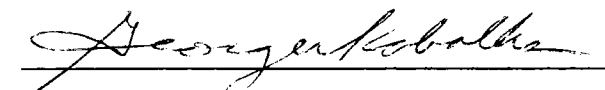
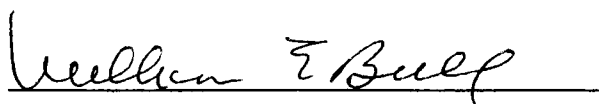


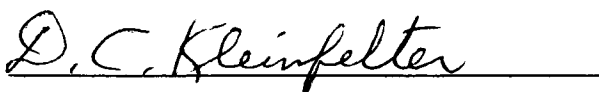
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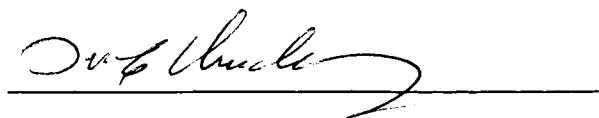
I am submitting herewith a dissertation written by Steven James Lambert entitled "The Design, Synthesis, and Radioiodination of Some Selected Biologically Active Molecules for Use As Potential Medical Imaging Agents". I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Chemistry.


Dr. 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

The Design, Synthesis, and Radioiodination of Some Selected Biologically Active
Molecules for Use As Potential Medical Imaging Agents

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Steven James Lambert

December 1994

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DEDICATION

This thesis is dedicated to my mother

Mrs. Elizabeth Lambert

and

my late father

Warren George Lambert Ph.D.

who provided financial, tutorial, and emotional support, as well
as love and encouragement, which without, the completion of this work
would not have been possible. I will always appreciate and love them both.

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Last but not least, I would like to extend my sincerest appreciation to Dr. P. C. Srivastava, Dr. F. F. Knapp, and the technical support personnel of the Oak Ridge National Laboratory.

ABSTRACT

The research presented in this dissertation involves the synthesis of organic molecules designed to contain the important structures present in known biologically active compounds. The design procedure allowed for the insertion of a radioactive isotope of iodine into the molecule. The inclusion of radioiodine presented a synthetic challenge which was initially met by utilizing a synthetic procedure involving the hydroboration of an alkyne followed by iodination. This procedure, however, was not appropriate for certain classes of molecules which necessitated the development of a new radioiodination procedure involving the addition of radioactive iodine monochloride to alkynes. All the compounds discussed were designed such that the radioactive iodine was placed in a position which would least likely affect the molecule's biological activity. Finally, the radioactive compounds were synthesized and biologically tested. Several of the compounds exhibited significant activity in bio-distribution studies.

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CHAPTER 1. RADIOACTIVE ISOTOPES USED FOR IMAGING

A. INTRODUCTION

A.1 HISTORY OF ISOTOPES

When the artificial production of radioactive isotopes was announced over 45 years ago¹, it caused a tremendous sensation among chemists and physicists but little attention was given to medical applications. However, within a short period of time, physiologists found that the new radioactive substances provided powerful tools for the study of metabolic processes. In the ensuing years, many applications became apparent. For example, in 1939 radioactive phosphorus was used as a substitute for whole body x-irradiation in the treatment of certain blood discrasias². At about the same time the value of radioactive iodine for thyroid function studies was demonstrated and within a short period of time the material was used to treat toxic goiter. By 1940 there was considerable literature dealing with medical uses of radioactive isotopes. But there was no suggestion they would eventually be used in standard diagnostic and therapeutic procedures. This was a consequence of the fact that the new substances were obtainable only from cyclotrons, usually at great cost, and in such a state of chemical impurity as to require considerable chemical manipulation before they could be used. In 1946 the discovery of the chain reacting pile (or nuclear reactor) changed the picture and isotopes became available for non-military purposes. In July of 1946, the Atomic Energy Commission (AEC) announced the availability of certain isotopes for medical use, at a much lower cost than cyclotron-produced products; within six months, more than 30 institutions acquired over 100 shipments of radioactive materials with some physicians using very large quantities of

isotopes³. At present thousands of institutions are authorized to obtain and experiment with radioactive substances.

A.2 MEDICAL USES OF ISOTOPES

There are basically two large categories for the use of isotopes in medicine to the benefit of human beings: therapy and tracer studies. Therapy involves the use of the radioactive substance to alter the biological system with radioactive emissions usually killing or inactivating a group of cells or certain tissues. Tracer studies, which include medical imaging, is a much larger category and uses radioactivity to obtain information about the function of an organ or biological system without in altering its state or condition, in any way, 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 sophisticated radiation detecting devices. The data collected, when processed by a computer, can give a detailed picture of the function of the system and insight into a metabolic biological process. Unfortunately, as of this date, the number of agents available is relatively small.

A.3 THE REQUIREMENTS FOR SUCCESSFUL IMAGING AGENTS

The development of radioactive agents for medical imaging is a relatively new field. In order for a compound to be a successful candidate for medical imaging it must meet very stringent physical, structural, and chemical properties. It must be soluble in the tissue to be imaged or it must be passively or actively transported into that area. Very often this involves enzyme mediated carrier systems which impose not only physical and chemical restraints on the molecule

but also stereochemical constraints. Receptor imaging, where the molecule fits like a key in a lock, is an example where these constraints severely limit the number of organic molecules which could be successful candidates. When possible, organic chemists design compounds which resemble the biological molecules that naturally function in the system. A well known, and often used, compound for brain imaging is 2-[^{18}F]-fluorodeoxyglucose. Resembling glucose, a natural sugar metabolized by the brain, the radioactive compound "fools" the enzyme carrier systems and is transported into the brain where it enters the metabolic pathway as if it were glucose. Since the more active areas of the brain have a higher metabolic rate, these areas become concentrated with radioactivity and detection leading to imaging becomes possible. It is understandable why this compound is successful. The only difference between it and glucose is the replacement of one OH by a fluorine atom. Unfortunately, many labeling procedures drastically alter the physical and chemical properties of the molecule by altering its structure and can render the agent useless as an imaging or a metabolic tracing agent. However, if the molecule can be labeled in a position removed from the functional groups responsible for receptor or metabolic activity, then the biological activity may be retained and a successful agent for medicinal use may result.

A.4 IODINE-125: A CONVENIENT ISOTOPE FOR USE IN DEVELOPING SYNTHETIC PROCEDURES

Since their introduction to the science community, the number and availability of radioisotopes have dramatically increased and many procedures and reagents have been developed for use with the various isotopes. During the research and development stage of a medical imaging agent, experience has

proven it best to use an isotope which is relatively safe and easy to handle. Once the procedure for the synthesis and utility of the agent have been established, an isotope more suitable for imaging is used.

After surveying the available isotopes, [^{125}I]-iodine is often chosen by researchers for two reasons. It is relatively safe to work with because it has comparatively low energy emissions and yet it has a high energy emission isotope which is [^{131}I]-iodine. Once the synthetic procedures have been determined with ^{125}I , the ^{131}I isotope can be substituted without changing the reaction conditions. Another important advantage of using ^{125}I is that it possesses a long half-life (60 days) which eliminates time constraints when developing chemical reaction procedures. Since ^{125}I is widely used in the early stages of development of imaging agents, a large number of reagents and procedures (chemical reactions) have been developed. The various methods are reviewed in the following section.

B. RADIOIODINATION TECHNIQUES AND REAGENTS

B.1 RADIOIODINATION REAGENTS AVAILABLE FOR LABELING

An iodination reagent is generally chosen after consideration of the type of substrates present in the molecule. Radioactive iodine is usually provided in the sodium iodide form in a solution of sodium hydroxide to prevent oxidation of the iodide to molecular iodine, which is volatile and would result in a loss of activity and an increased danger of contamination. More often than not, this necessitates the chemical transformation of the iodide into a reactive iodinating species so that the desired reaction with substrate can occur. Other than sodium iodide, the reagents used for iodination are molecular iodine, hydroiodic acid, iodine

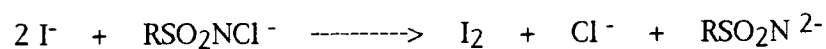
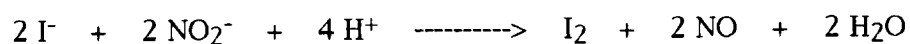
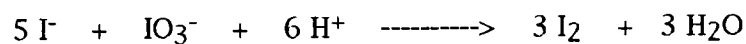
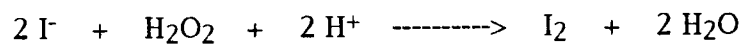
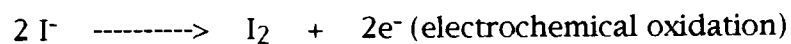
monochloride, methyltriphenoxyphosphonium iodide, and organic N-iodo compounds.

Sodium iodide, one of the most stable iodine compounds, may be used without alteration and also serves as the starting material for all other iodinating agents. Elemental iodine, perhaps the next simplest iodinating reagent, can be formed from sodium iodide by electrochemical oxidation⁴ or chemical oxidation with hydrogen peroxide under acidic conditions⁵, potassium iodate under acidic conditions⁶, sodium nitrite under acidic conditions⁷, chloramine-T under neutral conditions⁸, and enzymatic oxidation by lactoperoxidase⁹ (Scheme 1).

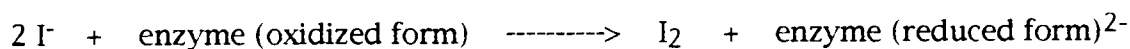
Hydroiodic acid is prepared by adding water to "cold" iodine containing red phosphorus. A gas is produced (HI) which is purified by passage over red phosphorus and then passed through a series of cold traps. The hydroiodic acid is then passed into a final trap containing radioactive sodium iodide. The mixture is allowed to warm until melting allows the iodides to exchange. The labeled hydroiodic acid is then purified by distillation¹⁰ (Scheme 2).

Iodine monochloride (ICl) has been used to chlorinate, iodinate, and chloriodinate a wide variety of organic compounds on a preparative scale. It has also been used as an analytical reagent for the determination of the degree of unsaturation in substances such as fats, oils, and rubbers. Iodine monochloride also displays a strong propensity for complex formation. A large number of adducts formed between iodine monochloride and organic donors such as amines, sulfides, and ethers have been characterized.

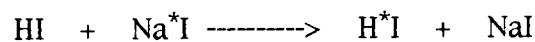
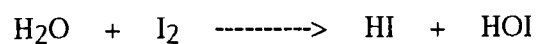
Iodine monochloride is a reddish-brown solid with a melting point of 27 °C and a boiling point of 97-100 °C with some decomposition. The commercially available reagent is usually prepared by passing a stream of chlorine gas over iodine until the calculated gain in weight has been achieved. The product is then



R = p-tolyl



Scheme 1. Ionic reactions representing methods for the production of elemental iodine.



Scheme 2. Production of radiolabeled hydroiodic acid.

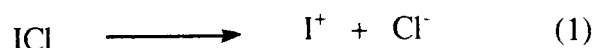
purified by fractional crystallization or by low temperature distillation.

There are several examples of reactions where iodine monochloride is generated *in situ*. Systems which have proven successful are *tert*-butyl hypochlorite and iodide, chloramine-T and a source of iodide, dichloramine-T and a source of iodide, antimony (V) chloride and iodine, copper (II) chloride and iodine in acetonitrile, and N-chlorosuccinimide and a source of iodide. In certain cases, the liberation of free iodine monochloride has not been proven though its presence is strongly suspected.

Iodine monochloride is unstable in water and is easily hydrolyzed to hydrochloric acid and hypoiodous acid, with the latter rapidly disproportionating into iodic acid or hydroiodic, depending on the pH of the solution. ICl solutions in non-polar solvents such as chloroform, cyclohexane, petroleum ether, and carbon disulfide, are usually more stable than the pinkish solutions obtained in polar solvents such as alcohols, ethers, acetone, and pyridine.

Iodine monochloride in the vapor state is irritating to the entire respiratory tract and can severely burn the eyes; the liquid is also corrosive to the skin. Appropriate protection must therefore be used while working with this reagent.

Iodine monochloride halogenations are influenced strongly by the dielectric constant of the reaction medium. Dissociation of the molecule may occur in two ways: heterolytically which causes iodination, or homolytically which causes chlorination with some accompanying iodination (Scheme 3). Shulek and Burger have discussed the effects of the dielectric constant of the reaction medium on these types of reactions^{11,12}. Solvents with a high dielectric constant assist the heterolytic iodinating process, while those of low dielectric constant



Scheme 3. Dissociation of iodine monochloride.

promote homolytic dissociation. Iodine monochloride has been estimated to dissociate heterolytically in solvents with a dielectric constant greater than 3.8. There are exceptions to this generalization which are dependent on the substrate molecule. Even in a non-polar solvent, iodine monochloride may heterolytically dissociate with the assistance of a polar or polarizable substrate.

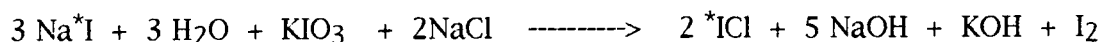
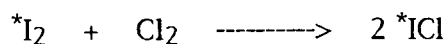
Iodine monochloride, being a mixed halide, is a more reactive iodinating reagent than iodine. This increased reactivity is due to the molecule's significantly polarized bond caused by the more electronegative chlorine atom which renders the "iodine portion" of the molecule an effective electrophile (Figure 1).

Radioactive iodine monochloride may be prepared by several methods. One method involves oxidation of radioactive iodine by its introduction, via distillation, into an ampule containing chlorine at low temperature. After removal of the excess chlorine under low pressure, the radioactive iodine monochloride is condensed via the use of a dry ice/acetone bath. This method has been utilized to produce radioactive carrier-free iodine monochloride¹³. Other methods



Figure 1. Iodine monochloride, a polarized molecule.

involve oxidation of the radioiodide with a suitable oxidizing agent such as chloramine-T, N-chlorosuccinimide, or potassium iodate solution containing sodium chloride^{10,11,12} (Scheme 4). These methods are particularly useful since the iodine monochloride can be produced *in situ*¹⁴. The substrate may or may not be present during this initial reaction.



Scheme 4. Methods for producing iodine monochloride.

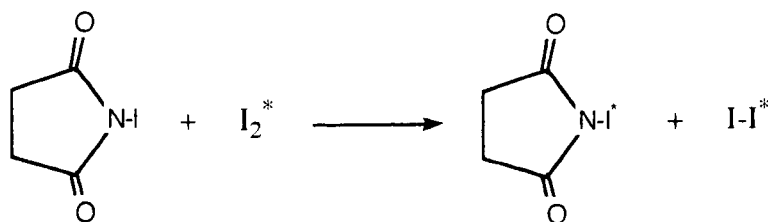
Though the activity of the iodinating reagent is diluted, radioactive iodine monochloride can also be prepared by simply mixing "cold" iodine monochloride with the radioactive sodium iodide¹⁵. An iodide exchange occurs and labeled iodine monochloride is formed. The substrate is added subsequently for final reaction with the radioactive iodine monochloride.

An iodinating agent, methyltriphenoxyphosphonium iodide, is prepared by refluxing triphenoxyphosphine with methyl iodide in the absence of moisture. After precipitation with ether, the labeled reagent is prepared by reaction of methyltriphenoxyphosphonium iodide (the precipitate) with labeled iodine in dioxane¹⁶ (Scheme 5).



Scheme 5. Synthesis of radioiodinated methyltriphenoxyphosphonium iodide.

Organic N-iodo compounds are also used for iodination reactions. The most widely used organic N-iodo compound is N-iodosuccinimide. It is prepared by reacting the silver salt of succinimide with molecular iodine in acetone. The labeled reagent is then prepared by the reaction of radioactive iodine with the resulting N-iodosuccinimide which allows exchange of iodine¹⁷ (Scheme 6).



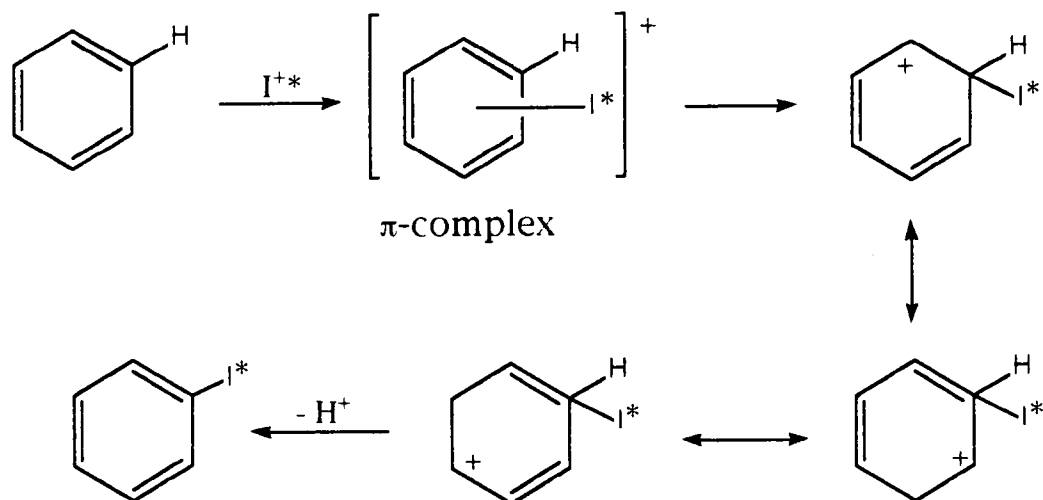
Scheme 6. Iodine isotopic exchange using N-iodosuccinimide.

The previously listed reagents are those most commonly used to effect radioiodination. The choice of the iodinating reagent depends on the reaction desired, which is generally determined by the functional groups in the substrate. The reagents and examples of their use will be discussed in the next section. The iodination reactions are divided into reaction types for clarity.

B.2 REACTIONS USED TO AFFECT IODINATION

AROMATIC SUBSTITUTION

In electrophilic aromatic substitution, the substrate contains an aromatic ring sufficiently activated to react with an electrophilic iodinating reagent. The electrophile may exist as a positive ion (I^+ , most likely associated with the anion or solvent) or a dipole ($I^+ \cdots X^-$). As the electrophile approaches the aromatic ring a π -complex is formed followed by attachment of the electrophile to the ring to form a cyclic, non-aromatic carbocation stabilized by mesomerism (Scheme 7).

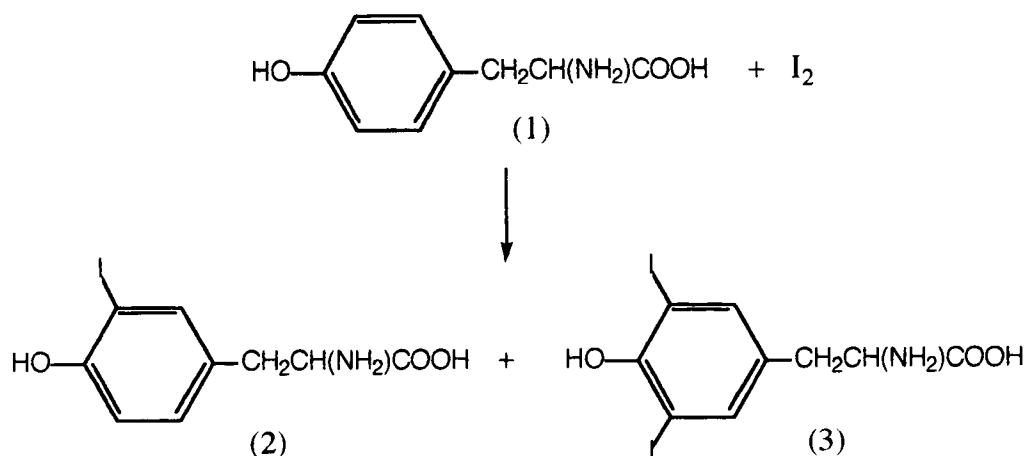


Scheme 7. An iodination reaction including resonance structures resulting from electrophilic attack on an aromatic ring.

Aromaticity is normally regained by the loss of a proton. Reactivity and orientation of the substitution reaction are dependent upon the groups attached to the ring. Activating groups increase the rate of reaction and lead to ortho/para substitution whereas deactivating groups reduce the rate and result in meta

substitution, except for the halogens which direct ortho/para. These effects are due to stabilization or destabilization of the transition state via electron donation to or withdrawal from the positively charged ring, respectively. Orientation may also be influenced by steric factors in multisubstituted ring systems.

Molecular iodine can be used to iodinate aromatic ring systems but only in the presence of a Lewis acid catalyst such as iron metal or aluminum halides. Silver perchlorate^{18,19} and the copper (II) halides²⁰ are particularly effective catalysts. During the iodination reaction, the hydroiodic acid that is produced can behave as a reducing reagent. If not removed, the hydroiodic acid will reduce the product iodobenzene, rendering the reaction useless. If the substrate is an aromatic amine, the hydroiodic acid forms an insoluble salt with the product and the reaction proceeds. Very often, however, an oxidizing reagent such as nitric acid, manganese (II) oxide, mercury (II) oxide, hydrogen peroxide, or chloramine-T is used as a co-reactant. When the substrate contains a phenolic or anilinic group, the aromatic ring is significantly activated and the monoiodo and diiodo products are obtained. For example, tyrosine (1) contains a phenolic group and, upon labeling with iodine, produces the monoiodo (2) and diiodo (3) products²¹ (Scheme 8). In some instances, the ratio of monoiodination to diiodination products may be influenced by the use of an appropriate buffer system²² and/or careful control over reaction conditions such as temperature and time²³.



Scheme 8. Iodination of an aromatic ring with molecular iodine.

Iodine monochloride behaves in much the same manner as iodine except it is more reactive due to its polarized bond and, upon iodination, the reducing agent (hydroiodic acid) is not formed. This eliminates the need for an oxidizing agent. The increased reactivity of iodine monochloride is exemplified in its reaction with tyrosine. At room temperature, the reaction is complete in two minutes²⁴, whereas the reaction of tyrosine with iodine at room temperature requires eight to ten hours²³.

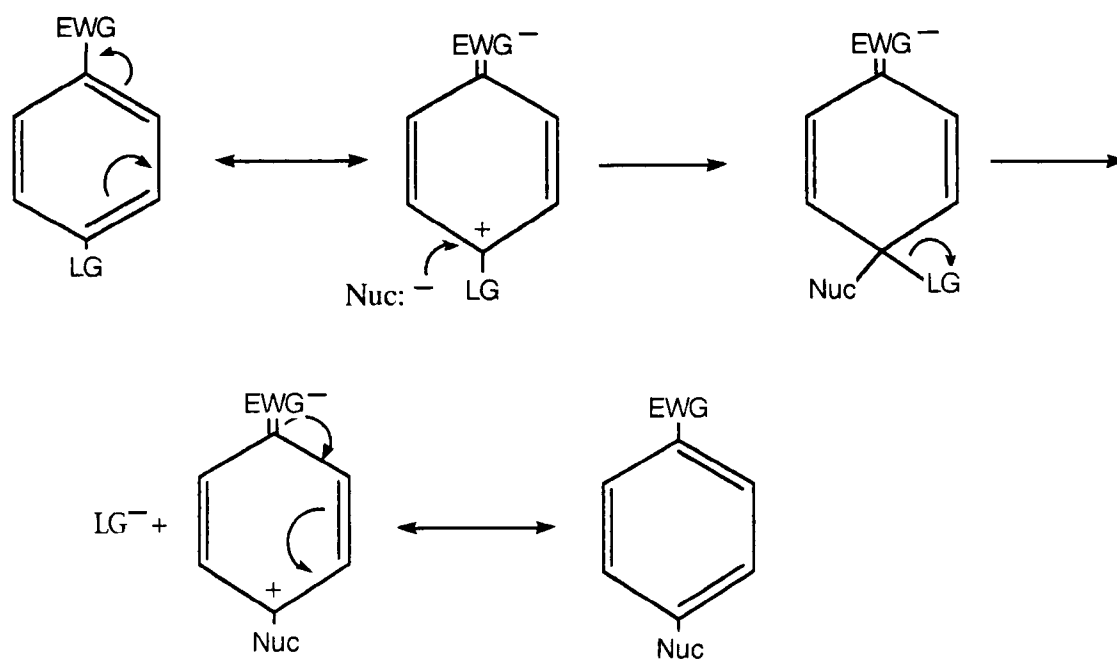
Since the reactivity of iodine monochloride is much greater than that for iodine, control over the monoiodination to diiodination ratio is much more difficult. No matter what the ratio of iodine monochloride to tyrosine is, a mixture of monoiodination and diiodination products are always formed^{22,25}. If the major product is to contain only one iodine atom, reactions involving very rigid stereochemical constraints must exist. These conditions exist when the enzyme lactoperoxidase is used. When tyrosine is iodinated using this enzyme, a stereospecific complex involving the amino group is involved as evidenced by the

fact that D-tyrosine is iodinated much faster than L-tyrosine²⁶. If the aromatic ring already contains one iodine, electrophilic attack of a second iodine is more difficult presumably due to steric constraints. Therefore when tyrosine is iodinated using lactoperoxidase in a phosphate buffer system at pH 7, iodination of tyrosine is 20 times faster than that of iodotyrosine²⁴.

Nucleophilic aromatic substitution can occur *via* a $S_{Ar}2$ reaction involving a Meisenheimer complex (substitution of a deactivated aromatic ring), an aryne intermediate, or *via* diazonium chemistry. Mechanistically, they may also be categorized as addition-elimination, elimination-addition, and radical or electron transfer processes, respectively. Unlike nucleophilic substitution at saturated carbon, aromatic nucleophilic substitution rarely, if ever, occurs in a single step; that is, intermediates are involved.

The mechanism for nucleophilic aromatic substitution of a deactivated ring may be characterized as involving a polarized aromatic ring system in which the electron density lies toward the electron withdrawing group, EWG (Scheme 9). This places a positive charge in the ortho and para positions of the ring. Since the leaving group LG is in the para position, nucleophilic substitution occurs here. Because the substitution occurs para, the other resonance forms are not shown in Scheme 9. Had the leaving group been in either ortho position, nucleophilic substitution would have occurred at the ortho position.

For radioiodinations, the nucleophile in Scheme 9 would be a radioiodide ion. All electron withdrawing groups, because of the mesomeric and inductive effects, favor this mechanism of nucleophilic aromatic substitution. Steric effects ortho to the electron withdrawing group contribute little to the reaction. This is because nucleophilic attack occurs perpendicular to the ring. The mechanism is sometimes referred to as an $S_{Ar}2$ mechanism since it is bimolecular, but, in



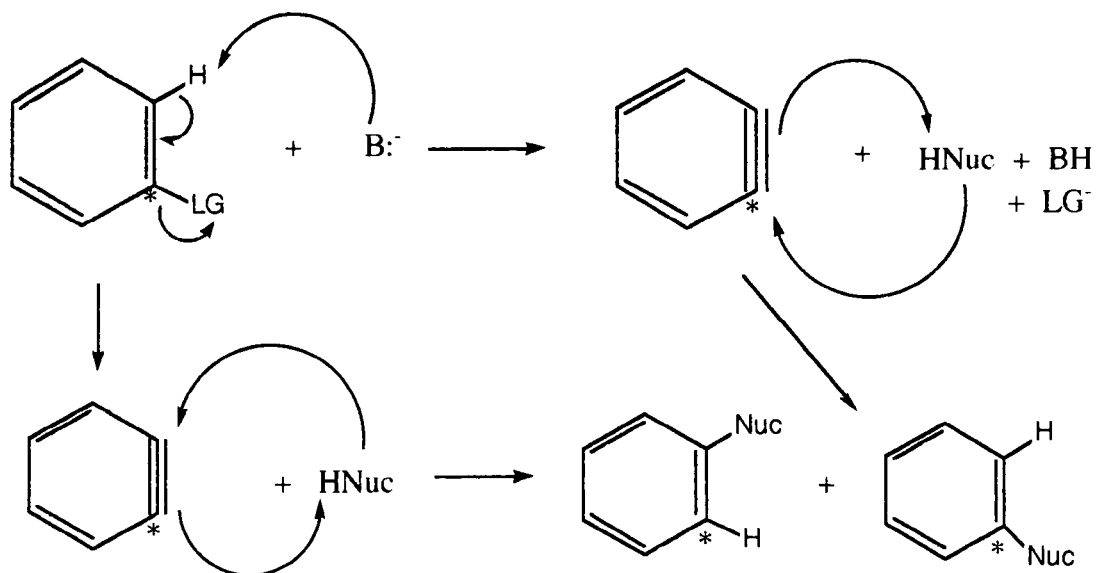
Scheme 9. Nucleophilic attack on an aromatic ring.

contrast to aliphatic nucleophilic substitution, the bonds of the leaving group and nucleophile are not broken and formed at the same time.

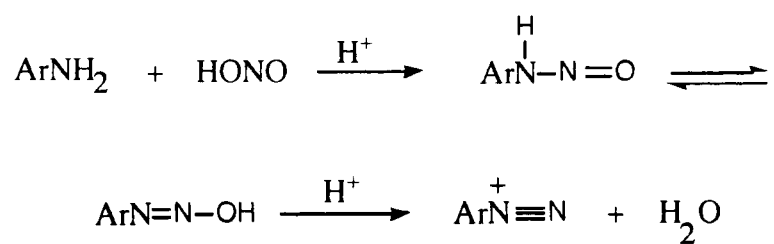
Nucleophilic aromatic substitution via an aryne intermediate occurs primarily with monohalogenated aromatic rings, containing no electron withdrawing groups, and in a strongly basic medium. One interesting feature of this reaction is that the nucleophile (entering group) does not necessarily occupy the position of the leaving group. The use of radiolabeled carbon compounds has suggested that the reaction occurs by a mechanism that involves elimination followed by addition (Scheme 10)^{27,28}. A strong base, B^- , abstracts a proton ortho to the leaving group while the resulting negative charge formation on the ring is dissipated by the simultaneous ejection of the leaving group (anion). This forms an aromatic compound containing a triple bond called an aryne (benzyne in Scheme 10). An aryne is extremely reactive due to ring strain on the sp hybridized carbon atoms, and it readily reacts with the nucleophile (Nuc can be iodide). The nucleophile may form a bond with the radiolabeled carbon ($^*C$) or the carbon atom ortho to it. If both carbons ortho to the leaving group are substituted, no reaction occurs.

Aryl diazonium ions are usually prepared by reaction of an aniline with nitrous acid generated *in situ* from a nitrite salt²⁹. Unlike aliphatic diazonium salts, which decompose readily to form nitrogen and a carbocation, aryl diazonium salts are sufficiently stable to exist at room temperature and those with a nonnucleophilic counter ion can be isolated. The steps involved in the formation of a diazonium ion are the addition of NO^+ to the nucleophilic amino group followed by the elimination of water (Scheme 11).

The usefulness of the aryl diazonium intermediates results from the excellence of nitrogen as a leaving group. There are three basic mechanisms for

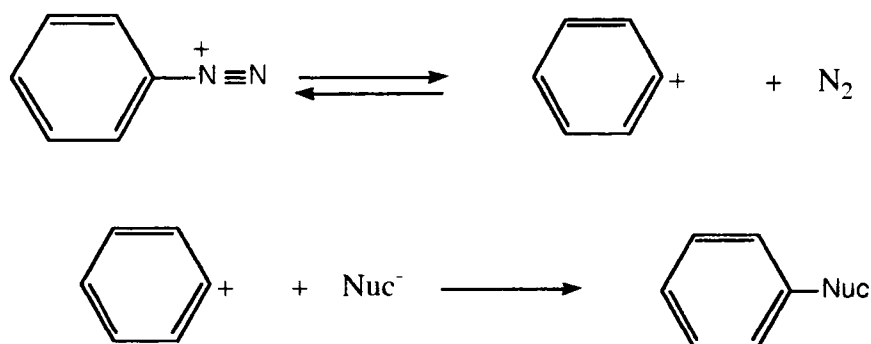


Scheme 10. Nucleophilic aromatic substitution *via* an aryne intermediate.



Scheme 11. Diazotization of an aromatic amine.

the nucleophilic aromatic substitution of a diazonium ion intermediate. The one most consistent with radioiodinations follows the S_N1 mechanism where nitrogen leaves with the resulting formation of an aryl carbocation^{30,31}. The aryl carbocation is especially unstable because the vacant sp^2 orbital lies in the plane of the ring and therefore is unable to stabilize the charge by distributing it over the p-orbitals of the ring (Scheme 12). This makes the aryl carbocation extremely vulnerable to any nucleophiles in solution, iodide for example. The S_N1 mechanism for these reactions is supported by the facts that the rate is dependent only on the concentration of diazonium ion and the first step is reversible^{32,33,34}. The reversibility of the first step is exemplified by the isolation of the intermediate $Ar-N^{15}N^+$ from the solution originally containing $Ar-^{15}NN^+$. Solvent effects, isotope effects, and substituent effects are also in agreement with a rate determining unimolecular decomposition of the aryl diazonium ion³⁴.



Scheme 12. Nucleophilic displacement of nitrogen from an aromatic ring.

Ortho- and para-iodobenzoic acids can be labelled by nucleophilic aromatic substitution with sodium radioiodide in aqueous hydrochloric acid, pH 6-6.5,

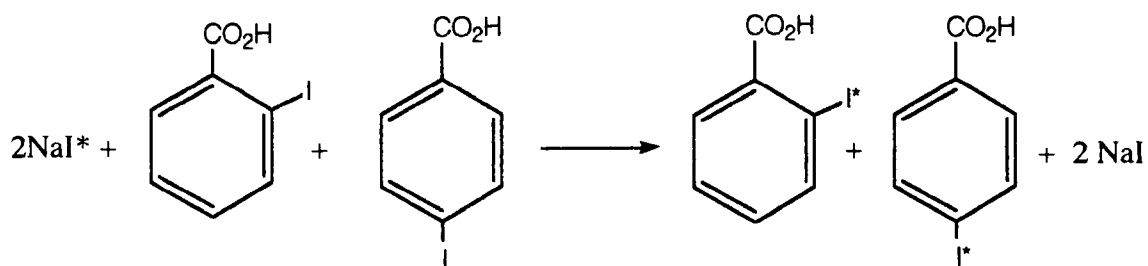
after two hours at 100 degrees^{35,36} (Scheme 13). Sodium nitrite is added in order to oxidize any reducing agent formed in the reaction mixture. The weakly acidic conditions are maintained in order to keep the carboxyl groups protonated.

Another example of nucleophilic aromatic substitution is the replacement of the amino group of aniline with radioiodide (Scheme 14). Aniline is diazotized with sodium nitrite and hydrochloric acid at 0 degrees³⁷. Sodium radioiodide is added and the reaction mixture allowed to warm to room temperature.

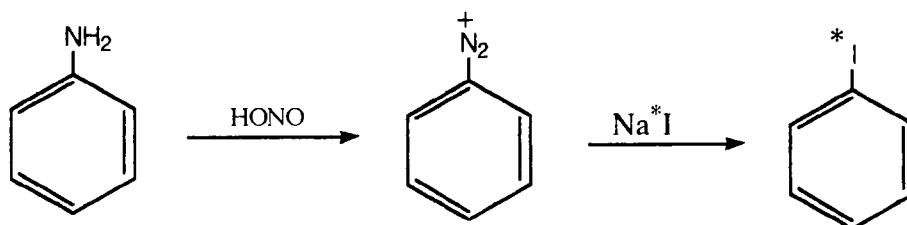
ELECTROPHILIC ADDITIONS TO DOUBLE BONDS

Electrophilic additions to double bonds can be characterized as Lewis acid-base reactions in which the halogenation reagent behaves as an acid and the double bond acts as the base. The electrophile approaches the double bond and forms a pi-complex which collapses into a positively charged ion. The nucleophilic part of the reagent subsequently becomes attached to the positively charged ion. Iodine monochloride and hydroiodic acid react in this manner. Iodine reacts in a similar manner but may require polarization by adding an electrophilic reagent such as aluminum chloride or boron trifluoride. Even the solvent can polarize the I-I bond sufficiently such that it reacts with the double bond.

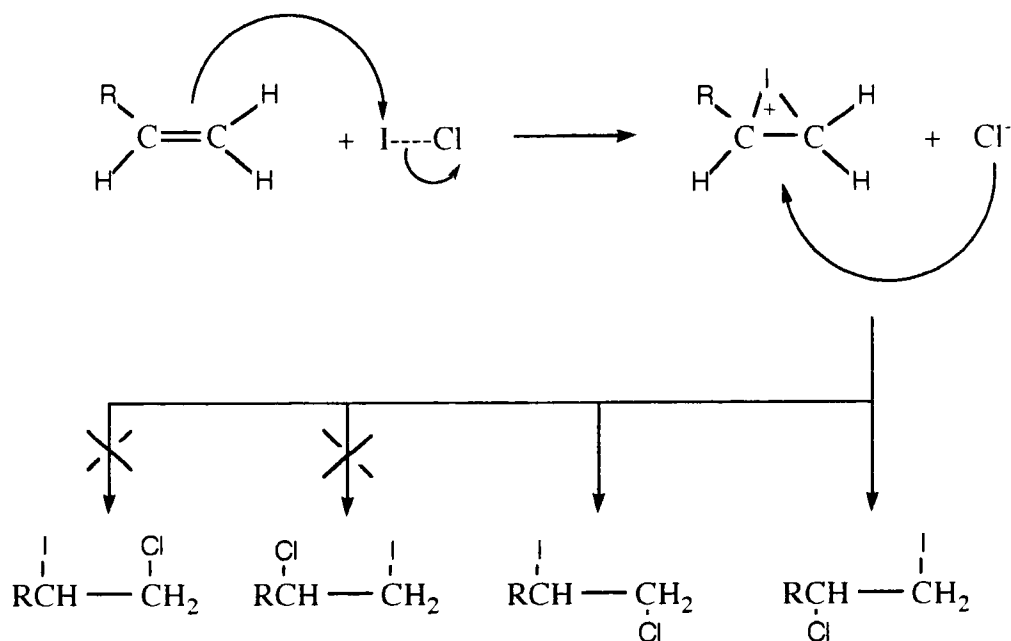
The orientation of an electrophilic addition of an unsymmetrical reagent, such as iodine monochloride, to an unsymmetrical double bond can lead to the formation of four different products (Scheme 15). The formation of the cis-products are, however, generally precluded by the initial formation of a cyclic iodonium ion which sterically blocks a syn addition of chloride ion. Therefore only two products are observed, both formed by overall anti-addition. Most



Scheme 13. Iodine isotopic exchange.



Scheme 14. Radioiodination *via* diazotization.

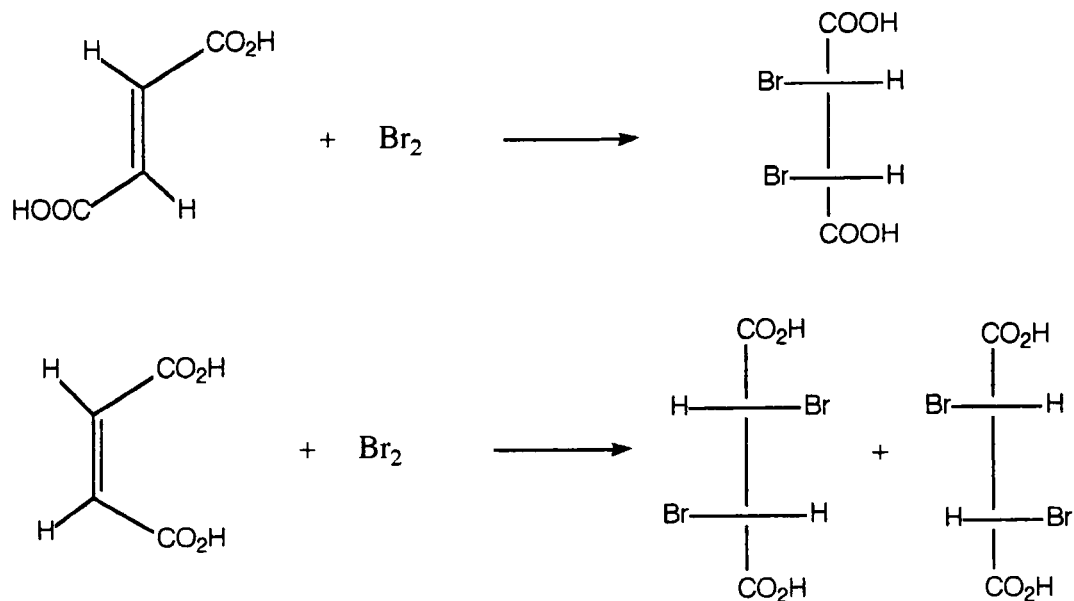


Scheme 15. Iodine monochloride addition to an alkene.

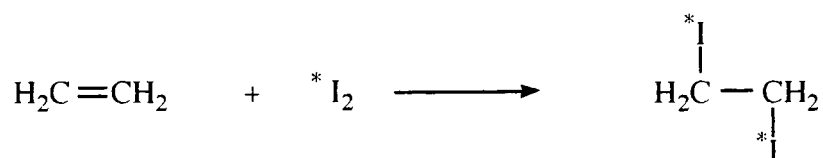
electrophilic additions to double bonds follow this mechanism. However, substituent and solvent effects can change the mechanism leading to more than two addition products. The stereochemistry of the addition is exemplified by the addition of bromine to E- and Z-maleic acid (Scheme 16). When bromine adds to E-maleic acid, only one product is formed, the meso compound. However when Z-maleic acid is brominated, an enantiomeric pair is formed.

Iodine reacts with alkenes in a fashion analogous to bromine except more slowly, and an equilibrium exists between the iodinated product and the starting materials³⁸⁻⁴⁰. The rate of iodination increases as the number of alkyl substituents on the double bond increases; the rate decreases as the number of electronegative substituents on the double bond increases. The electrophilicity of iodine can be increased by the use of Lewis acids and by using polar solvents such as acetic acid, alcohols, and nitrobenzene⁴¹. The vicinal diiodo compounds formed *via* the electrophilic addition are unstable at room temperature and decompose by eliminating iodine. The decomposition is further catalyzed by the presence of iodine⁴¹⁻⁴⁴.

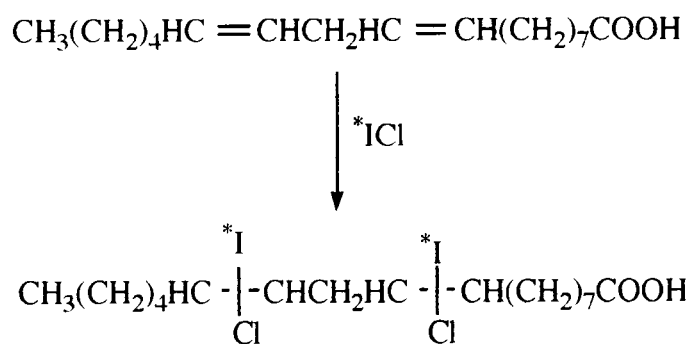
As an example, radioactive 1,2-diiodoethane may be prepared by distilling radioiodine into an ampule, cooled with liquid nitrogen, followed by the addition of ethylene⁴⁴. The reaction requires 12 hours at room temperature (Scheme 17). However, radioiodine monochloride reacts rapidly and quantitatively with linoleic acid in ether solution (Scheme 18) with multiple products being formed. Hydroiodic acid may be added in a similar fashion but is seldom employed since it must first be prepared from sodium radioiodide.



Scheme 16. Stereospecific addition of bromine to malonic acid.



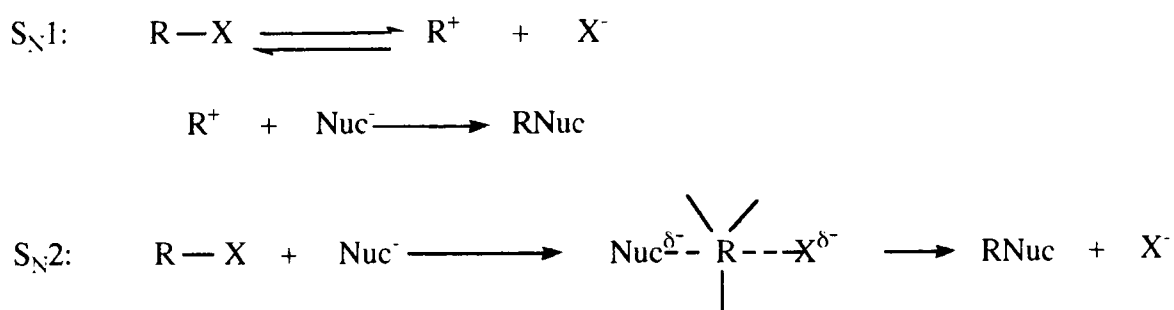
Scheme 17. Radioiodination of ethene.



Scheme 18. Chlororadioiodination of linoleic acid.

ALIPHATIC NUCLEOPHILIC SUBSTITUTION

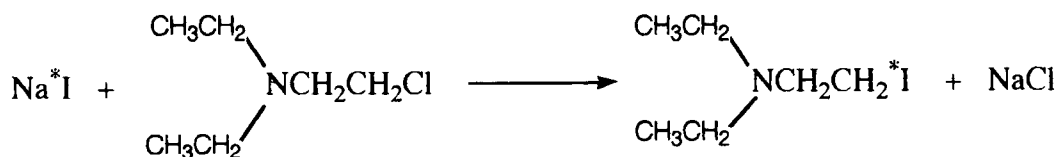
There are two basic mechanisms proposed for aliphatic nucleophilic substitution: S_N1 and S_N2 . The S_N1 mechanism is characterized by the formation of a carbocation. The rate of the reaction is dependent upon the rate of formation of the carbocation which depends upon the substituents and the solvent. The rate increases as the number of electron releasing substituents is increased and when polar solvents are used. Both factors stabilize the carbocation intermediate. The rate of an S_N2 reaction is dependent upon the concentration of both nucleophile and substrate. The mechanism is also characterized by complete inversion of configuration (Scheme 19).



Scheme 19. Mechanisms of aliphatic nucleophilic substitution.

For many aliphatic nucleophilic radioiodinations, radioiodide can be used as commercially supplied. In this form, iodide ion is the nucleophile. For example, 2-chloro-N,N-diethylamine incorporated radioiodide up to 70% when it reacted with sodium radioiodide in boiling acetone for eight minutes⁴⁵ (Scheme 20). Depending upon the structure of the substrate, many problems can occur

with aliphatic nucleophilic substitutions. Examples include the possible rearrangement of the intermediate carbocation, elimination of HX (E1 or E2) from the substrate, and stereochemical inversion.

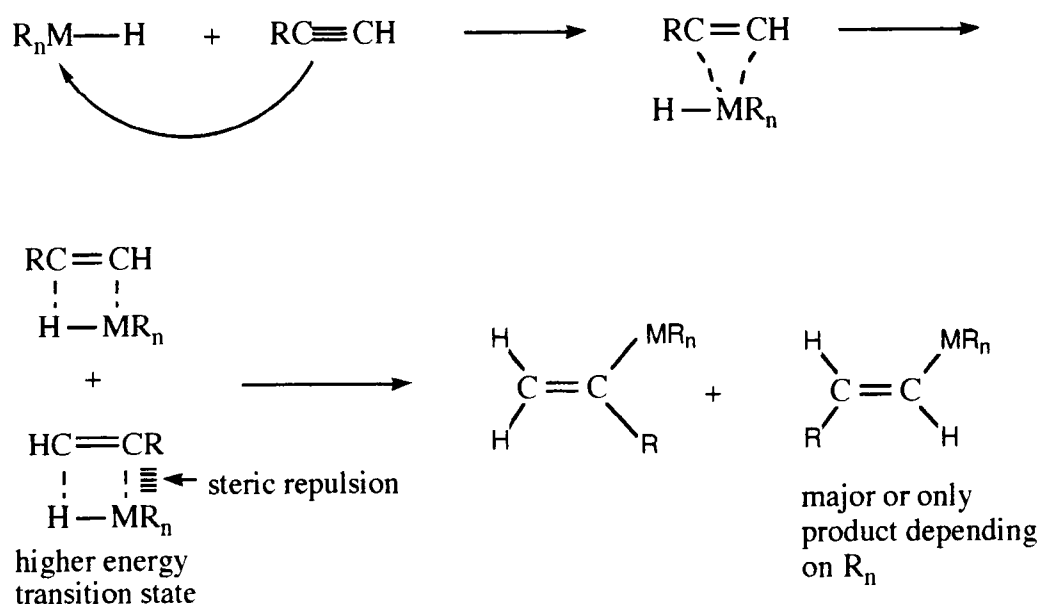


Scheme 20. Nucleophilic radioiodination.

