R. *J. Nucl. Med.* **1984**, *26*, 1327.

12. Herscheid, J. D. M.; van Rijn, C. J.; Visser, G. W. M.; Hoekstra, A. *J. Labelled. Cmpd. Radiopharm.* **1984**, *21*, 1192.

13. Ishiwata, K.; Ido, T.; Nakanishi, H.; Iwata, R. *Appl. Radiat. Isot.* **1987**, *38*, 463.

14. Shiue, C-Y.; Fowler, J. S.; Wolf, A. P.; Alexoff, D.; MacGregor, R. R. *J. Labelled Cmpds. Radiopharm.* **1985**, *22*, 503.

15. Oberdorfer, F.; Hofman, E.; Maier-Borst, W. *J. Labelled Cmpd. Radiopharm.* **1988**, *25*, 999.

16. Eakins, M. N.; Palmer, A. J.; Waters, S. L. *Int. J. Appl. Radiat. Isot.* **1979**, *30*, 695.

17. Tewson, T. J.; Welch, M. J. *J. Org. Chem.* **1978**, *43*, 1090.

18. Shiue, C-Y.; Arnett, C. D; Wolf, A. P. *Eur. J. Nucl. Med.* **1984**, *9*, 77.

19. Cacace, F.; Wolf, A. P. *J. Amer. Chem. Soc.* **1978**, *100*, 3639.

20. Firnau, G.; Chirakal, R.; Garnett, E. S. *J. Labeled Cmpd. Radiopharm.* **1984**, *23*, 1228.

21. Adam, M. J.; Ruth, T. J.; Grierson, J. R.l Abeysekera, B.; Pate, B. D. *J. Nucl. Med.* **1986**, *27*, 1462.

22. Chirakal, R.; Brown, K. L.; Firau, G.; Garrett, E. S. Hughes, D. W. Sayer, B. G.; Smith, R. W. *J. Fluorine Chem.* **1984**, *37*, 267.

23. Coenen, H. H.; Moerlein, S. M. *J. Fluorine Chem.* **1989**, *36*, 63.

24. Diksic, M.; Farrokhzad, S. *J. Nucl. Med.* **1985**, *26*, 1314.

25. Speranza, M.; Shiue, C-Y.; Wolf, A. P.; Wilbur, D. S.; Angelini, G. *J. Fluorine Chem.* **1985**, *30*, 97.

26. Speranza, M.; Shiue, C-Y.; Wolf, A. P.; Wilbur, D. S.; Angelini, G. *J. Chem. Soc., Chem. Comm.* **1984**, 1448.

27. Adam, M. J.; Pate, B. D.; Ruth, T. J.; Berry, J. M.; Hall, L. D. *J. Chem. Soc., Chem. Comm.* **1981**, 733.

28. Clark, J. C.; Silvester, D. J. *Int. J. Appl. Radiat. Isot.* **1966**, *17*, 151.

29. Luxen, A.; Barrio, J. R.; Bida, G. T.; Satyamurthy, N. *J. Labelled Cmpd. Radiopharm.* **1986**, *23*, 1066.

30. Mislankar, S. G.; Gildersleeve, D. L. Wieland, D. M.; Massin, C. C.; Toorongian, S. A.; Wieland, D. M. *J. Nucl. Med.* **1987**, *28*, 624.

31. Mislanker, S. G.;Gildersleeve, D. L.; Wieland, D. M.; Massin, C. C.; Mulholland, G. K.; Toorongian, S. A. *J. Med. Chem.* **1989**, *31*, 362.

32. Visser, G. W. M.; Halteren, B. W.; Herscheid, J. D. M.; Brinkman, G. A.; Hoekstra, A. *J. Chem. Soc., Chem. Comm.* **1984**, 655.

33. Finger, G. C.; Kruse, C. W. *J. Amer. Chem. Soc.* **1956**, *78*, 6034.

34. Arnett, C. D.; Wolf, A. P.; Shiue, C-Y.; Fowler, J. S.; MacGregor, R. R.; Christman, D. R.; Smith, M. R. *J. Nucl. Med.* **1986**, *27*, 1878.