IODODEMETALLATIONS

Iododemetalation is one of the more convenient and efficient methods for radioiodinations. The hydrometallated compounds are usually thermodynamically stable, and the compounds react readily with radioiodinating agents such as radioiodine monochloride, generated *in situ*, to give acceptable radiochemical yields.

The hydrometallation reaction may be characterized as the *syn*-addition product of a metal hydride, (MH), with a substrate containing a double or triple bond (Scheme 21). Depending on the metal, tin for example, and reaction conditions, *anti*-addition may also be observed due to a radical mechanism. For most hydrometallations, however, *syn*-addition is observed. The most widely used metals for hydrometallation, and subsequent iododemetalation, are boron, tin, aluminum, and mercury. The metals may be bonded to a variety of alkyl groups (R_n). As the size of the alkyl groups increases, the percentage of product



Scheme 21. Regiochemistry of metal hydride additions to alkynes.

with the metal bonded to the least substituted carbon atom increases. The regioselectivity of these reactions can be explained in terms of steric factors (Scheme 21). When the metal hydride is bonded to bulky alkyl groups, the metal is sterically hindered from becoming attached to the more highly substituted unsaturated carbon.

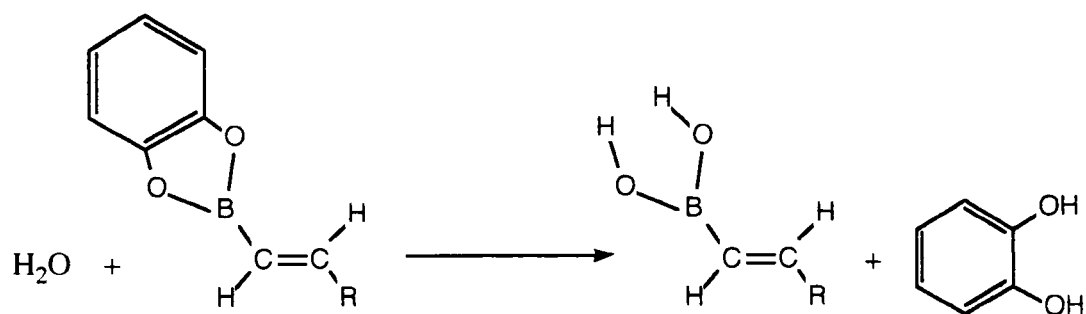
One of the more efficient methods of iodination involves hydroboration followed by iododeboration. Replacement of boron is rapid in the presence of base⁴⁶. The best yields are obtained using sodium methoxide in methanol⁴⁷. If less basic conditions are required, the use of iodine monochloride and sodium acetate give good yields⁴⁸. It should be noted that since the halogen atom replaces the boron atom, the regioselectivity of these reactions is opposite to that observed by direct addition of the hydrogen halide. Terminal olefins give primary halides.

Since alkynes are reactive toward hydroboration agents, the most useful procedures involve the addition of a disubstituted borane to an acetylene (Scheme 22). Catecholborane (benzo-1,3-dioxo-2-borole) is a particularly useful reagent for hydroboration of acetylenes⁴⁹. The adducts derived from catecholborane and an acetylene can be hydrolyzed to the corresponding vinylboronic acid with water. Dibromoborane in dimethylsulfide is also an acceptable reagent for the preparation of vinylboronic acids. The intermediate vinylboronic acid is very stable and can be kept for long periods of time. Since the hydroboration occurs in a *syn* fashion and the iodinolysis occurs with retention of alkene geometry, the iodides resulting have the E configuration⁵⁰(Scheme 23).

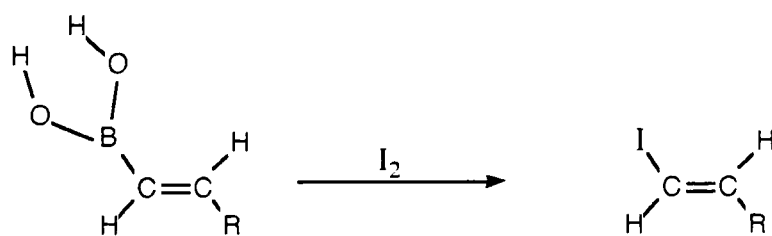
Although the boranes are reducing agents, the reductions are sufficiently slow that ester, cyano, and nitro groups do not interfere with hydroboration. On the other hand, ketones, aldehydes, carboxylic acids, and tertiary amide groups are reduced rapidly and such reductions can be competitive with hydroboration⁵¹. These functional groups must be protected before being subjected to hydroborations.

LABELLING WITH COUPLING REAGENTS

Sometimes the molecule which is to be labelled does not contain the appropriate functional groups necessary for labelling by the methods already discussed. In these cases, labelling with a coupling reagent must be performed. This labelling method is used extensively to prepare tracers for radioimmunoassays. The molecule to be labeled is coupled with a compound which is particularly reactive towards the iodinating reagent. Proteins are often employed because they have aromatic amino acid residues and are thus easily labelled. The coupling is carried out in such a manner that no change in the structure of the



Scheme 22. Hydrolysis of catechol boronic acid ester to the corresponding vinyl boronic acid.



Scheme 23. Iodination of a boronic acid.

original molecule occurs and the stereochemistry of the functional groups is retained. The portion of the molecule which is coupled must be as far removed as possible from the functional groups responsible for the molecule's biological activity. The derivatives generally used are tyrosine methyl ester (TME), tyramine, histamine, and histidine (Figure 2).

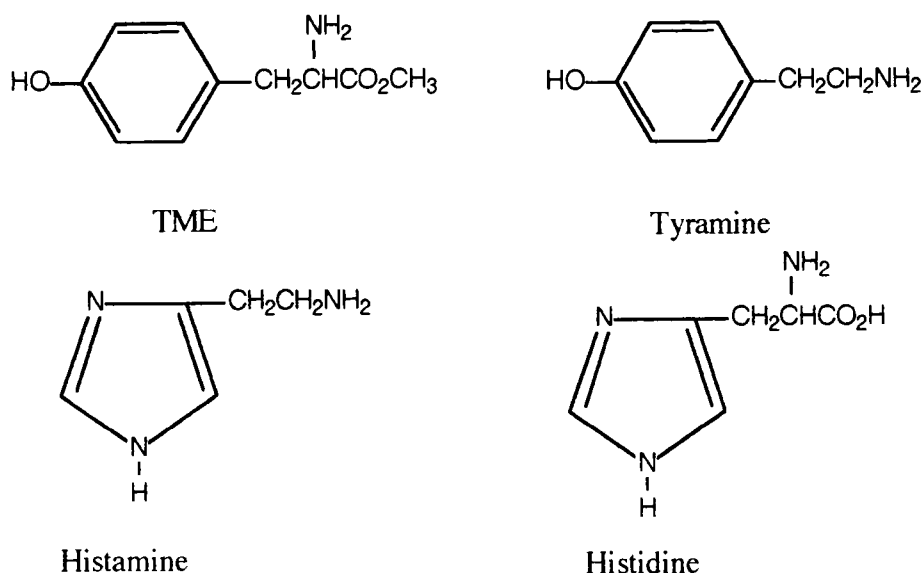


Figure 2. Commonly used coupling agents.

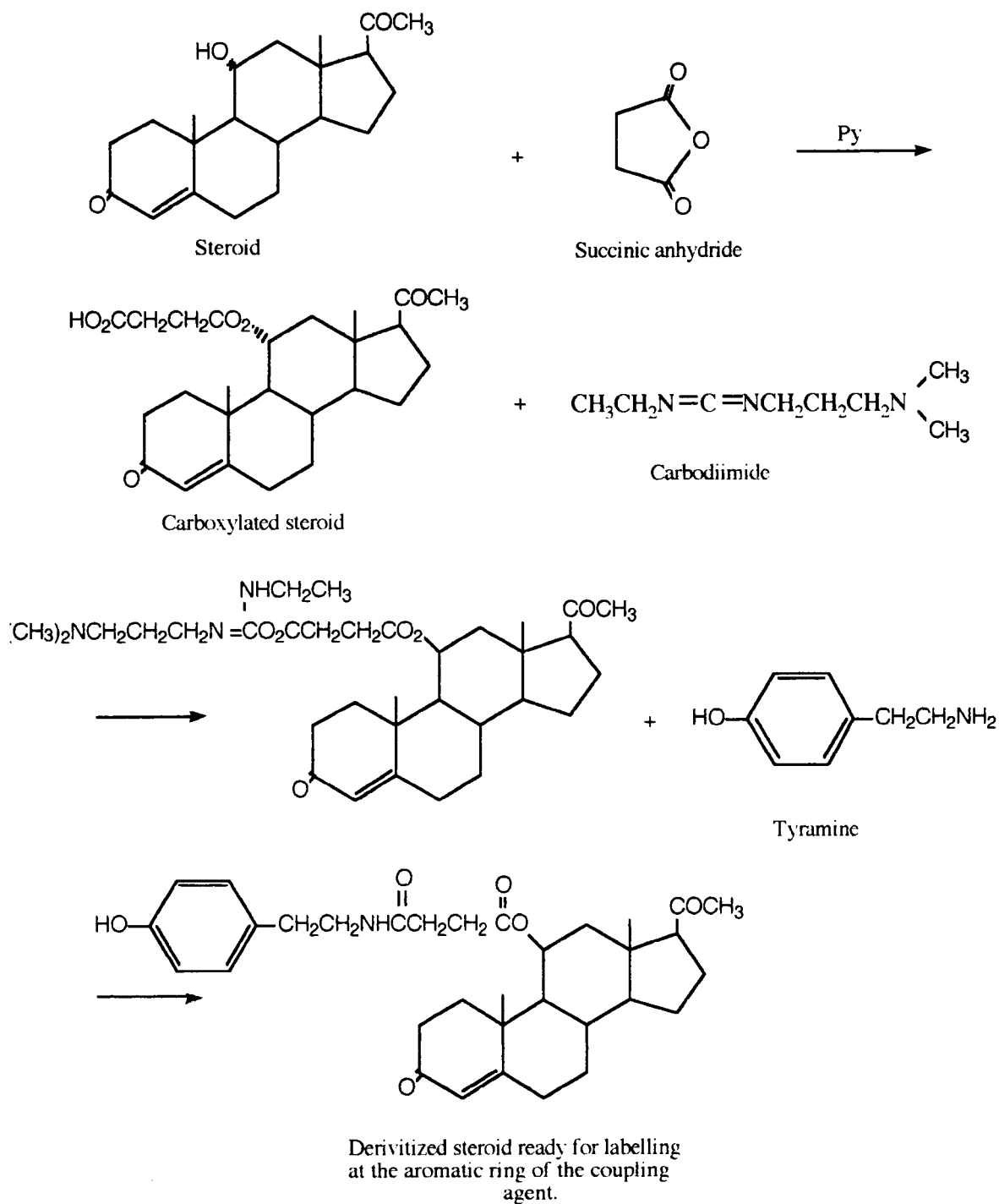
If the molecule to be labelled does not contain a carboxyl group, it is converted to one that does. The molecule is then linked to a coupling reagent and subsequently labeled. If the molecule does not contain a carboxyl group, various methods may be used to introduce it. For example, succinic anhydride reacts with a hydroxyl group and forms the corresponding hemisuccinate. Carboxymethoxyamine reacts with the carbonyl group of an aldehyde or ketone to

form the O-carboxymethyl oxine. To form the amide bond to the coupling reagent, isobutylchloroformate or carbodiimide can be used. The derivitization of 11- α -hydroxyprogesterone is an example of the process (Scheme 24).

B.3 THE INITIAL SELECTION OF AN IODINATION PROCEDURE FOR THIS STUDY

The methods discussed are those most commonly used for the introduction of radioiodine into molecules. As mentioned earlier, the goal of radioiodinations is to introduce the radioiodine into the molecule at a position far removed from the functional groups responsible for the molecule's biological activity. This is important because iodine is a large atom and its introduction into a molecule can effect its physical, structural, and chemical properties. It is hoped that these changes are not sufficient to effect the molecule's interactions with the biological environment being investigated.

After careful consideration of substrates, iodination reagents, and techniques, the initial iodination method chosen for this study was hydroboration of an alkyne functional group followed by iododeboration. If the substrate did not contain the necessary alkyne, the molecule was redesigned and synthesized to contain one. The optimal goal was considered to be the synthesis of a biologically active molecule containing a biologically stable alkenyl radioiodide moiety. An important consideration in the design of radioiodinated compounds for biological studies is their *in vivo* stability. Even though radioactive alkyl iodides are readily prepared, they are not ideal agents because they undergo facile *in vivo* deiodination which results in accumulation of activity in the thyroid⁵² and blood⁵³. Aryl and alkyl radioiodides were thus excluded because of their size and instability, respectively. All of the radioiodinations described, including a newly developed radioiodination technique, depend upon



Scheme 24. Synthesis of a "steroid-coupling agent" derivative, a radioiodinated steroid agent precursor.

the presence of an alkyne function which ultimately provides an alkenyl radioiodide.

B.4 THE GOAL OF THIS STUDY

Enormous challenges face the organic chemist who synthesize, label, and biologically test novel compounds which can be used for the diagnosis and treatment of diseases or the illucidation of some biological processes. The goal of this thesis is to synthesize, label, and test molecules designed to mimic known biologically active molecules for use as agents for medical imaging or biological studies. The initial choice of an iodination procedure was the hydroboration-iodination technique but, as will be discussed, because this method was not appropriate for certain molecules, a new iodination technique was developed. The emphasis of this dissertation is on the synthetic organic chemistry and the novel labeling procedure developed as a result of this work.

As discussed previously, the radioactive isotope used for all labeling experiments was ^{125}I . This isotope is commonly used for many preliminary labeling experiments due to its relative safety in handling. The relatively long half-life, sixty days, makes the synthesis, storage, and biological testing of the new agent more convenient.

Even though ^{125}I is relatively safe, all radioactive isotopes are hazardous to a certain extent. For this reason when radioactive agents are developed, the chemistry is performed first using a "cold" isotope, one which is not radioactive. Once the chemical procedures are established the radioactive isotope is substituted for the "cold" one. Therefore, this study is presented in four basic sections: 1) the design, synthesis, and "cold" iodination of agents using the hydroboration-iodination technique; 2) the development of a new iodination procedure, iodine monochloride addition to alkynes containing multifunctional groups; 3) the design, synthesis, and "cold" iodination of agents using the new procedure; 4) the description of the radioiodination procedures and the test

results of the individual radioiodinated agents.

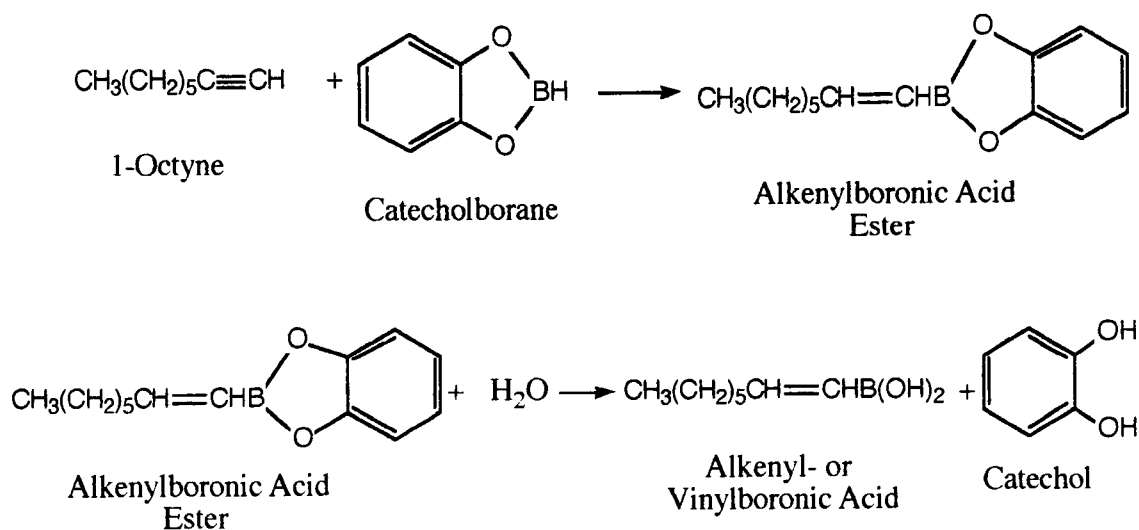
CHAPTER 2. SYNTHESIS OF IODINATED AGENTS USING THE HYDROBORATION-IODINATION PROCEDURE

A. INTRODUCTION

Prior to the synthesis and radioiodination of multifunctional molecules, a procedure using a "test" substrate was required. In order to synthesize and label compounds efficiently, the technique chosen should produce a stable intermediate which will readily react with an iodinating reagent. As noted earlier, hydroboration followed by iododeboration meets these requirements.

Catecholborane was chosen as the hydroborating agent due to its efficient reactivity with triple bonds. The hydroboration sequence can be accomplished by reacting catecholborane (1,3,2-benzodioxaborole) with the alkyne⁵⁴. When catecholborane is added to the alkyne, the hydroboration occurs in a *syn* fashion, producing an alkene with the *E* configuration⁵⁵. Furthermore, the vinylboronic acid is easily and quickly converted to the vinyl iodide when it reacts with iodine monochloride generated in situ. In order to develop a method for radioiodination, 1-octyne was used as the test substrate for hydroboration followed by iodination (Scheme 25).

Catecholborane reacts with 1-octyne at slightly elevated temperatures in THF to form the corresponding hydroborated adduct via a *syn* addition. It was not necessary to isolate this intermediate since the addition of water to the reaction mixture hydrolyzes the intermediate to form the (*E*)-octenylboronic acid and catechol. Unfortunately, separation of the product from the catechol proved to be difficult even with the use of column chromatography. Therefore fractional



Scheme 25. Synthesis of a vinylboronic acid.

precipitation was used to prepurify the product. This consisted of dissolving the boronic acid in THF followed by the dropwise addition of water and collection of the white flocculant precipitate. This procedure, followed by column chromatography, provided the product with sufficient purity for analysis. The presence of boron in the molecule was obvious due to the absence of a resonance for C-1 in the ^{13}C NMR spectrum, a consequence of the quadropolar nature of the boron atom. Both proton resonances, however, for the alkene hydrogens are visible.

The alkenylboronic acid was converted, in good yield, to the vinyl iodide by its reaction with sodium iodide and chloramine-T. (Sodium iodide reacts with chloramine-T to form iodine monochloride *in situ*.) The complexation of boron with a Lewis base assisted the subsequent migration of the alkenyl moiety to I^+ , thereby providing the desired vinyl iodide in good yield. Purification by column chromatography yielded 1-octenyl iodide as white crystals. Analysis by ^1H NMR

confirmed the *E* geometry by revealing a trans proton coupling constant of 16 Hz, while the ^{13}C NMR spectrum indicated the presence of iodine as evidenced by the large upfield shift observed for C-1. The remainder of both spectra was consistent with the product. The hydroboration-iodination sequence was then utilized to prepare a series of biologically active iodovinyl reagents.

B. SYNTHESIS OF AN IODINATED LIPOSOMAL MARKER USING THE HYDROBORATION-IODINATION PROCEDURE

B.1 INTRODUCTION

Liposomes have attracted a great deal of scientific interest in two quite distinct applications. First, liposomes provide an excellent model for cell membranes, allowing exploration of questions concerning membrane lipid chemistry, lipid-protein interactions, transport phenomena, and ligand binding to membranes⁵⁶. Second, and most pertinent to this research, was the development of liposomes as controlled delivery systems for therapeutic agents. In this regard a wide variety of pharmaceuticals have been entrapped or encapsulated in liposomes; these include antitumor agents, antibiotics, anti-inflammatory substances, and drugs acting on the central nervous system⁵⁷. A liposomal marker would, therefore, be an invaluable tool for the design of liposomes. It would aid the researcher in gaining knowledge as to the location and fate of the newly designed and synthesized liposome. Before the liposomal marker could be designed, however, some properties and characteristics of liposomes had to be considered.

A liposome is defined as any structure composed of lipid bilayers that enclose a volume (Figure 3). The lipid is not necessarily a phospholipid, but this

is the most commonly used component. Liposomes are generally characterized by their lipid composition in terms of stoichiometric proportions. When phospholipids are dispersed in an aqueous phase, a heterogeneous mixture of vesicular structures is usually formed, most of which contain multiple lipid bilayers forming concentric spherical shells. These types of liposomes were

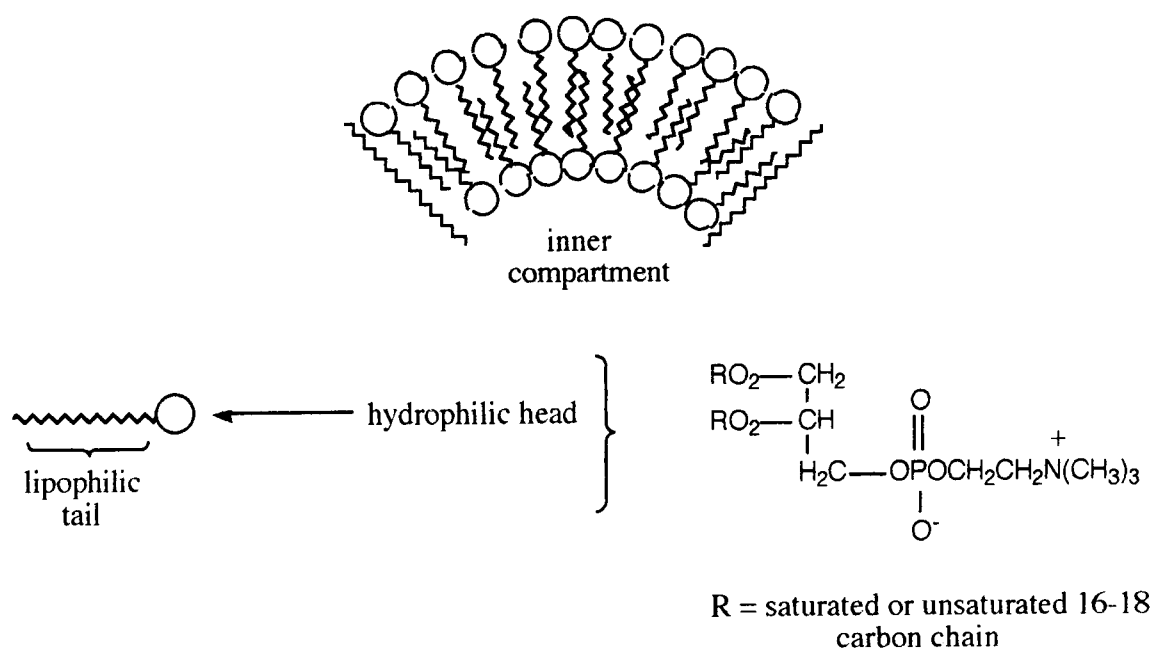


Figure 3. A section of a typical liposome.

first prepared by Bangham and co-workers⁵⁸ and are now referred to as multilamellar vesicles (MLVs). When this lipid dispersion is sonicated, the MLVs are reduced to much smaller structures in the size range of 25-50 nm diameter. These are termed small unilamellar vesicles (SUVs) since they contain only a single bilayer. Depending on the liposome's composition and method of preparation,

large unilamellar vesicles (LUVs) can also be prepared. There are two important parameters of liposome preparations that are functions of vesicle size and concentration: captured volume, the volume enclosed by a given amount of lipid expressed as liters of liquid entrapped per mole of total lipid, and encapsulation efficiency, the fraction of aqueous compartment sequestered by the lipid bilayers. Both of these parameters are very important depending on the intended use of the liposome. For example, if liposomes are to be used for intracellular delivery of an entrapped compound, an LUV preparation with a high captured volume will deliver a much greater volume per lipid mass than a SUV preparation. Furthermore, if the encapsulated compound is rare or costly, it is desirable to maximize the amount trapped in the liposome volume.

It is obvious that polar or hydrophilic drugs will be found in the internal aqueous compartments of liposomes, while lipophilic or amphipathic drugs can partition into the liposome membrane. Thus, brief sonication of liposomes containing water soluble drugs causes rapid release of all the entrapped material. By contrast, sonication of liposomes containing lipophilic drugs results in slow and partial release, indicating that the drug is associated with the stable liposome membrane. The ability of liposomes to entrap various types of pharmaceuticals makes them very important and potentially useful as a drug delivery system since they interact intimately with membranes at the cellular level.

There are several different mechanisms by which liposomes interact with cells. The first is fusion of liposomes with cells which is defined as the unification of the outermost lipid bilayer of the liposome with the plasma membrane of the cell which necessarily requires the mixing of the liposomal aqueous contents with the cytoplasm of the cell. Another important criterion for fusion is the mixing of the liposomal lipids with the plasma membrane lipids. Perhaps the most

convincing evidence for liposome-cell fusion comes from the reports that biologically active substances encapsulated in liposomes can be introduced into the interior of the cells⁵⁹. Some of these substances are impermeable to cell membranes and can express their biological effects only when delivered to the cell interior.

The second mechanism is adsorption, which involves the stable attachment of liposomes on the cell surface. The attached liposomes may be intact or modified by the medium or cell components. It is not clear whether the adsorbed liposomes can subsequently undergo endocytosis, fusion, or other types of interactions with cells⁶⁰.

The third mechanism involves lipid exchange, which utilizes the transfer of liposomal lipids to the cell membrane with concomitant transfer of cellular lipids to the liposome membrane. This transfer is not accompanied by the transfer of liposome contents into cells. This phenomenon of cellular lipid transfer to liposomes was first observed by Pagano and Huang⁶¹. The transfer of cholesterol, a common component of liposomes and cells, has been observed to take place by this mechanism⁶². Many studies have used this approach to elucidate the role of plasma membrane cholesterol in cellular functions such as cell proliferation and the transport activities of cells⁶³.

The fourth mechanism is endocytosis which involves the ingestion of intact liposomes by cells through a process resembling the phagocytosis of particles by cells. The extent of endocytotic activity depends on the type of cell and the contents of the liposome membrane. For example, the highly phagocytic macrophage cells endocytose liposomes with little selectivity while the endocytotic activity of more selective cells may be increased by the presence of glycolipids in the liposomal membrane⁶⁴.

The last form of cell-liposome interaction is cell-induced liposome leakage. Depending on the constitution of the liposomal membrane, it may be induced to leak when in the presence of cells by adsorbing certain lipoproteins onto the surface of the liposome⁶⁵.

B.2 THE CHEMICAL DESIGN OF A LIPOSOMAL MARKER

By examining the various mechanisms of cell-liposome interactions, the potential of liposomes as a drug delivery system becomes obvious. However, many problems are associated with this application of liposomes. The mechanism of interaction depends on many factors such as the liposome contents, the method of preparation, and the nature of the biological system in which the liposomes will be in contact. Perhaps the most important factor in using liposomes as a drug delivery system is knowledge relating to where the drug is released in the biological system (i.e., whether at the site of injection/implant or at tissue, cellular, or intracellular sites). Since this aspect depends on the liposome's method of preparation and size, liposomal preparations must be tested and a method developed for determining their ultimate positions within the biological system.

In this study, a liposomal marker was developed and designed based on the constitution of a typical liposomal membrane. After administration of the marked liposomes to a biological system, the system was dissected into components and the radioactivity determined in each by the use of a scintillation counter.

Since cholesterol is a common component of (and stably associated within) the liposomal membrane, the liposomal marker was designed to contain a cholesterol nucleus (Scheme 26). Attached to the cholesterol nucleus of the marker is a long unbranched hydrocarbon chain. This mimics the hydrocarbon

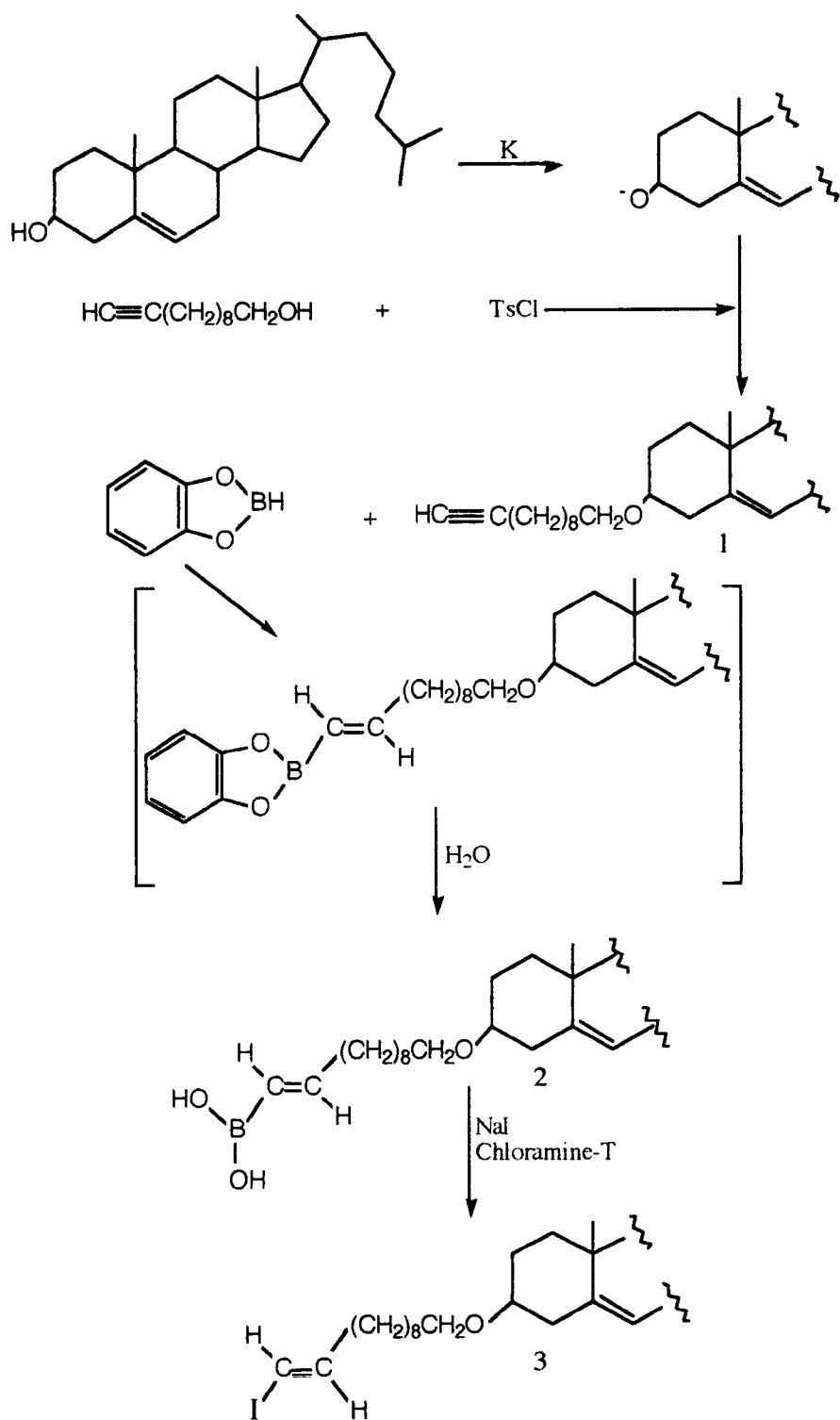
chains of the phospholipids and increases the marker's solubility in the liposomal membrane by lipophilic interactions with the long hydrocarbon "tails" of the phospholipids. The hydrocarbon chain is attached to the cholesterol nucleus by an ether linkage. This provides a very stable attachment without adding significant polarity to the molecule. The long hydrocarbon chain contains a terminal alkenyl group which is attached to an iodine atom. The alkenyl group is selected for attachment to iodide because it is more resistant to *in vivo* deiodination. The terminal alkenyl iodide was derived from the corresponding terminal alkyne *via* the hydroboration-iododeboration sequence developed previously in our lab.

B.3 SYNTHESIS OF A LIPOSOMAL MARKER

In order to connect the hydrocarbon chain with the cholesterol nucleus, an ether linkage was chosen. Since ω -undecynyl alcohol is commercially available, it was used as the alkylating agent by converting it to the tosylate. The tosylate, ω -undecynyl tosylate, was prepared according to literature procedures⁶⁶ using ω -undecynyl alcohol (commercially available) and tosyl chloride in pyridine and chloroform. The product was contaminated by a compound which had a very similar R_f value on silica gel thin layer chromatography plates using various solvent systems. After repeated silica gel chromatography, the by-product was isolated and identified, by ^1H and ^{13}C NMR, as the tosylate of ethyl alcohol. A careful inspection of the reagents and solvents revealed the contaminant, ethyl alcohol, to be a stabilizing agent added to commercially available chloroform. The reaction was repeated with chloroform which had been purified by alumina column chromatography. This provided the product in good yield and it was easily purified by silica gel column chromatography.

The Williamson ether synthesis was used to synthesize the ω -undecynyl cholesteryl ether (Scheme 26). The alkoxide of cholesterol was prepared by the reaction of potassium with cholesterol in benzene. The reaction mixture became quite thick, which necessitated the use of an external stirring device and the addition of more solvent. The conversion of cholesterol to the corresponding alkoxide was complete after 24 hours at reflux temperature. The tosylate, ω -undecynyl tosylate, in benzene was added drop-wise to the alkoxide and the reaction mixture was stirred for 48 hours at reflux temperature. The resulting thick mixture slowly disappeared leaving a salt precipitate in its place. Evaporation of the solvent provided a residue which was chromatographed to provide ω -undecynyl cholesteryl ether (1) in an 85% yield. The compound was identified via elemental analysis and ^1H and ^{13}C NMR spectroscopy. Five singlets were present in the ^1H NMR spectrum corresponding to the five methyl groups of the cholesterol nucleus as well as a triplet at δ 3.44 which was consistent with the CH_2 attached to the oxygen atom. The ^{13}C NMR data included peaks at δ 141.10, 121.35 and 84.62 consistent with the cyclic olefinic carbons and the internal alkyne carbon, respectively. Interestingly, the external alkyne carbon and the CH_2 carbon attached to the oxygen atom were superimposed at δ 68.07. This was determined by the off-resonance decoupled ^{13}C NMR spectrum which included both a doublet and a triplet resonance, respectively, centered at this frequency.

The hydroboration sequence was accomplished by reacting catecholborane (1,2-benzodioxaborole) with the alkyne. The corresponding hydroborated product was not isolated. Water was added dropwise to the mixture, which led to the formation of the hydrolyzed catecholborane adduct, the corresponding vinyl boronic acid (2), as a white precipitate. Unfortunately catechol, a by-product of the reaction, precipitates with the vinylboronic acid, thereby making purification



Scheme 26. Synthesis of a liposomal marker.

difficult. However, the majority of the catechol was removed by dissolving the residue several times in THF and reprecipitating the product by the drop-wise addition of water. After chromatographing the resulting white flocculant material on a silica gel column, the vinylboronic acid was obtained in a 69% yield. The resulting boronic acid was stable to the atmosphere and could be stored under refrigeration for long periods.

The identification of the product was confirmed by both ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum included resonances at δ 5.30, 6.57 and 6.93, consistent with the alkene carbons and the acidic protons of the vinyl boronic acid, respectively. The ^{13}C NMR spectrum demonstrated only one alkene carbon resonance at δ 150.82 due to the internal alkene carbon. The terminal alkene carbon resonance was not observed due to the boron nuclear quadrupole moment's effect on the relaxation time of this atom's nucleus. Obtaining a correct elemental analysis of this compound was difficult due to the propensity of the boronic acid to coordinate with water. If an attempt to dry the elemental sample was made by heating it under vacuum, the boronic acid dehydrated to form a boronic acid ester. It is therefore advisable not to dry boronic acids prior to storage.

The final product was readily obtained by dissolving the boronic acid in aqueous THF containing sodium iodide and a mild oxidizing agent, then stirring the reaction mixture for 2 hours⁶⁷. Extraction with ether, followed by concentration, provided the crude product which was purified by silica gel chromatography to yield the vinyl iodide (3) as white crystals in a 70% yield.

The stereochemistry of the vinyl iodide was confirmed by ^1H NMR spectroscopy. The terminal alkene proton resonated at δ 5.95, generating a doublet with a coupling constant of 14.5 Hz. This relatively large coupling

constant is consistent with vicinal alkene protons which are trans to one another. The inclusion of iodine into the molecule was demonstrated by ^{13}C NMR spectrum, which revealed a resonance at δ 74.34 corresponding to the external alkene carbon atom attached to iodine. This large upfield shift is due to the heavy atom effect of iodine which shields the nucleus of the carbon to which it is attached. The other alkene carbon atom resonance was in the "alkene region" at δ 146.85. All other resonances, ^1H and ^{13}C , were consistent with the proposed structure.

C. SYNTHESIS OF AN IODINATED SPIROPERIDOL ANALOG USING THE HYDROBORATION-IODINATION PROCEDURE

C.1 INTRODUCTION

One aspect of the study of human brain function, to which intense research effort has been devoted, is the elucidation of the role of cerebral dopaminergic receptors in diseases of neurotransmission. Biochemical studies have indicated alterations in the population of cerebral dopaminergic D_2 receptors in diseases such as schizophrenia, Parkinson's disease, Huntington's chorea, tardive dyskinesia, and the aging process⁶⁸. The prevalence of these diseases in the general population necessitates the need for non-invasive, clinically useful methods for the measurement of cerebral dopaminergic receptor concentrations. A successful imaging agent would be utilized by physicians involved in treating disease and physiologists doing research in cerebral morphology, biochemistry, and function.

The ability to image dopamine-rich areas of the brain by positron emission tomography (PET) and single photon emission computerized tomography (SPECT) has increased the need for receptor-specific radiolabeled pharmaceuticals.

Several agents which have proven successful for imaging cerebral dopaminergic D₂ receptors are [⁷⁵Br]-bromperidol, [⁷⁷Br]-bromperidol, and several spiroperidol analogs such as N-(3-[¹⁸F]fluoropropyl)spiroperidol, [¹⁸F]-N-methylspiroperidol, and [¹²⁵I]iodoalkenyl-substituted spiroperidol⁶⁹ (Figure 4). Spiroperidol is used in the treatment of neuropsychiatric disorders and has a high affinity for D₂ dopamine receptors. This makes radiolabeling of spiroperidol analogs an attractive method for the production of successful agents for imaging and related studies. Three different spiroperidol analogs were designed and were radiolabeled using two different iodination procedures, the hydroboration-iodination technique and the novel radioiodination procedure described in the next chapter. Only the derivative iodinated by the hydroboration-iodination procedure will be discussed here.

C.2 THE CHEMICAL DESIGN OF A SPIROPERIDOL ANALOG

All spiroperidol analogs designed to date include substituents on the piperidine ring nitrogen, a site of the molecule believed to be involved with receptor binding. It seems probable that relieving steric hindrance around this site could increase binding to the receptor site, thereby leading to a superior agent. One design which eliminates steric hindrance around the ring nitrogen is the 1-phenyl-1,3,8-triazaspirodecanone moiety, a pharmacophore of spiroperidol. In order to increase the lipophilicity of the molecule, an aliphatic carbon chain was added to the lactam nitrogen. The carbon chain also contained a terminal alkyne function to provide a site for iodination far removed from the 1-phenyl-

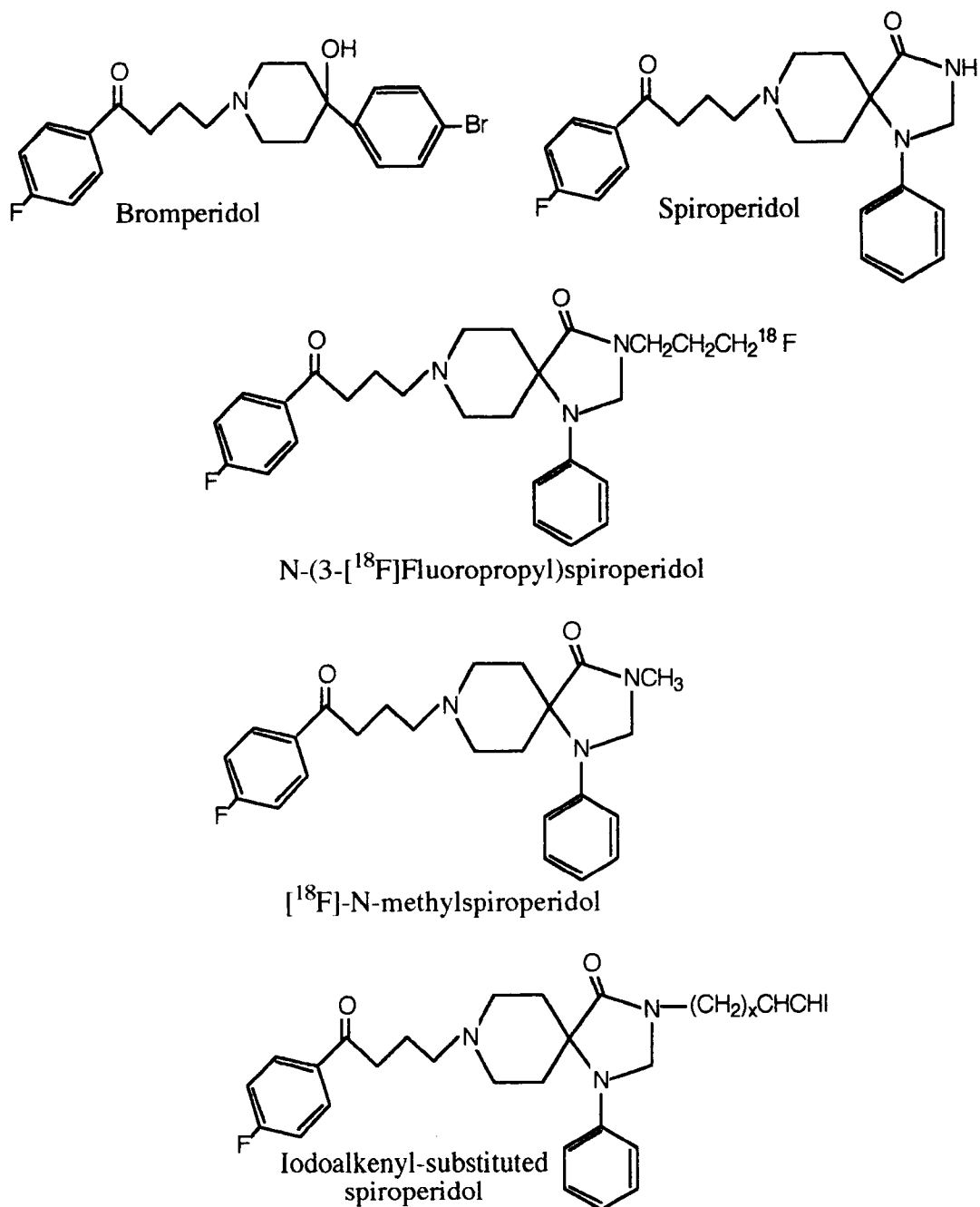


Figure 4. Dopamine receptor active agents.

1,3,8-triazaspirodecanone pharmacophore. After iodination, the analog would be 3-(*E*-1-iodopenten-5-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (Figure 5).

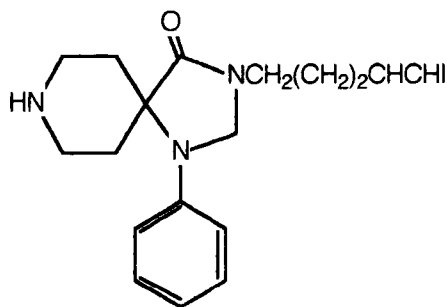


Figure 5. 3-Iodopentenyl-1-phenyl-1,3,8-triazaspirodecanone

C.3 SYNTHESIS OF AN IODINATED SPIROPERIDOL ANALOG

The alkylating agent was prepared from commercially available 5-iodo-1-pentyne. Since the alkylation reaction occurs readily, it was decided to iodinate 5-iodo-1-pentyne first. An excellent method used earlier involves the hydroboration-iodination sequence. Therefore, 5-iodo-1-pentyne was allowed to react with catecholborane, followed by hydrolysis with water, to provide the 5-iodo-1-penten-1-ylboronic acid (Scheme 27). The vinylboronic acid derivative was not purified but used directly. The vinylboronic acid was dissolved in aqueous THF containing sodium iodide, and chloramine-T or N-chlorosuccinimide (NCS) was added to provide the in situ generation of ICl. The product was extracted into ether and characterized. The ^1H NMR spectrum (see Appendix I) contained the characteristic doublet and the superimposed doublet of triplets in the alkene region for the terminal and internal vinylic protons, respectively. The

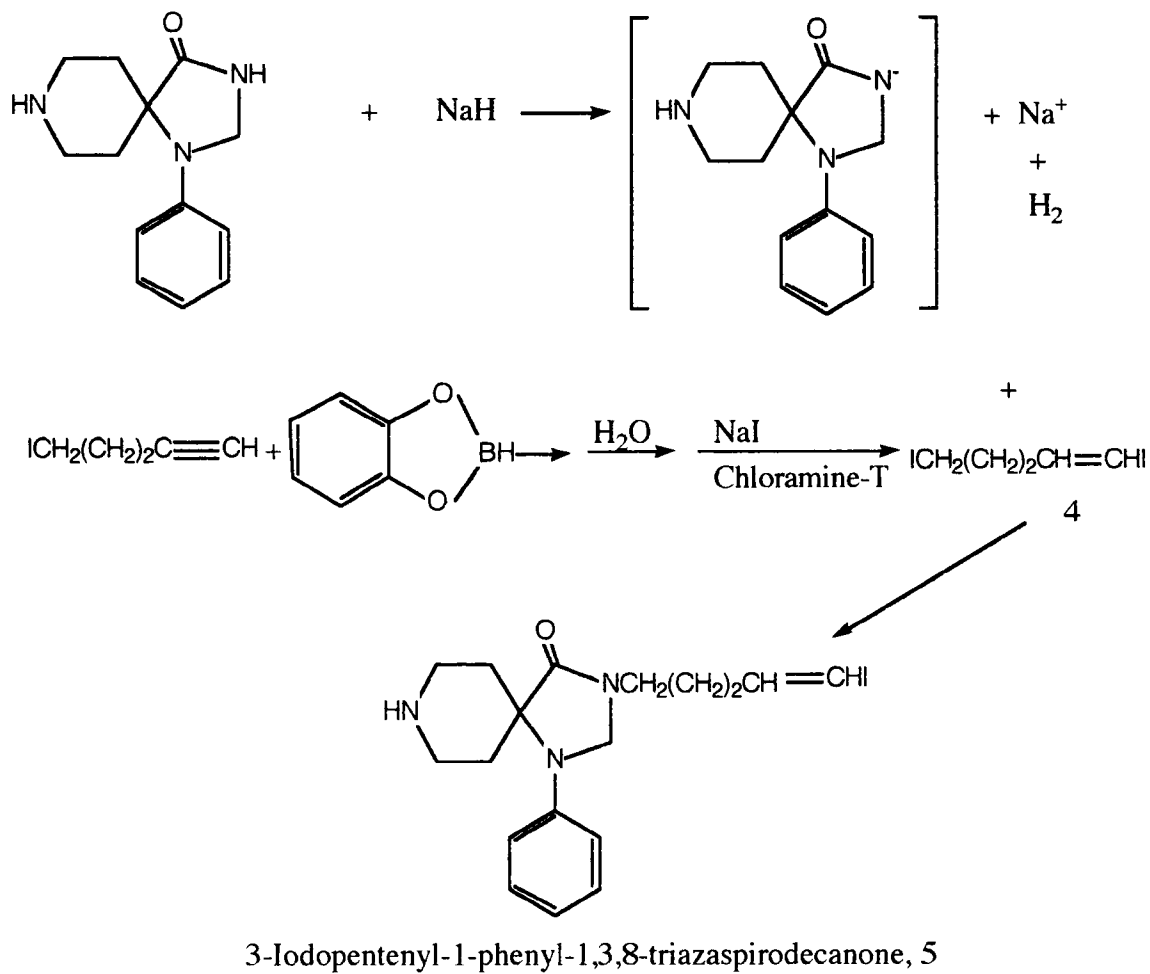
^{13}C NMR spectrum (see Appendix I) contained the CHI carbon resonance shifted upfield from the alkene region due to the heavy atom effect of iodine. The remainder of both spectra was consistent with (*E*)-1,5-diiodo-1-pentene (4). When radiolabeled, this molecule contains the desired vinyl radioiodide which was subsequently used to alkylate 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. The alkylation was accomplished by mixing sodium hydride in DMF with the spiro-compound followed by the addition of 1,5-diiodo-1-pentene. The reaction proceeded at room temperature to provide 3-((*E*)-1-iodopenten-5-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (5) in good yield. None of the 8-iodopentenyl iodide derivative substituted on nitrogen of the piperidine ring could be isolated, indicating alkylation of the piperidine nitrogen was not a competing reaction. Both ^1H and ^{13}C NMR spectra were similar to the spectra of the starting materials minus the lactam hydrogen resonance.

D. SYNTHESIS OF AN IODINATED MYOCARDIAL IMAGING AGENT USING THE HYDROBORATION-IODINATION PROCEDURE

D.1 INTRODUCTION

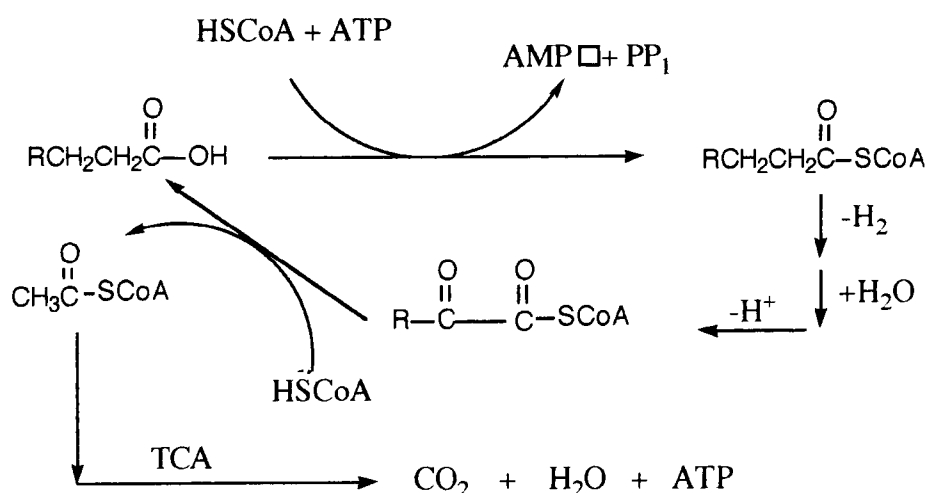
Since the myocardium is an organ essential for life and its cessation causes instantaneous death, new methods of diagnosis and treatment for a variety of myocardial malfunctions are continually being researched. These methods include metabolic and imaging studies using a variety of radiolabeled compounds⁷⁰.

In this study, the biological needs of the heart were exploited in the design of the potential imaging agents. The myocardial energy demands are met by the metabolism of various biomolecules such as acetone, lactic acid, and many fatty acids. After considering the synthetic possibilities, a radiolabeled fatty acid was



Scheme 27. Synthesis of a spiroperidol analog.

designed as the target molecule for myocardial uptake experiments. The fatty acids are a major source of energy for the myocardium. After the fatty acid is transported from the blood into the individual myocardial cells, metabolism begins by activation of the molecule by its conversion into the corresponding coenzyme A derivative (Scheme 28). The Coenzyme A derivative is then degraded to acetyl CoA by successive β -oxidations, resulting in the removal of two-carbon fragments. The resulting acetyl CoA fragments are then metabolized via the tricarboxylic acid cycle (TCA or Krebs cycle). These oxidations provide energy for the heart in the form of ATP which is utilized in muscle contraction.



Scheme 28. Enzyme mediated metabolism of fatty acids in the heart.

D.2 THE CHEMICAL DESIGN OF A MYOCARDIAL IMAGING AGENT

Previous studies indicate that the fatty acids which demonstrate the greatest myocardial uptake are those containing a chain of seventeen carbon atoms attached to the carboxylic acid group⁷¹. Since the vinyl radioiodinated

derivatives are relatively stable to *in vivo* deiodination, the radioiodinated analog of oleic acid, 9(10)-[^{125}I]iodooleic acid, was designed as a potential myocardial imaging agent. The precursor to this agent must contain an alkyne instead of the alkene. Due to previous success, the hydroboration-iododeboration sequence was the procedure of choice to effect the iodination.

D.3 SYNTHESIS OF AN IODINATED MYOCARDIAL IMAGING AGENT

Because boranes reduce carboxylic acid groups, the commercially available stearolic acid was protected by esterification prior to hydroboration. When stearolic acid was refluxed with methanol containing a catalytic amount of concentrated sulfuric acid, only one product, methyl stearolate (6), was formed (Scheme 29). The triple bond was unreactive under these reaction conditions. The only other component of the reaction mixture, after evaporation of the solvent and removal of the catalyst, was the starting material, stearolic acid. Since the product was easily separated from the starting material and the triple bond was retained, this quick and simple method of esterification was preferred over other methods, such as esterification using diazomethane. The product was easily identified by the use of ^{13}C and ^1H NMR. These spectra very closely resembled the starting material with the addition of a proton resonance at δ 3.7, integrating for 3 protons, and a carbon resonance corresponding to the methyl group carbon. As expected, the alkyne carbon resonances were still present at δ 80.2 and 80.0.

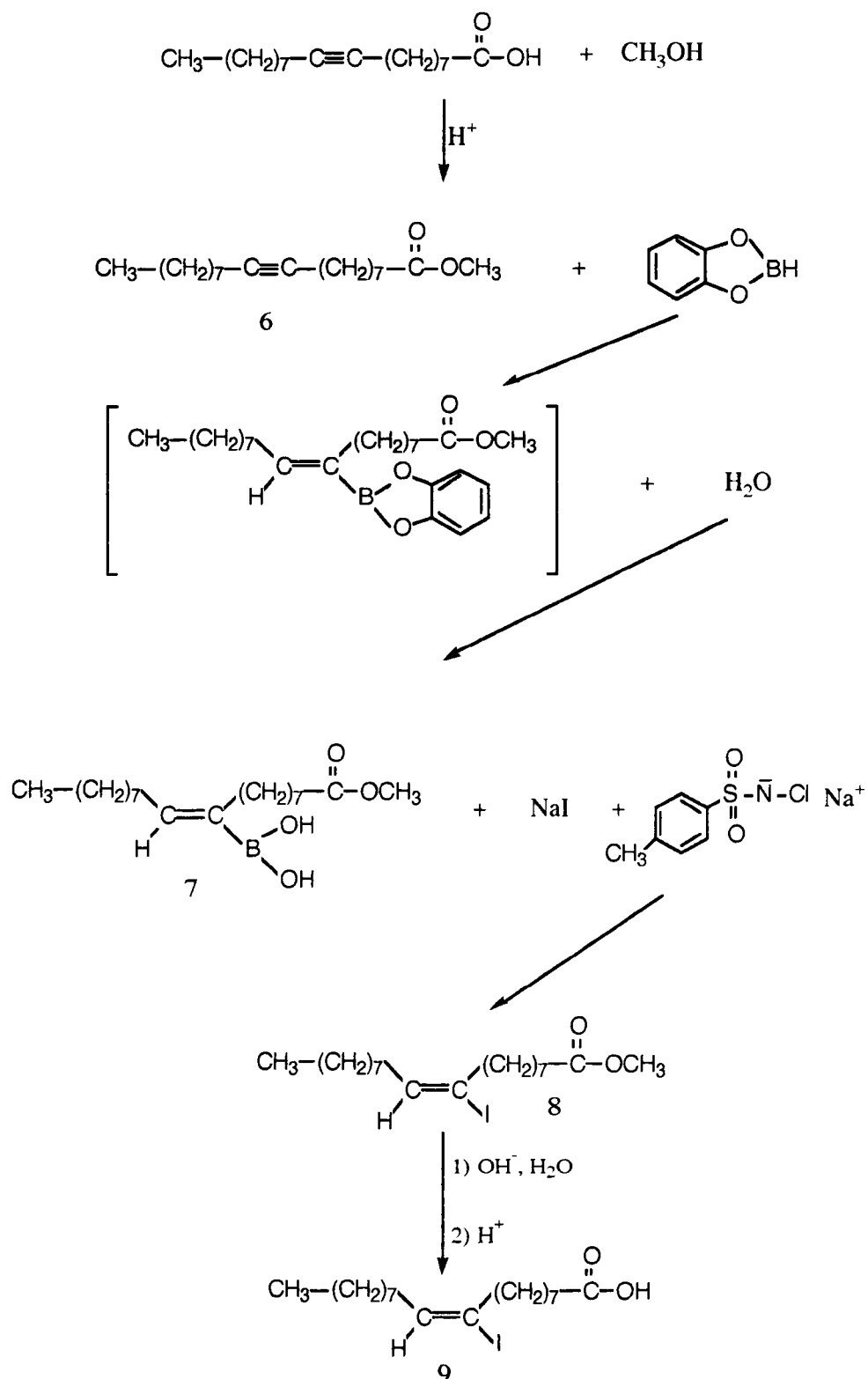
Methyl stearolate was allowed to react with catecholborane at reduced temperature, after which the reaction mixture was allowed to slowly warm to room temperature. The hydroborated intermediate was not isolated. Instead, water was added to the re-cooled stirred mixture. After extraction with ether, an oil remained which completely decomposed to leave a white crystalline precipitate

when stored between 5 and 10 °C overnight. The addition of water to the oil appeared to slow its decomposition. If used immediately, the oil was sufficiently stable for TLC, ^1H , and ^{13}C NMR analyses though some decomposition was observed during the analyses, i.e., formation of a white crystalline precipitate in the NMR tubes.

TLC analysis (silica gel) of the oil immediately after isolation indicated the formation of a single product (a single spot). When the TLC experiment was repeated using product which had been stored, the initial spot was absent and several decomposition products had formed as indicated by the "appearance" of several additional spots. The initial product was confirmed to be the desired vinylboronic acid by NMR analysis (7).

The ^1H NMR spectrum was consistent with the structure of the desired vinylboronic acid as evidenced by the presence of a triplet at δ 6.6 with a coupling constant typical of a vinyl proton's spin-spin interactions with the adjacent methylene protons. The integration value of the triplet was one. The ^{13}C NMR spectrum was even more definitive. The absence of the alkyne carbon resonances and the presence of only two alkene carbon resonances centered at δ 174.3 were especially noteworthy. Each alkene carbon resonance corresponds to the vinylic carbon attached to hydrogen of each of the two regioisomers. The two resonances of the regioisomeric alkene carbons attached to boron are not observed due to the boron's nuclear quadrupole moment effect on the nuclear relaxation time of the attached carbon. As expected, the absence of these alkene carbon resonances is indicative of their attachment to boron.

The instability of the vinylboronic acid precluded its purification by silica gel column chromatography even though it was sufficiently stable for silica gel TLC analysis. For this reason, the crude vinylboronic acid (oil) was used immediately



Scheme 29. Synthesis of an iodinated fatty acid.

after preparation and without purification. The instability of this compound is presumably responsible for the very poor yield of < 15%. The use of a different hydroborating agent, dibromoborane, had no significant effect on the outcome of the reaction or yield of the desired vinylboronic acid.

After preparation, the vinylboronic acid was immediately taken up into aqueous THF. After cooling, sodium iodide and chloramine-T were added followed by stirring for 1 hour. The organic layer was separated and chromatographed to provide a very poor yield of what was presumed to be the vinyl iodide (8). The ^1H NMR contained two triplets in the alkene region at δ 5.6 and 6.2 both of which integrated for one proton, presumably the vinyl hydrogen of both isomers. The ^{13}C NMR data was inconclusive. Hydrolysis with base followed by acidification provided what was presumed to be the free acid (9) by ^1H NMR analysis.

E. A CRITIQUE OF THE HYDROBORATION-IODINATION PROCEDURE

The hydroboration-iodination technique is an excellent method for iodinating the alkyne function of simple molecules. Unfortunately when applied to the methyl ester of stearolic acid, the method provided unstable intermediates, poor yields, and complex reaction mixtures which complicated purification. The technique also proved to be inadequate for molecules containing amines. When catecholborane was mixed with an amine, a very exothermic reaction occurred which provided a darkened and "tarry" mixture. The TLC analysis of the reaction mixture indicated the formation of multiple decomposition products. The hydroboration of the alkyne is presumably precluded by the reaction of catecholborane, a Lewis acid, with the nitrogen of the amine, a Lewis base. Since many biologically active molecules contain Lewis bases, such as amines, the

hydroboration-iodination technique was abandoned and a method was sought such that molecules with multifunctional groups could be iodinated.

CHAPTER 3. THE DEVELOPMENT OF AN ALTERNATE IODINATION PROCEDURE

A. INTRODUCTION

A.1 THE NECESSITY FOR AN ALTERNATE IODINATION PROCEDURE

The hydroboration-iodination procedure was efficient for iodinating the alkyne function of the steroid derivative and the spiroperidol analog discussed previously. However, the method proved inadequate for the iodination of the unsaturated fatty acid ester. Since the procedure was successful for other molecules containing ester functions, it is not clear why it failed for the esterified fatty acid. Nevertheless, further experimentation demonstrated the procedure's inability to iodinate molecules containing amine functional groups. The problem with the technique became obvious when catecholborane reacted with propargylamine. Upon addition, the reaction mixture became very hot and decomposition products were formed as indicated by TLC analysis. Other amines, such as N-propargyl-4-phenylpiperidine, reacted with catecholborane in a similar manner. Since amines constitute a ubiquitous and important class of pharmacologically active bio-molecules and a general iodination technique for compounds containing multifunctional groups was desired, the hydroboration-iodination technique was discarded. Therefore an alternate iodination procedure was developed.

A.2 SELECTION OF IODINE MONOCHLORIDE AS THE IODINATING REAGENT

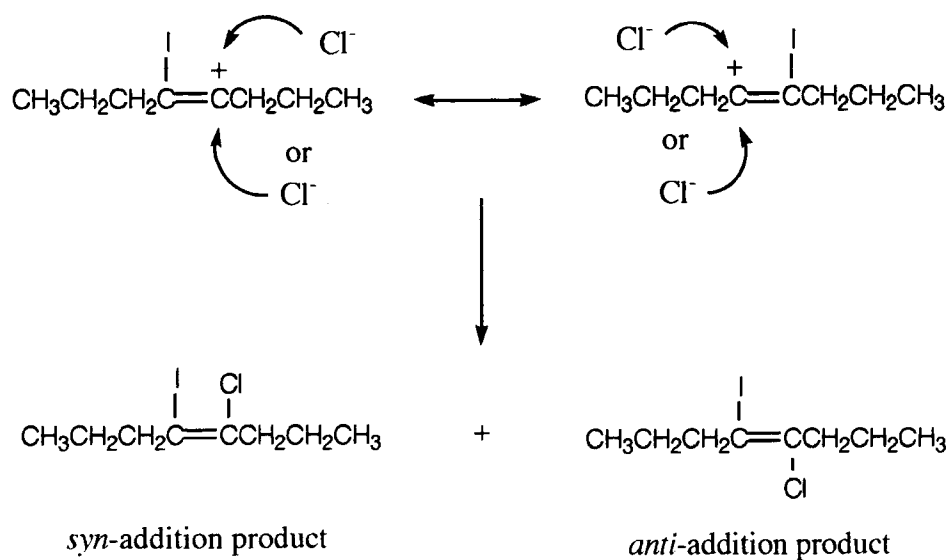
During the iodination reaction in the hydroboration-iodination procedure,

iodine monochloride is formed in situ from the reaction of sodium iodide and chloramine-T. If iodine monochloride were to add across the triple bond of alkynes containing multifunctional groups, a new route to the desired vinyl iodide would be available. Such a reaction would also eliminate the hydroboration reaction as an added step and allow direct conversion of the alkyne to the alkenyl iodide. Initially it was thought that iodination of triple bonds would be slow. This however was not the case. A short study of iodine monochloride and its additions to alkynes was initiated in order to develop a general iodination procedure which could subsequently be applied to radioiodinations.

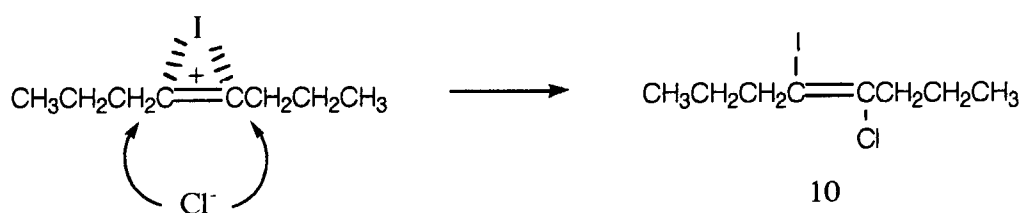
B. CHLOROIODINATION OF SIMPLE ALKYNES

When iodine monochloride reacted with 4-octyne in THF, one product was isolated by vacuum distillation, (*E*)-4-chloro-5-iodo-4-octene (10). Since the solvent was THF, which has a relatively high dielectric constant, heterolytic dissociation of the iodine monochloride was expected and the chloriodinated alkene was produced. If an open iodonium ion intermediate were formed, some of the *syn*-addition product would also have been expected (Scheme 30). The presence of only a single product was indicative of the formation of a cyclic iodonium ion intermediate which underwent nucleophilic attack by chloride (Scheme 31). Being a symmetrical alkyne, the chloride anion can attach to either carbon and form the same product. The cyclic iodonium ion explains the observation that only one product formed as well as the *anti*-stereospecificity of the reaction.

The ^{13}C spectrum revealed the presence of both iodine and chlorine atoms in the product molecule. The carbon attached to iodine resonates at δ 99.5 while the carbon attached to chlorine resonates much further down field at δ 131.7.



Scheme 30. The product possibilities of ICl addition to 4-octyne.



Scheme 31. Mechanism of ICl addition to 4-octyne.

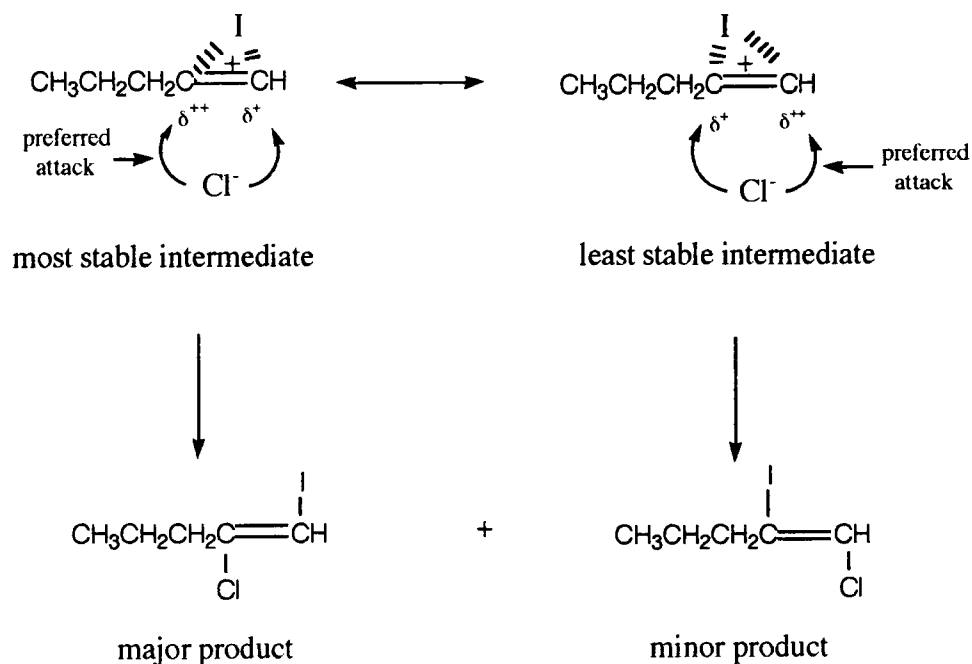
The ^1H spectrum also indicated the presence of two different methylene groups attached to carbons number four and five by the presence of two triplets centered at δ 2.3 and 2.6 corresponding to the protons attached to C-I and C-Cl, respectively.

While the reaction of iodine monochloride with symmetrical alkynes in polar solvents generally formed one product, reactions with unsymmetrical alkynes produced two products. Thus, the reaction of 1-pentyne with iodine monochloride in THF yielded two products, which could be separated and purified by fractional distillation. The products were, as expected, indicative of the cyclic iodonium transition state. The addition occurred with *anti*-stereospecificity forming the (*E*)-1-iodo-2-chloro- (11) and the (*E*)-1-chloro-2-iodo-1-pentene (12) in a ratio of two to one, respectively. This ratio is due to the unsymmetrical nature of the cyclic transition state (Scheme 32).

The intermediate with the majority of the positive charge on carbon number two is the more stable. This is due to the attenuating effect of the carbon chain attached to carbon number two on the positive charge. The least stable transition state is the one with the majority of charge on the terminal carbon since there is less attenuation of the charge. The major product, therefore, was the one in which iodine was attached to the terminal carbon atom while the minor product was the one with chlorine attached to the terminal carbon atom.

Since two isomers were formed as a result of the addition, a method for the identification of these compounds was proposed which was an extension to the original Markovnikov designation. It states that "the Markovnikov isomer is the one in which the more electrophilic part of the electrophile is attached to the carbon atom whose sum of Taft's constants (σ^*) is the more positive"⁷².

Taft's constant "sigma star" (σ^*) is a substituent constant that relates the



Scheme 32. Mechanism of ICl addition to 1-pentyne.



Figure 6. Transition states for acid and base catalysis of carboxylic acid esters.

polar effect of R relative to the standard CH₃ on the acid and base catalyzed hydrolyses of aliphatic carboxylic acid esters. Their respective transition states resemble those depicted in Figure 6. It has been postulated that any difference between substituent effects in the acid and base catalyzed reactions must be attributed to polar effects, which clearly influence the oppositely charged transition states differently. A substituent R with electron withdrawing properties would destabilize the acid catalyzed transition state while stabilizing the base catalyzed transition state. This would have the effect of decreasing the rate of the acid catalyzed reaction while increasing the rate of the base catalyzed reaction. The Taft equation is:

$$\log (k/k_0)_B - \log (k/k_0)_A = \rho^* \sigma^*$$

where k is the hydrolytic rate constant for the ester RCOOR', k₀ the value for the corresponding acetate, A and B the acid and base catalyzed reactions, respectively, and ρ* is a constant. As the electron withdrawing capacity of R increases, it follows from the equation that σ* increases. Therefore Taft's constant may be viewed as the quantitative electron withdrawing capacity of substituent R. The constant is also very useful for predicting the relative rates of reactions other than the hydrolysis of esters where the substituent is in proximity to the reaction center. An example is the addition of iodine monochloride to alkynes where Taft's constant not only gives a prediction of the relative rates, but can also be used to predict the regiochemistry of the addition when using unsymmetrical alkynes. The addition of iodine monochloride to 1-pentyne is an example. The σ*

values for the proton and alkyl substituent are 0.49 and -0.12, respectively. Therefore the major regioisomer formed is the terminal iodide.

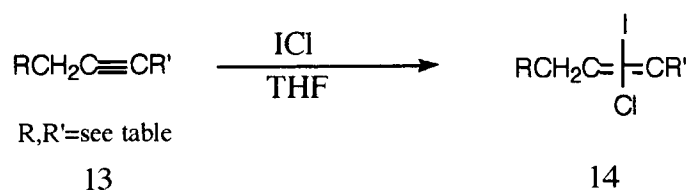
However, the rule has been unsuccessful in predicting the ratio of regioisomers formed when iodine monochloride is added to terminal alkynes bearing substituents with varying σ^* values. For example, when iodine monochloride is added to terminal (perfluoroalkyl)alkynes⁷³, phenylacetylenes⁷⁴, and ethyl 3-butynoate⁷⁵, the iodine would be predicted to attach to the internal carbon atom since it has the greater σ^* value. This is not in accord with experimental observation; these reactions give, regiospecifically, the terminal iodides. Because σ^* values are not available for all substituents described in this work, the original Markovnikov (M) and anti-Markovnikov (A) designations are used.

C. CHLOROIODINATION OF ALKYNAMINE HYDROCHLORIDES

During the development of the new ICl radioiodination procedure, several alkynamine hydrochlorides were iodinated since there was interest in radioiodinating organic amines. In each case, varying degrees of regioselectivity were observed, depending on the nature of the nitrogen substituents. As expected, each addition yielded the chloriodinated (E)-alkene in which chlorine was attached to the terminal carbon atom, as predicted by the extended Markovnikov rule. Due to the inconsistencies of this rule regarding iodine monochloride additions to alkynes, the factors controlling the regiochemistry of these reactions were investigated. Based on the experimental results, the regioselectivity and rates of these reactions were found to be dependent upon the inductive effect of the positively charged nitrogen.

Treatment of the hydrochlorides of alkynamines 13 with iodine

monochloride in anhydrous THF at reflux temperature provided the corresponding (*E*)-chloroiodoalkenes 14 in good yield (Scheme 33). However, reaction of the free base 13 provided a more complex mixture containing unreacted starting material and therefore a substantial decrease in product yield. This is presumably due, in part, to the strong coordination complex amines form with Lewis acids as described previously. While symmetrical alkynes provided



Scheme 33

only one isomer, the unsymmetrical alkynes 13a-c consistently provided two regioisomers in varying amounts. Typical reactions with substrates, yields, and regioisomeric ratios are listed in Table 1.

The hydrochloride products were converted to the free bases for purification by preparative silica gel chromatography (TLC) and were obtained as oils which were unstable and decomposed slowly. Some chloriodinated alkenes were converted to the more stable hydrochloride salt in order to obtain analytical-grade samples. The positions of iodine and chlorine in alkenes 14 were determined by ^{13}C and ^1H NMR data collected for the free amines (Table 2).

The ^1H NMR spectra of (A)-14a-c (see Appendix I) contained vinyl proton resonances downfield from those of the corresponding (M)-14a-c isomers. This is expected since a vinyl proton geminal to chlorine should resonate downfield

Table 1. Chloriodination of alkynamine hydrochlorides with iodine monochloride.

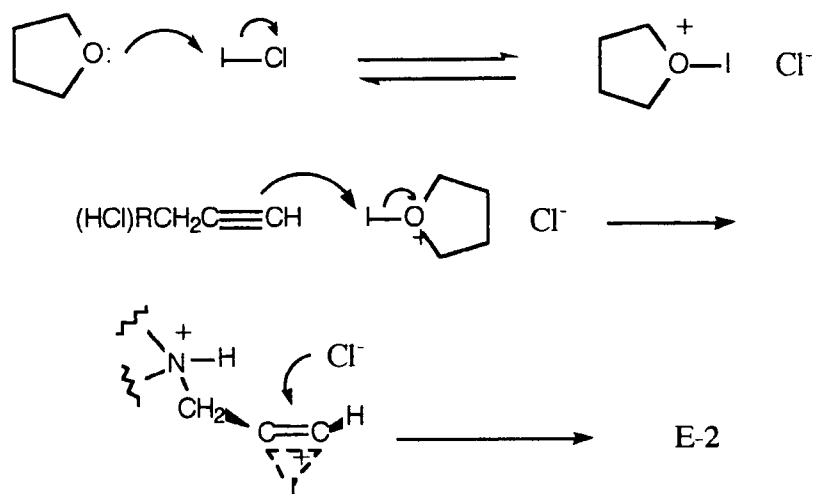
hydrochloride of 13	ICl addition product 14	
	isolated yield, %	regioisomeric ratio (A/M)
a, R = NH ₂ ; R' = H	22	4
b, R = CH ₂ N<chem>C1CCNCC1</chem>-Ph; R' = H	71	2
c, R = CH ₂ CH ₂ N<chem>C1CCNCC1</chem>-Ph; R' = H	72	0.5

Table 2. Characterization of the chloriodoalkenes.

product 14	isomer	¹ H NMR	¹³ C NMR	
			I	T
a, R = NH ₂ ; R' = H	A	6.50	107 (s)	121 (d)
	M	6.38	138 (s)	73 (d)
b, R = CH ₂ N<chem>C1CCNCC1</chem>-Ph; R' = H	A	6.49	100 (s)	120 (d)
	M	6.33	136 (s)	74 (d)
c, R = CH ₂ CH ₂ N<chem>C1CCNCC1</chem>-Ph; R' = H	A	6.46	102 (s)	119 (d)
	M	6.29	139 (s)	73 (d)

from the vinyl proton geminal to iodine⁷³⁻⁷⁵. The ^{13}C NMR data further supported the assignment of the positions of iodine and chlorine in alkenes 14a-c (see Appendix I). The ^{13}C NMR spectra of products derived from symmetrical alkynes contained resonances with chemical shifts typical of internal alkene carbon atoms bearing iodine and chlorine⁷⁶ and were consistent with those of the (A)-2 and (M)-2 isomers, respectively. Furthermore, the terminal carbons of the (M)-2 isomers exhibited a large upfield shift from the alkene region due to the heavy atom effect of iodine and appeared as doublets in the off-resonance decoupled ^{13}C NMR spectra. The (A)-2 isomers displayed doublets at 119-121 ppm, indicating the terminal carbons attachment to chlorine. The presence of *E* and *Z* isomers in the mixture was ruled out on the basis of the large difference in chemical shifts of the internal and external alkene carbons between the A and M isomers in the ^{13}C NMR spectra. The *E* assignment was based on the high anti-stereoselective nature of iodine monochloride additions to terminal alkynes and the close agreement of the ^{13}C NMR data with that reported for (*E*)-chloriodoalkenes^{77,78}.

The anti stereospecificity of these reactions supports the cyclic iodonium ion intermediate previously proposed for electrophilic additions to terminal alkynes⁷⁹⁻⁸². In the case of 13a-c, the reaction provided the same yield and regioisomeric ratio whether performed in the presence (or absence) of light or hydroquinone. These observations, therefore, can be used to exclude a radical mechanism. The ionic nature of the reaction was expected since the dielectric constant of 7.58 for THF is sufficiently high⁸³ to promote homolytic cleavage of the I-Cl bond⁸⁴. The hydrochloride substrate would enhance this process by increasing the ionic strength of the mixture. Since solvents with available lone pairs readily form complexes with iodine monochloride⁸⁵⁻⁸⁷ (Scheme 34), it is



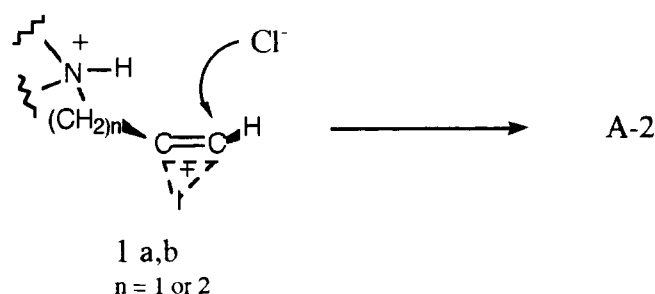
Scheme 34. Proposed mechanism of ICl addition to propargylamine hydrochlorides.

likely that a hypoiodite iodinating species is involved. The *in situ* formation of this species would account for the high heat of solvation observed when mixing iodine monochloride with THF. Mechanistically, the electrophilic attack by the iodinating species could form a bridged iodonium ion intermediate, which upon subsequent anti attack by chloride would form the (*E*)-chloroiodoalkene (Scheme 34).

The regioselectivity observed in product 14a can be explained in terms of an inductive effect. For these reactions the Markovnikov rule is obeyed since σ^* for N^+CH_2 is 1.9 and σ^* for H is 0.457⁸⁸. Accordingly, the major product contains internally bound iodine, the A isomer. To determine if the regiochemistry was fixed by inductive effects, 13b and 13c were synthesized and allowed to react with iodine monochloride. The A/M ratio was observed to be 2 and 0.5, respectively.

These results support an inductive regiocontrol mechanism. The inductive

effect of the positively charged nitrogen results in unsymmetrical bonding of iodine in the intermediate cyclic iodonium ion. The iodine bonding to the internal carbon would be greater and would place the majority of the positive charge on the terminal carbon, which is an energetically favored situation since the positively charged centers are maximally separated as shown in Scheme 35. Since the positive charge accumulates on the terminal carbon atom, it becomes more susceptible to nucleophilic attack by chloride, resulting in preferential formation of the A isomer. This preference is maximal for cyclic iodonium ion 1a as exemplified by an A/M ratio of 4.

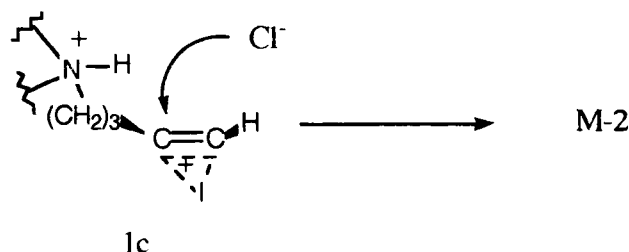


Scheme 35. Addition of ICl to form the A regioisomer.

In contrast, when the nitrogen is substituted, such as in 13b, the inductive effect of the positive charge on the iodonium ion intermediate is somewhat attenuated, resulting in increased bonding between the external carbon atom and iodine. This leads to increased M isomer formation, decreasing the A/M ratio to 2.

Increasing the distance between the reaction center and the positively

charged nitrogen increases M-isomer formation by further increasing iodine bonding to the terminal carbon atom. In progressing from 13b to 13c, the majority of charge moves from the external to the internal carbon atom, resulting in an A/M ratio change from 2 to 0.5, respectively (Scheme 36).



Scheme 36. Addition of ICl to form the M regioisomer.

In accordance with this progression, the absence of an inductive effect, as in the addition of ICl to 1-octyne, results in regiospecific formation of the M isomer^{89,90}. When these reactions were performed at room temperature, the dependency of the rate of reaction on the inductive effect was reflected in the product yield. All reactions were performed under conditions identical to those described earlier with the exception that room temperature was used. The results are listed in Table 3.

The rate of product formation of 14c > 14b > 14a is in the expected order of the relative stabilities of the iodonium ion intermediates. This is expected since the nitrogen of 13a has no substituents to attenuate the positive charge as is the case for 13b and 13c. The destabilization of the transition state results in decreased product formation as indicated by a yield of only 9% for 13a, whereas

the nitrogen-substituted compounds 13b and 13c give higher yields. Stability is added to the transition state of 13b and 13c by increasing the distance between

Table 3. Chloriodination of alkynamine hydrochlorides with iodine monochloride at room temperature.

hydrochloride of 13	ICl addition product 14		
	isolated yield, %	regioisomeric ratio (A/M)	relative rate
a, R = NH ₂ ; R' = H	9	4.5	1
b, R = CH ₂ N  Ph; R' = H	38	1.8	4.2
c, R = CH ₂ CH ₂ N  Ph; R' = H	50	0.5	5.6

the positive nitrogen and the iodonium ion, which results in significantly higher yields. This order of reactivity also suggests that formation of the iodonium ion is the rate-determining step since factors that decrease the inductive effect result in an increased rate via increased iodonium ion stability. The regiochemistry observed at room temperature is not significantly different from that obtained at elevated temperatures.

D. THE IODINE MONOCHLORIDE ADDITION REACTION AS AN ALTERNATE METHOD FOR IODINATION.

The addition of ICl to alkynes occurred readily and efficiently even in the presence of amines protected as the hydrochloride. The experiments

demonstrated the inability of ICl to react cleanly with the free base amines. This was, presumably, due to the reaction or complexation of ICl with the lone pair of electrons on the amine nitrogen. However, this was not a problem since any amine can be easily converted to the corresponding hydrochloride and then chloriodinated. The new procedure was used for the iodination of the remaining compounds. Since the reaction was successful for molecules containing amines, no problems were expected with ICl reacting with other functional groups because amines are the strongest organic Lewis bases.

CHAPTER 4. SYNTHESIS OF IODINATED AGENTS USING THE NEW IODINE MONOCHLORIDE ADDITION PROCEDURE

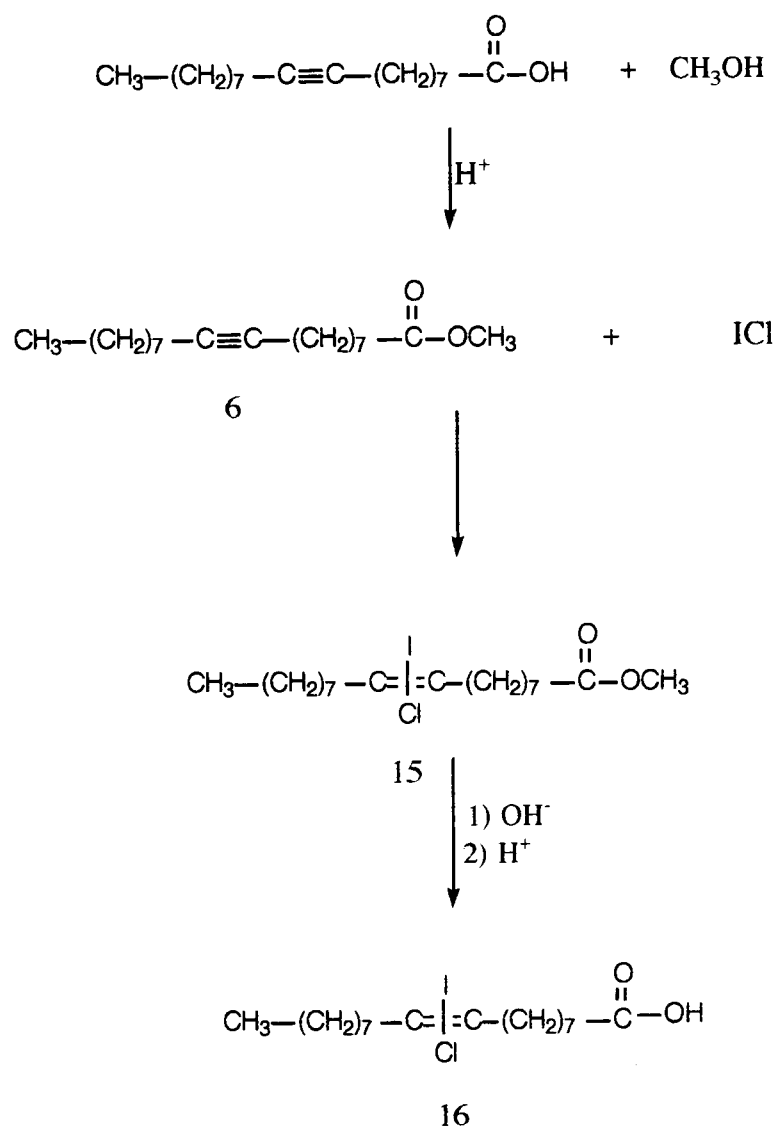
A. SYNTHESIS OF IODINATED MYOCARDIAL IMAGING AGENTS

A.1 SYNTHESIS OF IODINATED STEAROLIC ACID

The importance of fatty acids as myocardial imaging agents was discussed in Chapter 2, section E. Since the iodine monochloride addition to alkynes containing amines was shown to be successful, it was decided to apply the method to the same fatty acid designed in Chapter 2, section D, stearolic acid.

When iodine monochloride reacted with stearolic acid in a variety of solvents, e.g. THF, petroleum ether, and dioxane, the reaction mixture turned dark and as many as six products were formed. However, when methyl stearolate was reacted with ICl in THF, one major product formed (15) as indicated by TLC analysis (Scheme 37). This product formed by the *anti*-addition of iodine monochloride⁹¹. Since the molecule is not symmetrical, the product actually consisted of two geometrical isomers which were impossible to separate by ordinary purification techniques.

The ^{13}C NMR spectrum of the product mixture revealed two resonances at δ 99.0 and 99.4 which were consistent with alkene carbons attached to iodine. These resonances corresponded to C-9 and C-10 of the two geometrical isomers. The C-9 and C-10 of the two isomers attached to chlorine resonated at δ 131.4 and 131.7 consistent with alkene carbons attached to chlorine. The methylene carbons, C-7 and C-11, adjacent to the alkene carbons also demonstrated a downfield shift. The partially superimposed triplets, present in the ^1H NMR spectrum of the starting material, were resolved and shifted down-field to



Scheme 37. Synthesis of a chloriodinated fatty acid.

δ 2.2 and 2.5 after the addition of iodine monochloride. The remainder of the spectrum was consistent with the expected products.

The corresponding carboxylic acid (16) was obtained by base hydrolysis using aqueous methanolic sodium hydroxide followed by acidification. The product was purified by silica gel column chromatography using 1% methanol in chloroform as eluant. Both ^{13}C and ^1H NMR spectra were consistent with the hydrolysis in that the methyl carbon and the methyl singlet, respectively, were absent. Additionally, the carboxylic acid proton resonance was present as a broad peak centered at δ 9.1.

The addition of ICl to the alkyne function of methyl stearolate provided a successful method for a straightforward iodination and a method which lead to the desired alkenyl iodide. It also eliminated the hydroboration reaction which was required for iodination of the cholesterol and the spiroperidol derivatives discussed in Chapter 2. Since the method was successful for iodinating methyl stearolate, it was also used to iodinate a stearolic acid ester analog, which upon hydrolysis gave another fatty acid derivative designed for myocardial imaging.

A.2 THE CHEMICAL DESIGN AND SYNTHESIS OF IODINATED α -METHYLSTEAROLIC ACID

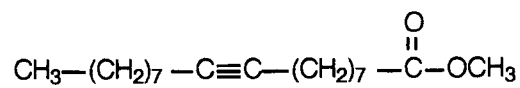
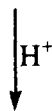
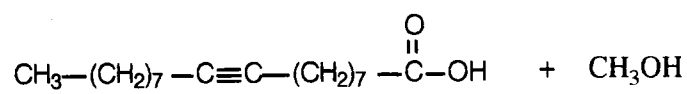
As outlined in Scheme 28, Chapter 2, page 50, fatty acids are taken into the heart tissue and enter the metabolic pathway. Within this oxidative pathway are mechanisms which involve the abstraction of both of the α -protons in the initial metabolic steps. If these steps were inhibited, by replacing at least one of the α -hydrogens with a methyl group, it follows that the fatty acid would accumulate in the metabolic pathway within the heart. When the radioiodinated derivative of the fatty acid was used, radioactivity would then also accumulate in

the heart enhancing the imaging process. Therefore the α -methyl- derivative of stearolic acid was chosen as a candidate for the iodination procedure.

The addition of ICl to the alkyne function of unprotected carboxylic acids proved to be an inefficient reaction. This was observed when ICl was reacted with stearolic acid. Therefore the methyl ester of stearolic acid was synthesized. The esterification was necessary since the carboxylic acid group must be protected prior to the methylation reaction. Methyl stearolate was synthesized as described in Chapter 2; then it was α -methylated by reacting the corresponding enolate with methyl iodide (Scheme 38). The α -methylation (17) was confirmed by the ^1H NMR spectroscopy which resulted in a spectrum similar to methyl stearolate with the exception of a doublet at δ 1.10 corresponding to the methyl group coupled to the remaining α -proton. The ^{13}C NMR spectrum included an additional peak corresponding to the α -methyl group.

When α -methyl stearolate (17) reacted with ICl in THF, the corresponding addition products (18) were formed in good yield, paralleling the results obtained using methyl stearolate. As with the ICl addition to methyl stearolate, the ^{13}C NMR spectrum of the α -methyl derivatives revealed the presence of two isomers, each containing iodine and chlorine attached to sp^2 -hybridized carbon atoms. The ^1H NMR spectrum also supported the presence of isomers due to the downfield shifted methylene groups adjacent to the alkene carbons.

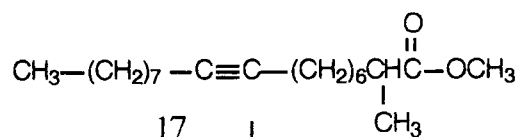
In order to regenerate the carboxylic acids (19), the addition products reacted with a methanolic solution of sodium hydroxide followed by acidification. All spectra indicated the presence of the free carboxylic acid while retaining the iodine and chlorine from the previous reaction.



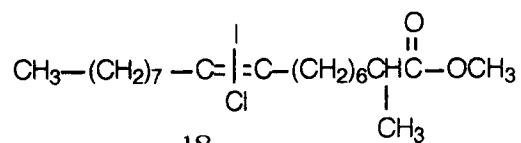
6

1) BuLi

2) CH₃I

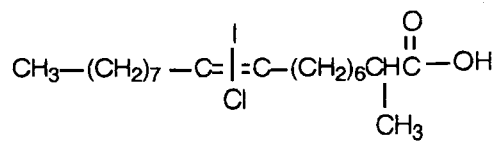


17



18

1) OH⁻
2) H₃O⁺



19

Scheme 38. Chloriodination of an α -methyl branched fatty acid ester.

B. THE INITIAL ASSESSMENT OF THE IODINE MONOCHLORIDE ADDITION PROCEDURE

The addition of ICl to the alkyne function of the carboxylic acid esters proceeded efficiently, in good yield, and provided the desired biologically stable vinyl iodides. The vinyl iodides are also chemically stable since they were unaffected by the refluxing base which was necessary to regenerate the carboxylic acids. Though the study demonstrated that ICl reacts with the free carboxylic acid to produce multiple products (Chapter 3), this problem was solved by protecting the carboxylic acid as an ester prior to the chloriodination. The ICl addition procedure was used for the iodination of the remaining agents.

C. SYNTHESIS OF IODINATED PHARMACOLOGICALLY ACTIVE CNS AGENTS

C.1 INTRODUCTION

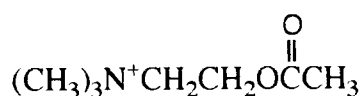
Drugs which exert their primary effect on the central nervous system (CNS) constitute the most widely used group of pharmacologically active agents. Centrally acting drugs are responsible for a broad spectrum of effects that are indispensable to therapeutics. Highly selective effects on symptoms such as pain and fever are very familiar, and debilitating neurological syndromes as diverse in etiology as epilepsy and Parkinsonism can be effectively controlled by CNS drug therapy.

Current knowledge of the CNS is incomplete. It is, therefore, necessary to devise methodology which will give insight into the processes which govern CNS functioning. Radiolabelling can provide a powerful tool for studying these processes by elucidating a metabolic pathway or an anatomical structure. Since iodine monochloride addition to alkynes proved to be an effective method of

iodinating amines, several CNS active drugs containing amine functionalities or their derivatives were chosen as candidates for iodination using the new ICl addition procedure.

C.2 THE DESIGN AND SYNTHESIS OF THE IODINATED CHOLINOMIMETIC AGENTS TREMORINE AND A TREMORINE ANALOG

Information is transmitted through the body by means of the nervous system which contains nerve cells called neurons. Since the neurons are not physically connected, an impulse is transmitted from one to the other by chemical transmitters which must pass through a space called the synaptic cleft. Acetylcholine (Figure 7) is one of these conductors and any compound with similar pharmacological action is termed parasympathomimetic or cholinomimetic.



Acetylcholine

Figure 7. The natural chemical neurotransmitter acetylcholine.

One such related compound is oxotremorine (Figure 8). It is a synthetic drug that has proven extremely valuable as an investigative tool in the search for anti-Parkinsonism drugs and in other studies of the pharmacology of the central nervous system. Tremorine is another example of a centrally acting cholinomimetic which is pharmacologically and structurally similar to

oxotremorine. Radioiodinated tremorine could possibly be used to image or elucidate features of the acetylcholinergic nervous system. Therefore, tremorine was chosen as the substrate for iodine monochloride addition.

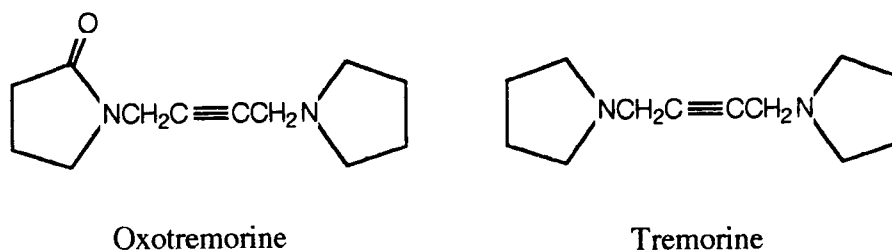
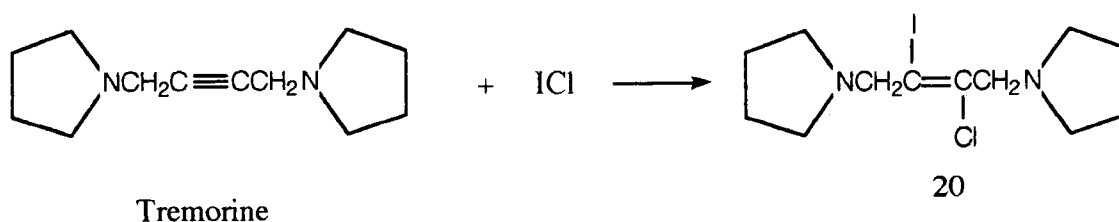


Figure 8. The cholinomimetic agents oxotremorine and tremorine.