35. Attina, M.; Cacace, F.; Wolf, A. P. *J. Chem. Soc., Chem. Comm.* **1983**, 108.

36. Barrio, J. R.; Satyanurthy, N.; Ku, H.; Phelps, M. E. *J. Chem. Soc., Chem. Comm.* **1983**, 433.

37. Cacace, F.; Speranza, M.; Wolf, A. P.; Fowler, J. S. *J. Labelled Cmpds. Radiopharm.* **1981**, *18*, 1721.

38. Cacace, F.; Speranza, M.; Wolf, A. P.; MacGregor, R. R. *J. Fluorine Chem.* **1982**, *21*, 145.

39. Kilbourn, M. R.; Welch, M. J.; Dence, C. S.; Tewson, T. J.; Saji, H.; Maeda, M. *Int. J. Appl. Radiat. Isot.* **1984**, *35*, 591.

40. Shiue, C-Y.; Watanabe, M.; Wolf, A. P.; Fowler, J. S.; Salvadori, P. *J. Labelled Cmpd. Radiopharm.* **1984**, *21*, 533.

41. Maeda, M.; Fukumura, T.; Kojima, M. *J. Labelled Cmpd. Radiopharm.* **1986**, *23*, 1104.

42. Maeda, M.; Fukumura, T.; Kojima, M. *Chem. Pharm. Bull.* **1985**, *33*, 1301.

43. Angelini, G.; Speranza, M.; Wolf, A. P.; Shiue, C-Y. *J. Fluorine Chem.* **1985**, *27*, 177.

44. Ding, Y-S.; Shiue, C-Y.; Fowler, J. S.; Plenevaux, A.; Wolf, A. P. *J. Nucl. Med.* **1989**, *30*, 753.

45. Lemaire, C.; Guillaume, M.; Christiaens, L. *J. Nucl. Med.* **1989**, *30*, 752.

46. Kilbourn, M. R.; Dence, C. S.; Welch, M. J.; Mathias, C. J. *J. Nucl. Med.* **1987**, *28*, 462.

47. Siler, D. Isotopes: "Essential Chemistry and Applications II"; Amersham International PLC: Cardiff, UK, 1987; Chapter 2.

48. Cohen, N. C.; Blaney, J. M.; Humblet, C.; Gund, P. and Barry, D.C. *J. Med. Chem.* **1990**, *33*, 883.

49. Seevers, R.H. and Cousell, R. E. *Chem. Rev.* **1982**, *82*, 575.

50. Jones, R. L. *J. Chem. Soc.* **1952**, 4080.

51. Hlavka, J, J, and Buyske, D. A. *Nature (London)* **1960**, *186*, 1064.

52. Fowler, J. S.; MacGregor, R. R.; Wolf, A. P.; Ansari, A. N.; Atkins, H. L. *J. Med. Chem.* **1976**, *19*, 356.

53. Beirerwaltes, W. H.;Weiland, D. M.;Ice, R. D.; Seabold, J. E.; Sarkar, S. D.; Gill, S. P.; Moskey, S. T. *J. Nucl. Med.* **1976**, *17*, 998.