Since tremorine already contains an alkyne function, it was used without chemical modification. Tremorine was converted to the (di)hydrochloride salt followed by reaction with iodine monochloride in THF to produce only one product, as indicated by TLC (Scheme 39). The starting material was completely consumed and the product was converted to the free amine which made purification and identification procedures more efficient.



Scheme 39. The addition of ICl to tremorine.

The resulting oil was confirmed to be the iodine monochloride addition product, 20, by ^1H and ^{13}C NMR spectroscopy. The spectra included a down field

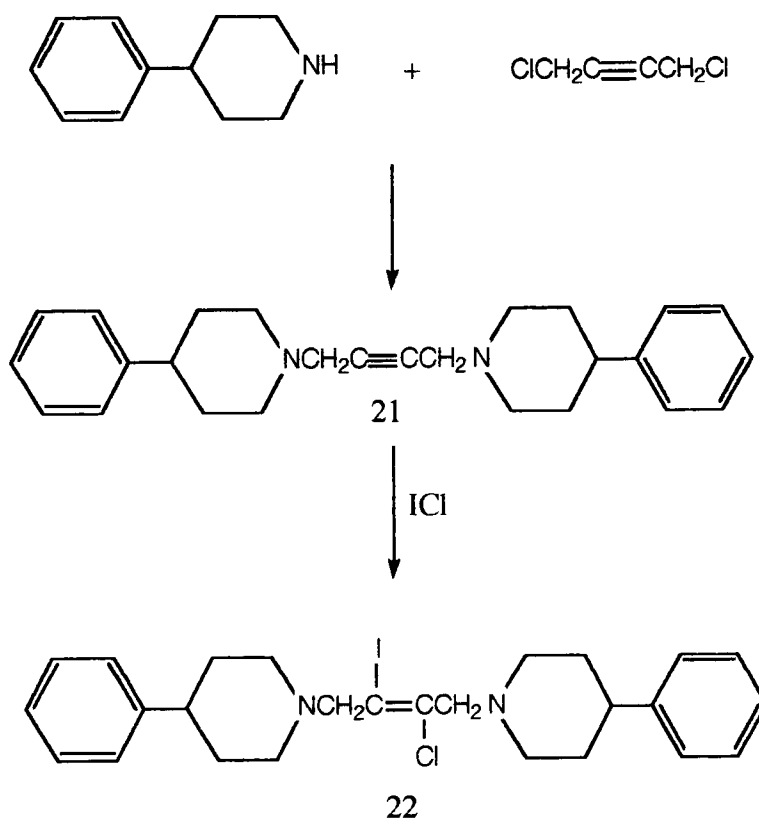
shift for the methylene hydrogens and the appearance of singlets at δ 101 (C-I) and 131 (C-Cl), respectively.

An analog of tremorine was synthesized in which the pyrrolidine rings were replaced with the 4-phenylpiperidine ring system. The 4-phenylpiperidine ring system is a very important pharmacophore found in many centrally acting compounds. It also increases the lipophilicity of the molecule, which presumably results in greater CNS solubility. This is because the CNS is composed primarily of fat-containing tissue. Therefore this analog was synthesized and chloriodinated.

The 4-phenylpiperidine derivative of tremorine was synthesized by reaction of excess amine, four equivalents, with 1,4-dichloro-2-butyne (Scheme 40). Using an excess of this magnitude provided much better product yields than did adding triethylamine as a base to absorb the acid produced from the reaction. The presence of one product was indicated by TLC analysis. After basification, the product was extracted into ether and purified by silica gel column chromatography to provide 1,4-di-(4-phenylpiperidinyl)-2-butyne (21) as fine white crystals.

Since 4-phenylpiperidine is relatively expensive, as compared to other basic amines, triethylamine and pyridine for example, it was easily recovered from the reaction mixture during the purification procedure, column chromatography. Because the product had a much higher R_f value than the starting amine, it was eluted from the column first using methanol/chloroform as the eluant. After elution of the product was complete, the alcohol content of the eluant was increased and the starting material/base, 4-phenylpiperidine, rapidly eluted from the column. Evaporation of the solvent resulted in 100% recovery of the excess amine.

The ^1H NMR spectrum confirmed the presence of two 4-phenylpiperidine



Scheme 40. The synthesis of a chloriodinated tremorine analog.

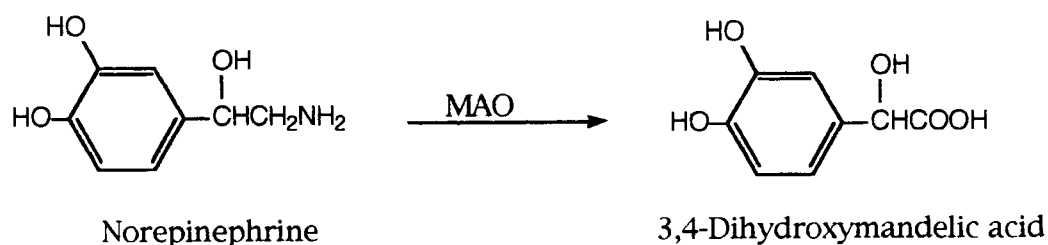
ring systems in the molecule by indicating a large singlet at δ 7.22 which integrated for ten protons. The ^{13}C NMR spectrum indicated the symmetrical nature of the molecule by exhibiting one singlet for the sp hybridized carbon atoms.

After conversion to the (di)hydrochloride, 1,4-di-(4-phenylpiperidiny)-2-butyne reacted with iodine monochloride, and the product was isolated by column chromatography to provide the addition product 22 as white crystals. This was unusual as most of the iodine monochloride addition products previously synthesized were oils when converted to the free amines.

The ^1H and ^{13}C NMR spectra (see Appendix I) were very similar to the iodine monochloride addition product derived from tremorine with exception of the phenyl groups. The methylene hydrogens' peaks in the ^1H NMR spectrum were separated and shifted downfield in comparison to the starting material, and the carbons attached to iodine and chlorine in the ^{13}C NMR spectrum exhibited their characteristic peaks.

C.3 SYNTHESIS OF THE IODINATED MONOAMINE OXIDASE INHIBITOR PARGYLINE

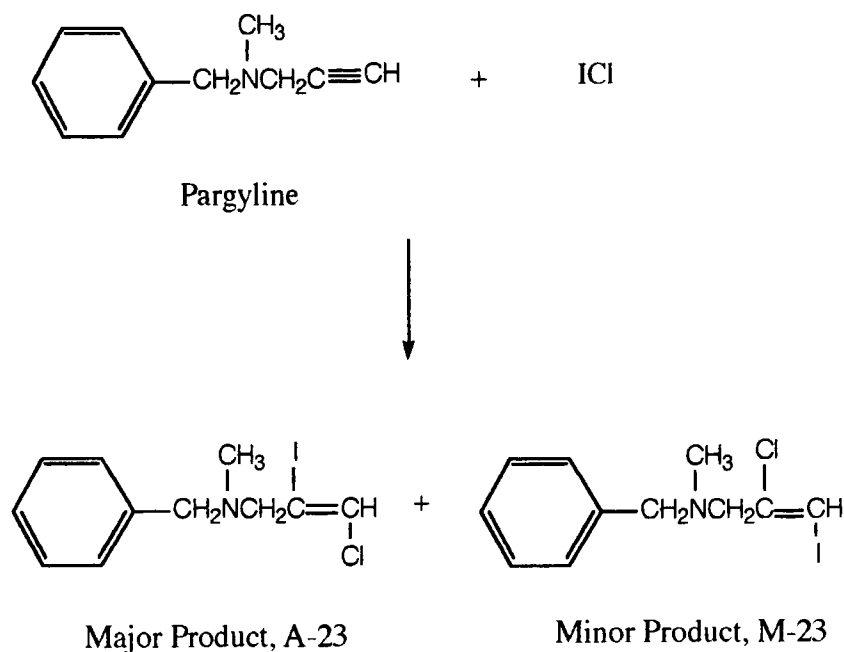
Pargyline is a monoamine oxidase (MAO) inhibitor. Monoamine oxidase is involved in the metabolism of certain primary endogenous amines in the body, as shown in Scheme 41.



Scheme 41. Synaptic inactivation of norepinephrine.

When the MAO enzyme is blocked, amines such as norepinephrine can accumulate in tissues. Excessive accumulation of norepinephrine interferes with the transmission of nerve impulses across the ganglia. Suicide enzyme inactivators, such as pargyline, are interesting enzymatically and pharmacologically because they bind irreversibly to target enzymes. Several propynylamines are suicide inactivators of MAO⁹²⁻⁹⁵, and the ³H and ¹⁴C labeled analogs have been synthesized for enzyme studies^{96,97}. The ¹¹C pargyline derivative has also been synthesized and the results indicate that the compound reflects the MAO concentration in the brain, heart, and lungs. However, in spite of pargyline's high specificity for MAO, ¹¹C-pargyline displayed mainly nonspecific binding, which necessitates the construction of compounds which are more specific for the enzyme⁹⁸. If more specific agents can be discovered and subsequently labeled, much information about MAO and nerve function could be gained. Therefore pargyline was selected as a substrate for iodine monochloride addition, Scheme 42.

As with tremorine and 1,4-di-(4-phenylpiperidinyl)-2-butyne, pargyline contains an alkyne function and was chloriodinated directly without modification. The hydrochloride salt of pargyline was used in the reaction with iodine monochloride. The product was purified by silica gel column chromatography to provide an excellent yield, 97%, of a mixture of both geometrical isomers, A-23 and M-23. The excellent yield was surprising due to the observation that pargyline hydrochloride was not completely soluble in THF. Because the isomers had similar R_f values, they were resolved by thin layer chromatography using a silica gel preparatory plate. The geometrical isomers were visualized by an ultraviolet lamp which delineated each compound facilitating removal of the silica gel from the plate prior to elution.

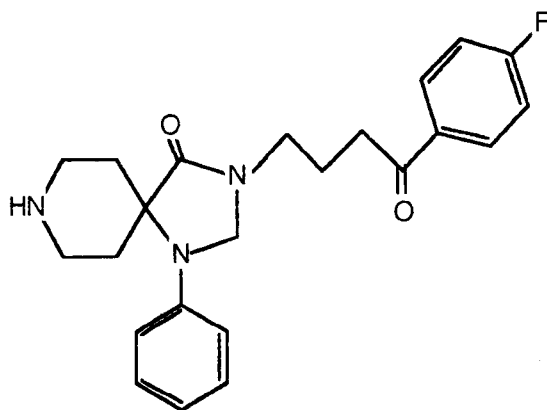


Scheme 42. The chloriodination of pargyline.

The ^1H NMR of the purified mixture of isomers was instrumental in determining the ratio of the terminal chloride isomer (A) to the terminal iodide isomer (M). The ratio of the alkenyl protons of the terminal chloride and terminal iodide isomers was four to one, respectively. The regioselectivity of the reaction can be explained by the asymmetric cyclic iodonium ion intermediate, as described earlier. This increases the rate of attack of chloride upon the terminal carbon thereby producing the A-isomer as the major product. Superimposition of the individual spectra produced a summation which was identical to the spectrum of the isomeric mixture. Both ^{13}C and ^1H NMR spectra (see Appendix I) were consistent with the addition of ICl to an unsymmetrical alkynamine hydrochloride as described in Chapter 3.

C.4 THE DESIGN AND SYNTHESIS OF IODINATED POTENTIAL DOPAMINE RECEPTOR ACTIVE SPIROPERIDOL ANALOGS

The importance of spiroperidol and radiolabeled spiroperidol analogs as potential dopamine receptor imaging agents was discussed in Chapter 2, section C. A pharmacophore is a part of a pharmacologically active molecule which is necessary for drug action. The 1-phenyl-1,3,8-triazaspirodecanone moiety of spiroperidol is a pharmacophore. Therefore, each derivative contains this group. One interesting design contains both butyrophenone and 1-phenyl-1,3,8-triazaspirodecanone groups connected via the lactam nitrogen instead of the piperidine nitrogen as in spiroperidol. This analog was termed pseudospiroperidol or pseudospiperone, Figure 9. The pseudospiperone design allows alkynylation of the piperidine nitrogen so subsequent ICl addition can be performed.

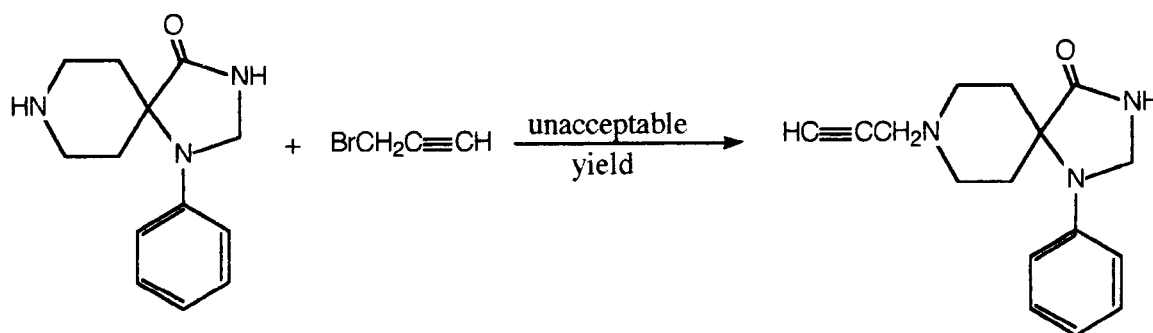


Pseudospiperone

Figure 9. In pseudospiperone, the lactam nitrogen is substituted instead of the piperidine nitrogen.

The pharmacophore of spiroperidol, 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, is commercially available and was utilized as the starting material. The

initial synthetic scheme involved propargylation of the starting material by stirring the spiro compound with an excess of propargyl bromide (Scheme 43). The reaction was extremely slow and inefficient regardless of the solvent or the base used to absorb liberated acid produced by the reaction. In each case, as much as 90% of the reaction mixture was composed of starting material.

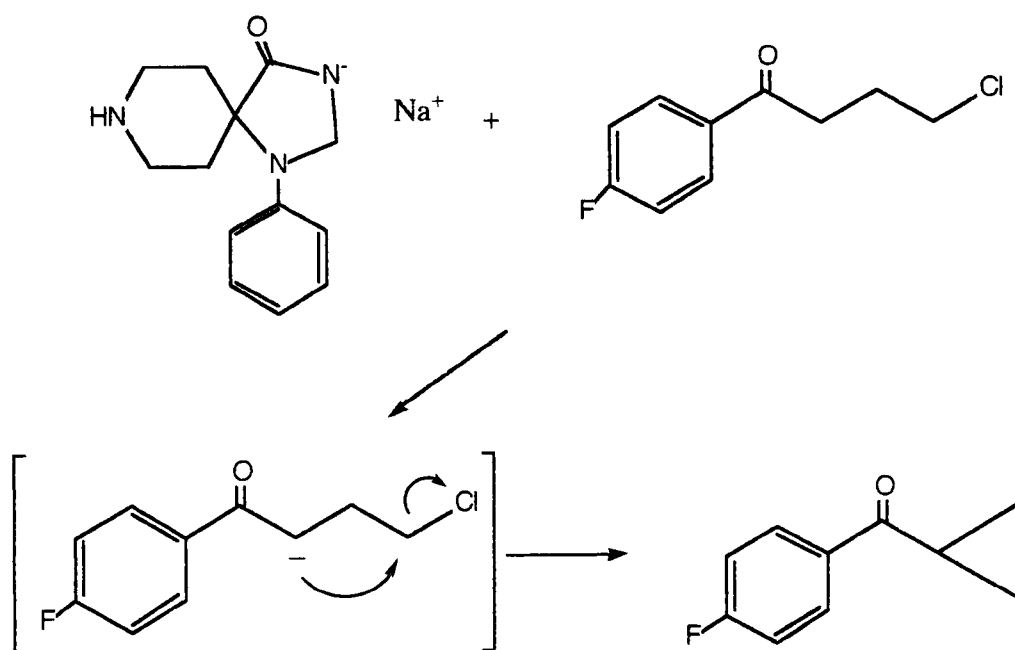


Scheme 43. Propargylation of the piperidine ring provided poor yields before alkylation.

The reason for the unacceptable yield is not clear since the alkynylation of the piperidine ring occurred readily in previous experiments. Nevertheless, propargylation was replaced as the initial step of the reaction scheme by alkylation.

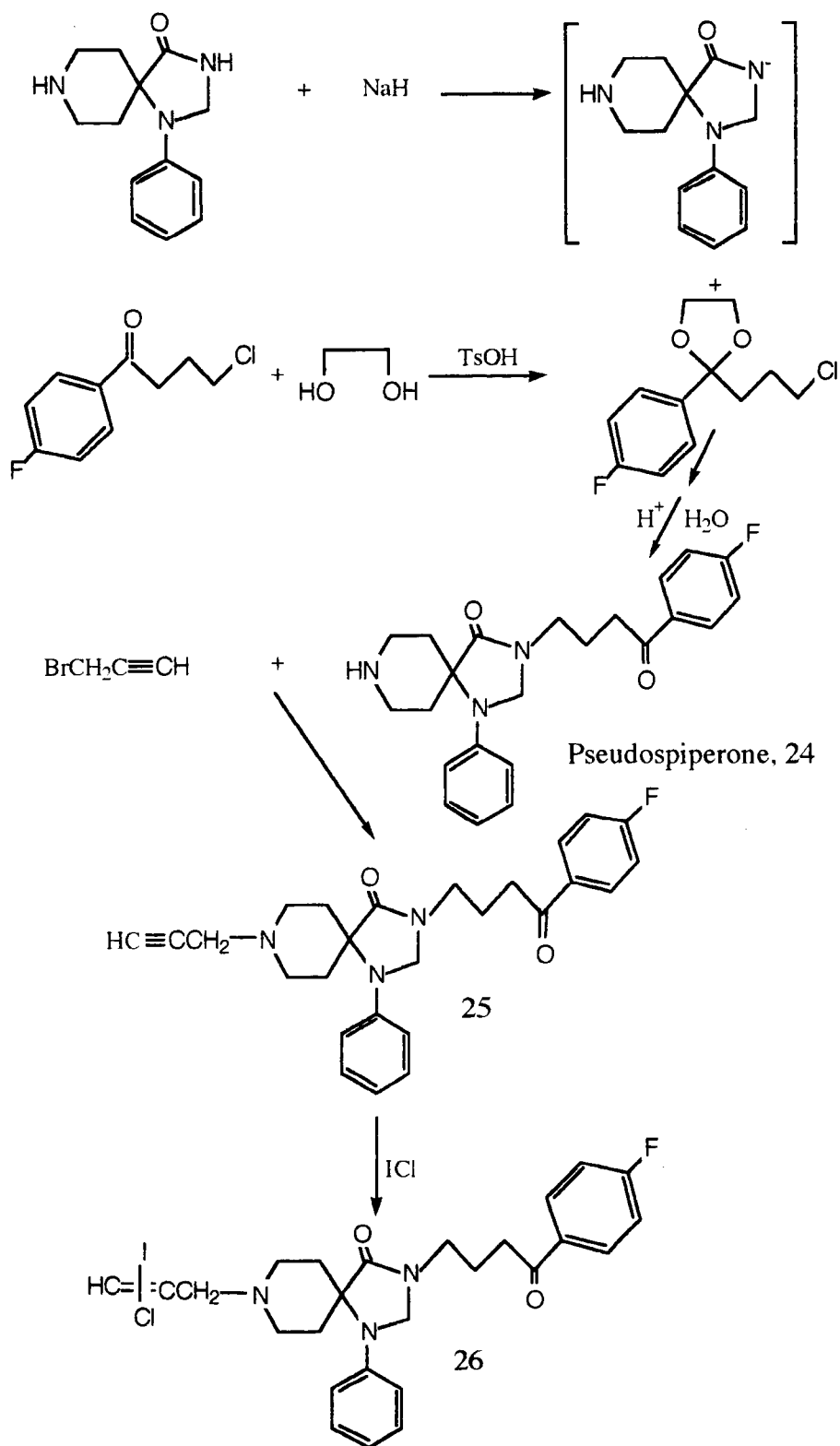
The spiro compound was dissolved in a suspension of DMF containing an oil dispersion of sodium hydride. After the evolution of hydrogen ceased, the alkylating agent, 4-chloro-4'-fluorobutyrophenone, was added and the mixture was stirred overnight. As expected, a salt precipitate was observed on the bottom of the reaction container, indicating a reaction had taken place. After quenching with water, thin layer chromatography (TLC) analysis of the reaction mixture indicated complete transformation of the alkylating agent into a higher R_f product while the spiro compound remained entirely unchanged. Though the product of the reaction was not isolated and characterized, it was assumed that 4-chloro-4'-

fluorobutyrophenone was converted to the corresponding enolate followed by cyclization to form cyclopropyl-p-fluorophenyl ketone, Scheme 44. If the formation of the enolate with subsequent cyclization was occurring instead of alkylation, the problem could be avoided by converting the carbonyl group to a



Scheme 44. Attempted alkylation of the lactam nitrogen resulted in intramolecular cyclization of the alkylating agent.

ketal. Therefore, the alkylating agent, 4-chloro-4'-fluorobutyrophenone, was refluxed in benzene containing ethylene glycol and a catalytic amount of p-toluenesulfonic acid. This provided the ethylene ketal in excellent yield, which was then used as the alkylating agent. The TLC analysis of the reaction mixture, after quenching, indicated consumption of the spiro compound as well as the ketal. The crude product was isolated and refluxed with aqueous methanol containing hydrochloric acid. This regenerated the carbonyl group and provided



Scheme 45. Synthesis of chloriodinated N-propargylpseudospiperone.

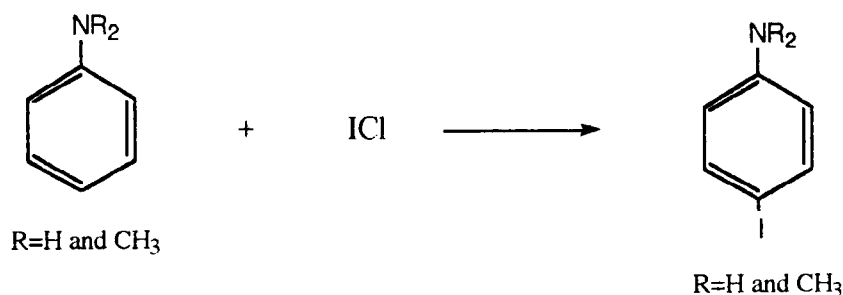
pseudospiperone (24) in greater than 50% yield. The identification of pseudospiperone was accomplished through the use of ^1H and ^{13}C NMR spectra and TLC analysis. The ^1H NMR spectrum was nearly indistinguishable from that of spiroperidol except for the presence of the amine proton resonance which was superimposed on one of the piperidine ring proton resonances at δ 1.6. In order to confirm the nature of this additional peak, D_2O was added to the NMR tube to allow proton exchange with the NH deuterium. The resulting ^1H NMR spectrum lacked the additional NH peak of the original spectrum. Therefore the site of alkylation was the lactam nitrogen. The ^{13}C NMR spectrum was consistent with the assigned structure for pseudospiperone and included two carbonyl and two aromatic ring resonances. The aromatic ring, which contained fluorine, complicated the aromatic region of the spectrum and the individual peak assignments. This is due to the fact that fluorine has a nuclear spin of $1/2$ and splits every carbon in the aromatic ring with coupling constants ranging from 10 to 256 hertz. Since both ^1H and ^{13}C NMR spectra resembled those of spiroperidol, additional evidence from TLC analysis was necessary. When spiroperidol was spotted next to pseudospiperone (and the plate developed), the difference in the two compounds was obvious. Spiroperidol had an R_f value of 0.85 while pseudospiperone had a value of 0.17 in the methanol/chloroform solvent system.

The propargylation of the piperidine ring nitrogen was accomplished by mixing pseudospiperone with propargyl bromide at a slightly elevated temperature. This provided N-propargylpseudospiperone (25). A notable feature of the propargylation reaction was the fact that the yield was poor, 12%, if 1-phenyl-1,3,8-triazaspirodecanone was used as the starting material. However, if the alkylation of the lactam nitrogen with the butyrophenone moiety was performed first, the yield of the subsequent propargylation product dramatically

increased to 74%. The reason for the difference in yields is not clear. The presence of the propargyl group on the piperidine ring of pseudospiperone was best demonstrated by the ^1H NMR spectrum, which included a doublet and triplet with a small coupling constant characteristic of the propyne system.

After conversion to the hydrochloride, N-propargylpseudospiperone reacted with ICl in THF. After refluxing the reaction mixture for 1.5 hours, the ICl addition product (26) was obtained in good yield. Several TLC experiments with various solvent systems led to the conclusion that the regioisomers were essentially inseparable. Therefore, the regioisomers were analyzed as a mixture. The ^{13}C NMR spectrum displayed the CI peaks at δ 79.8 and 101.5, and the CCl peaks at δ 121.5 and 133.0 thereby confirming that the addition of ICl gave two separate regioisomers. The A/M ratio of regioisomers was difficult to determine because the integration of the vinyl H peaks overlapped the aromatic ring integrations. Nevertheless, the remainder of the ^1H NMR spectrum was consistent with the assigned structures for the regioisomers.

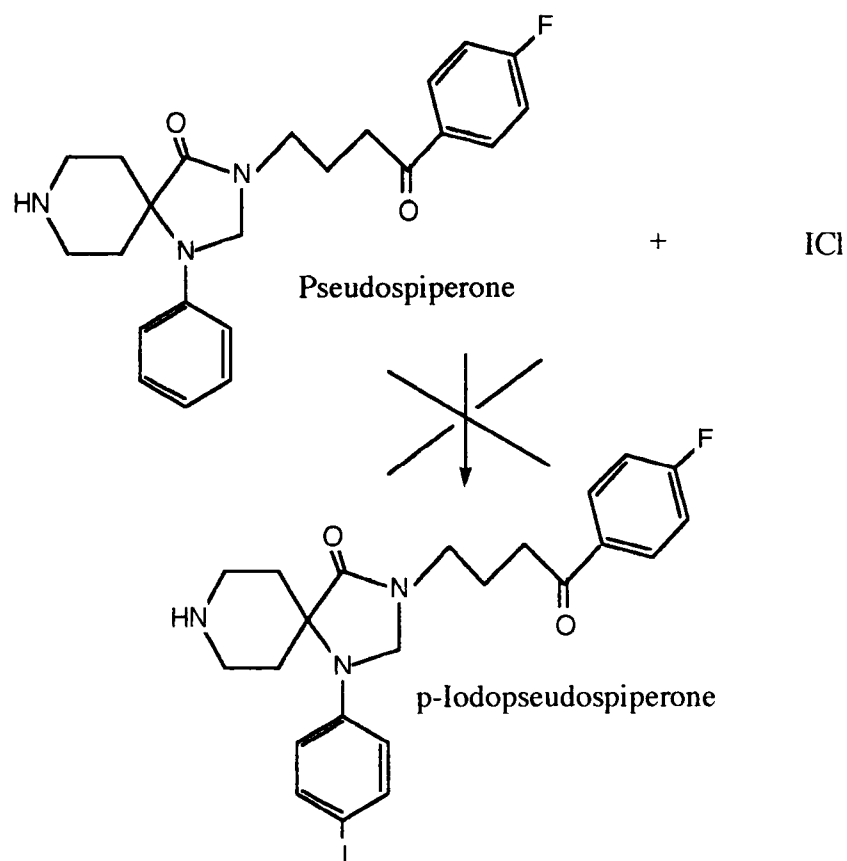
An interesting aspect of the iodine monochloride addition was the fact that the aromatic ring was not iodinated. Iodine monochloride will not iodinate aromatic rings unless they are activated. For example, when ICl reacted with aniline, N-methylaniline, or N,N-dimethylaniline, the mono-substituted p-iodo-aniline derivatives were readily produced in each case (Scheme 46). The formation of para-, mono-substitution products was confirmed by ^1H NMR. The aromatic protons resonated and integrated as expected; a distorted and finely split doublet generated by an AA'XX' system, in the aromatic region integrated for four protons. Since these reactions proceeded readily, it was not clear why the aromatic ring of N-propargylpseudospiperone was not iodinating. In fact, when pseudospiperone was refluxed in a solution of ICl in THF for an extended period of



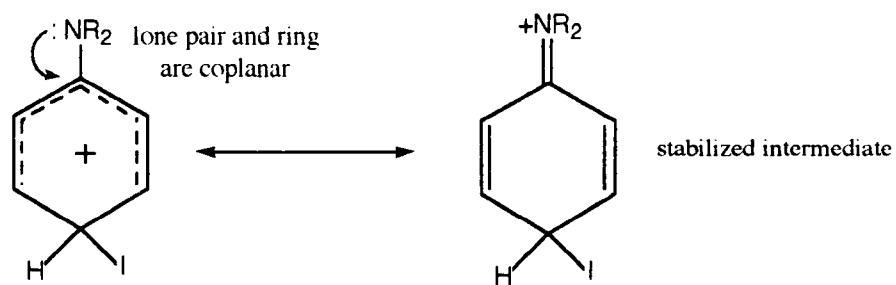
Scheme 46. Iodination of anilines with ICl.

time (Scheme 47), only the starting material could be isolated from the reaction mixture. An explanation for the unreactivity of the aromatic ring in the triazaspirodecanone moiety was made clear when a space model of pseudospiperone was constructed. In order for the aromatic ring to be activated, as in the case of aniline and the N-substituted anilines, the lone pair of electrons on the sp^2 -hybridized nitrogen must lie in the same plane as the π -system of the aromatic ring (Scheme 48). This is the only way the electrons of nitrogen can delocalize the developing positive charge of the transition state in the substitution reaction. The space model of pseudospiperone reveals that the aromatic ring of the triazaspirodecanone moiety is twisted out of the plane of the nitrogen's lone pair of electrons due to steric repulsion from the piperidine ring (Figure 10). Therefore the ring is no more activated towards substitution than a benzene ring, which is unreactive with ICl under ordinary reaction conditions.

It has been determined that the ring nitrogen of pharmacophores containing a piperidine ring system is necessary for pharmacological activity. The nitrogen of the piperidine ring presumably coordinates with Lewis acid sites of the receptor, and the ring carbons provide appropriate spacing between the nitrogen and other reactive sites of the molecule. The product obtained from N-



Scheme 47. Attempted iodination of pseudospiperone with ICl.



Scheme 48. Stabilized reaction intermediate for electrophilic iodination reaction.

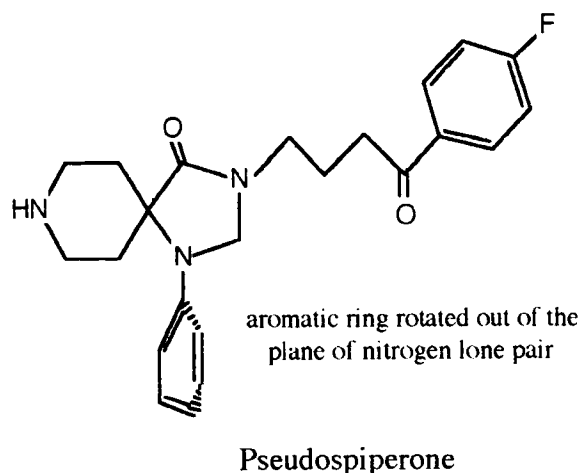
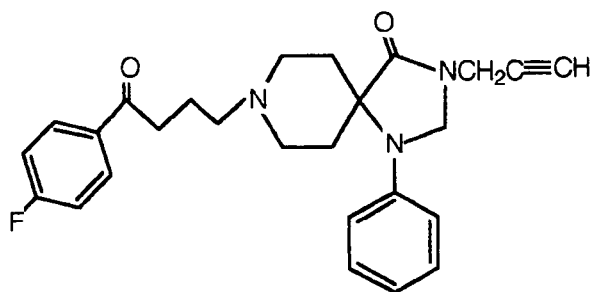


Figure 10. Position of aromatic ring in pseudospiperone.

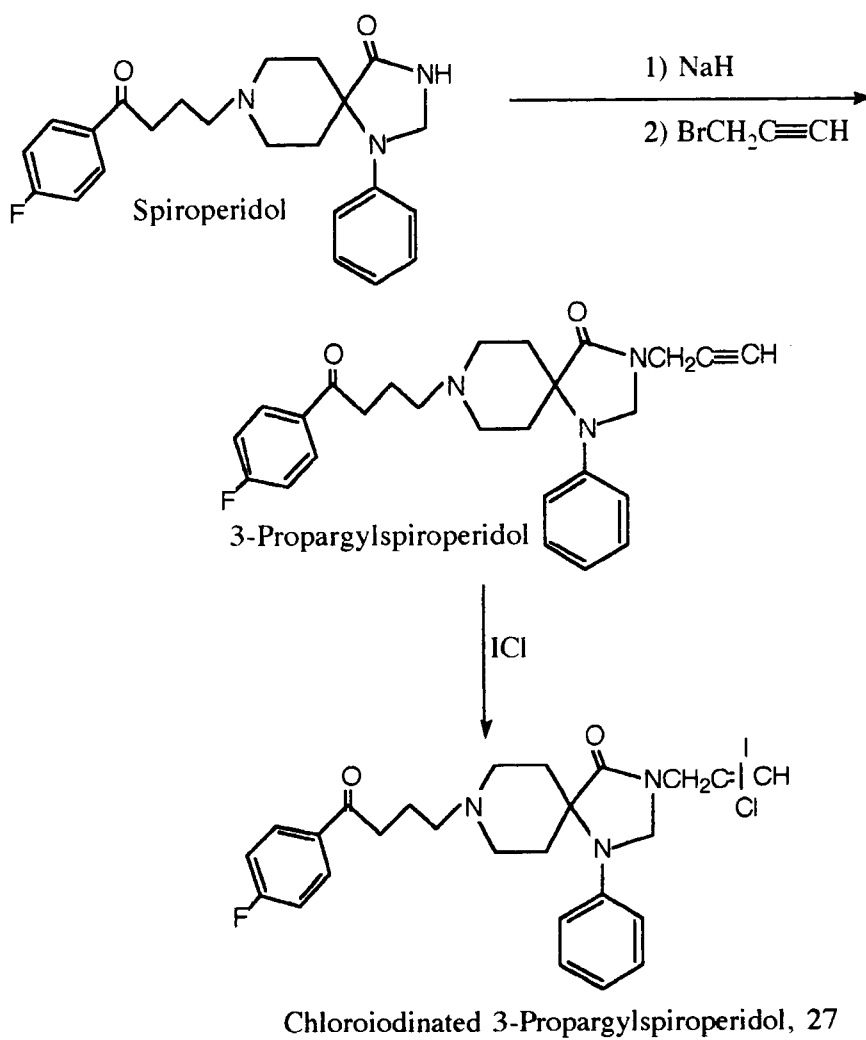
agent. In order to test this theory, the alkylation site was moved to the lactam nitrogen while the butyrophenone moiety was retained on the piperidine ring nitrogen as in spiroperidol. Subsequent chloriodination would thus be far removed from the piperidine nitrogen. Therefore, 3-propargylspiroperidol (Figure 11) was synthesized according to the literature procedure, and was subsequently iodinated using the iodine monochloride addition procedure.

Spiroperidol is commercially available and was used as the starting material. Spiroperidol was dissolved in DMF containing sodium hydride and the mixture was stirred until the evolution of hydrogen gas ceased (Scheme 49). Propargyl bromide was added and the resulting mixture was stirred overnight. This provided propargylated spiroperidol in good yield. The ^1H and ^{13}C NMR spectra were similar to those of N-propargylpseudospiperone, including the propargyl and butyrophenone resonances. When N-propargylspiroperidol hydrochloride was refluxed in a solution of THF containing ICl, the addition product was formed in better than 60% yield (27). The A/M isomeric ratio was different from those of the propargylamine hydrochlorides, being less than one.



3-Propargylspioperidol

Figure 11. Propargylation at the 3-position of spioperidol.



Scheme 49. Synthesis of chloriodinated 3-propargylspioperidol.

A 1:1.3 ratio was obtained by integrating the resonances in the ^1H NMR spectrum for the CH and CHCl protons. The difference in the ratio was expected since the positively charged nitrogen present in the propargylamine hydrochlorides is not a factor in the regiochemistry of the addition because the propargyl group is attached to a lactam nitrogen. This indicates that the most stable reaction intermediate is the one in which the positive charge resides mostly on the internal carbon atom. Subsequent attack by chloride on the intermediate then leads to the Markovnikov (M) addition product.

C.5 THE DESIGN AND SYNTHESIS OF IODINATED BARBITURIC ACID DERIVATIVES

The barbiturates exert a general depressant effect on the cerebrospinal axis. These drugs depress neuronal activity, as well as skeletal muscle, smooth muscle, and cardiac muscle activity⁹⁹. Depending on the compound, dose, and route of administration, the barbiturates can produce different degrees of depression such as sedation, hypnosis, or anesthesia. Like other general depressants of the central nervous system, barbiturates modify the mechanism of synaptic transmission rather than neuronal conduction. These drugs reduce the excitability of the post-synaptic cell by altering the permeability of the cell membrane¹⁰⁰.

Barbiturates exert their action on the central synaptic transmission process of the reticular activating system, and the cerebral cortex becomes deactivated¹⁰¹. Barbiturates are anti-depolarizing blocking agents, because they prevent the generation of excitatory post-synaptic potential. These drugs raise the threshold and extend the refractory period of the post-synaptic cell. The depressant activity of barbiturates can be followed very well on the electroencephalogram. The cerebral electrical activity of normal individuals

increases with emotional tension, anxiety, or consumption of a central nervous system stimulant¹⁰². The increased cerebral electrical activity is caused by intensified reticular activation of the cortex. Administration of barbiturates in sufficient doses has a general calming effect, which is clearly observed on the electroencephalogram. Cerebral electrical activity returns to normal as a result of the suppressing influence of barbiturates on reticular activation. Since barbiturates exert their activity preferentially on the reticular activating system, it is reasonable to assume this area of the brain contains receptors which are specific for barbiturates. Several radiolabeled barbituric acid derivatives have been synthesized and biologically tested¹⁰³. The radiolabeled agents demonstrated good solubility in the brain tissue and relatively long retention. However, the agents also demonstrated nonspecific activity and distributed themselves evenly throughout the brain. Even as the agents "washed out", they maintained an even distribution throughout the brain tissue. Because of the good brain perfusion demonstrated by the agents, synthesis of receptor specific barbiturate derivatives is an important pursuit. In order to design potential candidates for labeling, the structure activity relationships need to be considered.

Thousands of barbiturates have been synthesized on a trial and error basis¹⁰⁴. On the basis of these derivatives, several clear correlations between structure and activity have emerged. The pharmacologically active barbiturates are classified as 5,5-disubstituted barbituric acids, 5,5-disubstituted thiobarbituric acids, and 1,5,5-trisubstituted barbituric acids¹⁰⁵(Figure 12).

One of the barbiturates described in this thesis is classified as a 5,5-disubstituted barbituric acid. It contains an ethyl group as one of the substi-

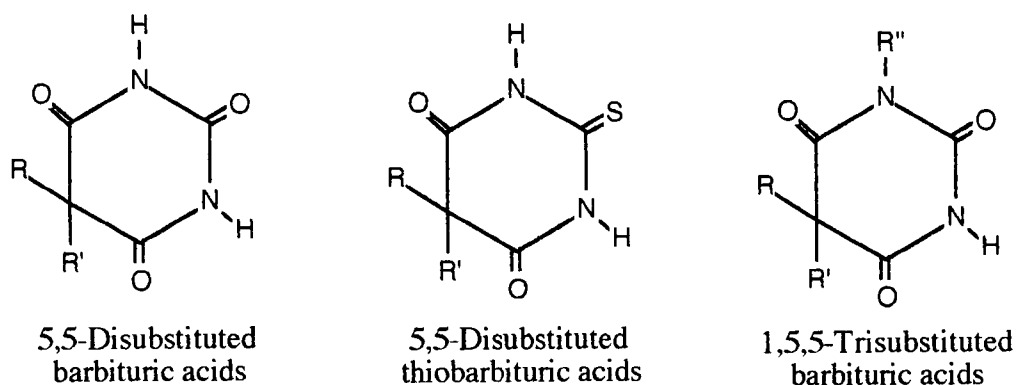


Figure 12. Pharmacologically active barbituric acid derivatives.

tients analogous to phenobarbital, a 5,5-disubstituted barbituric acid derivative ($R = \text{phenyl}$, $R' = \text{ethyl}$). The ethyl group imparts the pharmacological property of increasing the length of action due to increased lipophilicity, which is important for solubility in brain tissue. The other substituent is the propargyl group, which contains the necessary alkyne function for ICl addition. The candidate for iodination was, therefore, 5-ethyl-5-propargylbarbituric acid (Figure 13).

For the synthesis of 5-ethyl-5-propargylbarbituric acid, diethyl ethylmalonate, which is commercially available, was used as the starting material. When diethyl ethylmalonate was dissolved in ethanol containing sodium ethoxide and propargyl bromide was added, the dialkylated diethyl malonate (28) was formed in good yield (Scheme 50). The ^1H NMR spectrum was indicative of the product: it revealed the triplets and quartets produced by the diethyl ester protons and the methylene's ethyl group protons. The diethyl ester and the ethyl group proton resonances integrated for ten and five protons, respectively. The triplet and doublet of the propargyl's alkyne proton and methylene protons, respectively, were present and exhibited their characteristically small coupling constant.

systems consisting of a solvent and a strong base were tested including ethanol and sodium ethoxide. However very little product could be isolated. The most successful reaction system found was DMF and sodium hydride. Diethyl ethylpropargylmalonate was added to a solution of DMF containing a 60% sodium hydride oil emulsion and urea. After heating and stirring overnight, the 5,5-disubstituted barbituric acid (29) was formed in a yield of only 15%. Despite the poor yield, the product was easily isolated and purified by silica gel chromatography. The simplicity of the ^1H NMR spectrum of the product was expected but, nevertheless, interesting. The only resonances are those of the substituents, the characteristic doublet and triplet of the propargyl group's alkyne proton and methylene protons, respectively, the triplet and quartet of the ethyl group, and a broad resonance at δ 10.35 corresponding to the acidic protons of the barbituric acid ring. The ^{13}C NMR spectrum was also consistent with the product's structure.

The iodination was performed in refluxing THF containing ICl. The ICl addition formed two regioisomers (30) as expected. The proton integrations from the ^1H NMR spectrum (see Appendix I) were used to calculate the yield of each regioisomer. The A/M ratio was much smaller, 0.7, than previous additions to propargylamine hydrochlorides, because the alkyne group in this molecule is not attached to any electron withdrawing groups. The A/M regioisomeric ratio, therefore, resembled that of an ICl addition to a simple alkyne, discussed in Chapter 3. The ^{13}C NMR spectrum (see Appendix I) was not typical in the alkene region presumably due to its position relative to the shielding and deshielding zones of the barbituric acid ring. For example, the internal CI resonance was further upfield than usual, δ 82.4, and the internal CCl resonance was further downfield than usual, δ 149.7.

The second barbituric acid derivative synthesized was also disubstituted at the 5 position. The propargyl group was retained in this design because of the success in iodinating it in the previous barbituric acid derivative. The other substituent was chosen to be the p-fluorobutyrophenone moiety of spiroperidol. The p-fluorobutyrophenone group is a pharmacophore of spiroperidol which contributes to its dopamine receptor activity. It was, therefore, postulated that its inclusion into the barbituric acid molecule would yield an agent which might be specific for dopamine receptors in the reticular activating system. The resulting barbituric acid derivative is shown in Figure 14. Diethyl malonate was utilized

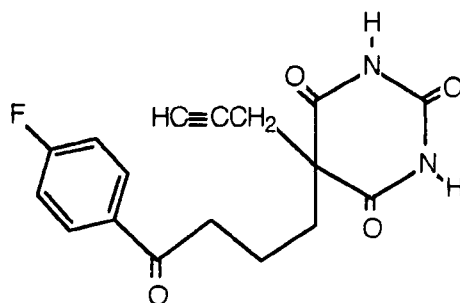
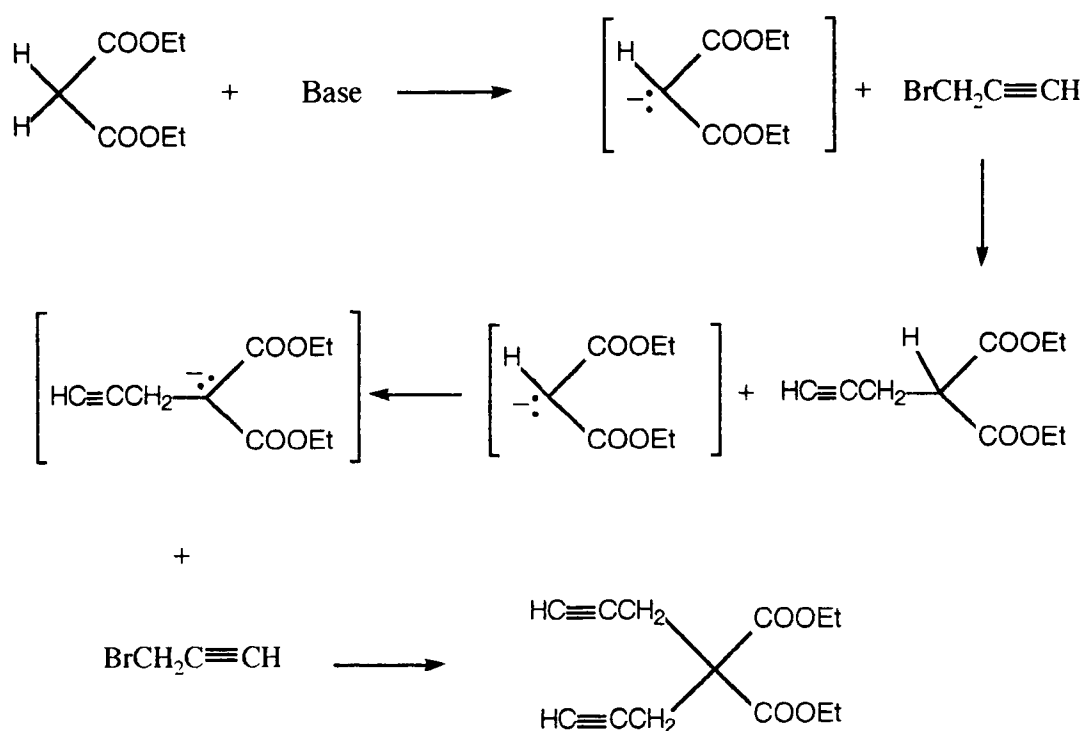


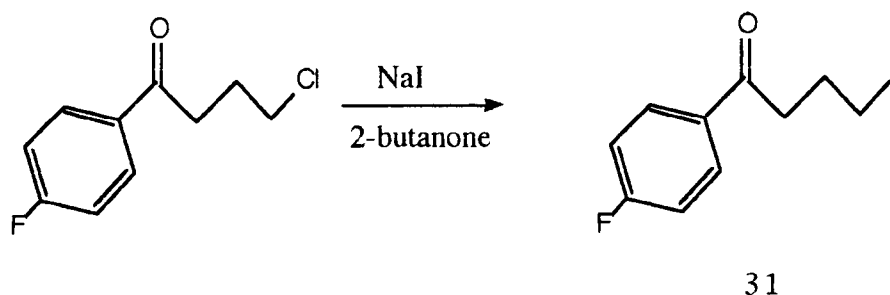
Figure 14. A propargylated barbituric acid derivative containing the butyrophenone pharmacophore.

as the starting material. Since 4-chloro-4'-fluorobutyrophenone is more expensive than propargyl bromide, the propargylation reaction was attempted first. Several solvent systems and bases were used for the propargylation reaction. When diethyl malonate was dissolved in DMF or ethanol, a strong base added, (sodium hydride or sodium) and propargyl bromide added drop-wise, the product mixture contained three components, the starting material, the mono-propargylated derivative, and the di-propargylated derivative. Presumably, the



Scheme 51. Propargylation and dipropargylation of diethyl malonate.

enolate of diethyl malonate formed and was alkylated to give the mono-substituted diethyl malonate, which equilibrated with some of the unsubstituted enolate (Scheme 51). The mono-substituted enolate ion then underwent a second substitution to form the di-substituted product. The problem of di-substitution was solved by changing the order of substitution. Diethyl malonate was added to a suspension of sodium hydride in DMF. After the evolution of hydrogen gas ceased, 4-chloro-4'-fluorobutyrophenone was added and the reaction mixture was heated and stirred overnight. This provided only the mono-alkylated product in less than satisfactory yield, 22%. The yield of the mono-alkylated product was increased to 77% by converting the alkylating agent to the 4-iodo- derivative (Scheme 52).



Scheme 52. Transhalogenation.

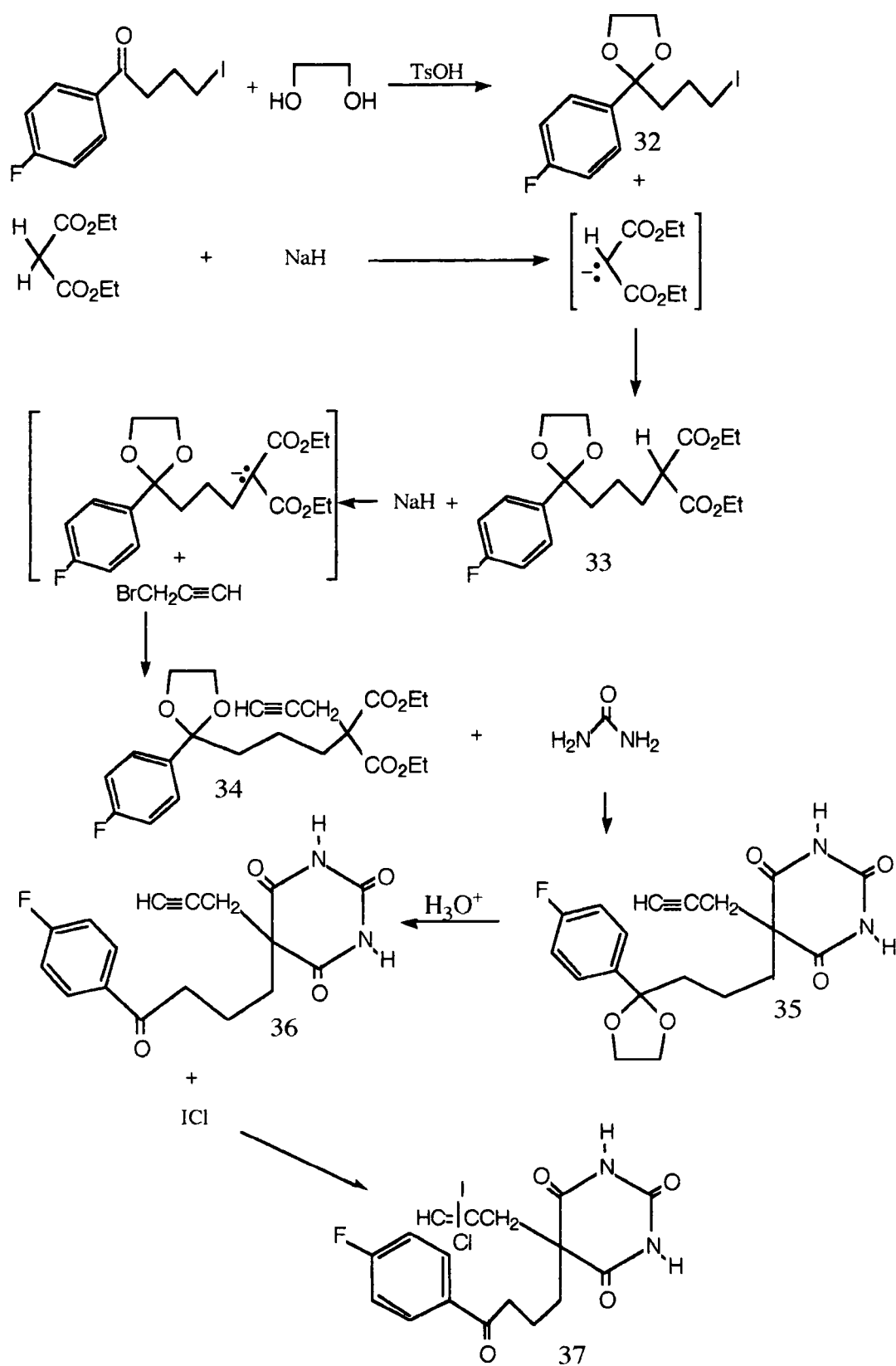
The desired conversion was accomplished by refluxing 4-chloro-4'-fluorobutyrophenone in 2-butanone containing an excess of sodium iodide. The reaction proceeded in good yield, but the product (31) was unstable and had to be used immediately for optimum yield in the subsequent reaction. Though the alkylation of diethyl malonate with 4-iodo-4'-fluorobutyrophenone proceeded

efficiently and in good yield, as did the subsequent propargylation reaction, the ring closure reaction proceeded in very poor yield when the carbonyl group of the butyrophenone was not protected. Therefore the initial alkylation was performed with the ethylene ketal of 4-iodo-4'-fluorobutyrophenone which proceeded in an acceptable yield, 55%, to provide the monoalkylation product (33, Scheme 53). The ^1H NMR spectrum of 33 exhibited the characteristic triplet and quartet of the ethyl groups, and the triplet of the alpha tertiary hydrogen of the malonate portion split by the adjacent CH_2 , proving that only mono-substitution took place. The ^{13}C NMR spectrum included the characteristic splitting pattern of the aromatic carbons due to the spin-spin interactions with the fluorine atom, discussed previously in Section C.4.

The second alkylation with propargyl bromide was performed in DMF using sodium hydride as the base. The reaction was carried out under the same conditions as the first alkylation and provided the di-alkylated product (34) in good yield. Both NMR spectra included resonances indicative of the di-substituted malonate, i.e. diethyl ester, propargyl, and the ethylene ketal groups.

The ring closure reaction was accomplished by dissolving the di-substituted malonate in a suspension of sodium hydride in DMF containing urea. After heating, the ring closure occurred providing the barbituric acid (35) but in poor yield, 16%. If the yield was based on consumed starting material, the yield increased to 69%. The ring closure was confirmed by the ^1H NMR spectrum, which included resonances of the barbituric acid protons at δ 10.50 as well as the absence of the ethyl ester proton resonances.

The carbonyl group of the butyrophenone moiety was regenerated by refluxing the ketal in acidic aqueous methanol. This provided the butyrophenone-propargyl di-substituted barbituric acid (36) derivative in good



Scheme 53. Synthesis of an iodinated barbituric acid derivative.

yield as indicated by the appearance of a carbonyl resonance at δ 170.3 in the ^{13}C NMR spectrum and the absence of the doublet of multiplets, corresponding to the ethylene ketal, in the ^1H NMR spectrum. The non-protected carbonyl reagent was used in the subsequent chloriodination reaction.

The chloriodination reaction was performed in refluxing THF containing an excess of ICl . The addition yielded both A and M regioisomers (37) and in approximately the same A/M ratio as found in the previous chloriodinated barbituric acid, 0.7. A regioisomeric ratio less than one was expected since the most stable intermediate is the one in which the positive charge resides on the internal, secondary, carbon atom. When chlorine adds to the more stable intermediate, the M addition product prevails.

C.6 THE DESIGN AND SYNTHESIS OF IODINATED SYNTHETIC OPIATE DERIVATIVES

When centrally acting analgesics are considered, morphine immediately comes to mind. Its widespread use, beginning with its discovery in the early nineteenth century and increasing with its total synthesis in 1952¹⁰⁶, has been based upon its depressant action on the central nervous system, resulting in increased tolerance to pain. Chemical dissection of the morphine molecule has led to many active derivatives including the accidental discovery of 4-phenylpiperidines¹⁰⁷ while attempting to synthesize antispasmodic drugs related to cocaine. One of the phenylpiperidines, meperidine, had one-fifth to one-eighth the analgesic potency of morphine¹⁰⁸. Further examination demonstrated that it behaved as all narcotic analgesics, that is it produced respiratory depression and a dependence syndrome, but possibly less sedation. At first such compounds appeared to bear no structural similarity to morphine. Upon closer scrutiny, however, it was perceived that in meperidine and its congeners the B, C,

and D rings of morphine had been opened, leaving the A and E rings intact (Figure 15).

From the various narcotic analgesics synthesized, it was recognized that common structural features existed between the molecules. This in turn led to the hypothesis of an opiate receptor in the brain. Since narcotic and synthetic narcotic analgesics are such an important class of compounds, it would be advantageous to discover their mechanism of action and specific location of

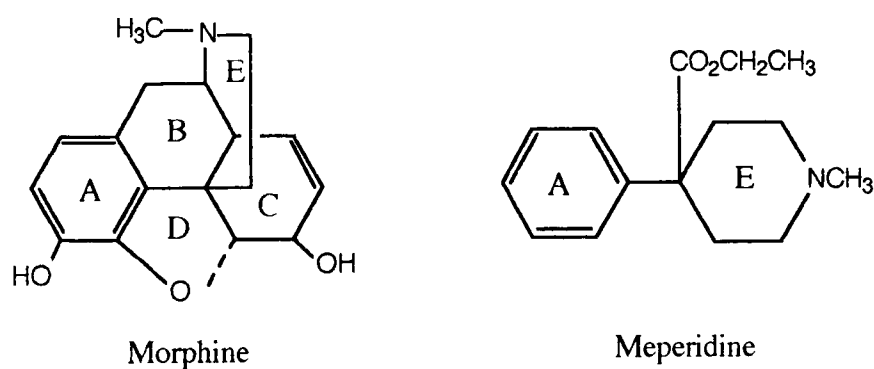


Figure 15. A structural comparison of morphine and meperidine.

action. Therefore, imaging or locating opiate receptor rich areas of the brain could lead to important discoveries about brain function, especially as related to the perception of pain. The important structural features responsible for synthetic narcotic activity were considered in the design, synthesis and iodination of the two target 4-phenylpiperidine derivatives.

The most straightforward 4-phenylpiperidine derivative targeted was 4-phenyl-1-propargylpiperidine. This structure contains the pharmacophore 4-phenylpiperidine present in morphine and meperidine as the A and E rings. The propargyl group is present to supply an alkyne function for ICl addition and to

increase lipophilicity. This simple compound was the first to be synthesized and iodinated (Figure 16).

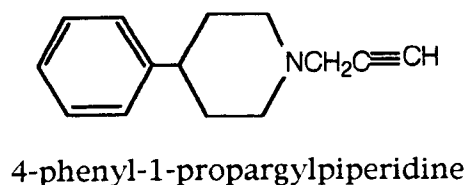
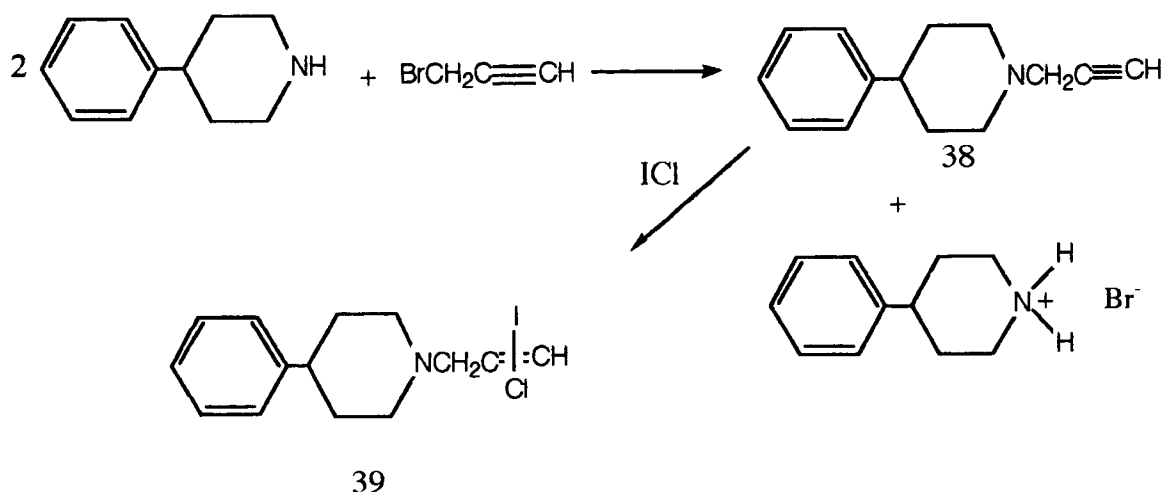


Figure 16. Propargylated 4-phenylpiperidine.

The pharmacophore, 4-phenylpiperidine, is commercially available and was used as the starting material. The propargylation was performed by dissolving a 2:1 molar excess of 4-phenylpiperidine in methanol containing propargyl bromide and heating for several days (Scheme 54). This procedure provided the product in twice the yield when compared to the procedure using one equivalent of 4-phenylpiperidine and a base, such as triethylamine or pyridine, to absorb the liberated acid. Though 4-phenylpiperidine is more expensive than triethylamine or pyridine, it is more efficient to use in excess because it is easily recovered in the purification process. Both ^1H and ^{13}C NMR spectra were consistent with 4-phenyl-1-propargylpiperidine (38).

One aspect concerning the propargylation or alkylation of a secondary amine, not previously mentioned, is the ease of purification of the product. The



Scheme 54. Chloriodination of N-propargyl-4-phenylpiperidine.

alkylation of the secondary nitrogen eliminates hydrogen bonding between the product and the stationary phase of the column, silica gel, facilitating separation and thus purification. In an alcohol/chloroform solvent system, the secondary amine has a typical R_f value of approximately 0.1 while the tertiary amine has a value of approximately 0.8. When the components of a mixture exhibit a large separation on TLC analysis, column chromatography will usually provide a quick and efficient method of separation and purification.

When 4-phenyl-1-propargylpiperidine was converted to the corresponding hydrochloride and added to a refluxing solution of THF containing ICl, the chloriodinated product (39) was formed in good yield, 68%. The A/M regioisomeric ratio was typical of an iodonium ion intermediate in which the positive charge resides mainly on the terminal carbon atom due to the positively charged nitrogen. Subsequent attachment of chloride to the carbon atom furthest from the positively charged nitrogen yields the A isomer. This intermediate, and place of chloride attachment, is approximately five times more likely as suggested

by an A/M regioisomeric ratio of five to one, which was determined by integration of the vinyl protons in the ^1H NMR spectrum of the mixture. The regioisomers were separated by preparatory TLC and analyzed individually. The ^{13}C off-resonance decoupled NMR spectrum of the A isomer included a singlet at δ 102.0 and a doublet at δ 121.1, indicating the attachment of iodine and chlorine to the internal and external carbon atoms, respectively. The ^1H NMR spectrum of the A isomer confirmed this assignment by exhibiting a resonance at δ 6.65 corresponding to the hydrogen attached to the terminal carbon, which is also attached to chlorine. The ^{13}C off-resonance decoupled NMR spectrum of the M isomer, in comparison, had a singlet at δ 134.8 and a doublet at δ 75.7, indicating chlorine and iodine attachment to the internal and external carbon atoms, respectively. The ^1H NMR spectrum of the M isomer included its vinyl proton resonance at δ 6.56, upfield in comparison to the A isomer's vinylic proton, indicating the terminal carbon atom's attachment to iodine. The mass spectral data for the A and M isomer were essentially the same. Both exhibited molecular ion peaks at m/e 361 (^{35}Cl). The isolation and analysis of the individual regioisomers added additional evidence to the assignment of structures to the previously synthesized regioisomeric mixtures which were inseparable.

The next 4-phenylpiperidine derivative designed, synthesized, and iodinated resembled meperidine. An ethoxycarbonyl group was added to the 4-position of the N-propargyl-4-phenylpiperidine design. This structure is identical to that of meperidine except the N-methyl group is replaced with an N-propargyl group (Figure 17).

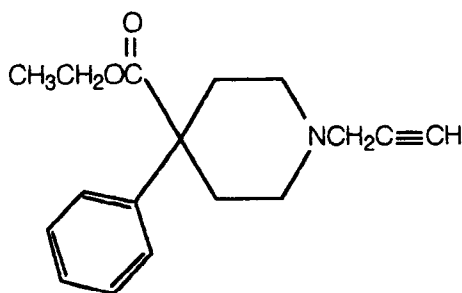
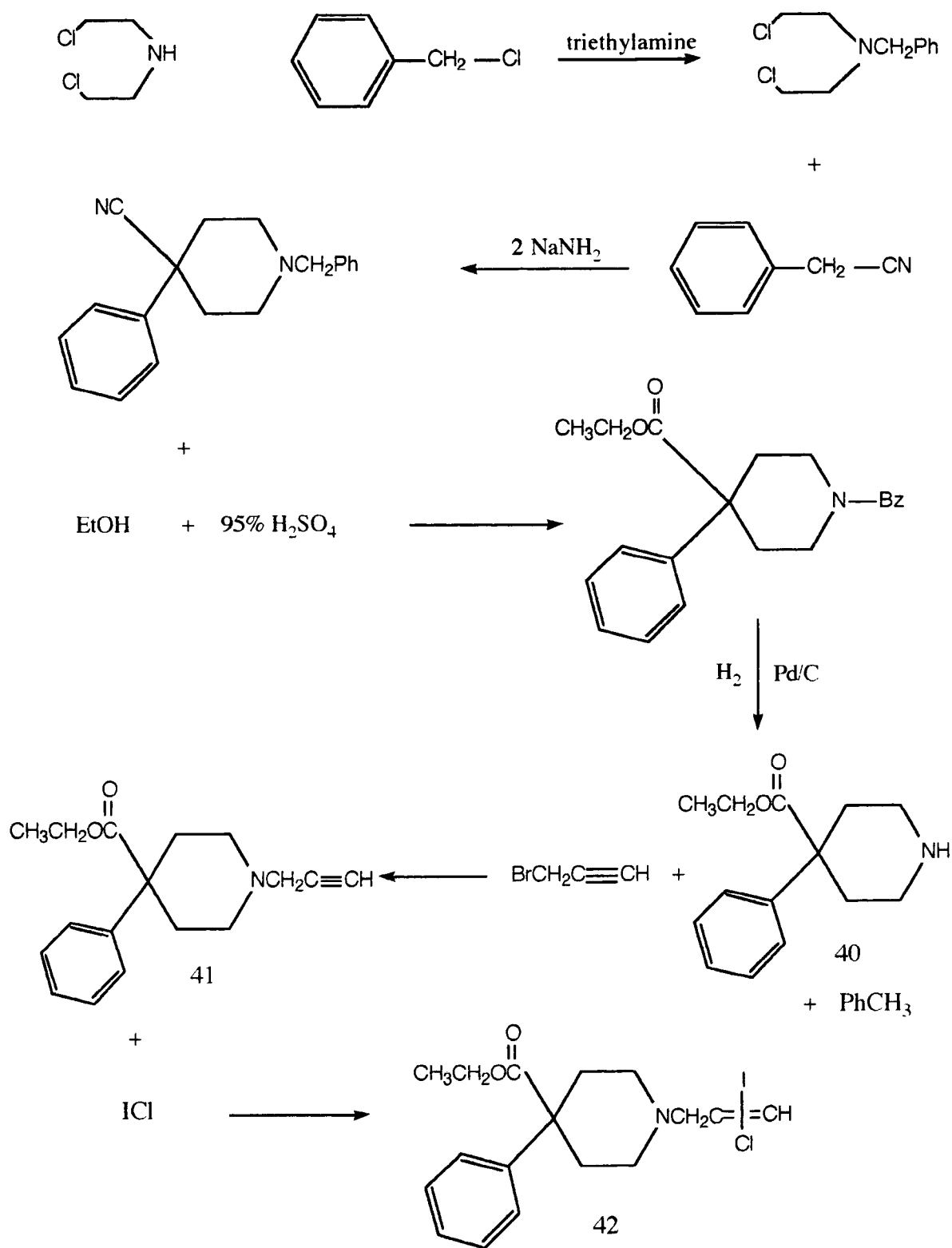


Figure 17. Propargylated meperidine.

There are various procedures for synthesizing the 4-substituted-4-phenylpiperidine ring system. The method of Eisleb¹⁰⁹ was used to synthesize 4-ethoxycarbonyl-4-phenylpiperidine (Scheme 55). This derivative can also be synthesized from commercially available 4-cyano-4-phenylpiperidine. The amine, bis-(2-chloroethyl)amine reacted with benzyl chloride to provide the tertiary benzylamine in good yield. Both chlorinated amines, the starting material and product, are very corrosive to the skin and should be handled with extreme caution. The ring closure reaction was accomplished by mixing the tertiary amine with benzyl cyanide and slowly adding an excess of sodium amide (at least two equivalents) at a rate to maintain a reduced temperature. Great care should be exercised during this procedure because there is an induction period and, if the addition is too fast, the reaction can proceed at an explosive rate. If the addition is performed at a rate sufficient to maintain reduced temperatures, slightly higher than room temperature, the reaction provides 1-benzyl-4-cyano-4-phenylpiperidine. The nitrile was converted to the corresponding ethyl ester, 1-benzyl-4-ethoxycarbonyl-4-phenylpiperidine, by esterification with ethanol and sulfuric acid. In order to propargylate the piperidine ring, the benzyl group had to be removed by catalytic



Scheme 55. Chloriodination of N-propargylmeperidine.

hydrogenation in acidic methanol over a palladium/carbon catalyst. This produced the debenzylated amine (40) in good yield which was also synthesized by refluxing the commercially available 4-cyano-4-phenylpiperidine in a 50% ethanol/sulfuric acid mixture. The piperidine nitrogen was then alkynylated with propargyl bromide to produce 4-ethoxycarbonyl-4-phenyl-1-propargylpiperidine (41). The ethyl and propargyl protons were observed at their expected resonance values in the ^1H NMR spectrum and exhibited their characteristic splitting patterns and coupling constants. The alkyne carbons of the propargyl substituent were characterized by resonances and splitting patterns in the ^{13}C off-resonance decoupled NMR spectrum which exhibited a singlet at δ 79.1 and a doublet at δ 73.0 corresponding to the internal and external alkyne carbons, respectively. After converting 4-ethoxycarbonyl-4-phenyl-1-propargylpiperidine to the corresponding hydrochloride, chloriodination was performed in refluxing THF containing ICl. The addition formed both regioisomers (42) in an expected A/M ratio of approximately five to one. The relatively large A/M regioisomeric ratio is due to the unsymmetrical nature of the most stable iodonium ion intermediate similar to the one described on page 109. This configuration allows for maximal separation of both positive charges, as discussed in Chapter 3.

In the case of chloriodinated 4-ethoxycarbonyl-4-phenyl-1-propargylpiperidine, the regioisomers were inseparable by ordinary chromatography techniques. Therefore the regioisomers were characterized as a mixture. Both ^1H and ^{13}C NMR spectra were consistent with the approximate five to one A/M ratio of ICl addition product.

CHAPTER 5. THE SYNTHESIS OF THE RADIOIODINATED AGENTS AND THE SUBSEQUENT BIOLOGICAL TESTING PROCEDURE.

A. INTRODUCTION

As described in the previous chapters, all of the synthetic procedures were used to prepare non-radiolabeled iodinated derivatives. This chapter details the substitution of radioiodine for the "cold" iodine used in the iodination reactions. The isotope used for all "hot" experiments was [^{125}I]iodine for reasons discussed in Chapter 1. When [^{125}I]iodine is commercially purchased, it is supplied as the [^{125}I]iodide of sodium with an activity of ~ 20 millicuries (mCi) in 100 microliters (μL) of a 0.1 M solution of sodium hydroxide. For the iodination reactions, [^{125}I]iodine in the form of iodide must be oxidized to the mixed halogen [^{125}I]iodine monochloride. In this form, it possesses the necessary electrophilic properties to add to triple bonds and to substitute the boron of a boronic acid. The following sections describe the general procedures used for the radioiodination of the agents using the hydroboration-[^{125}I]iodination procedure and the new chloro-[^{125}I]iodination of alkynes procedure. Both procedures may be performed under no-carrier-added or carrier-added conditions. No-carrier-added, or carrier-free, means that the radioiodination is performed without the addition of "cold" sodium iodide while carrier-added refers to the addition of "cold" sodium iodide to the reaction mixture. When no-carrier-added conditions are used, most of the product molecules contain "hot" iodine. These conditions are used when an agent is being tested for receptor activity and a high specific activity (most iodine atoms in the molecules are "hot") reagent is needed. Carrier-added conditions produce a reagent that has a lower specific activity but is satisfactory for certain purposes, such as testing the agent as a perfusion agent

or performing a biodistribution test. The advantage of performing a carrier-added experiment is the radiochemical yield is usually significantly higher. However, the no-carrier-added reagent is usually superior because it can be used for receptor site studies, whereas the carrier-added reagent is inappropriate. This is due to the fact that receptor sites exist in very low concentrations. Both of the radioiodination procedures to be discussed are very useful because they can be used to produce no-carrier-added agents in good radiochemical yields. If a carrier-added experiment is desired, both of the following procedures can be modified by adding either sodium iodide or iodine monochloride to the reaction mixture to provide a carrier-added agent.

B. RADIOIODINATION USING THE HYDROBORATION- $[^{125}\text{I}]$ IODINATION PROCEDURE

The alkyne function of the agent was converted to the corresponding boronic acid using catecholborane followed by hydrolysis as described in Chapter 2. For the radioiodination, the vinylboronic acid was used in very low concentration, approximately 20 μL of a 0.5 M solution in 50% aqueous THF, and was mixed with 80 μL of THF and 5 μL of sodium $[^{125}\text{I}]$ iodide (~ 5 mCi). With stirring, a solution of chloramine-T or N-chlorosuccinimide (NCS), 25 μL of a 0.5 M solution in 50% THF, was then added and the reaction mixture was capped and allowed to stir at room temperature in the absence of light for 1 hour. The reaction product was then extracted into chloroform or ether (usually 4 X 1 mL was sufficient). The extracts were combined, dried with a suitable drying agent (anhydrous magnesium sulfate), reduced in volume with a stream of nitrogen or argon, and spotted onto a silica gel thin layer chromatography plate. Since the product was formed in an amount too small for chemical analysis, the

radioiodinated product was spotted adjacent to an authentic sample of the "cold" iodinated compound in such a way as to allow for a small amount of overlap or double spot. The plate was developed in an appropriate solvent mixture and the radioactive band corresponding to the authentic sample was scraped and eluted to provide the radioiodinated product in a 17-50% radiochemical yield.

Another procedure used to insure the authenticity of the radioiodinated agents was to repeat the above procedure on a large scale using "cold" sodium iodide and analyze the products. In all cases, TLC analyses indicated the radioiodinated agents corresponded to the authentic "cold" iodinated compounds.

C. RADIOIODINATION USING THE NEW CHLOROIODINATION OF ALKYNES

PROCEDURE

For this procedure, the alkyne was used as the substrate without modification. The most important aspect of this procedure involved the production of a neutral solution of [^{125}I]iodine monochloride in anhydrous THF. This was accomplished by adding 5 μL (~5 mCi) of commercially available sodium [^{125}I]iodide in 0.1 N sodium hydroxide to a reaction vial containing 50 μL THF. To neutralize the solution an equal volume (5 μL) of 0.1 N hydrochloric acid was added with stirring. At this stage, the solution consisted of a neutral mixture of sodium [^{125}I]iodide and sodium chloride in aqueous THF. To oxidize the [^{125}I]iodide to [^{125}I]iodine monochloride, 25 μL of a 0.5 M solution of chloramine-T or NCS in THF was added and the mixture was stirred for 1 hour. Due to the extremely low concentration of [^{125}I]iodide, the reaction rate was very slow. Therefore, it was advisable to keep the volume of the mixture to a minimum, allowing some evaporation, but preventing complete dryness. The mixture was then loaded onto a column consisting of a Pasteur pipet packed with a

cotton plug, 1 cm of sand, 5 cm of silica gel, 3 cm of anhydrous magnesium sulfate, another cm of sand, and another cotton plug compressed such that the integrity of the layers was maintained (Figure 18). The column was eluted with 1-3 mL of anhydrous THF and the eluant was collected in a vial. A bulb was added to the top of the column and squeezed to force out the residual THF. This produced 1-3 mL of a solution of [^{125}I]iodine monochloride in anhydrous THF. The alkyne substrate, 25 μL of a 0.5 M solution in anhydrous THF, was added to the [^{125}I]iodine monochloride solution and the solution was refluxed for 1-2 hours under argon in the absence of light. During this time, small aliquots were removed to monitor the reaction's progress by TLC analysis. If the reaction mixture contained no product after 30 min, which occurred on several occasions, the reaction mixture was allowed to evaporate to a minimal volume but not to dryness. If dryness occurred, several drops of anhydrous THF were added. In each case, when the volume was reduced in this manner, the reaction would initiate. Because evaporation and volume are difficult to control, an oil bath set at 90 $^{\circ}\text{C}$ was used to provide reflux temperatures so if dryness occurred the reactants would not be destroyed by excessive heat. After cooling, the reaction mixture was partitioned between ether (3 mL) and water (2 mL). Sodium sulfite or sodium thiosulfate (several crystals) was added with stirring to destroy unreacted [^{125}I]iodine monochloride. The ether layer was isolated, dried (anhydrous magnesium sulfate), filtered, and reduced in volume by a stream of argon. The residue was spotted onto a silica gel TLC plate adjacent to an authentic sample of the "cold" compound in such a way as to allow a small amount of overlap. The plate was developed in an appropriate solvent and the radioactive band corresponding to the authentic sample was scraped and eluted to provide the chloro[^{125}I]iodinated product in good radiochemical yield, 20-70%.

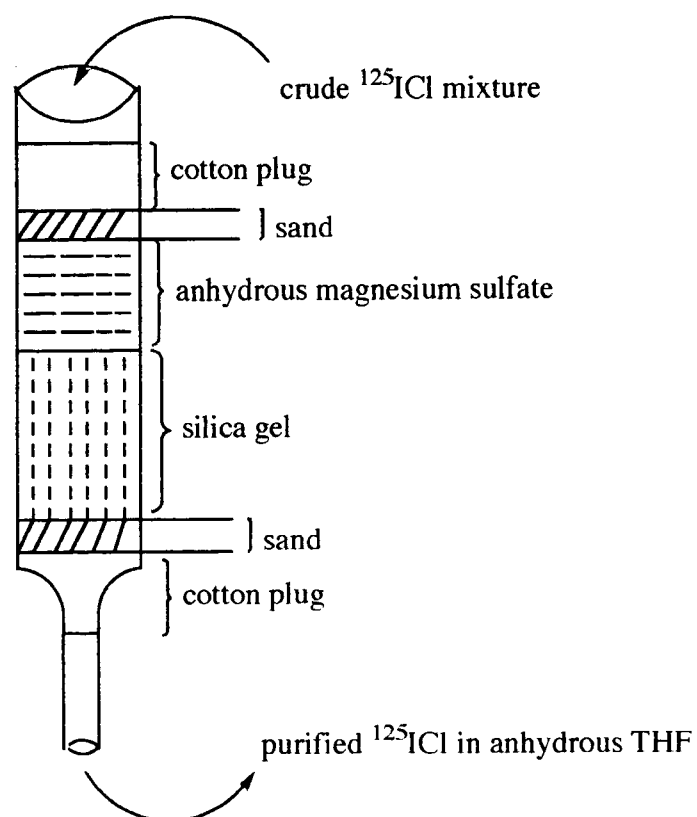


Figure 18. Purification column for $^{125}\text{I}\text{Cl}$ mixture.

Another procedure used to insure the authenticity of the radioiodinated agents, as mentioned previously, was to repeat the above procedure on a large scale using "cold" sodium iodide and analyzing the products. In all cases, TLC analyses indicate the radioiodinated agents corresponded to the authentic "cold" iodinated compounds.

D. BIODISTRIBUTION STUDIES USING LABORATORY ANIMALS

In order to prepare the radioiodinated products for injection into laboratory animals, the solvent was evaporated using a stream of argon and then the sample was dissolved in glycerol (2-5 drops depending on the substrate) and diluted with 0.9% saline to a concentration of $\sim 0.1\text{-}3\ \mu\text{Ci}$ per mL. To exclude particulates and bacteria, the solution was passed through a millipore filter. The radioiodinated agent ($0.1\text{-}3\ \mu\text{Ci}$) was then administered by intravenous injection into a lateral tail vein to metofane anesthetized female Fisher VAF rats. The rats were divided into 3 groups ($n=5$) and were allowed to consume food and water prior to the experiments. After injection, each group of rats was euthanized by cervical fracture following metofane anesthesia at three different time periods, usually 5, 30, and 60 minutes. The brain, heart, liver, lungs, and kidneys were removed, rinsed with saline, blotted dry, and weighed in tared vials; a blood sample was also taken. The samples were counted in a Packard Minaxi 5000 sodium iodide auto gamma counter. The experimental results were reported in graph form as mean % dose/gram vs minutes after injection. The biodistribution experimental results for the radioiodinated analogs of the reagents synthesized in this study are discussed in Chapter 6 and are presented in Appendix 2.

CHAPTER 6. CONCLUSIONS

A. SUMMARY OF RADIOIODINATION CHEMISTRY

A variety of biologically important molecules were efficiently labeled with radioiodine using two different techniques. It was found that when iodinating molecules which do not contain Lewis base functionalities, the hydroboration-iodination procedure can be used to synthesize high specific activity agents in good radiochemical yield. However, molecules containing multiple functional groups including Lewis base functionalities (amines) can be most efficiently radioiodinated using the new chloriodination of alkynes procedure. Both procedures require an alkyne function which must be present in the precursor molecule. The advantages of the new chloriodination of alkynes procedure are that molecules containing the alkyne function can be radioiodinated directly without hydrometallation and the reactions can be performed with multifunctional group containing molecules.

B. THE UTILITY OF BOTH RADIOIODINATION PROCEDURES

A possible disadvantage of the new ICl addition procedure is that, along with the introduction of iodine, chlorine is also added to the molecule. This may add steric bulk to the molecule and could hinder it from complexing with a receptor site on a cell wall or other intended sites, such as a receptor site on an enzyme in a metabolic pathway. However, it is also possible that the presence of the iodine alone may behave in such a manner as to preclude the agent's utility in the presence or absence of chlorine. Therefore when using either method for designing an agent, it is helpful to ascertain the structures of the molecules necessary for activity so the labeling site can be introduced into a position far

removed from these structures. Unfortunately, the only certain method for ascertaining whether or not the labeling group is inactivating the molecule is to synthesize, radioiodinate, and biologically test it.

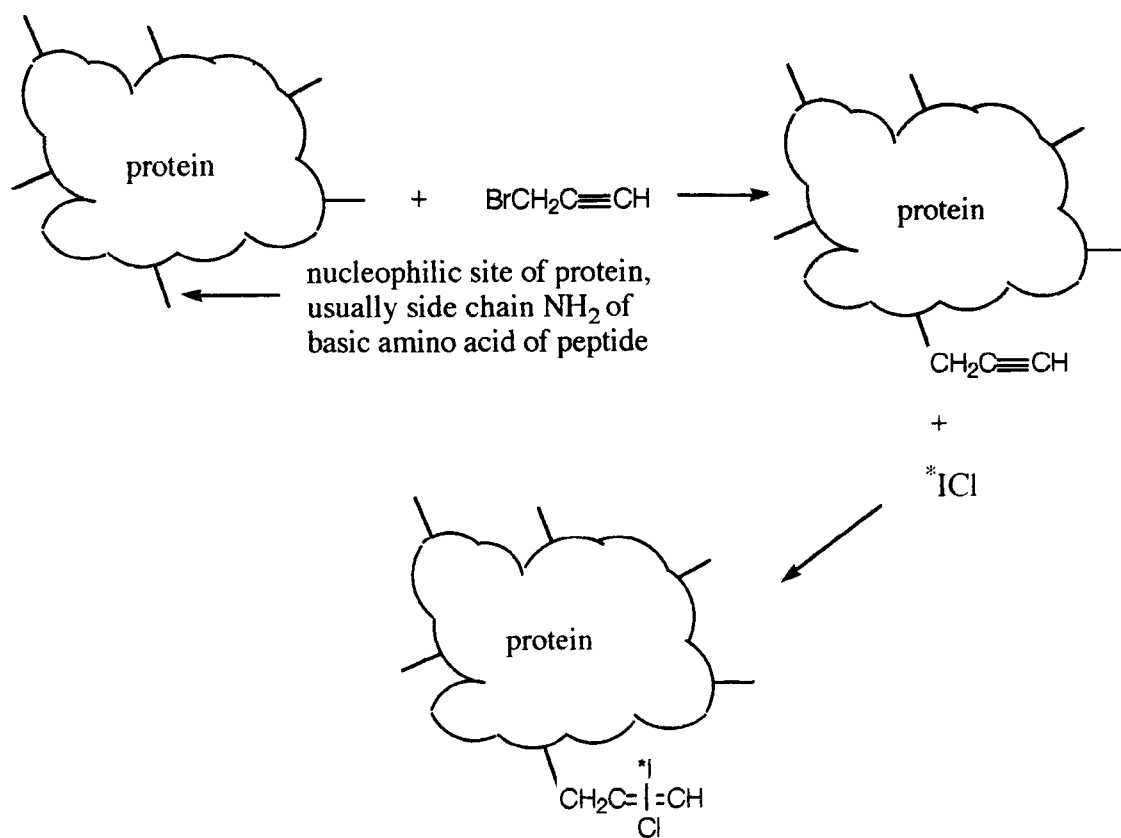
The results of this research also prove that the chloriodination of alkynes does not necessarily inactivate the molecule in question. For example, the fatty acids labeled by this procedure demonstrated good cardiac uptake and retention similar to the iodinated analogs and other radiolabeled fatty acids¹¹⁰ (see Appendix 2). Another interesting aspect of the chloriodination of alkynes procedure occurs when biologically testing radioiodinated compounds derived from unsymmetrical alkynes which produce two regioisomers, A and M. Some molecules produced radioiodinated regioisomers which could not be separated while other molecules, N-propargyl-4-phenylpiperidine and pargyline, produced regioisomers which were easily separated into the corresponding A and M isomers. It was initially expected that during the biodistribution studies there would be no significant difference between the regioisomers; this was not the case. The difference in biodistribution between the A and M regioisomers formed by the chloriodination of pargyline was quite pronounced (see Appendix 2). Both A and M regioisomers demonstrated acceptable brain tissue solubilities. However, the A regioisomer demonstrated superior brain solubility and brain to blood ratios, a property necessary for brain perfusion agents. The difference in biodistribution properties between the A and M regioisomers is presumably due to the increased fat solubility of the A regioisomer in comparison to the other tissues examined. This would account for the greater solubility of the A isomer in brain tissue because the brain is composed mainly of fats.

The success of the new chloriodination of alkynes procedure for the radioiodination of biologically interesting molecules has been proven in two

distinct aspects: 1) the chemistry of the chloriodination reaction has been applied to molecules containing multiple functional groups; 2) the chloriodination reaction has produced four potentially useful radioiodinated agents that exhibit the desired biological properties as demonstrated by biodistribution studies: three cardiac imaging agents, the two radioiodinated fatty acids and the 3-(iodopentenyl)-1,3,8-triazaspirodecanone derivative, and one potential brain perfusion agent, the A regioisomer of the ^{125}ICl addition product of pargyline, see Appendix 2.

C. FUTURE USES FOR THE NEW RADIOIODINATION PROCEDURE

In view of the procedure's success, the ICl addition procedure could be used as the radioiodination procedure of choice for radioiodination experiments. The chloriodination of alkynes procedure should be considered as a viable radioiodination alternative for molecules which resist radioiodination by existing techniques. Since the radioiodination and chlororadioiodination of alkynes adds bulk to any labeled molecule, the procedures may be most successful for labeling large molecules which contain bulky areas unrelated to the molecule's activity. Specifically, since radiolabeled proteins such as antibodies are important to many researchers, the radioiodination of proteins could be easily accomplished by using the chloriodination of alkynes procedure (Scheme 56). Current procedures for labeling proteins consists of using relatively large coupling agents followed by radioiodination (Chapter 1). Using the new radioiodination procedure developed in this research, the protein could be easily labeled by using propargyl bromide as a smaller coupling agent to couple with a nucleophilic site of the protein followed by ^{125}ICl addition to provide a high specific activity agent.



Scheme 56. Proposed method for labeling proteins using the ICl addition to alkynes procedure.

CHAPTER 7. EXPERIMENTAL SECTION

A. GENERAL METHODS

^1H and ^{13}C NMR spectra were recorded on a Jeol FX-90Q spectrophotometer and are reported in ppm relative to the internal standard tetramethylsilane (TMS=0) and deuterated chloroform ($\text{CDCl}_3=77.1$), respectively. Mass spectral data were obtained by electron impact (EI) and obtained on a ZAB-EQ hybrid mass spectrometer. Chromatography was performed on Analtech 1000- μm preparatory silica gel plates, using 0-15% methanol in chloroform as eluant, unless otherwise stated. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, TN. All starting materials, not synthesized, and solvents were purchased from commercial suppliers. All compounds were analyzed by ^1H and ^{13}C NMR. In addition, some representative compounds were also characterized by mass spectral and elemental analyses. Several of the ICl addition compounds were isolated as oils, which were found to be difficult to analyze, and for these cases the elemental analyses are not reported. All reactions using hazardous chemicals and reagents were performed in a well ventilated hood to minimize health hazards.

B. EXPERIMENTAL CONDITIONS FOR THE RATE STUDY DISCUSSED IN

CHAPTER 3

The alkynylamine hydrochloride 13 (0.22 mmol) was dissolved in THF (1 mL) containing ICl (0.26 mmol) and allowed to stand at room temperature protected from light for 2 h. The mixture was partitioned between water (5 mL) and chloroform (10 mL) while sodium thiosulfate was added until the color faded. The mixture was rendered basic by the addition of 1N sodium hydroxide (2 mL) followed by saturation with sodium chloride. The organic layer was collected and

the aqueous phase was further extracted with chloroform until it was free of product and starting material, as determined by thin layer chromatography. The extractions were combined, dried (anhydrous magnesium sulfate), filtered, and the solvent evaporated at room temperature until a constant mass was obtained upon weighing. In each case, 13a-c providing 14a-c, the mixture was an oil. The mass of the oil mixture was determined and recorded followed by ^1H NMR analysis of the oil mixture. The spectrum of each oil produced from the reaction mixture of 13a-c plus ICl was consistent with a mixture of starting material and product. If a chloroform peak was present, the mixture was evaporated until the peak was absent or insignificant. The proton integration was used to determine the molar ratio of product to starting material from which the yields and product distributions were calculated. Over 95% of the mass was accounted for except in the case where propargylamine volatilized with the solvent while its ICl addition product did not.

C. SYNTHETIC EXPERIMENTAL CONDITIONS

ω -Undecynyl cholesteryl ether (1)

Potassium metal (1.32 g; 33.7 mmol) and dry benzene (75ml) were added to a flame-dried, nitrogen flushed, 250 mL round-bottomed flask. The mixture was heated to 95 $^{\circ}\text{C}$ with vigorous stirring for 30 min. Cholesterol (15.0 g; 38.7 mmol) was added in portions followed by dry benzene (15 mL). After refluxing overnight, additional dry benzene (50 mL) was added to facilitate stirring followed by ω -undecynyl tosylate (10.9 g; 33.7 mmol) in dry benzene (20 mL). The mixture was refluxed under nitrogen for 48 h. The resulting thick solution was evaporated and the residue chromatographed on a silica gel (150 g) column, eluting with 4% ether/petroleum ether. This provided the ether (15.3 g; 85.0%) as white crystals

(ethanol): m.p. 40.5-41.5 °C; ^1H NMR (CDCl_3) δ 0.68, 0.83, 0.90, 0.95, 1.00 (s, 5 x CH_3), 1.10-2.50 (m, 45H, alkyl and alkynyl H), 3.12 (m, 1H, $-\text{OCH}$), 3.44 (t, 2H, $-\text{OCH}_2$), 5.35 (d, 1H, cyclic olefin); ^{13}C NMR (CDCl_3) δ 141.10 and 121.35 (olefinic carbons), 84.62 (internal alkyne), 78.93 ($-\text{OCH}$), 68.07 (superimposed external alkyne and $-\text{OCH}_2$). Anal. Calcd: C, 85.01; H, 12.01. Found: C, 84.80; H, 11.96.

11-Cholesterylundec-1-en-1-yl boronic acid (2)

A solution of ω -undecynyl cholesteryl ether (2.06 g; 3.81 mmol) was stirred under nitrogen at 60 °C overnight. After cooling the solution to room temperature, THF (28 mL) was added, followed by the dropwise addition of water (24 mL). The resulting mixture was stirred for 3 days. Water (150 mL) was added dropwise with vigorous stirring to precipitate the product. The crude product was collected and reprecipitated from THF (15 mL) using water, as described above, to facilitate the removal of catechol. The resulting white precipitate was collected and chromatographed on a silica gel (100 g) column eluting with 3% methanol/dichloromethane to provide the boronic acid (1.53 g, 69.2%): ^1H NMR (CDCl_3 : $\text{DMSO}-d_6$ = 9:1) δ 0.67, 0.82, 0.89, 0.94, 0.99 (s, 5 x CH_3), 1.10-2.65 (m, 44H, alkyl H), 5.30 (bd, 2H, superimposed $\text{HC}=\text{CHB}$ and cyclic olefinic H), 6.57 (m, 1H, $\text{HC}=\text{CHB}$), 6.93 (s, OH); ^{13}C NMR (CDCl_3 : $\text{DMSO}-d_6$ = 9:1) δ 150.82 ($\text{C}=\text{C}-\text{B}$), 140.72 and 121.19 (cyclic olefinic carbons), 78.61 ($-\text{OCH}$), 67.77 ($-\text{OCH}_2$). Anal. Calcd: C, 78.32; H, 11.59. Found: C, 78.95; H, 11.04.

ω -(*E*)-Iodoundecenyl cholesteryl ether (3)

To a solution of 11-cholesterylundec-1-en-1-yl boronic acid (0.10 g; 0.17 mmol) in THF (4.5 mL) and water (1 mL) was added aqueous 1 M sodium iodide (0.2 mL) and chloramin-T (0.08 g; 0.4 mmol) in 50% aqueous THF (1mL). The

mixture was stirred for 1.75 h. The resulting solution was extracted with ether (2 x 20 mL). The organic layer was collected, dried (MgSO₄), and concentrated under reduced pressure. The residue was chromatographed on a 5000 μ preparative TLC plate using 4% ether/petroleum ether as the eluant to provide the vinyl iodide (0.080 g; 70%) as white crystals (ethanol): m.p. 62.3-63.3 °C; ¹H NMR (CDCl₃) δ 0.60, 0.83, 0.90, 0.95, 1.00 (s, 5 x CH₃), 1.10-2.50 (m, 44H, alkyl H), 3.13 (m, 1H, -OCH), 3.44 (t, 2H, -OCH₂), 5.31 (d, 1H, cyclic olefin); 5.95 (d, 1H, J = 14.5 Hz, HC=CHI), 6.43 (m, 1H, HC=CHI); ¹³C (CDCl₃) δ 146.85 (C=CI), 141.27 and 121.47 (cyclic olefin), 79.05 (-OCH), 74.34 (C=CI), 68.22 (-OCH₂). Anal. Calcd: C, 68.65; H, 9.85. Found: C, 68.86; H, 9.98.