54. McFarlane, A. S. *Nature (London)* **1958**, *182*, 53

55. Prasada, Rao, M. D.; Padmanabha, J. *Indian J. Chem.* **1981**, *20A*, 133.

56. Hunter, W. M.; Greenwood, F. C. *Nature* **1962**, *194*, 405.

57. Hunter, R. *Proc. Soc. Exp. Biol. Med.* **1970**, *133*, 989.

58. Pierce Chemical Co. Rockford, IL 61105.

59. Ferguson, K. M.; Jeffcoate, S. L. *Acta Endocrinol., Supl.* **1979**, *225*, 130.

60. Moore, D. H.; Wolf, W. *J. Labelled Compd. Radiopharm.* **1978**, *15*, 443.

61. Morrison, M. M. *Methods Enzymol.* **1980**, *70*, 214.

62. Benenson, A.; Mersel, M.; Pinson, A.; Heller, M. *Ancl. Biochem.* **1980**, *101*, 57.

63. Bolton, A. E.; Hunter, W. M. *Biochem. J.* **1973**, *133*, 529.

64. Wood, F. T.; Wu, M. M.; Gerhart, J. C. *Anal. Biochem.* **1975**, *69*, 339.

65. Morrison, D. C. *J. Am. Chem. Soc.* **1952**, *74*, 4459.

66. Elias, H.; Arnold, C. Kloss, G. *Int. J. Appl. Radiat. Isot.* **1973**, *24*, 463.

67. Toth, G. *J. Chromatog.* **1978**, *172*, 527.

68. Elias, H.; Lotterhos, H. F. *Chem. Bec.* **1976**, *109*, 1580.

69. Sinn, H.; Maier-Borst, W.; Elias, H. *Int. J. Appl. Radiat. Isot.* **1978**, *30*, 511.

70. Counsell, R. E.;Weidert, J P.; Schwendner, S. W.; Szabo, S. M. *J. Nucl. Med.* **1982**, 47.

71. Mangner, T. J.; Wu, J. L.; Weiland, D. M.; Beierwaltes, W. H. *J. Nucl. Med.* **1981**, *22*, 12.

72. Mangner, T. J.; Wu, J. L. W. *J. Org. Chem.* **1982**, *47*, 1484.

73. Hochberg, R. B.; Rosner, W. *Proc. Natl. Accd. Sci.* U. S. A. **1980**, *77*, 328.

74. Maeja, M.; Shimoirsa, H.; Kanatsu, H.; Kojima, M. *Int. J. Appl. Radiat, Isot.* **1979**, *30*, 255.

75. Bo-Li, L.; Yu-Tai, J.; Maeta, M.; Kojima, M. *Radioisotopes* **1981**, *30*,391.

76. Wanek, P. M. *Radiochem. Radioanal. Lett.* **1981**, *46*, 401.

77. Boothe, T. E.; Campbell, J. A.; Djermouni, B.; Finn, R. D.; Gilson, A. J.; Ache, H. J. *Int. J. Appl. Radiat. Isot.* **1981**, *32*, 153.

78. Vogel, A. I. "A Textbook of Practical Organic Chemistry", 3rd ed.; Longmans, Green and Co.: New York, 1956; pp 590-600.

79. Wallach, O. *Liebigs An. Chem.* **1888**, *235*, 242.

80. Foster, N. I.; Dannals, R.; Burns, H. D.; Heindel, N. D. *J. Radioanal. Chem.* **1981**, *65*, 95.

81. Kabalka, G. W.; Varma, R. S. *Tetrahedron* **1989**, *45*, 6601.

82. Wilbur, D. S.; Anderson, K. W.; Stone, W. F.; O'Brien, H. A. *J. Labelled Compd. Radiopharm.* **1982**, *19*, 1171.

83. McKillop, A.; Fowler, J. S.; Zelesco, M. J.; Hunt, J. D.; Taylor, E. C.; McGilliuray, G. *Tetrahedron Lett.* **1969**, *29*, 2423.

84. Carlson, G. L. *Int. J. Appl. Radiat. Isot.* **1978**, *29*, 557.

85. Goodman, M. M.; Knapp, F. F.; kirsel, G.; Callahan, A. P. *J. Nucl. Med.* **1982**, *23*, 104.

86. Welh, M. J. *J. Am. Chem. Soc.* **1970**, *92*, 408.

87. El-Gashy, M.; Stocklin, G. *Radiochem. Radioanal. Lett.* **1974**, *18*, 281.

88. Molholland, G. K.; Hichwa, R. D.; Kilbourn, M. R.; Moskwa, J. *J. Labelled Cmpds. Radiopharm.* **1989**, *26*, 140.

89. Tewson, T. J.; Welch, M. J. *J. Org. Chem.* **1978**, *43*, 1090.

90. Wakseiman, C. *Fr. Actual. Chim.* **1986**, *9*, 20.

91. Kuhl, D. E.; Minoshima, S.; Fessler, J. A. *Neurology* **1996**, *52*, 399.

92. Wéden, L.; Eriksson, L.; Ingvar, M.; Parsons, S. M.; Rogers, G. A.; Stoner-Elanders, S. *Neurosci. Lett.* **1992**, *13*, 1.