5-Iodo-1-(*E*)-penten-1-yl iodide (4)

The vinyl boronic acid, 5-iodo-1-penten-1-yl boronic acid, (0.20 g; 0.84 mmol), was dissolved in 50% aqueous THF (6 mL). Sodium iodide (0.13 g; 0.84 mmol) was added followed by chloramine-T (0.20 g; 0.88 mmol) in aqueous THF (4 mL). The reaction was stirred for 2 hours in the absence of light at room temperature. Sodium thiosulfate was added until the color faded then the mixture was partitioned between 1 N NaOH (10 mL) and hexane (20 mL). The organic phase was isolated and the aqueous phase was extracted once more with hexane (20 mL). The organic fractions were combined, dried (MgSO₄), filtered, and the solvent evaporated to yield the vinyl iodide (0.18 g; 67%) as an oil of sufficient purity for subsequent reactions: ¹H NMR (CDCl₃) δ 2.17 (p, 2H, CH₂), 2.62 (2H, alkene CH₂), 3.26 (t, 2H, CH₂I), 6.08 (d, 1H, J = 16.5 Hz, HC=CHI), 6.48 (m, 1H, HC=CHI); ¹³C NMR (CDCl₃) δ 20.5, 36.9, 39.9 (alkyl CH₂'s), 144.5 (C=CI), 76.0 (C=CI).

1-Phenyl-3-[(*E*)-5-iodo-4-penten-1-yl]-1,3,8-triazaspiro[4.5]decan-4-one (5)

To a mixture of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.60 g; 0.26 mmol) in dry DMF (5 mL) was added sodium hydride (60% suspension, 10 mg; 0.26 mmol) in portions. The mixture was stirred for one hour then cooled to -30 °C followed by the addition of (*E*)-1,5-diiodo-1-pentene (0.080 mg; 0.26 mmol) in dry DMF (1 mL). The mixture was stirred for 45 minutes under argon then allowed to warm to room temperature overnight. The resulting mixture was evaporated at 40 °C *in vacuo*, then loaded onto a silica gel column, and gradient eluted with 0-10% methanol/chloroform. This provided the alkenylated spiro-compound (60 mg; 55%) as an off-white powder: ¹H NMR (CDCl₃) δ 1.62 (s, 1H, piperidine H), 1.69 (s, 2H, superimposed piperidine H and piperidine NH as indicated by the absence of the NH peak upon D₂O addition), 2.08 (m, 4H, superimposed CH₂C=O and CH₂ of pentene moiety), 2.35-3.83 (m, 10H, superimposed piperidine H's and NCH₂ and alkene CH₂ of pentene moiety), 4.65 (s, 2H, NCH₂N), 6.10 (d, 1H, J = 15.5 Hz, HC=CHI), 6.49 (m, 1H, HC=CHI) 6.90-7.39 (m, 5H, aromatic H); ¹³C NMR (CDCl₃) δ 26.5, 29.7, 33.5, 40.3, and 42.1 (piperidine C and CH₂'s, and alkyl CH₂'s), 60.6 and 63.7 (lactam NCH₂ and NCH₂N, respectively), 75.5 (C=CI), 115.3, 119.5, 129.6, 142.9 (aromatic C's), 144.9 (C=CI), 173.8 (C=O). Anal. Calcd: C, 50.81; H, 5.69; N, 9.88; Found: C, 51.02; H, 5.56; N, 9.72.

Methyl stearolate (6)

A mixture of methanol (200 mL), stearic acid (10.0 g; 36.3 mmol), and concentrated sulfuric acid (1 mL) were refluxed overnight. Ethyl acetate (200 mL) was added, followed by the dropwise (with stirring) addition of saturated aqueous NaHCO₃ until the evolution of gas ceased. The organic layer was isolated and the

aqueous phase extracted once more with ethyl acetate (100 mL). The organic fractions were combined, dried (MgSO₄), and evaporated to yield an oil (8.62 g; 79.1%) of sufficient purity for subsequent reactions: ¹H NMR (CDCl₃) δ 0.88 (t, 3H, CH₃), 1.26 (m, 22H, alkyl), 2.22 (m, 6H, superimposed 3 X CH₂'s), 3.69 (s, 3H, OCH₃); ¹³C NMR (CDCl₃) δ 51.29 (OCH₃), 79.97 and 80.22 (alkyne C's), 174.07 (carbonyl C).

Methyl (*E*)-9(10)-dihydroxyborostearate (7)

The compound was prepared according to the literature procedure of Kabalka et. al.¹¹¹

Methyl (*E*)-9(10)-iodostearate (8)

The compound was prepared according to the literature procedure of Kabalka et. al.¹¹¹

(*E*)-9(10)-iodostearic acid (9)

The compound was prepared according to the literature procedure of Kabalka et. al.¹¹¹

(*E*)-4-Chloro-5-iodo-4-octene (10)

To a mixture of iodine monochloride (2.63 g; 16.2 mmol) and THF (20 mL) was added 4-octyne (1.00 g; 9.00 mmol). The mixture was refluxed under argon for 2 h. After cooling, the mixture was partitioned between water (50 mL) and hexane (75 mL). Sodium bisulfite was added until the color faded then the organic layer was collected, dried (MgSO₄), filtered, and the solvent evaporated. The resulting dark oil was loaded onto a silica gel column and eluted with hexane

to provide a clear oil (1.86 g; 76.1%) which turns pink upon exposure to the atmosphere: ^1H NMR (CDCl_3) δ 0.95 (skewed triplets, 6H, CH_3 's), 1.59 (m, 4H, CH_2 's), 2.64 (t, 4H, alkene, CH_2 's); ^{13}C NMR (CDCl_3) δ 12.9 and 13.1 (CH_3 's), 20.7 and 22.0 (CH_2 's), 99.2 (C), 131.7 (C-Cl).

(E)-1(2)-Chloro-2(1)-iodo-1-pentene (11) (12)

To a solution of iodine monochloride (4.38 g; 27.0 mmol) in THF (8 mL) was added 1-pentyne (1.00 g; 14.7 mmol). The mixture was stirred at 40 °C under argon for 2 h. After cooling, the reaction mixture was partitioned between pet. ether (30 mL) and water (10 mL) while sodium bisulfite was added with stirring until the color faded. The organic phase was collected, dried (MgSO_4), filtered, and reduced to yield a dark orange oil. The oil was loaded onto a silica gel column and eluted with pet. ether. This yielded a clear oil (2.31 g; 68.6%) which was a mixture of the two regioisomers. The isomers were resolved by fractional distillation under vacuum to yield an M/A (11/12) regioisomeric pair: b.p. (M isomer, 11) = 43-44 °C (0.7 mm Hg); ^1H NMR (CDCl_3) δ 0.96 (t, 3H, CH_2CH_3), 1.62 (sextet, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2$), 2.50 (t, 3H, alkene CH_2), 6.30 (s, 1H, CH-I); ^{13}C NMR (CDCl_3) δ 12.95 (CH_2CH_3), 21.51 ($\text{CH}_3\text{CH}_2\text{CH}_2$), 46.16 (alkene CH_2), 72.62 (CH-I), 137.66 (C-Cl); b.p. (A isomer, 12) = 73-74 °C (0.7 mm Hg); ^1H NMR (CDCl_3) δ 0.94 (t, 3H, CH_2CH_3), 1.59 (sextet, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2$), 2.44 (t, 2H, alkene CH_2), 6.83 (s, 1H, CH-Cl); ^{13}C NMR (CDCl_3) δ 12.65 (CH_2CH_3), 19.96 ($\text{CH}_3\text{CH}_2\text{CH}_2$), 40.06 (alkene CH_2), 103.96 (C-I), 118.78 (CH-Cl). Anal. Calcd: C, 26.06; H, 3.50. Found: C, 25.75; H, 3.46.

Propargylamine (13a)

Propargylamine hydrochloride was purchased from a commercial supplier. The free base was obtained by partitioning the hydrochloride salt between 1N sodium hydroxide and ether. The ether layer was collected, dried (MgSO₄), and evaporated to provide the free base as an oil.

N-(3-Butyn-1-yl)-4-phenylpiperidine (13b)

The alkynylating agent 4-tosyl-1-butyne (1.0 g; 4.5 mmol), 4-phenylpiperidine (1.4 g; 8.9 mmol), and THF (10 mL) were mixed and heated to 60 °C in a capped reaction vial for overnight (crystals begin to form after 10 min). After cooling, 1 N NaOH (5 mL) and chloroform (10 mL) were added with stirring. The aqueous layer was extracted once more with chloroform (10 mL) then the organics were combined, dried (MgSO₄), filtered, and evaporated to yield a residue which was loaded onto a silica gel column and eluted with chloroform. This provided the product (0.71 g; 75%) as a clear oil: ¹H NMR (CDCl₃) δ 1.79 (m, 4H, piperidine), 1.96 (t, 1H, J = 2 Hz, alkyne H), 2.20-2.75 (m, 6H, piperidine and alkyl H), 2.98 (d, br, 2H, piperidine H), 7.23 (s, 5H, aromatic); ¹³C NMR (CDCl₃) δ 16.9, 33.3, 42.5, 53.9, 57.3 (piperidine and alkyl C), 69.0 (alkyne CH), 82.8 (alkyne C), 126.1, 126.7, 128.4 (aromatic CH's), 146.2 (aromatic C). Anal. Calcd: C, 83.05; H, 9.01; N, 6.87. Found: C, 82.99; H, 8.77; N, 6.77.

N-(4-Pentyn-1-yl)-4-phenylpiperidine (13c)

A mixture of 4-phenylpiperidine (3.0 g; 19 mmol), 5-chloropentyne (2.0 g; 19 mmol), THF (20 mL), and triethylamine (2.1 mL) were heated at reflux overnight. The resulting mixture was partitioned between chloroform (25 mL) and 1 N NaOH (20 mL). The organic fraction was collected, while the aqueous phase extracted once more. The extractions were combined, dried (MgSO₄),

filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and eluted with chloroform. This provided the pentynylated amine (1.4 g; 32%) as a clear viscous oil: ^1H NMR (CDCl_3) δ 1.62-2.55 (bm, 16H, piperidine, alkyl and alkyne H), 2.98 (bd, 2H, piperidine H), 7.20 (s, 5H, aromatic CH); ^{13}C NMR (CDCl_3) δ 16.5, 26.0, 33.5, 42.7, 54.3, 57.7 (piperidine and alkyl C), 68.4 (alkyne CH), 84.1 (alkyne C), 126.0, 126.6, 128.3 (aromatic CH 's), 146.3 (aromatic C). Anal. Calcd: C, 83.86; H, 9.33; N, 6.18. Found: C, 83.76; H, 9.26; N, 5.79.

N-Methyl-N-(4-pentyn-1-yl)-4-phenylpiperidinium iodide (13d)

A mixture of N-(4-pentyn-1-yl)-4-phenylpiperidine (0.20 g; 0.88 mmol), methyl iodide (0.50 g; 3.5 mmol), and ether (5 mL) were stirred at room temperature overnight. The mixture was evaporated *in vacuo* and the residue dissolved in chloroform (50 mL). A drying agent (MgSO_4) was added to assure dryness and the mixture was filtered. The filtrate was evaporated to dryness and the resulting residue was suspended in ether (50 mL). The suspension was filtered and the residue washed several times with ether to provide the ammonium salt (0.19 g; 62%) as a white powder: ^1H NMR (DMSO-d_6) δ 1.70-3.05 (bm, 9H, piperidine, and alkyl H), 3.13 (t, 1H, alkyne H), 3.45 (bm, 9H, CH 's attached to N), 7.35 (s, 5H, aromatic CH); ^{13}C NMR (DMSO-d_6) δ 18.5, 28.5, 37.4, 47.7, 59.6, 59.9, 63.8 (piperidine and alkyl C), 69.4 (alkyne CH), 83.1 (alkyne C), 125.8, 126.4, 129.4 (aromatic CH 's), 146.1 (aromatic C).

N-((*E*)-2(3)-Chloro-3(2)-iodo-2-propen-1-yl)amine (14a)

Propargylamine hydrochloride (1.0 g; 11 mmol) was added to a mixture of iodine monochloride (2.7 g; 16 mmol) and THF (40 mL). The reaction mixture was

refluxed with stirring under argon for 2 h. After cooling, the mixture was partitioned between chloroform (40 mL) and 1 N NaOH (40 mL). Sodium thiosulfate was added until the color faded. The organic layer was isolated and the aqueous layer extracted once more with chloroform (20 mL) after saturation with NaCl. The organic fractions were combined, dried (MgSO₄), filtered, and the solvent evaporated to yield a dark colored oil which was loaded onto a silica gel column and eluted with chloroform. This provided a relatively poor yield (0.50 g; 21%) of a clear oil which decomposed readily to form a reddish-brown oil: ¹H NMR (CDCl₃) δ 1.61 (s, 2H, NH₂), 3.53 (s, 1.6H, CH₂ of A isomer), 3.62 (s, 0.4H, CH₂ of M isomer), 6.38 (s, 0.2H, vinyl H of M isomer), 6.50 (s, 0.8H, vinyl H of A isomer); ¹³C NMR (CDCl₃) δ 48.2 (t, CH₂ of A isomer), 50.1 (t, CH₂ of M isomer), 73.3 (d, CHI of M isomer), 107.4 (s, CI of A isomer), 121.1 (d, CHCl of A isomer), 138.1 (s, CCl of M isomer); MS m/e (M⁺, rel int) 217 (³⁵Cl) (100).

N-((*E*)-3(4)-Chloro-4(3)-iodo-3-buten-1-yl)-4-phenylpiperidine (14b)

To a solution of iodine monochloride (0.29 g; 1.8 mmol) and THF (6 mL) was added N-(3-butyne-1-yl)-4-phenylpiperidine hydrochloride (0.25 g; 1.0 mmol). The resulting mixture was heated to 60 °C under argon for 2 h in the absence of light. After cooling, the mixture was partitioned between 1 N NaOH (10 mL) and chloroform (20 mL). Sodium thiosulfate was added with stirring until the color faded. The aqueous layer was extracted twice with chloroform (20 mL) after saturation with NaCl. The organic layers were combined, dried (MgSO₄), filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and eluted with chloroform. This provided a pure mixture of both A and M isomers (0.27 g; 71%) as an oil, which could not be resolved by chromatography: ¹H NMR (CDCl₃) δ 1.65-3.15 (bm, 13H, piperidine and alkyl H),

6.32 (s, 0.3H, CHI), 6.49 (s, 0.7H, CHCl), 7.25 (s, 5H, aromatic H); ^{13}C NMR (CDCl_3), alkyl resonances overlap, δ 73.8 (d, CHI), 99.9 (s, CI), 120.0 (d, CHCl), 125.5, 127.8, 128.9 (d, aromatic CH), 136.1 (s, CCl), 146.5 (s, aromatic C); MS m/e (M^+ , rel int) 375 (^{35}Cl) (43).

N-((*E*)-4(5)-Chloro-5(4)-iodo-4-penten-1-yl)-4-phenylpiperidine (14c)

The amine hydrochloride, N-(4-pentyn-1-yl)-4-phenylpiperidine hydrochloride (0.60 g; 2.3 mmol), was added to a mixture of iodine monochloride (0.66 g; 4.1 mmol) and THF (15 mL). The reaction mixture was refluxed under argon for 2 h in the absence of light. After cooling, the reaction mixture was partitioned between 1 N NaOH (10 mL), water (40 mL), and chloroform (50 mL). Sodium thiosulfate was added with stirring until the color faded. The organic phase was collected and washed twice with water (30 mL). The organic fraction was dried (MgSO_4), filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and eluted with chloroform. This provided the desired product as a pure inseparable mixture of A and M isomers (0.64 g; 72%) as a viscous clear oil: ^1H NMR (CDCl_3) δ 1.65-3.15 (bm, 15H, piperidine and alkyl H), 6.29 (s, 0.7H, CHI), 6.46 (s, 0.3H, CHCl), 7.25 (s, 5H, aromatic H); ^{13}C NMR (CDCl_3), alkyl resonances overlap, δ 72.6 (d, CHI), 102.0 (s, CI), 118.9 (d, CHCl), 125.9, 126.8, 128.2 (d, aromatic CH), 137.7 (s, CCl), 146.0 (s, aromatic C); MS m/e (M^+ , rel int) 389 (^{35}Cl) (30).

N-Methyl-N-((*E*)-4(5)-Chloro-5(4)-iodo-4-penten-1-yl)-4-phenylpiperidinium iodide (14d)

The ammonium salt, N-methyl-N-(4-pentyn-1-yl)-4-phenylpiperidinium

iodide, (96 mg; 0.26 mmol) was added to a solution of iodine monochloride (51 mg; 0.31 mmol) in dry THF (2 mL). The mixture was refluxed under argon for 2 hours in the absence of light then partitioned between chloroform (50 mL) and water (30 mL). Sodium thiosulfate was added until the color faded, and the organic layer was collected, dried (MgSO₄), filtered, and the solvent evaporated. This provided the chloriodinated ammonium salt (54 mg; 37%) in the same regioisomeric ratio as the demethylated hydrochloride salt: ¹H NMR (DMSO-d₆) δ 1.25-3.15 (bm, 9H, piperidine and alkyl H), 3.29-3.99 (bm, 9H, CH's attached to N), 6.45 (s, 0.7H, CHI), 6.55 (s, 0.3H, CHCl), 7.25 (s, 5H, aromatic H); ¹³C NMR (DMSO-d₆), alkyl resonances overlap, δ 82.9 (CHI), 101.0 (Cl), 121.2 (CHCl), 125.7, 126.7, 128.3 (aromatic CH), 134.9 (C₂Cl), 147.0 (aromatic C).

Methyl (*E*)-9(10)-chloro-10(9)-iodostearate (15)

Methyl stearolate (0.25 g; 0.85 mmol) was added to a mixture of iodine monochloride (0.28 g; 1.7 mmol) and THF (7 mL). The mixture was brought to reflux with stirring under argon for 3 h. The reaction mixture was partitioned between ether (50 mL) and water (20 mL). Sodium bisulfite was added until the color faded. The organic layer was collected, dried (MgSO₄), filtered, and the solvent evaporated to yield a dark colored oil which was loaded onto a silica gel (30 g) column and gradient eluted with 0-1% ether/pet. ether. This provided the title compound as a clear oil (0.26 g; 68%) which discolors upon contact with the atmosphere: ¹H NMR (CDCl₃) δ 0.89 (t, 3H, CH₃), 1.30 (m, 22H, alkyl), 2.32 (t, 2H, CH₂COOCH₃), 2.64 (t, 4H, alkene CH₂'s), 3.67 (s, 3H, OCH₃); ¹³C NMR (CDCl₃) δ 51.50 (OCH₃), 99.09 and 99.42 (alkene C-I), 131.46 and 131.73 (alkene C-Cl), 174.29 (carbonyl C). Anal. Calcd: C, 49.95; H, 7.50. Found: C, 49.88; H, 7.53.

(*E*)-9(10)-Chloro-10(9)-iodostearic acid (16)

The iodochlorinated ester, methyl 9(10)-chloro-10(9)-iodostearate, (0.20 g; 0.44 mmol) was added to a mixture of 1 *N* NaOH (2 mL; 2 mmol) and methanol (4 mL). The mixture was brought to reflux for 35 min. The mixture was then acidified by the dropwise addition of 1 *N* HCl (3 mL; 3 mmol) followed by extraction with two portions of ethyl acetate (20 mL). The organic fractions were combined, dried (MgSO₄), filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and eluted with 1% methanol/chloroform. This provided the iodochlorinated carboxylic acid as an oil (0.12 g; 61%): ¹H NMR (CDCl₃) δ 0.83 (t, 3H, CH₃), 1.23 (m, 22H, alkyl), 2.30 (t, 2H, CH₂COOCH₃), 2.58 (t, 4H, alkene CH₂'s), 9.05 (broad s, carboxylic acid H); ¹³C NMR (CDCl₃) δ 98.99 and 99.37 (alkene C-I), 131.36 and 131.66 (alkene C-Cl), 180.88 (carbonyl C). Anal. Calcd: C, 48.82; H, 7.28. Found: C, 49.13; H, 7.61.

Methyl α-methylstearolate (17)

Anhydrous THF (10 mL) was chilled in a dry-ice acetone bath under argon followed by the addition of lithium diisopropylamide, LDA, (6.4 mL; 1.1 *M* soln.) in hexane. Methyl stearolate (2.0 g; 6.8 mmol) in anhydrous THF (5 mL) was added dropwise at a rate sufficient to maintain the reduced temperature. After this addition, the mixture was stirred for 10 min. Methyl iodide (1.2 g; 8.2 mmol) in anhydrous THF (2mL) was added dropwise with stirring. After the addition, the mixture was allowed to warm to room temperature and stirred overnight. The resulting mixture was partitioned between ethyl acetate (50 mL) and 1 *N* HCl (20 mL). The organic layer was isolated, dried (MgSO₄), filtered, and the solvent evaporated to yield an oily residue which was loaded onto a silica gel column and eluted with 1.5% ether/hexane. This provided the title compound as

a clear oil (1.3 g; 61%): ^1H NMR (CDCl_3) δ 0.85 (t, 3H, CH_2CH_3), 1.15 (d, 3H, CHCH_3), 1.29 (m, 26H, alkyl H), 2.12 (distorted t, 4H, alkene CH_2 's), 2.39 (m, 1 H, CHCH_3), 3.65 (s, 3H, OCH_3); ^{13}C NMR (CDCl_3) δ 39.9 (CHCH_3), 51.5 (OCH_3), 80.0 and 80.2 (alkyne C's), 177.1 (carbonyl C). Anal. Calcd: C, 71.85; H, 10.28. Found: C, 72.07; H, 10.51.

Methyl (*E*)-9(10)-chloro-10(9)-iodo-2-methylstearate (18)

Methyl α -methylstearolate (0.60 g; 2.0 mmol) was added to a solution of dry THF (10 mL) containing iodine monochloride (0.57 g; 3.5 mmol) under argon and shielded from light. The mixture was refluxed for 2 h, cooled, and then partitioned between ethyl acetate (20 mL) and water (10 mL). Sodium bisulfite was added with stirring until the color faded. The organic layer was isolated, dried (MgSO_4), filtered, and the solvent evaporated to yield a dark oily residue which was loaded onto a silica gel column and eluted with chloroform. This provided the chloriodinated product as an oil (0.57 g; 62%): ^1H NMR (CDCl_3) δ 0.89 (t, 3H, CH_2CH_3), 1.15 (d, 3H, CHCH_3), 1.31 (m, 26H, alkyl H), 2.40 (m, 1 H, CHCH_3), 2.65 (t, 4H, alkene CH_2 's), 3.64 (s, 3H, OCH_3); ^{13}C NMR (CDCl_3) δ 51.3 (OCH_3), 99.0 and 99.2 (alkene C-I), 131.0 and 131.2 (alkene C-Cl) 176.9 (carbonyl C). Anal. Calcd: C, 50.79; H, 8.15. Found: C, 51.10; H, 8.37.

(*E*)-9(10)-Chloro-10(9)-iodo-2-methylstearic acid (19)

The chloriodinated ester, methyl (*E*)-9(10)-chloro-10(9)-iodo-2-methylstearate, (0.45 g; 0.96 mmol) was added to a solution of 1 N NaOH (4 mL; 4 mmol) and methanol (8 mL). The mixture was refluxed for 2 h. After cooling, 1 N HCl (6 mL; 6 mmol) was added followed by extraction with ethyl acetate (20 mL). The organic layer was isolated and the aqueous phase extracted once more with

with ethyl acetate (10 mL). The organics were combined, dried (MgSO₄), filtered, and the solvent evaporated. The resulting residue was loaded onto a silica gel preparatory plate (1000 u) and developed with 6% methanol/chloroform. This provided the free acid as an oil (0.23 g; 52%) which discolored upon contact with the atmosphere: ¹H NMR (CDCl₃) δ 0.83 (t, 3H, CH₂CH₃), 1.23 (d, 3H, CHCH₃), 1.32 (m, 26H, alkyl H), 2.42 (m, 1 H, CHCH₃), 2.63 (t, 4H, alkene CH₂'s), 9.12 (broad s, carboxylic acid H); ¹³C NMR (CDCl₃) δ 98.9 and 99.1 (alkene C-I), 130.6 and 130.9 (alkene C-Cl) 179.9 (carbonyl C). Anal. Calcd: C, 46.71; H, 6.17. Found: C, 47.02; H, 6.50.

1,1'-((*E*)-2-Chloro-3-iodo-2-buten-1,4-diyl)dipyrrolidine (20)

To a solution of iodine monochloride (4.38 g; 27.0 mmol) in THF (8 mL) was added tremorine dihydrochloride (3.89 g; 14.7 mmol). The mixture was stirred at reflux under argon for 2 h. The mixture was then partitioned between ether (40 mL) and 1 N NaOH (45 mL). Sodium sulfite was added until the color faded. The organic layer was collected and the aqueous layer extracted once more with ether (40 mL) after saturation with sodium chloride. The organic fractions were combined, dried (MgSO₄), filtered, and the solvent evaporated to provide a dark colored oil. The oily residue was loaded onto a silica gel column and eluted with 10% methanol/chloroform. This yielded the title compound (3.47 g; 72.7%) as a clear oil: ¹H NMR (CDCl₃) δ 1.80 and 2.61 (m, br, 16H, pyrrolidine H), 3.57 (s, 2H, CH₂Cl), 3.73 (s, 2H, CH₂CCl); ¹³C NMR (CDCl₃) δ 23.85, 23.93, 53.59, 53.75 (t, nonequivalent pyrrolidine rings), 63.4 (t, CH₂Cl), 65.6 (t, CH₂CCl), 100.6 (s, Cl), 130.6 (s, CCl). Anal. Calcd: C, 40.64; H, 5.68; N, 7.90. Found: C, 40.86; H, 5.77; N, 7.67.

1,4-Bis(4-phenylpiperidiny1)-2-butyne (21)

To a solution of 4-phenylpiperidine (0.52 g; 3.3 mmol) in methanol (6 mL) was added 1,4-dichloro-2-butyne (0.10 g; 0.81 mmol). The mixture was heated at 60 °C for 2 h. The reaction mixture was reduced in vacuo and partitioned between 1 N NaOH (5 mL) and ether (15 mL). The organic fraction was collected and the aqueous phase saturated with NaCl followed by one more extraction with ether (15 mL). The organic fractions were combined, dried (MgSO₄), filtered, and the solvent evaporated. The residue was loaded onto a silica gel column and eluted with 4% methanol/chloroform. This provided the substituted butyne (0.20 g; 67%) as a viscous oil: ¹H NMR (CDCl₃) δ 1.84 (m, br, 8H, piperidine H's), 2.10-2.62 (m, br, 6H, piperidine H's), 2.99 (d, br, 4H, piperidine H's), 3.35 (s, 4H, 2 X CH₂C), 7.20 (s, 10H, aromatic H's); ¹³C NMR (CDCl₃) δ 33.3, 42.2, 47.6, 53.2 (piperidine C's and 1,4-C's), 80.1 (alkyne C's), 126.1, 126.7, 128.3, 146.1 (aromatic C's). Anal. Calcd: C, 83.02; H, 8.68. Found: C, 82.78; H, 8.52.

1,1'-((*E*)-2-Chloro-3-iodo-2-buten-1,4-diyl)bis(4-phenylpiperidine) (22)

To a solution of iodine monochloride (0.10 g; 0.62 mmol) in THF (2 mL) was added 1,4-bis(4-phenylpiperidiny1)-2-butyne dihydrochloride (0.15 g; 0.34 mmol) and the mixture refluxed for 2 h under argon. The mixture was partitioned between 1 N NaOH (10 mL) and chloroform (10 mL). Sodium bisulfite was added until the color faded then the organic phase was collected. After saturation with NaCl, the aqueous phase was extracted once more with chloroform (10 mL). The organic layers were combined, dried (MgSO₄), filtered, and the solvent evaporated to yield an oil which was purified by silica gel column chromatography eluted with chloroform. This provided the iodochlorinated alkene (0.10 g; 70%) as a clear oil: ¹H NMR (CDCl₃) δ 1.65-3.16 (m, 18H,

piperidine), 3.44 (s, 2H, CH_2Cl), 3.67 (s, 2H, CH_2CCl), 7.23 (s, 10H, aromatic); ^{13}C NMR (CDCl_3) δ 33.5 (t, equivalent C-3, C-5, and C'-3, C'-5 of piperidine rings), 42.7 (d, equivalent C-4 of piperidine rings), 53.8 and 54.0 (t, nonequivalent C-2, C-6, and C'-2, C'-6 of piperidine rings), 65.6 (t, CH_2Cl), 68.5 (t, CH_2CCl), 103.0 (s, Cl), 126.2 (d, aromatic), 127.0 (d, aromatic), 128.5 (d, aromatic), 130.7 (s, CCl), 146.5 and 146.6 (s, nonequivalent C-1 and C'-1 of aromatic rings). Anal. Calcd.: C, 58.38; H, 6.03; N, 5.24. Found: C, 58.15; H, 6.15; N, 5.04.

N-Methyl-N-benzyl-N-((*E*)-2(3)-chloro-3(2)-iodo-2-propen-1-yl)amine (23)

To a solution of iodine monochloride (4.38 g; 27.0 mmol) and THF (8 mL) was added N-benzyl-N-methylpropargylamine (pargyline) hydrochloride (2.87 g; 14.7 mmol). The mixture was refluxed with stirring under argon for 2h. After cooling, the reaction mixture was partitioned between 1 N NaOH (45 mL) and ether (40 mL) followed by the addition of sodium thiosulfate until the color faded. The ether layer was collected and the aqueous phase extracted once more with ether (40 mL) after saturation with NaCl. The organic fractions were combined, dried (MgSO_4), filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and eluted with chloroform. This yielded a pure mixture of A and M isomers (4.56 g; 97.6%) in a ratio of 4:1, respectively, as an oil. The isomers (300 mg) were resolved by preparatory TLC using a silica gel plate (2000 μ) eluted with chloroform. The isomers were visualized with an ultraviolet lamp, scraped, and extracted with ether to provide each individual isomer: M isomer, oil; ^1H NMR (CDCl_3) δ 2.28 (s, 3H, CH_3), 3.45 (s, 2H, PhCH_2), 3.59 (s, 2H, CH_2CCl), 6.54 (s, 1H, CHI), 7.31 (s, 5H, Ar); ^{13}C NMR (CDCl_3) δ 41.5 (q, CH_3), 61.0 (t, CH_2CCl), 61.9 (t, PhCH_2), 74.4 (d, , CHI), 126.5, 127.5, 128.5 (d, aromatic C's), 135.0 (s, CCl), 138.5 (s, aromatic C); MS m/e (M^+ , rel int) 321 (^{35}Cl) (6.3); A

isomer, oil; ^1H NMR (CDCl_3) δ 2.20 (s, 3H, CH_3), 3.21 (s, 2H, CH_2Cl), 3.53 (s, 2H, PhCH_2), 6.63 (s, 1H, CHCl), 7.31 (m, 5H, aromatic); ^{13}C NMR (CDCl_3) δ 41.6 (q, CH_3), 60.0 (t, CH_2Cl), 61.2 (t, PhCH_2), 102.7 (s, Cl), 120.9 (d, CHCl), 127.1, 128.2, 129.0 (d, aromatic C's), 138.6 (s, aromatic C); MS m/e (M^+ , rel int) 321 (^{35}Cl) (6.2). Anal. Calcd: C, 36.90; H, 3.94; N, 3.91. Found (A and M mixture): C, 36.73; H, 4.15; N, 3.90.

1-Phenyl-8-(4'-fluorobutyrophenon-1-yl)-1,3,8-triazaspiro[4.5]decan-4-one
"Pseudospiperone" (24)

To a solution of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (1.8 g; 7.8 mmol) in dry DMF (50 mL) cooled to $-30\text{ }^\circ\text{C}$ under argon was added a 60% suspension of sodium hydride (0.32 g; 8.0 mmol) with stirring. After the evolution of hydrogen ceased (approximately 1 h), 4-chloro-4'-fluorobutyrophenone ethylene ketal (2.0 g; 7.8 mmol) in dry DMF (3 mL) was added dropwise followed by the addition of hexamethylphosphorotriamide (1.4 g; 7.8 mmol). The mixture was stirred for 30 min, allowed to warm to room temperature for 2 h, and then heated to $60\text{ }^\circ\text{C}$ for 1 h. The mixture was partitioned between saturated aqueous sodium bicarbonate (50 mL) and chloroform (50 mL). The organic layer was isolated and the aqueous layer was extracted once more with chloroform (50 mL). The organic layers were combined, dried (MgSO_4), filtered, and evaporated to yield a viscous oil. The oil was dissolved and refluxed for 1 h in a mixture of methanol (40 mL) and 1 N HCl (30 mL). After cooling, chloroform (60 mL) was added followed by the addition of sodium bicarbonate until gas evolution ceased. The organic layer was isolated and the aqueous phase extracted once more with chloroform (50 mL). The organic fractions were isolated, combined, dried (MgSO_4), filtered, and the solvent

evaporated to yield an oil which was loaded onto a silica gel column and was gradient eluted with 0-15 % methanol/chloroform. This provided the title compound (1.5 g; 47%) as beige-colored crystals: mp 76-78 °C; ^1H NMR (CDCl_3) δ 1.50 (s, 1H, piperidine H), 1.64 (s, 2H, superimposed piperidine H and piperidine NH as indicated by the absence of the NH peak upon D_2O addition), 2.17 (t, 2H, $\text{CH}_2\text{C=O}$), 2.25-3.34 (m, 8H, superimposed piperidine H 's and butyrophenone CH_2), 3.46 (t, 2H, lactam NCH_2), 4.73 (s, 2H, NCH_2N), 6.86-7.37 (m, 7H, aromatic H), 7.96 (m, 2H, aromatic CH 's ortho to aromatic CF); ^{13}C NMR (CDCl_3) δ 21.3, 28.7, 34.9, 40.2, and 41.4 (piperidine C and CH_2 's, and C-2 and C-3 of butyrophenone), 59.9 and 63.1 (lactam NCH_2 and NCH_2N , respectively), 115.5, 119.0, 129.2, 142.8 (aromatic C 's of spiro moiety), 116.2 (d, $J=18$ Hz, aromatic CH 's ortho to aromatic CF), 130.4 (d, $J=9$ Hz, aromatic CH 's meta to aromatic CF), 133.0 (d, $J=2$ Hz, aromatic C para to aromatic CF), 165.7 (d, $J=256$ Hz, aromatic CF), 174.1 (C=O of spiro moiety), 197.1 (C=O of p-fluorophenone moiety). Anal. Calcd: C, 69.85; H, 6.63; N, 10.63; F, 4.80; Found: C, 69.46; H, 6.76; N, 10.34; F, 4.69.

N-Propargylpseudospiperone (25)

A mixture of 1-phenyl-3-[1-(4'-fluorobutyrophenone)]-1,3,8-triazaspiro-[4.5]decan-4-one, pseudospiperone, (0.24 g; 0.65 mmol), an 80% solution of propargyl bromide in toluene (0.15 mL; 1.0 mmol), THF (15 mL), and triethylamine (1 mL) were stirred at room temperature overnight with stirring. The resulting mixture was partitioned between 1 N NaOH (5 mL) and chloroform (10 mL). The organic phase was isolated and the aqueous phase was extracted once more with chloroform (15 mL). The organic fractions were combined, dried (MgSO_4), filtered, and the solvent evaporated to yield an oil which was loaded onto

a silica gel column and gradient eluted with 0-10% methanol/chloroform. This provided the propargylated spiro compound (0.23 g; 85%) as a gum which solidified into an amorphous powder upon standing: ^1H NMR (CDCl_3) δ 1.53 (s, 1H, piperidine H), 1.68 (s, 1H, piperidine H), 2.08 (t, 2H, $\text{CH}_2\text{C}=\text{O}$), 2.27 (t, 1H, $J=2$ Hz alkyne H), 2.32-3.20 (m, 8H, superimposed piperidine H 's and butyrophenone CH_2), 3.29 (d, 2H, $J=2$ Hz, alkyne CH_2), 3.46 (t, 2H, lactam NCH_2), 4.71 (s, 2H, NCH_2N), 6.75-7.35 (m, 7H, aromatic H 's), 7.95 (m, 2H, aromatic CH 's ortho to aromatic CF); ^{13}C NMR (CDCl_3) δ 21.6, 29.3, 35.3, 40.4, 47.8, and 48.6 (piperidine C and CH_2 's, $\text{C}-2$ and $\text{C}-3$ of butyrophenone, and piperidine NCH_2CCH), 60.1 and 63.3 (lactam NCH_2 and NCH_2N , respectively), 72.9 (alkyne external CH), 79.3 (alkyne internal C), 115.6, 119.0, 129.1, 142.8 (aromatic C 's of spiro moiety), 115.5 (d, $J=18$ Hz, aromatic CH 's ortho to aromatic CF), 130.5 (d, $J=9$ Hz, aromatic CH 's meta to aromatic CF), 133.1 (d, $J=2$ Hz, aromatic C para to aromatic CF), 165.6 (d, $J=256$ Hz, aromatic CF), 174.3 ($\text{C}=\text{O}$ of spiro moiety), 197.2 ($\text{C}=\text{O}$ of p-fluorophenone moiety). Anal. Calcd: C, 72.03; H, 6.51; N, 9.69; F, 4.38; Found: C, 71.74; H, 6.47; N, 9.66; F, 4.68.

N-[(*E*)-2(3)-Chloro-3(2)-iodo-2-propen-1-yl]pseudospiperone (26)

To a mixture of THF (20 mL) and iodine monochloride (0.19 g; 1.2 mmol) was added *N*-propargylpseudospiperone hydrochloride (0.31 g; 0.66 mmol) and the resulting mixture was heated at reflux with stirring under argon in the absence of light for 45 minutes. After cooling, the mixture was partitioned between 1 *N* NaOH (10 mL) and ethyl ether (20 mL). Sodium thiosulfate was added with stirring until the color faded. The organic layer was isolated and the aqueous phase extracted twice more with ether (20 mL) after saturating the aqueous phase with sodium chloride. The organic fractions were combined, dried (MgSO_4),

filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and gradient eluted with 0-15% methanol/chloroform. This provided a mixture of the two isomers of the iodochlorinated alkene (0.17 g; 42%) as a tan colored oil which discolors rapidly, compared to similar compounds, upon exposure to the atmosphere. The isomers were inseparable using ordinary laboratory techniques and were therefore analyzed as a mixture: ^1H NMR (CDCl_3) δ 1.29 (s, 1H, piperidine H), 1.58 (s, 1H, piperidine H), 2.05 (t, 2H, $\text{CH}_2\text{C}=\text{O}$), 2.45-3.35 (m, 8H, superimposed piperidine H's and butyrophenone CH_2), 3.52 (t, 2H, lactam NCH_2), 3.71 (bs, 2H, propenyl CH_2), 4.69 (s, 2H, NCH_2N), 6.61 and 6.71 (s, 1H, vinylic H of terminal iodide and chloride, respectively), 6.95-7.35 and 7.41-7.58 (m, 7H, aromatic H's), 7.95 (m, 2H, aromatic CH 's ortho to aromatic CF); ^{13}C NMR (CDCl_3) δ 21.6, 25.6, 26.8, 28.8, 29.1, 30.3, 31.2, 35.2, 35.4, 38.7, 40.6, 44.6, and 48.9 (piperidine C and CH_2 's, C-2 and C-3 of butyrophenone, and piperidine NCH_2CCH), 59.8, 60.0, and 63.2, 63.3 (lactam NCH_2 and NCH_2N , respectively, both isomers), 79.8 and 101.9 (external and internal alkene Cl, respectively), 115.3, 115.6, 118.5, 119.0, 129.1, 129.3, 142.3, 142.8 (aromatic C's of spiro moiety, both isomers), 115.2 and 115.6 (d (two sets), $J=18$ Hz, aromatic CH 's ortho to aromatic CF, both isomers), 121.6 and 133.2 (external and internal alkene Cl, respectively), 130.5 and 130.9 (d (two sets), $J=9$ Hz, aromatic CH 's meta to aromatic CF, both isomers), 132.8 and 133.1 (d (two sets), $J=2$ Hz, aromatic C para to aromatic CF, both isomers), 165.2 and 165.6 (d (two sets), $J=256$ Hz, aromatic CF, both isomers), 174.5 and 174.8 ($\text{C}=\text{O}$ of spiro moiety, both isomers), 197.3 ($\text{C}=\text{O}$ of p-fluorophenone moiety). Anal. Calcd: C, 52.34; H, 4.73; N, 7.05; F, 3.19 Found: C, 51.93; H, 4.69; N, 6.79; F, 2.71.

3-Propargylspiroperidol (27a)

To a solution of spiroperidol (0.24 g; 0.60 mmol) in dry DMF (10 mL) cooled to -30 °C under argon was added a 60% suspension of sodium hydride (0.030 g; 0.80 mmol) with stirring. After the evolution of hydrogen ceased, approximately 30 min, an 80% solution of propargyl bromide in toluene (0.10 mL; 0.70 mmol) in dry DMF (3 mL) was added dropwise followed by the addition of hexamethylphosphorotriamide (0.14 g; 0.78 mmol). The mixture was stirred for 30 min, allowed to warm to room temperature for 2 h, and then heated to 60 °C for 1 h. The mixture was partitioned between saturated aqueous sodium bicarbonate (50 mL) and chloroform (50 mL). The organic layer was isolated and the aqueous layer was extracted once more with chloroform (50 mL). The organic layers were combined, dried (MgSO₄), filtered, and the solvent evaporated to yield a viscous oil which was loaded onto a silica gel column and gradient eluted with 0-15 % methanol/chloroform. This provided the title compound (0.15 g; 47%) as an amorphous beige-colored powder: ¹H NMR (CDCl₃) δ 1.50 (s, 1H, piperidine H), 1.62 (s, 1H, piperidine H), 2.11 (t, 2H, CH₂C=O), 2.32 (t, 1H, J=2 Hz alkyne H), 2.35-3.22 (m, 8H, superimposed piperidine H's and butyrophenone CH₂), 3.27 (d, 2H, J=2 Hz, alkyne CH₂), 3.44 (t, 2H, lactam NCH₂), 4.70 (s, 2H, NCH₂N), 6.74-7.34 (m, 7H, aromatic H's), 7.95 (m, 2H, aromatic CH's ortho to aromatic CF); ¹³C NMR (CDCl₃) δ 21.4, 29.1, 35.6, 40.9, 48.3, and 48.9 (piperidine C and CH₂'s, C-2 and C-3 of butyrophenone, and piperidine NCH₂CCH), 60.3 and 63.5 (lactam NCH₂ and NCH₂N, respectively), 73.2 (alkyne external CH), 78.8 (alkyne internal C), 115.4, 119.2, 128.8, 143.4 (aromatic C's of spiro moiety), 116.2 (d, J=18 Hz, aromatic CH's ortho to aromatic CF), 130.8 (d, J=9 Hz, aromatic CH's meta to aromatic CF), 133.0 (d, J=2 Hz, aromatic C para to aromatic CF), 165.8 (d, J=256 Hz, aromatic CF), 173.9 (C=O of spiro moiety), 196.9 (C=O of p-fluorophenone

moiety). Anal. Calcd: C, 72.03; H, 6.51; N, 9.69; F, 4.38; Found: C, 71.74; H, 6.47; N, 9.66; F, 4.68.

3-[(*E*)-2(3)-chloro-3(2)-iodo-2-propen-1-yl]spiropiperidol (27b)

To a mixture of THF (20 mL) and iodine monochloride (0.19 g; 1.2 mmol) was added 1-phenyl-3-propargyl-8-[3-(4'-fluorophenylcarbonyl)-1-propyl]-1,3,8-triazaspiro[4.5]decan-4-one, 3-propargylspiropiperidol, hydrochloride (0.31 g; 0.66 mmol) and the resulting mixture heated at reflux with stirring under argon in the absence of light for 45 minutes. After cooling, the mixture was partitioned between 1 N NaOH (10 mL) and ethyl ether (20 mL). Sodium thiosulfate was added with stirring until the color faded. The organic layer was isolated and the aqueous phase extracted twice more with ether (20 mL), after saturating the aqueous phase with sodium chloride. The organic fractions were combined, dried (MgSO₄), filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and gradient eluted with 0-15% methanol/chloroform. This provided an approximate 1:1 mixture of the two regioisomers of the chloriodinated alkene (0.33 g; 82%) as a brown colored oil which rapidly discolors, compared to similar compounds, upon exposure to the atmosphere. The regioisomers were inseparable using ordinary laboratory techniques and were therefore analyzed as a mixture: ¹H NMR (CDCl₃) δ 1.28 (s, 1H, piperidine H), 1.56 (s, 1H, piperidine H), 2.08 (t, 2H, CH₂C=O), 2.42-3.34 (m, 8H, superimposed piperidine H's and butyrophenone CH₂), 3.56 (t, 2H, lactam NCH₂), 3.69 (bs, 2H, propenyl CH₂), 4.71 (s, 2H, NCH₂N), 6.60 and 6.72 (s, 1H, vinylic H of terminal iodide and chloride, respectively), 6.93-7.34 and 7.41-7.59 (m, 7H, aromatic H's), 7.92 (m, 2H, aromatic CH's ortho to aromatic CF); ¹³C NMR (CDCl₃) δ 21.4, 25.2, 27.3, 29.1, 29.3, 30.3, 31.2, 35.4, 35.7, 38.9, 40.4, 44.2, and 48.6 (piperidine

$\underline{\text{C}}$ and $\underline{\text{CH}_2}$'s, $\underline{\text{C}}\text{-2}$ and $\underline{\text{C}}\text{-3}$ of butyrophenone, and piperidine $\text{N}\underline{\text{CH}_2}\text{CCH}$), 61.2, 61.4, and 63.1, 63.2 (lactam $\text{N}\underline{\text{CH}_2}$ and $\text{N}\underline{\text{CH}_2}\text{N}$, respectively, both isomers), 79.6 and 101.6 (external and internal alkene $\underline{\text{C}}\text{I}$, respectively), 114.8, 115.4, 118.8, 119.2, 129.0, 129.3, 141.8, 143.3 (aromatic $\underline{\text{C}}$'s of spiro moiety, both isomers), 115.6 and 115.9 (d (two sets), $J=17$ Hz, aromatic $\underline{\text{CH}}$'s ortho to aromatic CF, both isomers), 121.5 and 132.9 (external and internal alkene $\underline{\text{C}}\text{Cl}$, respectively), 131.1 and 131.2 (d (two sets), $J=10$ Hz, aromatic $\underline{\text{CH}}$'s meta to aromatic CF, both isomers), 132.7 and 133.3 (d (two sets), $J=2$ Hz, aromatic $\underline{\text{C}}$ para to aromatic CF, both isomers), 165.1 and 165.7 (d (two sets), $J=252$ Hz, aromatic $\underline{\text{C}}\text{F}$, both isomers), 174.7 and 174.9 (C=O of spiro moiety, both isomers), 198.6 (C=O of p-fluorophenone moiety). Anal. Calcd: C, 52.34; H, 4.73; N, 7.05; F, 3.19 Found: C, 51.93; H, 4.69; N, 6.79; F, 2.71.

Diethyl ethylpropargylmalonate (28)

Sodium (1.2 g; 50 mmol) was added to absolute ethanol (50 mL) under argon followed by the dropwise addition of diethyl ethylmalonate (8.0 g; 50 mmol). The mixture was stirred for 30 minutes then added dropwise to a solution of propargyl bromide (19 g; 130 mmol) in ethanol (20 mL) at a rate as to maintain a reduced temperature, below the boiling point of the solvent. After the addition, the reaction mixture was stirred overnight at room temperature. The mixture was partitioned between water (50 mL) and chloroform (50 mL). The organic phase was washed once more with water (50 mL) and the organic phase isolated, dried (MgSO_4), filtered, and the solvent evaporated to yield an oil which was loaded onto a silica gel column gradient eluted with hexane to 10% ethyl ether/hexane. This provided the dialkylated malonate (6.4 g; 79%) as an oil: ^1H NMR (CDCl_3) δ 0.88 (t, 3H, $\underline{\text{CH}_3}$ of ethyl malonate), 1.23 (t, 6H, $\underline{\text{CH}_3}$'s of ethyl esters), 1.95 (t, 1H, $J=2$

Hz, propynyl $\underline{\text{H}}$), 2.00 (q, 2H, $\underline{\text{CH}_2}$ of ethyl malonate), 2.81 (d, 2H, $\text{J}=2$ Hz, propargyl $\underline{\text{CH}_2}$), 4.23 (q, 4H, $\underline{\text{CH}_2}$'s of ethyl esters); ^{13}C NMR (CDCl_3) δ 10.2 ($\underline{\text{CH}_3\text{CH}_2}$ of malonate substituent), 14.5 ($\underline{\text{CH}_3\text{CH}_2}$ of ethyl esters), 34.5 ($\underline{\text{CH}_3\text{CH}_2}$ of malonate substituent), 37.5 ($\underline{\text{CH}_2}$ of propargyl moiety), 51.5 (methylene $\underline{\text{C}}$ of malonate), 61.2 ($\underline{\text{CH}_3\text{CH}_2}$ of ethyl esters), 71.8 (alkyne external $\underline{\text{CH}}$), 78.7 (alkyne internal $\underline{\text{C}}$). Anal. Calcd: C, 63.71; H, 6.77. Found: C, 63.39; H, 6.72.