93. Nordberg, A.; Lundqvist, H.; Hartvig, P. *Dement. Geriatr. Cogn. Disord.* **1997**, *8*, 78.

94. Iyo, M.; Nambe, H.; Fukushi, K.; Shinotoh, H.; Nagatsuka, S.; Suhara, T.; Sudo, Y.; Suzuki, K.; Irie, T. *Lancet* **1997**, *349*, 1805.

95. Pappata, S.; Tavitan, B.; Traykov, L.; Jobert, A.; Dalger, A.; Mangin, J. F.; Crouzel, C.; Giamberardino. *J. Neurochem.* **1996**, *67*, 876.

96. Schutshe, G. M.; Pierrat, F. A.; Kapples, K. J.; Cornfeldt, M. L.; Szewczak, M. R.; Huger, F. P.; Bores, G. M.; Haroutunian, V.; Davis, K. L. *J. Nuc. Med.* **1989**, *32*, 1805.

97. Knapp, M. J.; Knopman, D. S.; Solmon, P. R.; Pendlebury, W. W.; Davis, C. S.; Gracon, A. I. *J. Am. Med. Assoc.* **1994**, *271*, 985.

98. Farlow, M.; Gacon, S. I.; Hershey, L. A.; Lewis, K. W.; Sadowsky, C. H.; Dolan-Ureno, J. *J. Am. Med. Assoc.* **1992**, *268*, 2523.

99. Summers, W. K.; Koehler, A. L.; Marsh, G. M.; Tachiki, K.; Kling, A. *Lancet* **1989**, *1*, 729.

100. Marx, J. L. *Science* **1987**, *238*, 1041.

101. Woolf, T. F.; Pool, W. F.; Bjorge, S. M.; Chang, T.; Geol, O. P.; Purchase, C. F., II; Schroeder, M. C.; Kunze, K. L.; Trager, W. F. *Drug Metab. Dispos.* **1993**, *21*, 874.

102. Akula, M. R.; Longford, C. D. P.; Kabalka, G. W. *J. Labelled Compd. Radiopharm.* **1999**, *42*, S539.

103. Akula, M. R.; Kabalka, G. W. *J. Labelled Compd. Radiopharm.* **1997**, *40*, 83.

104. Tavitan, B.; Pappata, S.; Bonnet-Lours, S.; Prenant, C.; Jobert, A.; Crouzel, C.; Di Giamberardino, L. *Eur. J. Pharmacol.* **1993**, *236*, 229.

105. Planas, A. M.; Crouzel, C.; Hinnen, F.; Jobert, A.; Né, F.; Di Giamberardino, L.; Tavitan, B. *Neuroimage* **1994**, *1*, 173.

106. Mesulam, M. M.; Volicer, L.; Marquis, J. K.; Mufson, E. J.; Grenn, R. C. *Ann. Neurol.* **1986**, *19*, 144.

107. Bonnot-Lours, S.; Crouzel, C.; Prenant, C.; Hinnen, F. *J. Labelled Chem. Radiopharm.* **1993**, *33*, 277.

108. Mihara, M.; Ohnishi, A.; Tomono, Y.; Hasegawa, J.; Shimamura, Y.; Yamazaki, K.; Morishita, N. *Int. J. Clin. Pharmacol., Ther. Toxicol.* **1993**, *31*, 223.

109. Sugimoto, H.; Iimura, Y.; Yamanishi, Y.; Yamatsu, K. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 871.

110. Sugimoto, H.; Tsuchiya, Y.; Sugumi, H.; Higurashi, K.; Karibe, N.; Iimura, Y.; Sasaki, A.; Araki, S.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1992**, *35*, 4242.

111. Sugimoto, H.; Tsuchiya, Y.; Sugumi, H.; Higurashi, K.; Karibe, N.; Iimura, Y.; Sasaki, A.; Kawakami, Y.; Nakamura, T.; Araki, S.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1990**, *33*, 1880.

112. Irie, T.; Kukushi, K.; Namba, H.; Iyo, M.; Nagatsuka, S. 10th International Symposium on Radiopharmaceutical Chemistry, *Abstract*, **1993**, 458.

113. Irie, T.; Fukushi, K.; Ikota, N.; Namba, H.; Iyo, M.; Nagatsuka, S. *J. Labelled Chem. Radiopharm.* **1995**, *37*, 214.

114. Brown-Proctor, C.; Snyder, S. E.; Sherman, P. S.; Kilbourn, M. R. *Nuc. Med. Biol.* **1999**, *26*, 99.

115. Brufani, M.; Filocamo, L.; Lappa, S.; Maggi, A *Drugs Fut.* **1997**, *22*, 397.

116. Sugimoto, H.; Tsuchiya, Y.; Sugumi, H.; Higurashi, K.; Karibe, N.; Iimura, Y.; Sasaki, A.; Araki, S.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1992**, *35*, 4542.

117. Sugimoto, H.; Iimura, Y.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1995**, *38*, 4821.

118. Boekelheide, V.; Linn, W. J. *J. Am. Chem. Soc.* **1956**, *76*, 1286.

119. Pool, W. F.; Woolf, T. F.; Reily, M. D.; Caprathe, B. W.; Emmerling, M. R.; Jaen, J. C. *J. Med. Chem.* **1996**, *39*, 3014.

120. Bassato, M.; Corain, B.; Veronese, A. C.; D'Angeli, F.; Valle, G.; Zanotti, G. *J. Org. Chem.* **1984**, *49*, 4696.

121. Tsushima, T.; Sato, T.; Tsuji, T. *Tetrahedron. Lett.* **1980**, *21*, 3591.

122. Ido, T.; Wan, C. N.; Casella, V.; Fowler, J. S.; Wolf, A. P.; Reivich, M.; Kuhl, D. E. *J. Labelled Compd. Radiopharm.* **1978**, *14*, 175.

123. Tewson, T. J.; Welch, M. J. *J. Org. Chem.* **1978**, *43*, 1090.

124. Sugimoto, H.; Iimura, Y.; Yamanshi, Y.; Yamatsu, K. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 871.

125. Villalobos, A.; Blake, J. F.; Biggers, C. K.; Butler, T. W.; Chapin, D. S.; Chen, Y. L.; Ives, J. L.; Jones, S. B.; Liston, D. R.; Nagel, A. A.; Nason, D. M.; Neilsen, J. A.; Shalaby, I. A.; White, W. F. *J. Med. Chem.* **1994**, *37*, 2721.

126. Efange, S. M. N.; Michelson, R. H.; Knusel, B.; Hefti, F.; Boudreau, R. J.; Tennison, J. R. *Nucl. Med. Biol.* **1993**, *20*, 527.

127. Namavari, M.; Bishop, A.; Satyamurthy, N.; Bida, G.; Barrio, J. R. *Int. Radiat. Appl. Instrum.* **1992**, *43*, 989.

128. Chaki, H.; Yamabe, H.; Sugano, M.; Motita, S.; Bessho, T.; Tabata, R.; Saito, K.; Egawa, M.; Tobe, A.; Morinaka, Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1489.