5-Ethyl-5-propargylbarbituric acid (29)

Urea (1.81 g; 30.1 mmol) in dry DMF (10 mL) was added dropwise to a suspension of 60% sodium hydride (1.20 g; 30.1 mmol) in dry DMF (10 mL) under argon. After the evolution of gas ceased, diethyl ethylpropargylmalonate (6.87 g; 30.1 mmol) in dry DMF (5 mL) was added followed by stirring overnight at 60 °C. The resulting mixture was partitioned between water (150 mL) and ether (50 mL). The organic layer was discarded and the aqueous phase acidified with 1 N HCl (50 mL) and extracted with two portions of ether (2 X 70 mL). The organic fractions were combined, dried (MgSO_4), filtered, and the solvent evaporated to yield crystals (0.88 g; 15%) which were recrystallized from benzene: ^1H NMR (acetone- d_6) δ 0.98 (t, 3H, $\underline{\text{CH}_3}$ of ethyl substituent), 1.99 (t, 1H, $\text{J}=2$ Hz, propynyl $\underline{\text{H}}$), 2.08 (q, 2H, ethyl $\underline{\text{CH}_2}$), 2.78 (d, 2H, $\text{J}=2$ Hz, propargyl $\underline{\text{CH}_2}$), 10.35 (bs, barbituric acid $\underline{\text{H}}$'s); ^{13}C NMR (acetone- d_6) δ 11.6 ($\underline{\text{CH}_3\text{CH}_2}$ of ethyl substituent), 33.6 ($\underline{\text{CH}_3\text{CH}_2}$ of ethyl substituent), 38.2 ($\underline{\text{CH}_2}$ of propargyl moiety), 53.9 (methylene $\underline{\text{C}}$ of barbituric acid), 71.6 (alkyne external $\underline{\text{CH}}$), 79.2 (alkyne internal $\underline{\text{C}}$), 170.3 (barbituric acid carbonyl $\underline{\text{CO}}$). Anal. Calcd: C, 75.22; H, 7.93; N, 9.16; Found: C, 75.18; H, 7.97; N, 9.04.

5-Ethyl-5-[(*E*)-2(3)-chloro-3(2)-iodo-2-propen-1-yl]barbituric acid (30)

The substrate, 5-ethyl-5-propargylbarbituric acid, (0.30 g; 1.5 mmol) was added to dry THF (10 mL) followed by the dropwise addition of iodine monochloride (0.38 g; 2.3 mmol) in dry THF (2 mL). The reaction mixture was refluxed under argon for 2 hours. The resulting mixture was partitioned between ether (20 mL) and water (20 mL). Solid sodium thiosulfate was added until the color faded. The organic phase was collected and washed twice with water (20 mL), dried (MgSO₄), filtered, and the solvent evaporated to yield a mixture of isomers (0.49 g; 89%) which were inseparable by ordinary techniques: ¹H NMR (acetone-d₆ and CDCl₃) δ 0.91 (t, 3H, ethyl CH₃), 2.08 (q, 2H, ethyl CH₂), 3.06 and 3.23 (s, 2H, CH₂ adjacent to internal vinyl Cl and CCl, respectively), 6.80 and 7.82 (s, 1H, external vinyl CHI and CHCl, respectively), 10.41 (bs, barbituric acid H); ¹³C NMR (acetone-d₆ and CDCl₃) δ 9.2 (CH₃CH₂ of ethyl substituent), 33.8 (CH₃CH₂ of ethyl substituent), 47.6 (CH₂ of propenyl moiety), 56.2 (methylene C of barbituric acid), 79.1 and 82.4 (external CHI and internal Cl, respectively), 140.7 and 149.7 (external CHCl and internal CCl, respectively), 172.3 (barbituric acid carbonyl CO). Anal. Calcd: C, 30.32; H, 2.83; N, 7.86; Found: C, 30.28; H, 2.87; N, 7.74.

4-Iodo-4'-fluorobutyrophenone (31)

The butyrophenone, 4-chloro-4'-fluorobutyrophenone, (6.1 g; 5.0 mL; 30 mmol), sodium iodide (14 g; 90 mmol), and 2-butanone (120 mL) were stirred at reflux temperature overnight while being protected from light. The mixture was washed with water (100 mL) while sodium thiosulfate was added until the color faded. The mixture was washed three more times with water (100 mL) and the organic phase collected, dried (MgSO₄), filtered, and the solvent evaporated to

yield a dark-orange colored oil. The oil was loaded onto a silica gel column and eluted with 0-10% ether/hexane. This provided the transhalogenated compound (7.2 g; 81%) as a viscous clear oil which discolors rapidly upon exposure to the atmosphere: ^1H NMR (CDCl_3) δ 2.28 (p, 2H, CH_2), 3.26 (superimposed triplets, 4H, ICH_2 and $\text{CH}_2\text{C=O}$), 7.19 (t, 2H, aromatic CH), 8.06 (m, 2H, aromatic CH); ^{13}C NMR (CDCl_3) δ 20.5, 36.9, 39.9 (alkyl CH_2 's), 117.8 (d, $J=18$ Hz, aromatic CH 's ortho to aromatic CF), 132.6 (d, $J=9$ Hz, aromatic CH 's meta to aromatic CF), 135.5 (d, $J=2$ Hz, aromatic C para to aromatic CF), 168.8 (d, $J=256$ Hz, aromatic CF), 200.8 (carbonyl C). Anal. Calcd: C, 41.12; H, 3.45; Found: C, 41.43; H, 3.76.

4-Iodo-4'-fluorobutyrophenone ethylene ketal (32)

A mixture of 4-iodo-4'-fluorobutyrophenone (9.0 mL; 55 mmol), ethylene glycol (9.0 mL; 0.17 mol), p-toluenesulfonic acid (0.85 g), and benzene (400 mL) were refluxed overnight using a Dean-Stark trap in order to remove water. The organic phase was washed with saturated aqueous sodium bicarbonate until the evolution of gas ceased, dried (MgSO_4), filtered, and the solvent evaporated to provide a yellow oil (12 g; 91%). The yellow oil decomposes readily and must be used and/or analyzed immediately: ^1H NMR (CDCl_3) δ 1.85 (m, 4H, alkyl CH_2 's), 3.26(t, 2H, CH_2I), 3.79 (doublet of multiplets, 4H, ketal CH_2 's), 6.95 (t, 2H, aromatic CH), 7.35 (m, 2H, aromatic CH); ^{13}C NMR (CDCl_3) δ 26.7, 34.8, 42.5, 59.6 (alkyl and ketal CH_2 's), 115.9 (d, $J=18$ Hz, aromatic CH 's ortho to aromatic CF), 130.5 (d, $J=9$ Hz, aromatic CH 's meta to aromatic CF), 133.1 (d, $J=2$ Hz, aromatic C para to aromatic CF), 165.9 (d, $J=256$ Hz, aromatic CF), 197.1 (carbonyl C).

Diethyl 4'-fluorobutyrophenon-1-yl malonate (ethylene ketal) (33)

Diethyl malonate (3.3 g; 21 mmol) in dry DMF (5 mL) was added dropwise to a suspension of 60% oil dispersed NaH (0.84 g; 21 mmol) in dry DMF (15 mL) under argon. After the evolution of gas ceased, 4-chloro-4'-fluorobutyrophenone ethylene ketal (5.4 g; 22 mmol) was added followed by KI (0.13 g; 1.0 mmol) and the mixture stirred with heating to 80-90 °C overnight. After cooling, the mixture was partitioned between saturated aqueous sodium bicarbonate (50 mL) and chloroform (50 mL). The organic phase was washed three times with water (50 mL), dried (MgSO₄), filtered, and the solvent evaporated to yield an oil which was distilled to remove starting material. The residue was loaded onto a silica gel column and gradient eluted with hexane-10% ether/hexane. This provided the title compound (4.3 g; 55%) as an oil: ¹H NMR (CDCl₃) δ 1.20 (t, 6H, CH₃'s of ethyl groups), 1.85(m, 4H, superimposed butyrophenone CH₂'s, C-1 and C-2), 3.25 (t, 1H, methylene CH of malonate moiety), 3.82 (doublet of multiplets, 4H, ethylene ketal CH₂'s), 4.15 (q, 4H, CH₂'s of ethyl groups), 6.99 (t, 2H, aromatic CH's meta to aromatic CF), 7.39 (m, 2H, aromatic CH's ortho to aromatic CF); ¹³C NMR (CDCl₃) δ 13.5, 21.0, 27.5, 39.5, 51.5, 60.5, 64.0 (alkyl CH, CH₂'s and CH₃'s), 109.5 (ketal C), 114.5 (d, J=17 Hz, aromatic CH's ortho to aromatic CF), 127.3 (d, J=9 Hz, aromatic CH's meta to aromatic CF), 138.2 (d, J=2 Hz, aromatic C para to aromatic CF), 162.4 (d, J=256 Hz, aromatic CF), 168.9 (C=O's of diethyl malonate moiety). Anal. Calcd: C, 61.95; H, 6.84; F, 5.16; Found: C, 61.86; H, 7.07; F, 5.13.

Diethyl 4'-fluorobutyrophenon-1-yl propargyl malonate (ethylene ketal) (34)

The alkylated malonic ester, diethyl 4'-fluorobutyrophenon-1-yl malonate (ethylene ketal), (1.9 g; 5.1 mmol) in dry DMF (3 mL) was added to a suspension of

60% oil dispersed NaH (0.22 g; 5.6 mmol) in dry DMF (15 mL) under argon with stirring. After the evolution of gas ceased, the mixture was cooled to -30 °C after which 80% propargyl bromide (1.1 g; 7.6 mmol) in dry DMF (2 mL) was added dropwise so as to maintain the temperature. After the addition, the mixture was stirred for 30 minutes then allowed to gradually warm to room temperature and stirred overnight. The resulting mixture was partitioned between saturated aqueous sodium bicarbonate (30 mL), water (30 mL), and chloroform (30 mL). The organic phase was collected and washed three times with water (30mL). The organic phase was collected, dried (MgSO₄), filtered, and the solvent evaporated to yield the dialkylated malonic ester (1.4 g; 70%) as an oil: ¹H NMR (CDCl₃) δ 1.20 (t, 6H, CH₃'s of ethyl groups), 1.93 (m, 7H, butyrophenone CH₂'s , and propargyl CH), 2.75 (d, 2H, propargyl CH₂), 3.85 (doublet of multiplets, 4H, ethylene CH₂'s), 4.15 (q, 4H, CH₂'s of ethyl groups), 6.99 (t, 2H, aromatic CH's meta to aromatic CF), 7.40 (m, 2H, aromatic CH's ortho to aromatic CF); ¹³C NMR (CDCl₃) δ 13.5, 18.0, 22.5, 31.5, 40.5, 56.5, 61.0, 64.0 (alkyl CH₂'s and CH₃'s), 70.5 (propargyl CH), 78.5 (propargyl C), 109.5 (ketal C), 114.4 (d, J=17 Hz, aromatic CH's ortho to aromatic CF), 127.0 (d, J=9 Hz, aromatic CH's meta to aromatic CF), 138.0 (d, J=2 Hz, aromatic C para to aromatic CF), 161.5 (d, J=252 Hz, aromatic CF), 169.9 (C=O's of diethyl malonate moiety). Anal. Calcd: C, 65.01; H, 6.70; Found: C, 65.40; H, 6.81.

4'-Fluorobutyrophenon-1-yl propargyl barbituric acid (ethylene ketal) (35)

Urea (0.28 g; 4.6 mmol) in dry DMF (2 mL) was added dropwise to a suspension of 60% oil dispersed NaH (0.18 g; 4.6 mmol) in dry DMF (5 mL). After the evolution of gas ceased, diethyl 4'-fluorobutyrophenon-1-yl propargylmalonate (ethylene ketal) (1.4 g; 3.4 mmol) was added followed by heating to 70 - 80 °C

overnight. After cooling, the resulting mixture was partitioned between ether (20 mL), water (30 mL), and aqueous saturated sodium bicarbonate (15 mL). The aqueous layer was isolated, acidified (1N HCl), and extracted with ether (2 X 40 mL). The ether fractions were combined, dried (MgSO₄), filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and eluted with 5% methanol/chloroform. This provided the desired product (0.22 g; 16%) as a gum: ¹H NMR (acetone-d₆) δ 2.01 (m, 7H, butyrophenone CH₂'s , and propargyl CH), 2.78 (d, 2H, propargyl CH₂), 3.93 (doublet of multiplets, 4H, ethylene CH₂'s), 7.01 (t, 2H, aromatic CH's meta to aromatic CF), 7.45 (m, 2H, aromatic CH's ortho to aromatic CF) 10.50 (bs, barbituric acid H's); ¹³C NMR (acetone-d₆) δ 18.7, 23.1, 31.7, 40.7, 61.2, 63.8 (alkyl CH₂'s), 71.8 (propargyl CH), 79.2 (propargyl C), 110.5 (ketal C), 115.6 (d, J=17 Hz, aromatic CH's ortho to aromatic CF), 127.7 (d, J=10 Hz, aromatic CH's meta to aromatic CF), 138.0 (d, J=2 Hz, aromatic C para to aromatic CF), 161.5 (d, J=250 Hz, aromatic CF), 170.8 (C=O's of barbituric acid moiety). Anal. Calcd: C, 65.01; H, 6.70; Found: C, 65.40; H, 6.81.

4'-Fluorobutyrophenon-1-yl propargyl barbituric acid (36)

4'-Fluorobutyrophenon-1-yl propargyl barbituric acid (ethylene ketal) was dissolved and refluxed in acidic (HCl) aqueous methanol for 1 hour. Extraction of the reaction mixture with chloroform or ether provided the unprotected ketone in quantitative yield.

5-(4'-Fluorobutyrophenon-1-yl)-5-[(*E*)-2(3)-chloro-3(2)-iodo-2-propen-1-yl]barbituric acid (37)

4'-Fluorobutyrophenon-1-yl propargyl barbituric acid (ethylene ketal)

(0.10g; 0.27 mmol) was dissolved in THF (3 mL), followed by the addition of iodine monochloride (0.07 g; 0.43 mmol) in THF (1 mL). The reaction mixture was refluxed for two hours. After cooling, the mixture was partitioned between water (10 mL) and ether (15 mL). Sodium thiosulfate was added with stirring until the color faded. The ether layer was isolated and the solvent evaporated to yield a residue which was loaded onto a silica gel column and eluted with 5% methanol/chloroform. This provided the chloriodinated barbituric acid (0.12 g; 63%) as a brownish gum: ^1H NMR (acetone- d_6 and CDCl_3) δ 1.65 and 1.89 (m, 4H, butyrophene CH_2 , C-1 and C-2) 2.87 (t, 2H, $\text{CH}_2\text{C}=\text{O}$) 3.10 and 3.29 (s, 2H, CH_2 adjacent to internal vinyl Cl and CCl , respectively), 7.10 (t, 2H, aromatic CH 's meta to aromatic CF), 7.90 (m, 2H, aromatic CH 's ortho to aromatic CF); 6.82 and 7.81 (s, 1H, external vinyl CHI and CHCl , respectively), 10.55 (bs, barbituric acid H); ^{13}C NMR (acetone- d_6 and CDCl_3) δ 22.5, 31.6, 38.5 (alkyl CH_2 's), 48.6 (CH_2 of propenyl moiety), 56.8 (methylene C of barbituric acid), 79.7 and 83.2 (external CHI and internal Cl , respectively), 115.4 (d, $J=18$ Hz, aromatic CH 's ortho to aromatic CF), 130.5 (d, $J=9$ Hz, aromatic CH 's meta to aromatic CF), 133.0 (d, $J=2$ Hz, aromatic C para to aromatic CF), 141.7 and 150.7 (external CHCl and internal CCl , respectively), 166.3 (d, $J=252$ Hz, aromatic CF), 170.6 (barbituric acid carbonyl CO), 197.8 ($\text{C}=\text{O}$ of p-fluorophenone moiety). Anal. Calcd: C, 30.32; H, 2.83; N, 7.86; Found: C, 30.28; H, 2.87; N, 7.74.

N-Propargyl-4-phenylpiperidine (38)

Propargyl bromide (0.44 g; 3.7 mmol) was added to a mixture of 4-phenylpiperidine (1.2 g; 7.4 mmol) in methanol (10 mL). The reaction mixture was stirred at 60 $^\circ\text{C}$ for 2 h. After cooling, the reaction mixture was reduced *in vacuo* and partitioned between 1 N NaOH (5 mL) and ether (30 mL). The organic

phase was collected and the aqueous phase extracted once more with ether (30 mL) after being saturated with NaCl. The organic layers were combined, dried (MgSO_4), filtered, and the solvent evaporated to dryness. The residue was loaded onto a silica gel column and eluted with chloroform. This provided the propargylated amine (0.59 g; 80%) as white crystals: mp 69-70 °C; ^1H NMR (CDCl_3) δ 1.85 (m, 4H, piperidine), 2.25 (m, 4H, piperidine and alkyne H), 2.98 (d, br, 2H, piperidine H), 3.32 (d, 2H, $J = 2$ Hz, alkyne CH_2), 7.23 (s, 5H, aromatic); ^{13}C NMR (CDCl_3) δ 33.4 (t, piperidine CH_2), 42.1 (d, piperidine CH), 47.2 (t, piperidine CH_2), 53.0 (t, propargyl CH_2), 73.0 (d, alkyne CH), 79.1 (s, alkyne C), 126.1, 126.8, 128.4 (d, aromatic C 's), 146.1 (s, aromatic C). Anal. Calcd: C, 84.37; H, 8.60; N, 7.03. Found: C, 84.55; H, 8.96; N, 6.89.

1-((*E*)-2(3)-Chloro-3(2)-iodo-2-propen-1-yl)-4-phenylpiperidine (39)

The hydrochloride salt of *N*-propargyl-4-phenylpiperidine (0.11 g; 0.44 mmol) was added to a mixture of iodine monochloride (0.13 g; 0.81 mmol) and THF (4 mL). The mixture was refluxed for 2 h under argon. The reaction mixture was partitioned between 1 *N* NaOH (10 mL) and chloroform (20 mL). The organic layer was collected and the aqueous phase extracted again with chloroform (10 mL). The organic layers were combined, dried (MgSO_4), filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and eluted with 2% ether/pet. ether. The fractions containing the products with an R_f value of 0.6 and 0.8, TLC analysis using silica gel plates eluted with chloroform, were combined and evaporated to yield a mixture of the A and M isomers (0.12 g; 68%) in a ratio of 5:1, respectively, as an oil. The isomers were resolved by preparatory TLC using a silica gel plate (5000 ν) eluted with 2% ether/pet. ether. The isomers were visualized with an ultraviolet lamp, scraped, and extracted with ether to

provide each individual isomer: M isomer, oil; ^1H NMR (CDCl_3) δ 1.71-2.25 (m, 9H, piperidine H), 3.48 (s, 2H, CH_2CCl), 6.56 (s, 1H, CH), 7.25 (s, 5H, aromatic); ^{13}C NMR (CDCl_3) δ 33.5 (t, piperidine CH_2), 42.4 (d, piperidine CH), 53.8 (t, piperidine CH_2), 64.8 (t, CH_2CCl), 75.7 (d, CH), 125.6, 126.5, 127.1 (d, aromatic C's), 134.8 (s, CCl), 146.5 (s, aromatic C); MS m/e (M^+ , rel int) 361 (^{35}Cl) (47); A isomer, oil that crystallizes on standing, mp 51-52 $^\circ\text{C}$; ^1H NMR (CDCl_3) δ 1.65-2.12 (m, 9H, piperidine H), 3.26 (s, 2H, CH_2Cl), 6.65 (s, 1H, CHCl), 7.25 (s, 5H, aromatic); ^{13}C NMR (CDCl_3) δ 33.4 (t, piperidine CH_2), 42.5 (d, piperidine CH), 53.7 (t, piperidine CH_2), 61.3 (t, CH_2Cl), 102.0 (s, Cl), 121.1 (d, CHCl), 126.1, 126.9, 128.4 (d, aromatic C's), 146.4 (s, aromatic C); MS m/e (M^+ , rel int) 361 (^{35}Cl) (48). Anal. Calcd: C, 46.50; H, 4.74; N, 3.87. Found (A and M mixture): C, 46.86; H, 4.79; N, 3.81.

4-Ethoxycarbonyl-4-phenylpiperidine (40)

A mixture of 4-cyano-4-phenylpiperidine hydrochloride (1.0 g, 4.5 mmol), concentrated sulfuric acid (5g), and 95% ethanol (5 g) was refluxed for 3 h. After cooling, the reaction mixture was added dropwise to a stirring mixture of ether (50 mL) and cold aqueous sodium hydroxide (30 mL of water containing 3 g. of sodium hydroxide) at a rate so as to prevent refluxing the ether. After the addition, the aqueous layer was saturated with sodium chloride and the organic layer was collected, dried (MgSO_4), filtered, and the solvent evaporated to yield a residue which was loaded onto a silica gel column and eluted with chloroform to yield the ethyl ester (0.64 g, 61%) as an oil: ^1H NMR (CDCl_3) δ 1.21 (t, 3H, CH_3 of ethyl ester), 1.67 (bs, 1H, NH of piperidine), 1.81 (m, 4H, piperidine), 2.22 (m, 3H, piperidine), 2.96 (d, br, 2H, piperidine H), 4.17 (q, 2H, CH_2 of ethyl ester),

7.19 (s, 5H, aromatic); ^{13}C NMR (CDCl_3) δ 14.5 (q, CH_3 of ethyl ester), 33.9 (t, piperidine CH_2), 42.5 (d, piperidine CH), 46.8 (t, piperidine CH_2), 61.2 (t, CH_2 of ethyl ester), 125.9, 126.3, 128.1 (d, aromatic C's), 145.7 (s, aromatic C), 170.5 (s, $\text{C}=\text{O}$ of ethyl ester). Anal. Calcd: C, 80.37; H, 5.60; N, 3.03. Found: C, 80.55; H, 5.96; N, 3.89.

4-Ethoxycarbonyl-4-phenyl-1-propargylpiperidine (41)

A mixture of 4-ethoxycarbonyl-4-phenylpiperidine (0.53 g, 2.3 mmol), 80% propargyl bromide in toluene (0.32 g, 2.7 mmol), methanol (5 mL), and triethylamine (1 mL) was stirred at room temperature overnight. The resulting mixture was partitioned between ether (20 mL) and 1N sodium hydroxide (10 mL). The ether layer was collected, dried (MgSO_4), filtered, the solvent evaporated, and the residue loaded onto a 1000 μ silica gel preparatory plate and developed with 10% methanol/chloroform. This provided the propargylated piperidine (0.22 g, 36%) as an oil: ^1H NMR (CDCl_3) δ 1.22 (t, 3H, CH_3 of ethyl ester), 1.84 (m, 4H, piperidine), 2.22 (m, 4H, piperidine and alkyne H), 2.93 (d, br, 2H, piperidine H), 3.29 (d, 2H, $J = 2$ Hz, alkyne CH_2), 4.21 (q, 2H, CH_2 of ethyl ester), 7.22 (s, 5H, aromatic); ^{13}C NMR (CDCl_3) δ 14.6 (q, CH_3 of ethyl ester), 34.5 (t, piperidine CH_2), 43.1 (d, piperidine CH), 47.2 (t, piperidine CH_2), 53.4 (t, propargyl CH_2), 61.7 (t, CH_2 of ethyl ester), 72.7 (d, alkyne CH), 78.8 (s, alkyne C), 126.2, 126.7, 129.3 (d, aromatic C's), 145.8 (s, aromatic C), 171.1 (s, $\text{C}=\text{O}$ of ethyl ester). Anal. Calcd: C, 82.57; H, 6.54; N, 4.90. Found: C, 82.75; H, 6.90; N, 4.76.

1-((*E*)-2(3)-Chloro-3(2)-iodo-2-propen-1-yl)-4-ethoxycarbonyl-4-phenylpiperidine
(42)

A mixture of 4-ethoxycarbonyl-4-phenyl-1-propargylpiperidine hydrochloride (0.10 g, 0.32 mmol), THF (2 mL), and iodine monochloride (0.84 g, 0.52 mmol) was refluxed for 2 h in the absence of light and with stirring under argon. The resulting mixture was partitioned between ether (10 mL) and 1N sodium hydroxide (5 mL) while sodium thiosulfate was added until the color faded. The organic layer was isolated, dried (MgSO₄), filtered, the solvent evaporated, and the residue loaded onto a 1000 μ silica gel preparatory plate and developed with 10% methanol/chloroform. This provided an inseparable A/M (3.5/1) regioisomeric mixture of the chloriodinated product (0.10 g, 72%) as an oil which darkened upon exposure to the atmosphere and was analyzed as a mixture: ¹H NMR (CDCl₃) δ 1.24 (t, 3H, CH₃ of ethyl ester), 1.87 (m, 4H, piperidine), 2.26 (m, 3H, piperidine), 3.01 (d, br, 2H, piperidine H), 3.36 (bs, 2H, propenyl CH₂), 4.27 (q, 2H, CH₂ of ethyl ester), 6.58 and 6.69 (s, 1H, vinylic H of terminal iodide and chloride, respectively), 7.32 (s, 5H, aromatic); ¹³C NMR (CDCl₃) δ 13.9 (CH₃ of ethyl ester), 34.9(piperidine CH₂), 44.6 (piperidine CH), 47.5 (piperidine CH₂), 61.3 (CH₂Cl), 62.1 (CH₂ of ethyl ester), 64.8 (CH₂CCl), 76.8 and 102.0 (external and internal alkene CI, respectively), 121.4 (external CHCl), 126.2, 126.7, 129.3 (aromatic C's), 133.9 (internal CI), 145.8 (aromatic C), 171.1 (C=O of ethyl ester). Anal. Calcd: C, 52.77; H, 5.54; N, 4.70. Found: C, 52.75; H, 6.10; N, 4.56.

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APPENDICES

APPENDIX I

APPENDIX I

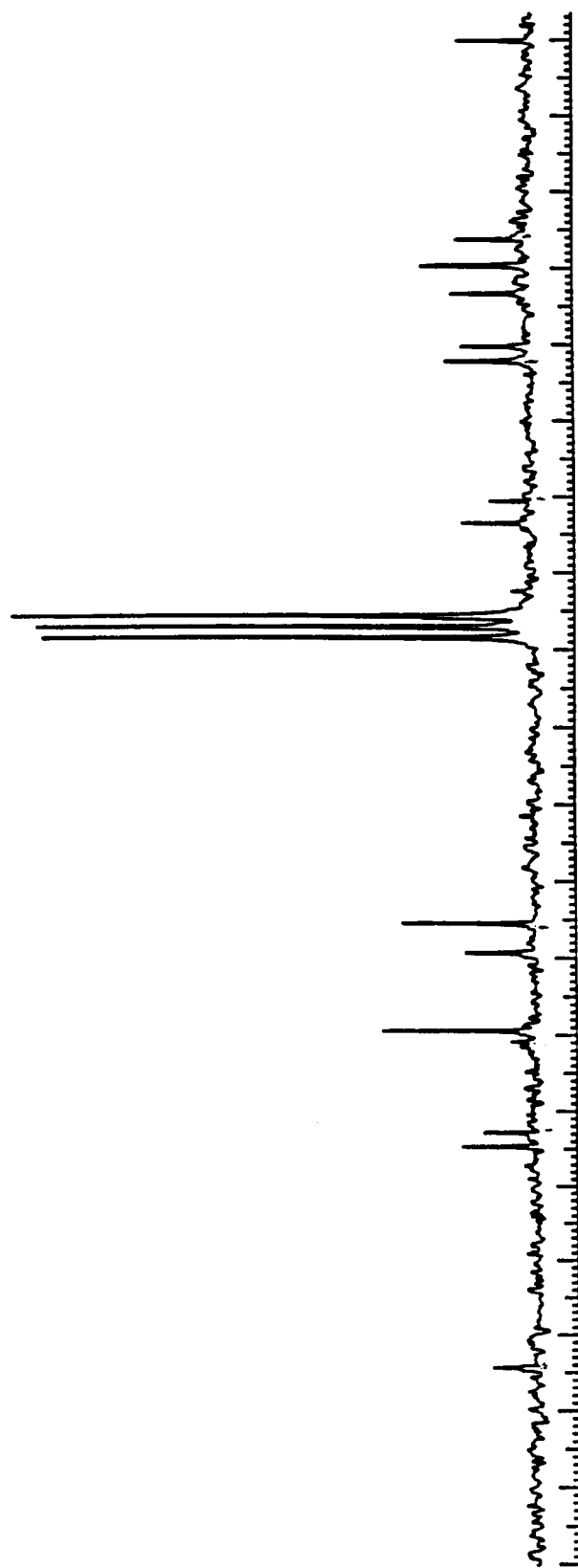
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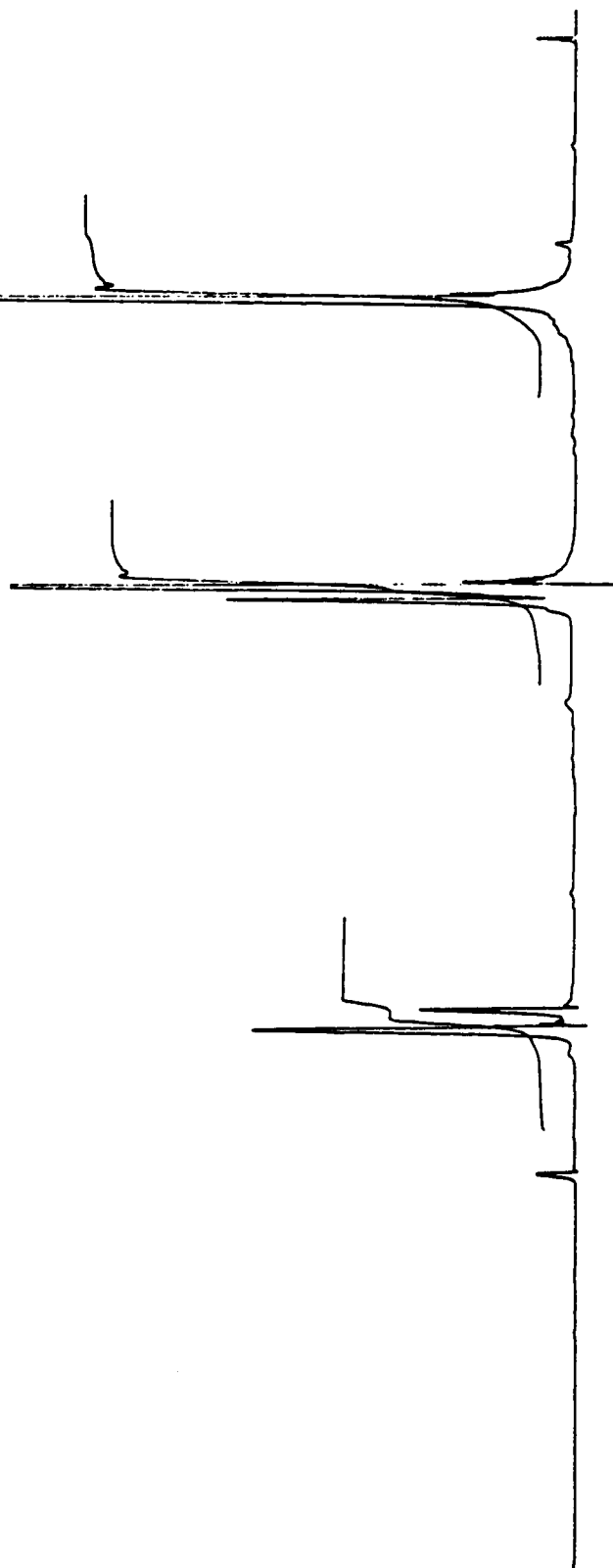
2. 1-Phenyl-3-[(E)-5-iodo-4-penten-1-yl)-1,3,8-triazaspiro[4.5]decan-4-one (5)

¹³C NMR; solvent: CDCl₃; reference: TMS = δ 0.



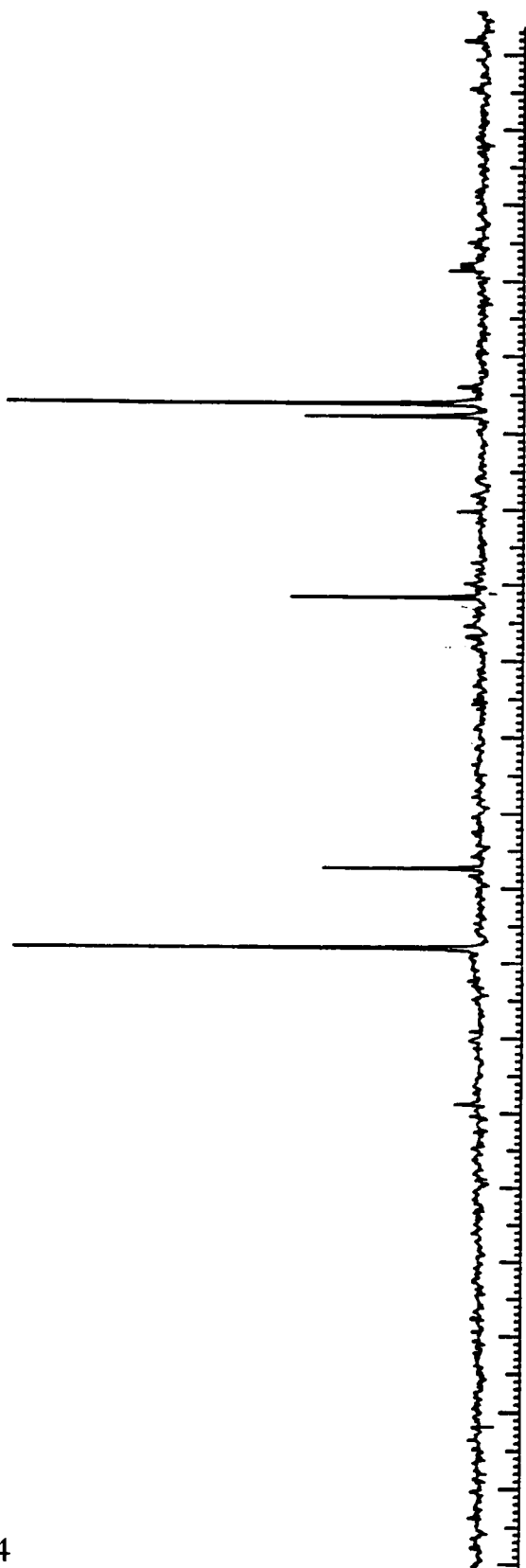
3. N-((E)-2(3)-Chloro-3(2)-iodo-2-propen-1-yl)amine
(14a)

¹H NMR; solvent: CDCl₃; reference: TMS = δ 0.



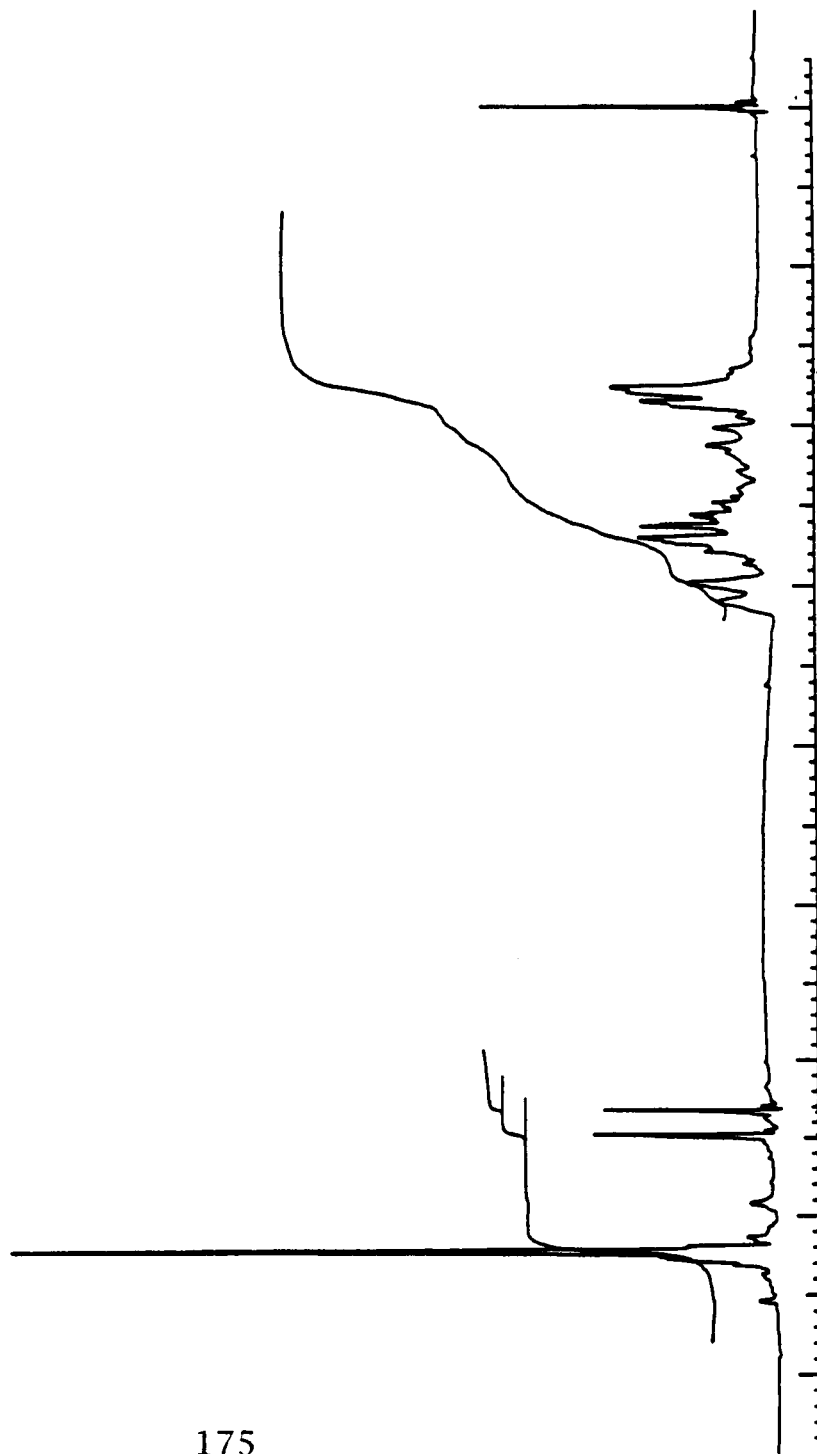
4. N-((E)-2(3)-Chloro-3(2)-iodo-2-propen-1-yl)amine
(14a)

^{13}C NMR; solvent: CDCl_3 ; reference: $\text{CDCl}_3 = \delta 77.1$



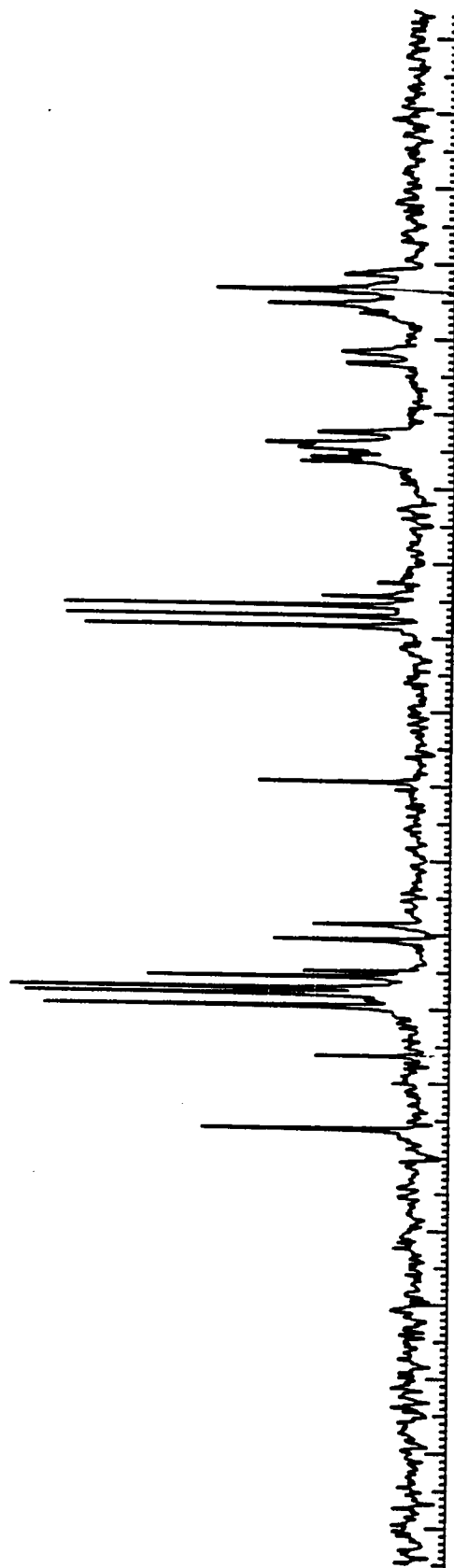
5. N-((E)-3(4)-Chloro-4(3)-iodo-3-buten-1-yl)-4-phenylpiperidine (14b)

^1H NMR; solvent: CDCl_3 ; reference: TMS = δ 0.



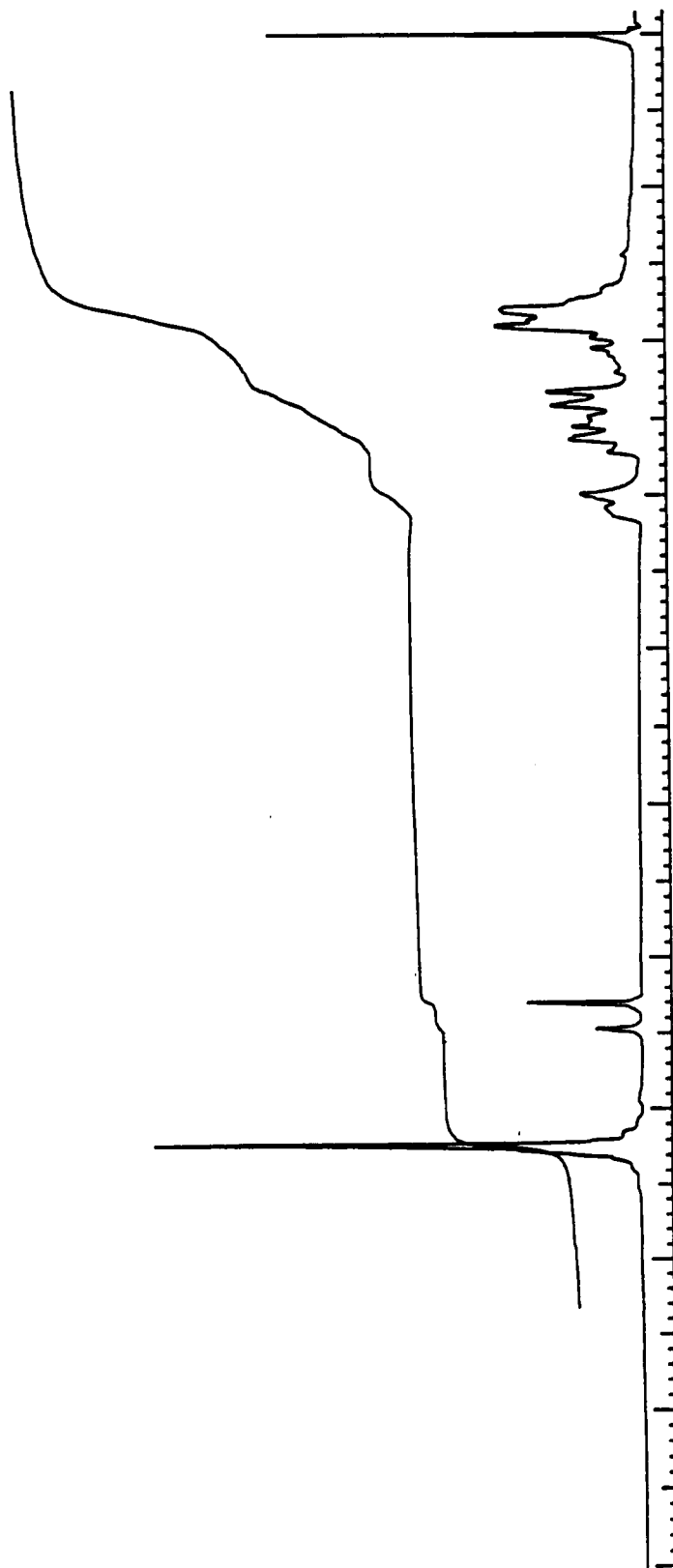
6. N-((E)-3(4)-Chloro-4(3)-iodo-3-buten-1-yl)-4-phenylpiperidine (14b)

¹³C off-resonance decoupled NMR; solvent: CDCl₃; reference: CDCl₃ = δ 77.1



7. N-((E)-4(5)-Chloro-5(4)-iodo-4-penten-1-yl)-4-phenylpiperidine (14c)

¹H NMR; solvent: CDCl₃; reference: TMS = δ 0.



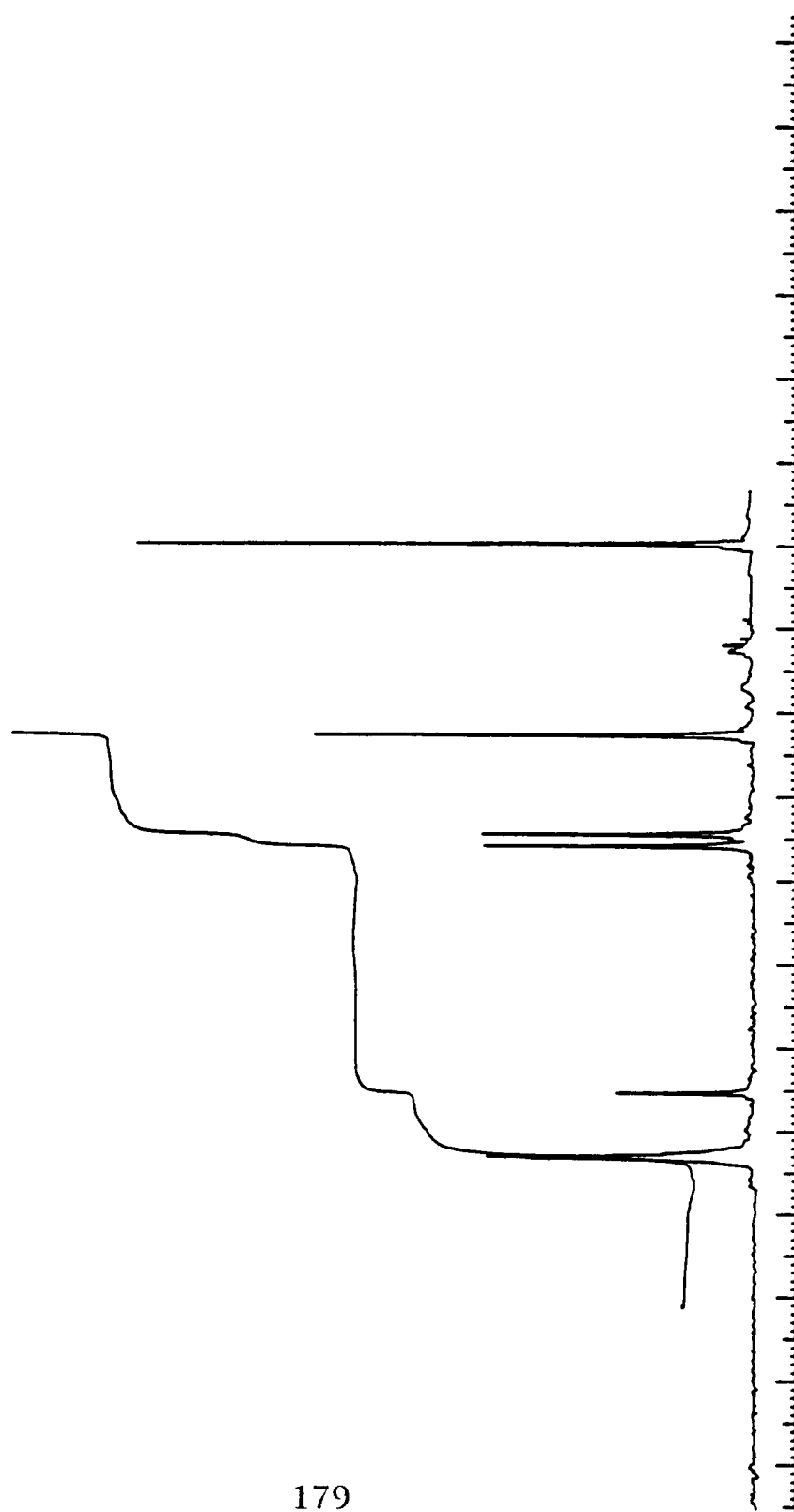
8. N-Methyl-N-benzyl-N-((E)-2(3)-chloro-3(2)-iodo-2-propen-1-yl)amine (23)

¹H NMR; solvent: CDCl₃; reference: TMS = δ 0.