129. Askew, B. C.; Bednar, R. A.; Bednar, B.; Claremon, D. A.; Cook, J. J. *J. Med Chem.* **1997**, *40*, 1779.

130. Moore, J. A.; Kornreich, L. D. *Tetrahedron. Lett.* **1963**, *20*, 1277.

131. Ciganek, E.; Tam, S. W.; Wright, A. S. US: 5356906.

132. Wiedermann, H. E. US: 3998828.

APPENDICES

Table A-1. Table of Chemicals and Suppliers

Compound	Supplier
Acetic Anhydride	Aldrich Chemical Company
Acetone	Fisher Chemical Company
Antharanilonitrile	Aldrich Chemical Company
Benzene	Aldrich Chemical Company
Chloroform	Aldrich Chemical Company
Chloroform-*d* 0.3 % TMS	Aldrich Chemical Company
Chloroform-*d* 0.5 % TMS	Isotec
Chloroform-*d* 1 % TMS	Isotec
Copper (I) chloride	Aldrich Chemical Company
1,3-Cyclohexandione	Aldrich Chemical Company
Cyclohexanone	Aldrich Chemical Company
Diethylaminosulfur trifluoride	Aldrich Chemical Company
Diethyl azidodicarbonate	Aldrich Chemical Company
Di-*t*-butyl dicarbonate	Aldrich Chemical Company
DMSO-d_6	Aldrich Chemical Company
Ether	Fisher Chemical Company
Ethyl Acetate	Fisher Chemical Company
Ethyl isonipecotate	Aldrich Chemical Company
Fluorine	National Welders and MG supplies
3-Fluorobenzyl bromide	Aldrich Chemical Company

Hexamethylditin	Aldrich Chemical Company
Hexanes	Fisher Chemical Company
Hydrochloride acid	Fisher Chemical Company
Iodine	Aldrich Chemical Company
3-Iodobenzyl bromide	Aldrich Chemical Company
Lithium Aluminum Hydride	Aldrich Chemical Company
Magnesium sulfate	Fisher Chemical Company
Methanol	Aldrich Chemical Company
Methanol	Aldrich Chemical Company
Methanol-d_4	Aldrich Chemical Company
Methylene Chloride	Fisher Chemical Company
Nirogen (Prepurified)	National Welders and MG supplies
O-18 Water	N. F. Chemicals
Peracetic acid	Aldrich Chemical Company
Phthalimide	Aldrich Chemical Company
4-Piperidineethanol	Aldrich Chemical Company
Potassium Carbonate	Fisher Chemical Company
p-Toluenesulfonic acid	Aldrich Chemical Company
Pyridine	Aldrich Chemical Company
Sodium [^{123}I]iodide	MDS Nordian
Sodium acetate	Aldrich Chemical Company
Sodium Carbonate	Fisher Chemical Company

Sodium chloride	Kroger
Sodium cyanide	Aldrich Chemical Company
Sodium hydroxide	Fisher Chemical Company
Sodium iodide	Aldrich Chemical Company
Sodium sulfate	Fisher Chemical Company
Sodium thiosulfate	Aldrich Chemical Company
Tetrahydrofuran	Fisher Chemical Company
Tetrakis(triphenylphosphine)palladium	Aldrich Chemical Company
Toluene	Fisher Chemical Company
Triethylamine	Aldrich Chemical Company
Trifluoroacetic acid	Aldrich Chemical Company
Triphenylphosphine	Aldrich Chemical Company
Zinc Chloride	Aldrich Chemical Company

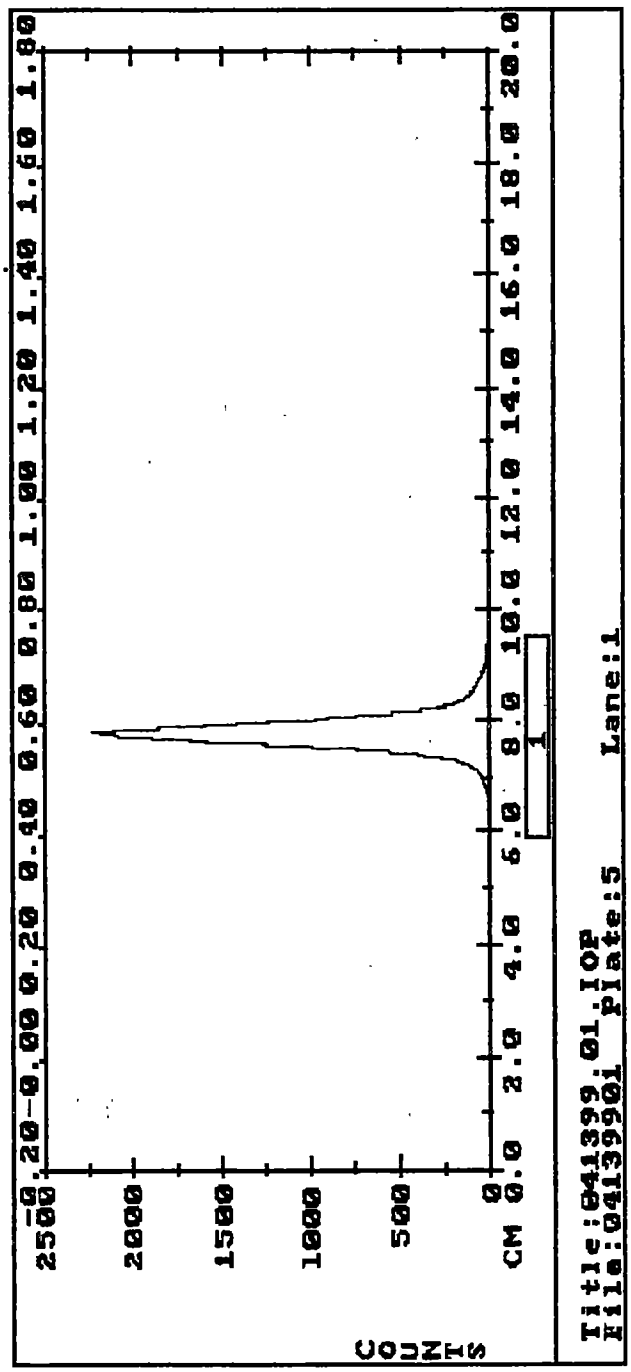


Figure A-1. Radio-TLC of Carrier-Added 1-(3-[^{123}I]Iodobenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine

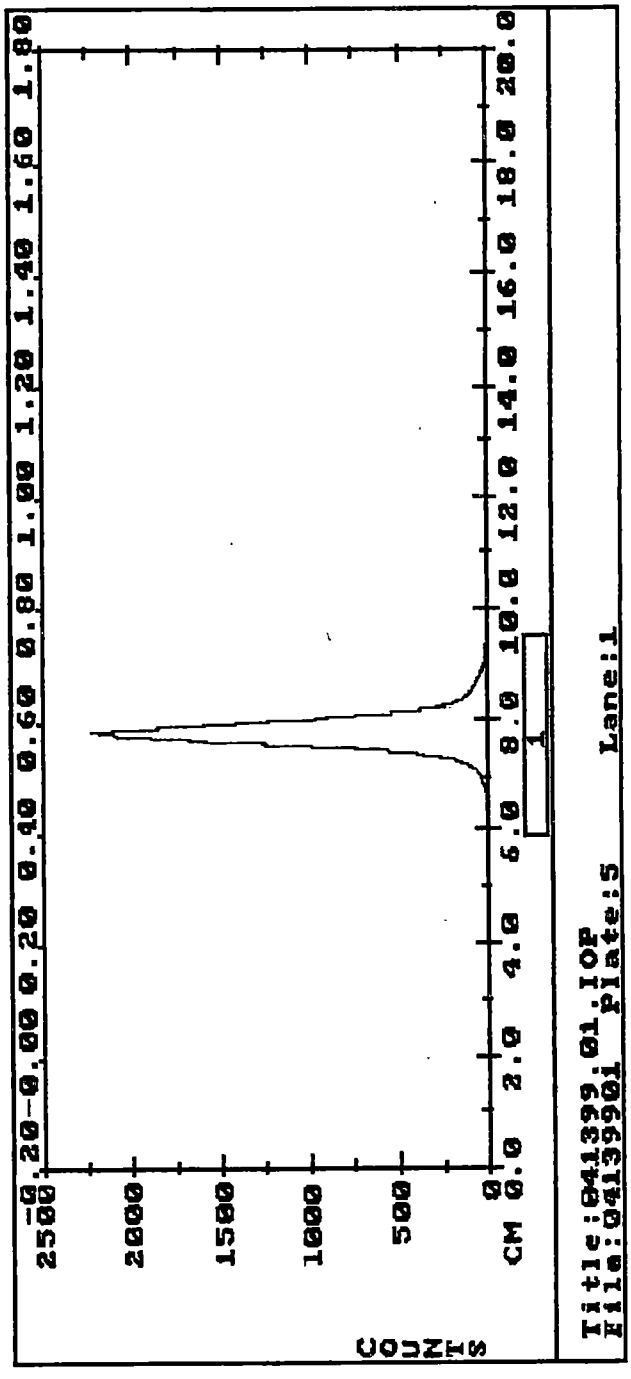


Figure A-2. Radio-TLC of No-Carrier-Added 1-(3-[^{123}I]Iodobenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine

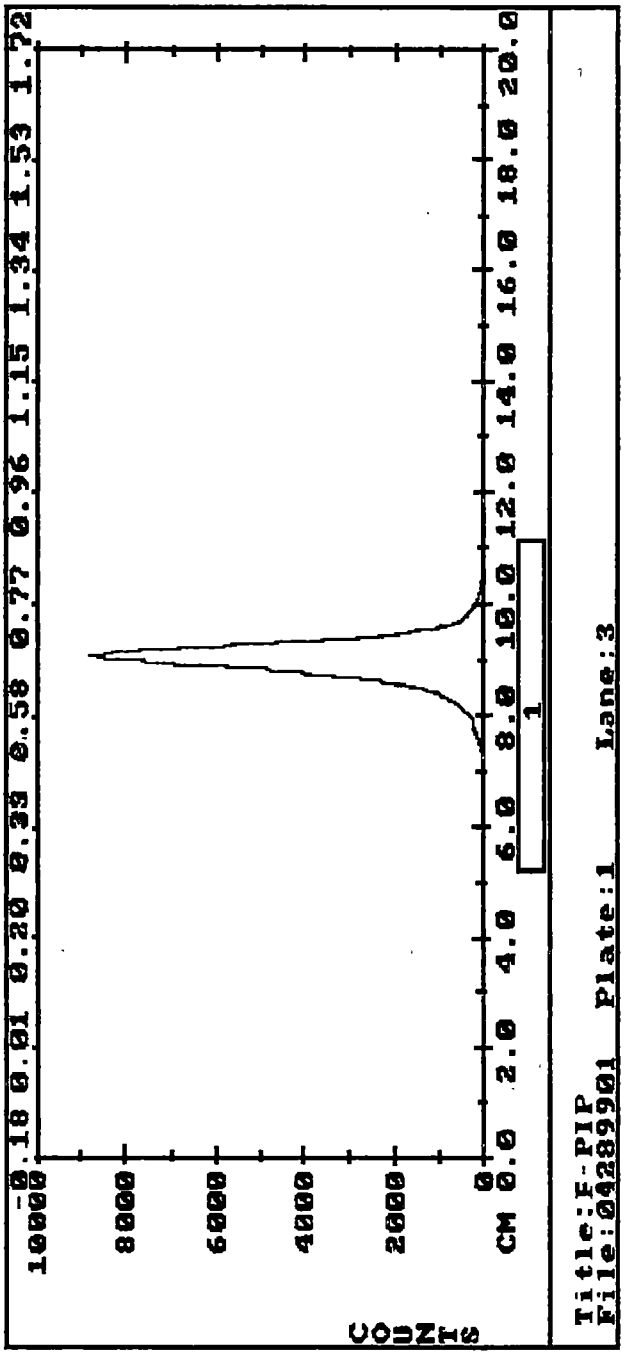


Figure A-3. Radio-TLC of Carrier-Added 1-(3-[^{18}F]Fluorobenzyl)-4-[2-(*N*-phthalimid-1-yl)ethyl]piperidine

VITA

George Albert Pacer was born on June 27, 1974 in Houston, Texas. He graduated from Notre Dame High School in June of 1992. He entered University of Tennessee, Knoxville in August 1992 where he received his Bachelor of Science in Chemistry Degree in May of 1996. In August of 1996 George began research under the direction of Dr. George W. Kabalka as a graduate student at The University of Tennessee, Knoxville. He graduated in August of 1999 and has accepted a position at Oak Ridge National Laboratory as a Research Associate.