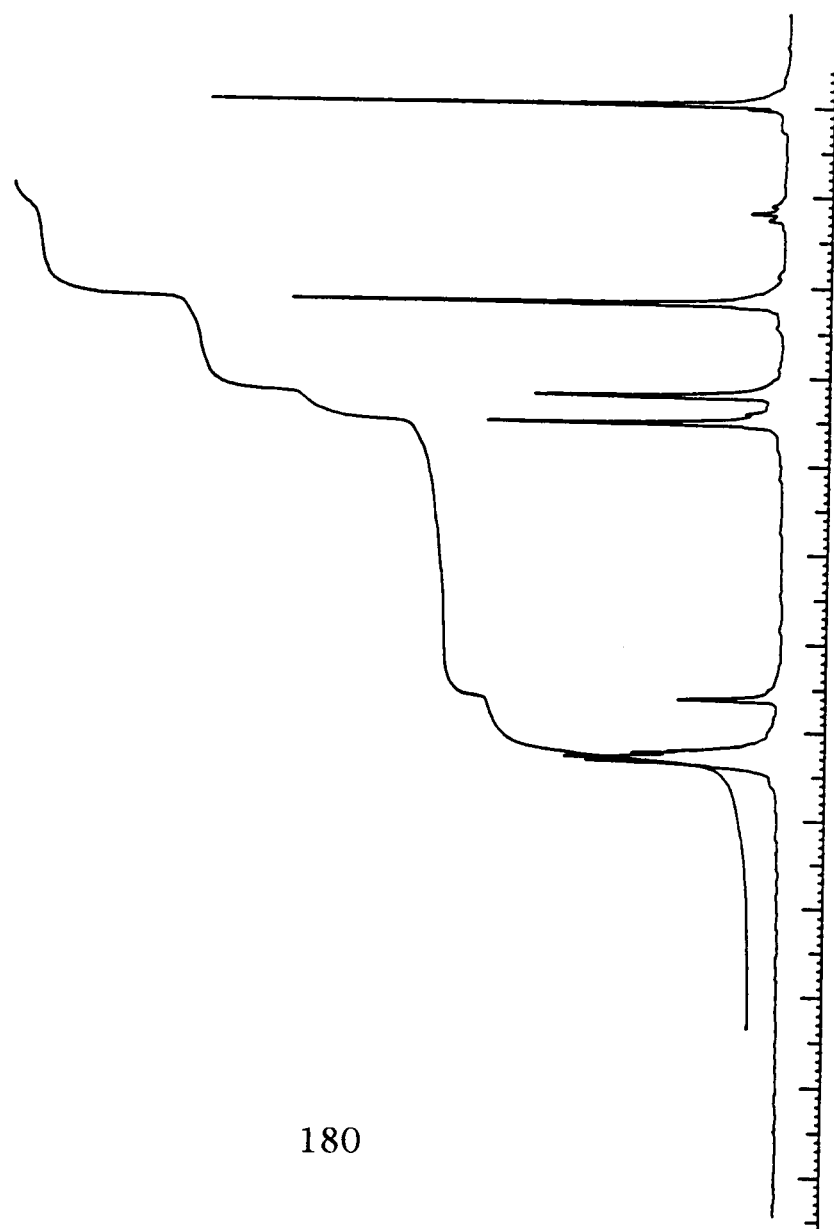
9. N-Methyl-N-benzyl-N-((E)-3-chloro-2-iodo-2-propen-1-yl)amine (23-A)

^1H NMR; solvent: CDCl_3 ; reference: TMS = δ 0.



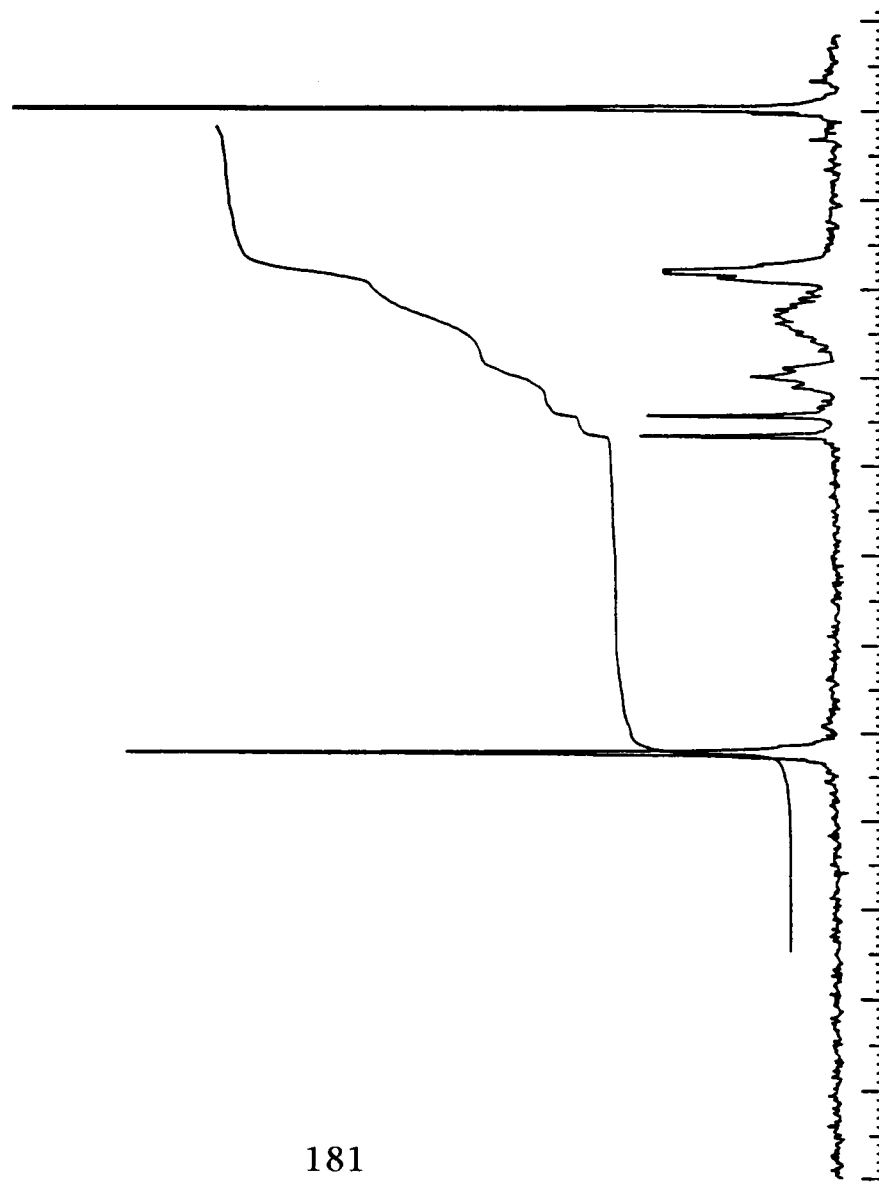
10. N-Methyl-N-benzyl-N-((E)-2-chloro-3-iodo-2-propen-1-yl)amine (23-M)

^1H NMR; solvent: CDCl_3 ; reference: TMS = δ 0.

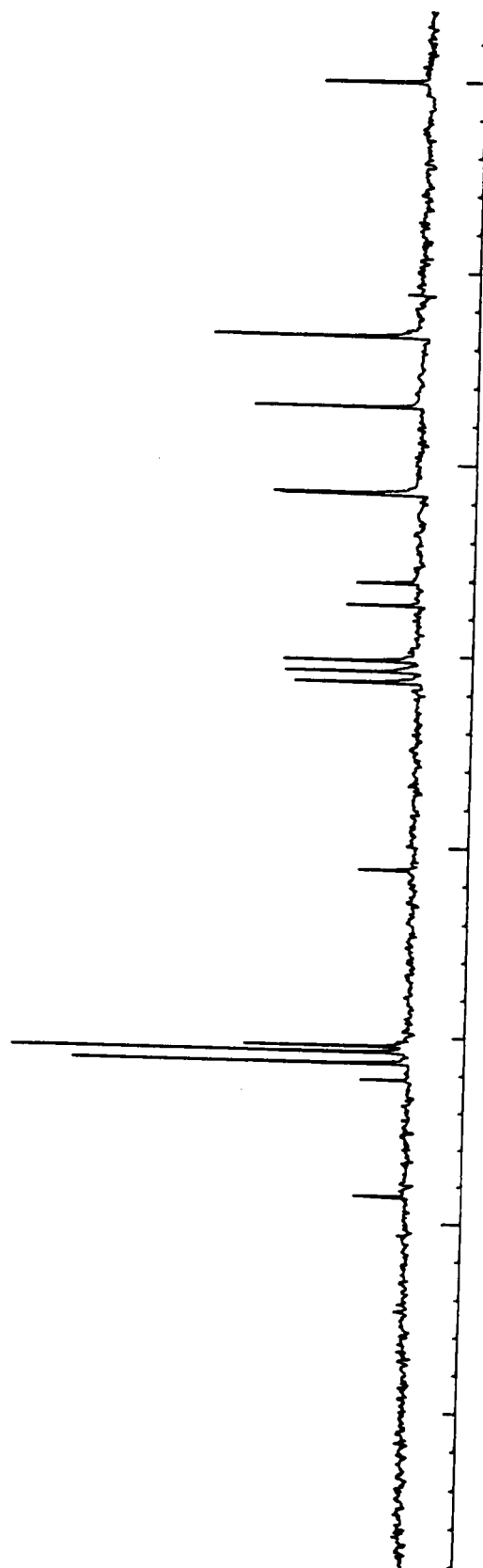


11. 1,1'-((E)-2-Chloro-3-iodo-2-buten-1,4-diyl)bis(4-phenylpiperidine) (22)

¹H NMR; solvent: CDCl₃; reference: TMS = δ 0.



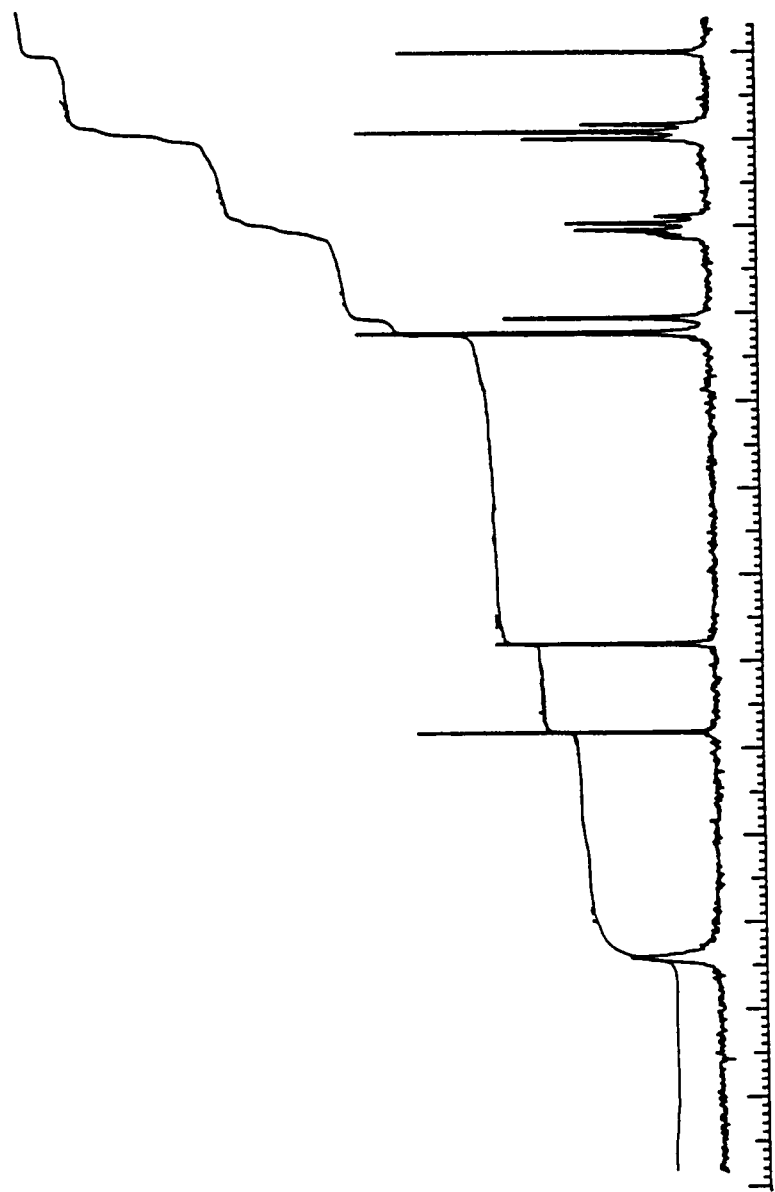
12. 1,1'-((E)-2-Chloro-3-iodo-2-buten-1,4-diyl)bis(4-phenylpiperidine) (22)
 ^{13}C NMR; solvent: CDCl_3 ; reference: $\text{CDCl}_3 = \delta 77.1$



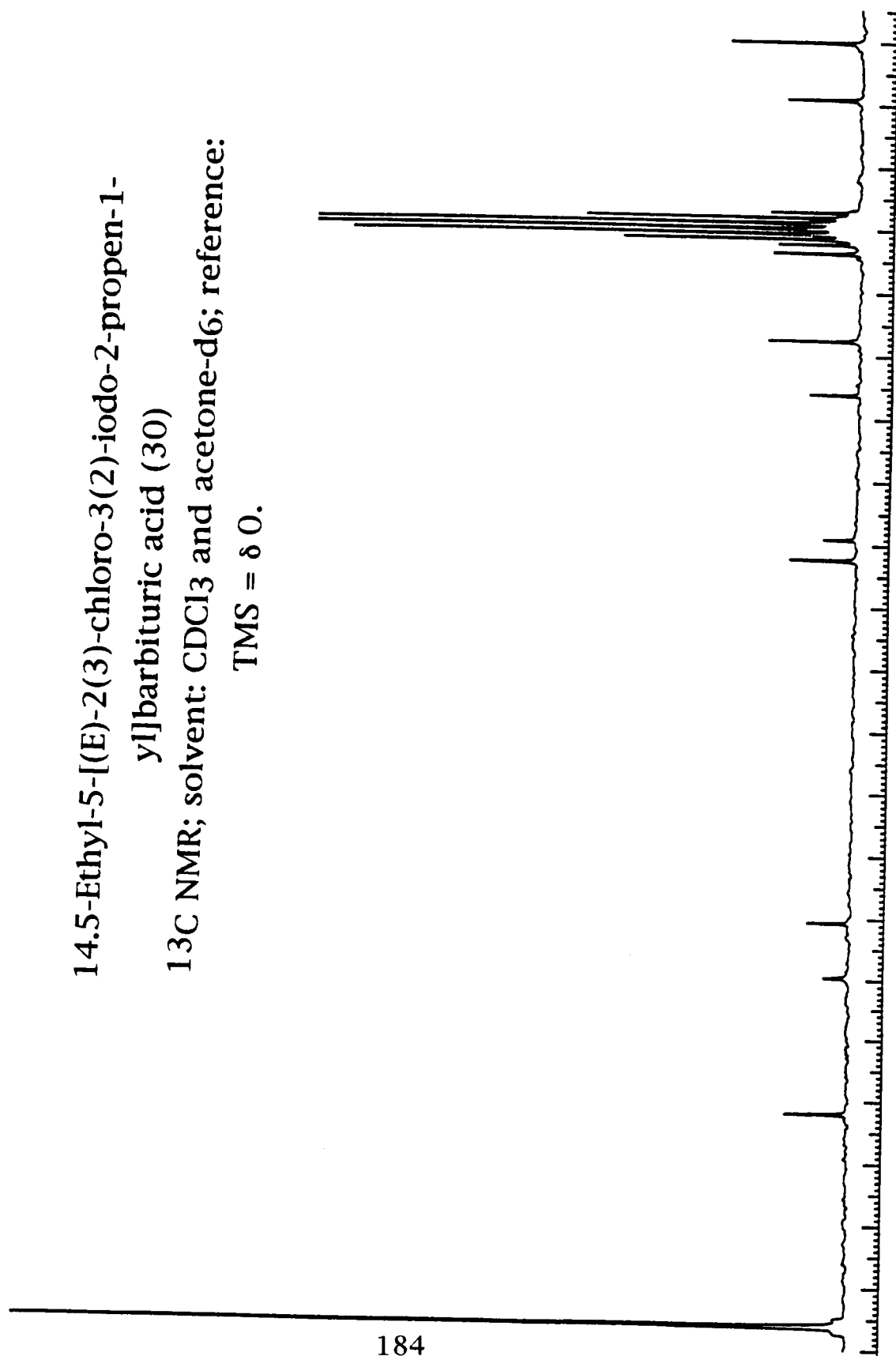
13. 5-Ethyl-5-[(E)-2(3)-chloro-3(2)-iodo-2-propen-1-yl]barbituric acid (30)

¹H NMR; solvent: CDCl₃ and acetone-d₆; reference:

TMS = δ 0.



14.5-Ethyl-5-[(E)-2(3)-chloro-3(2)-iodo-2-propen-1-yl]barbituric acid (30)
¹³C NMR; solvent: CDCl₃ and acetone-d₆; reference:
TMS = δ 0.



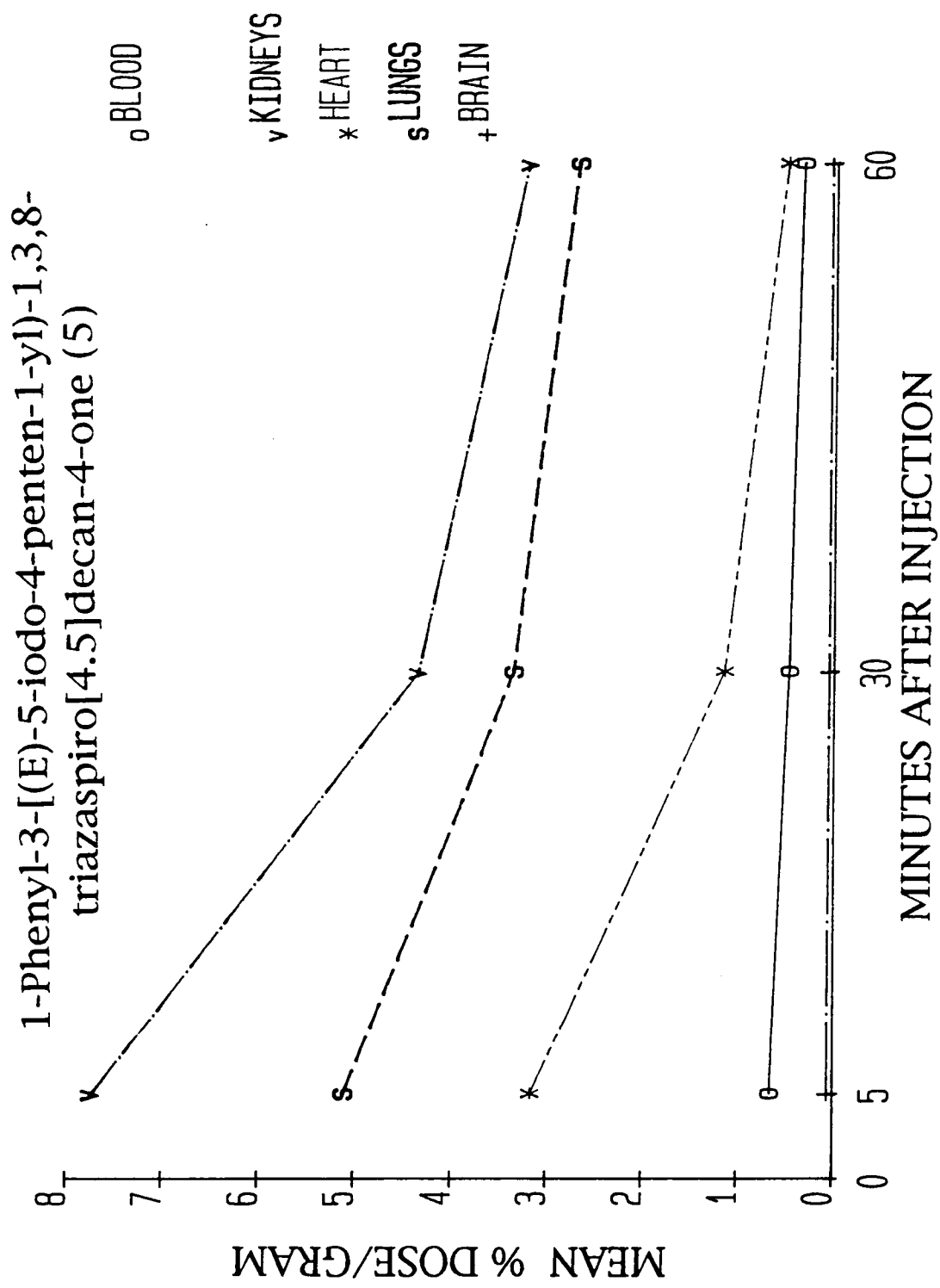
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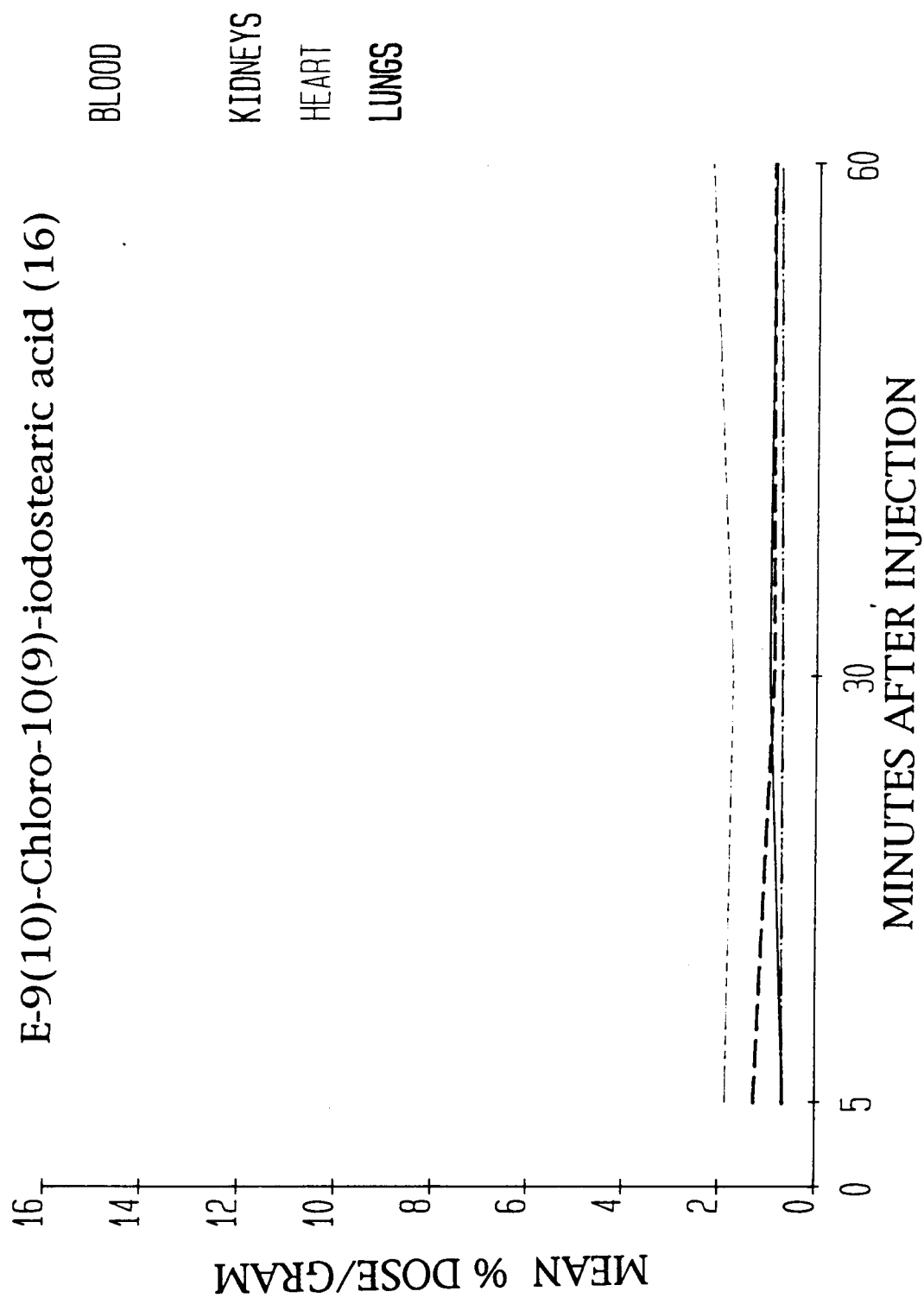
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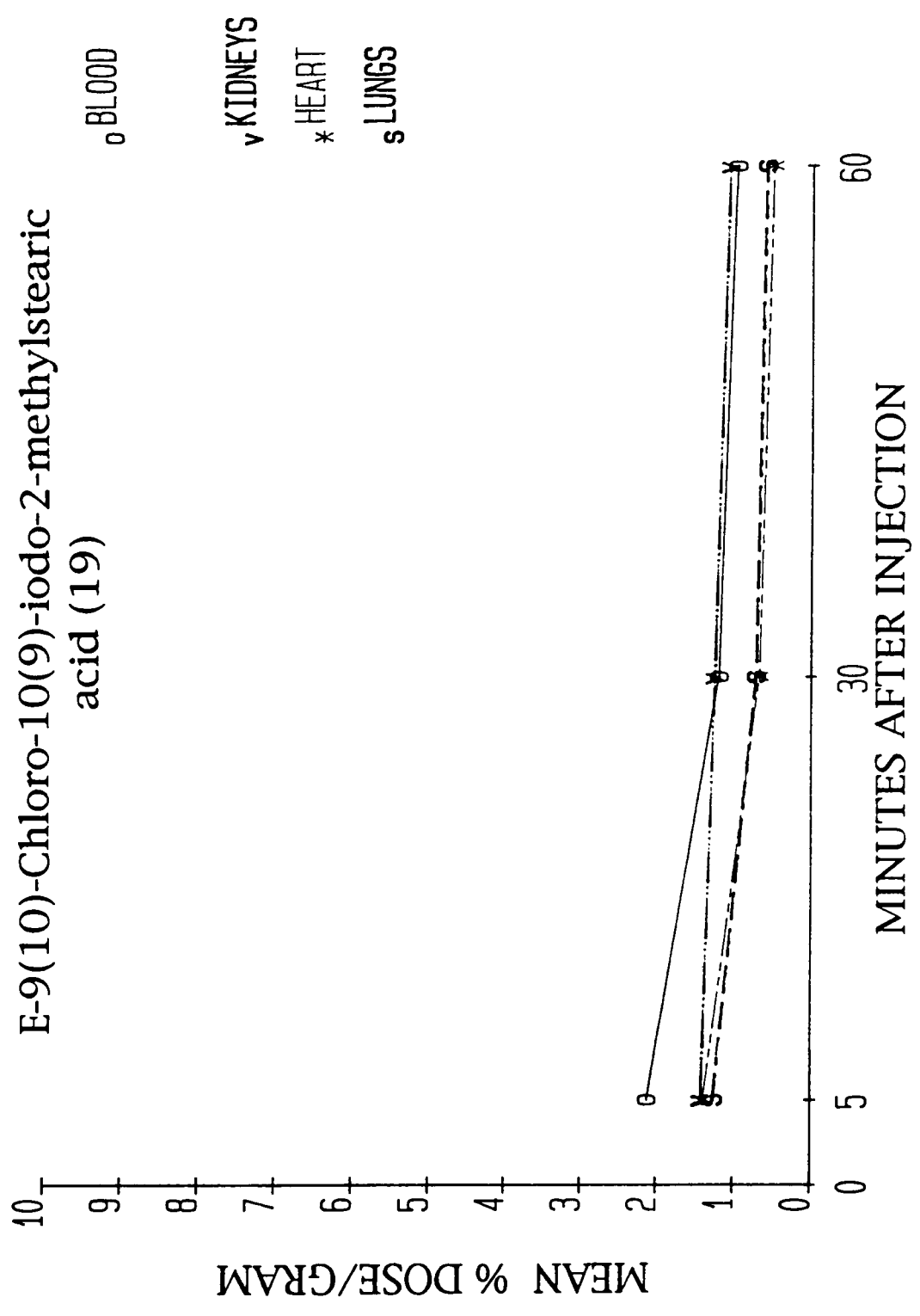
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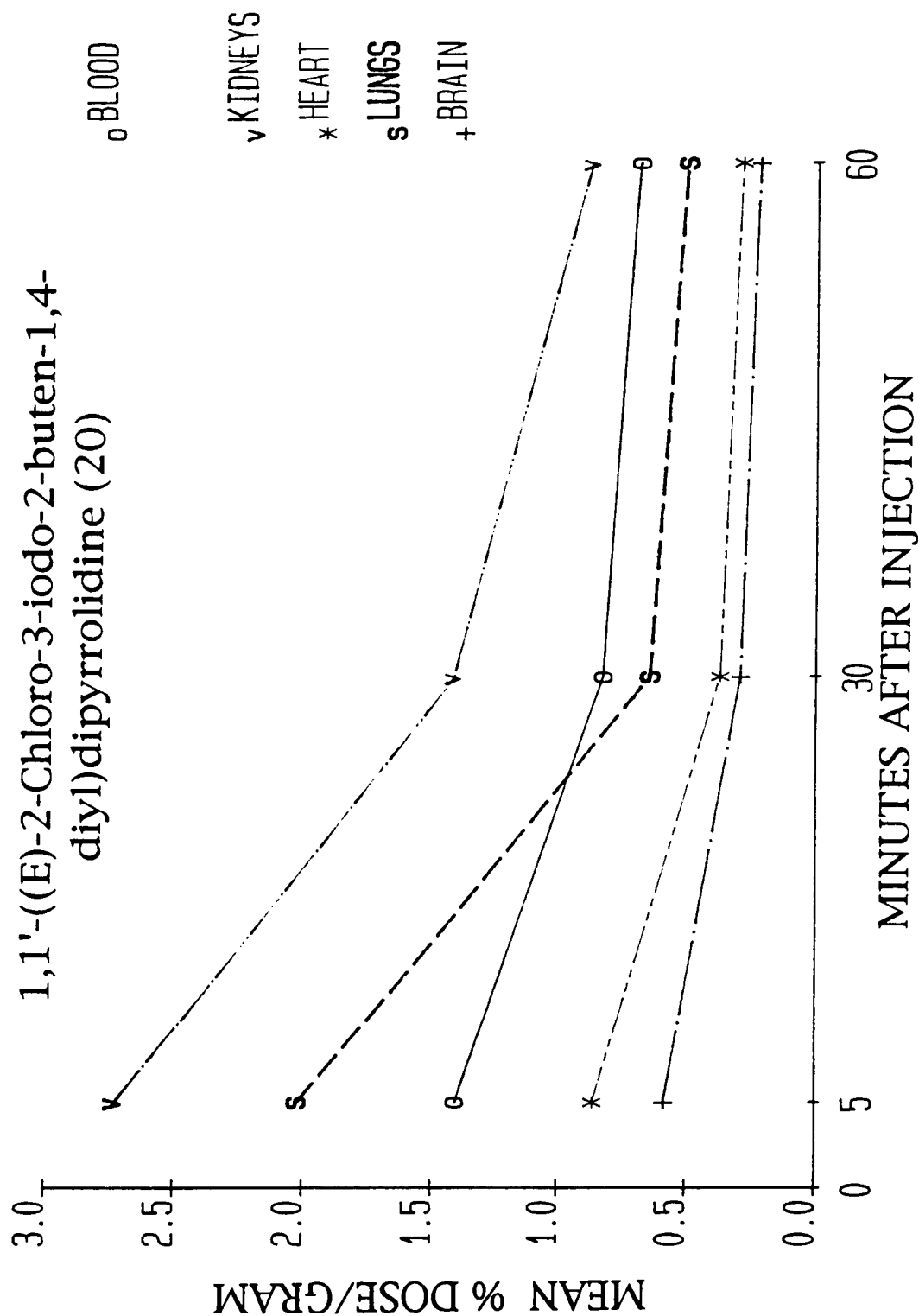
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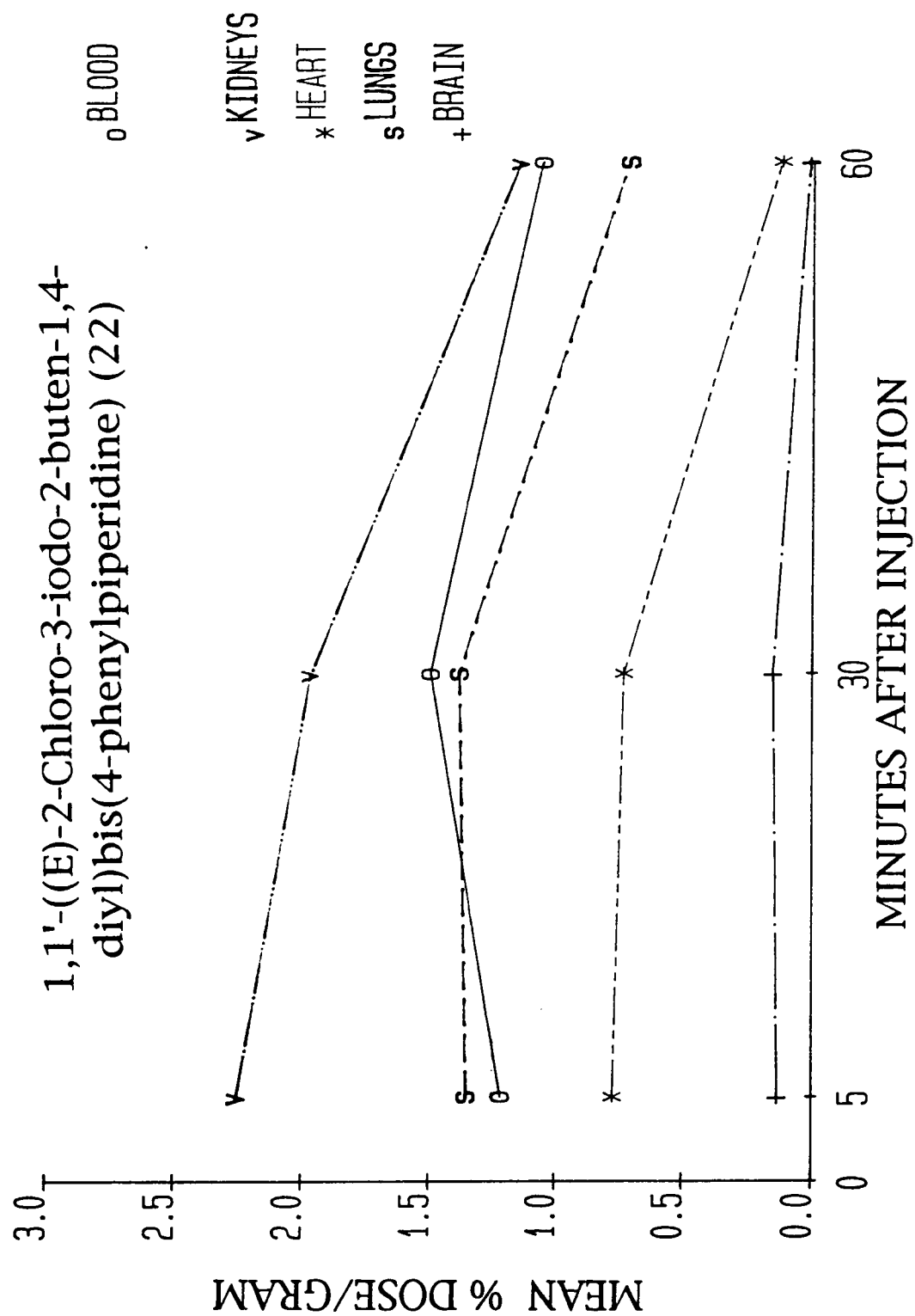
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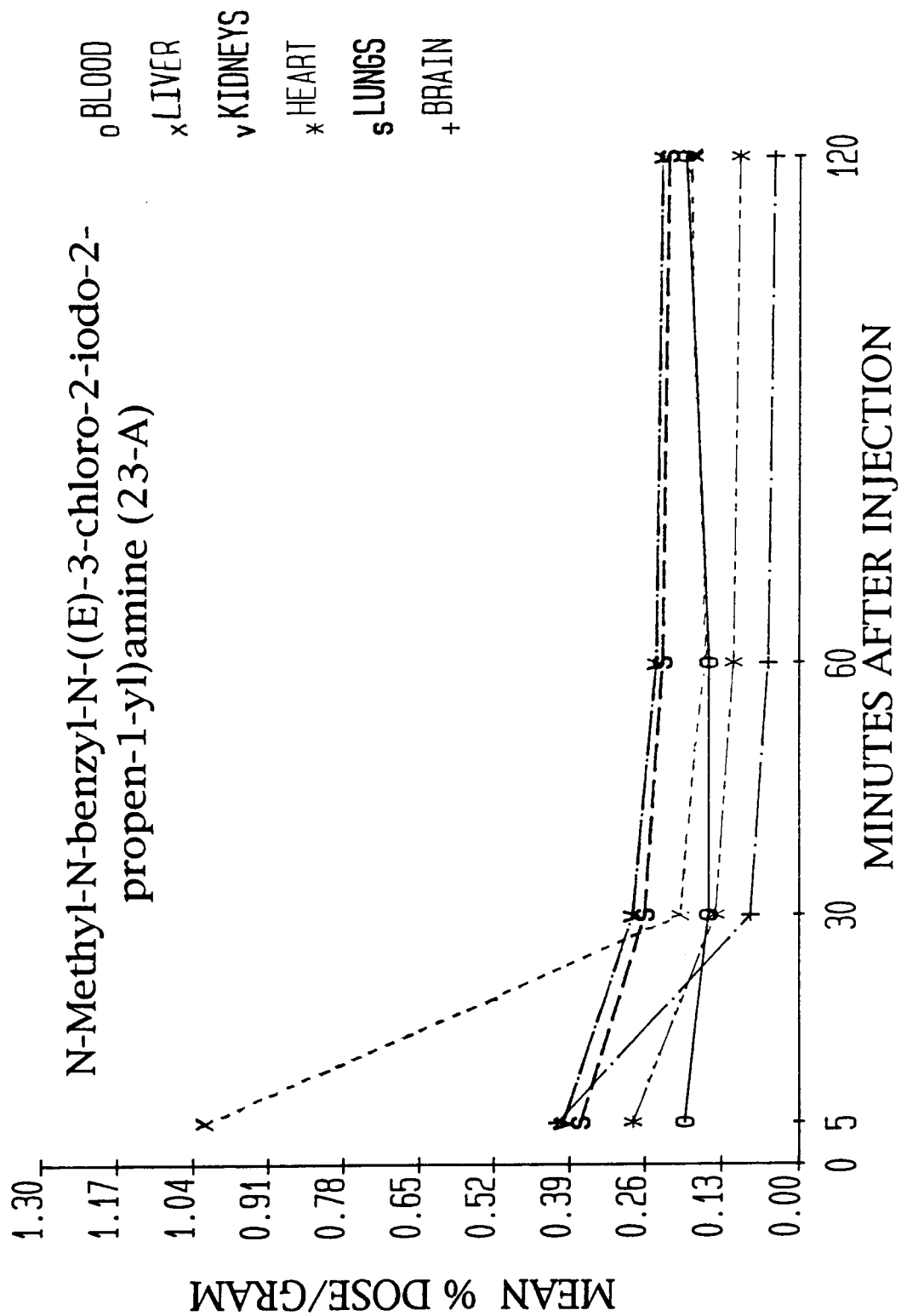


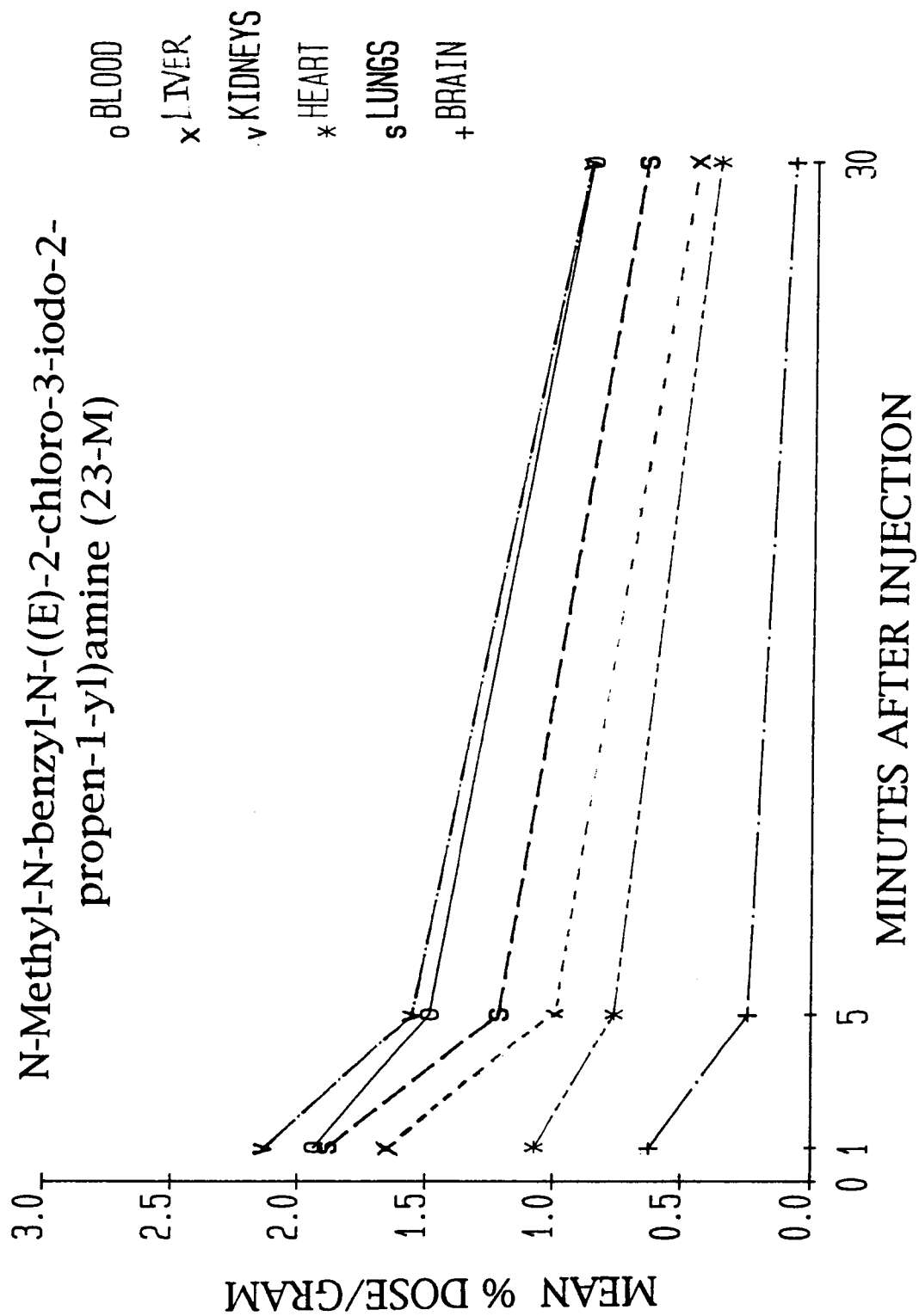


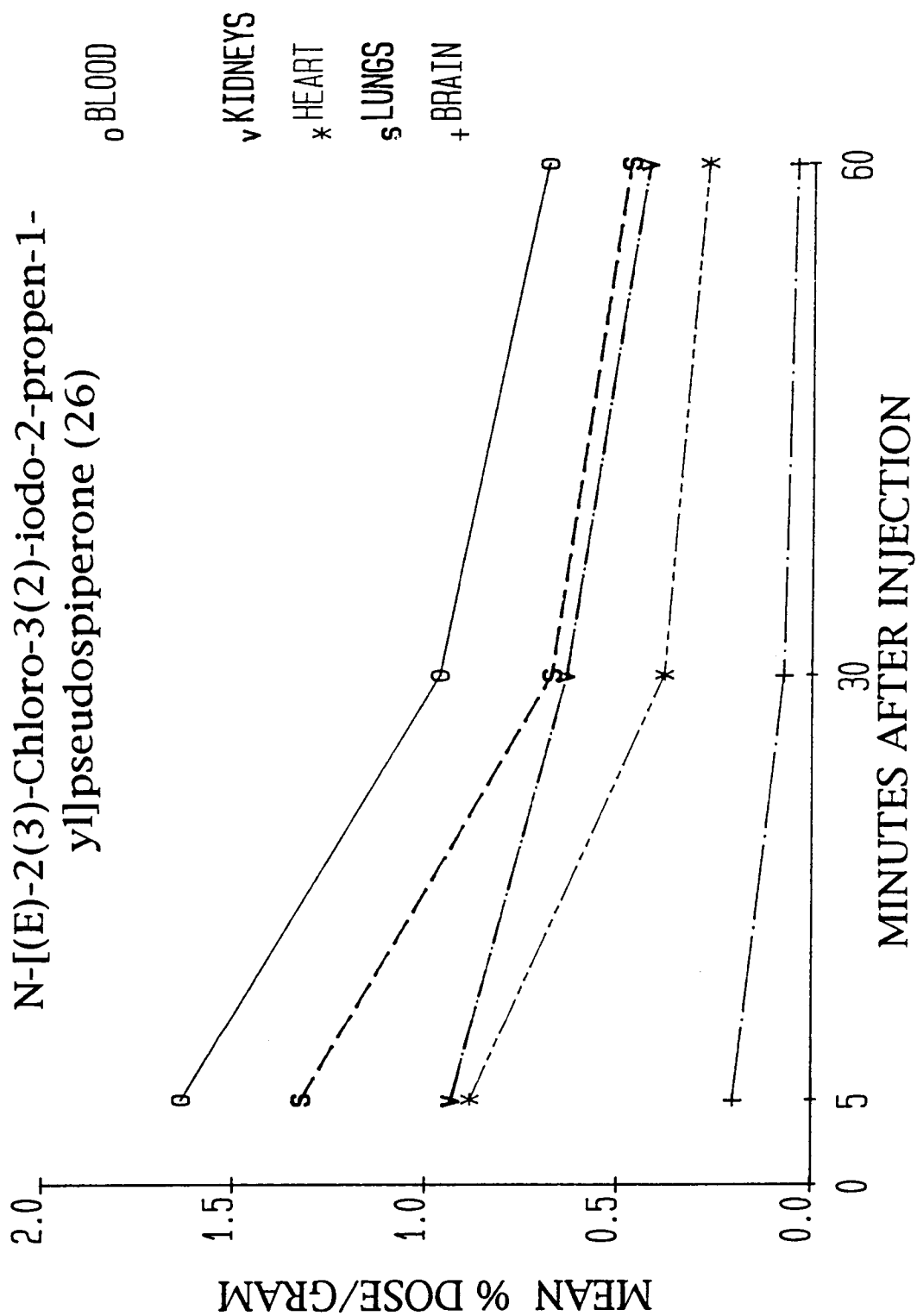


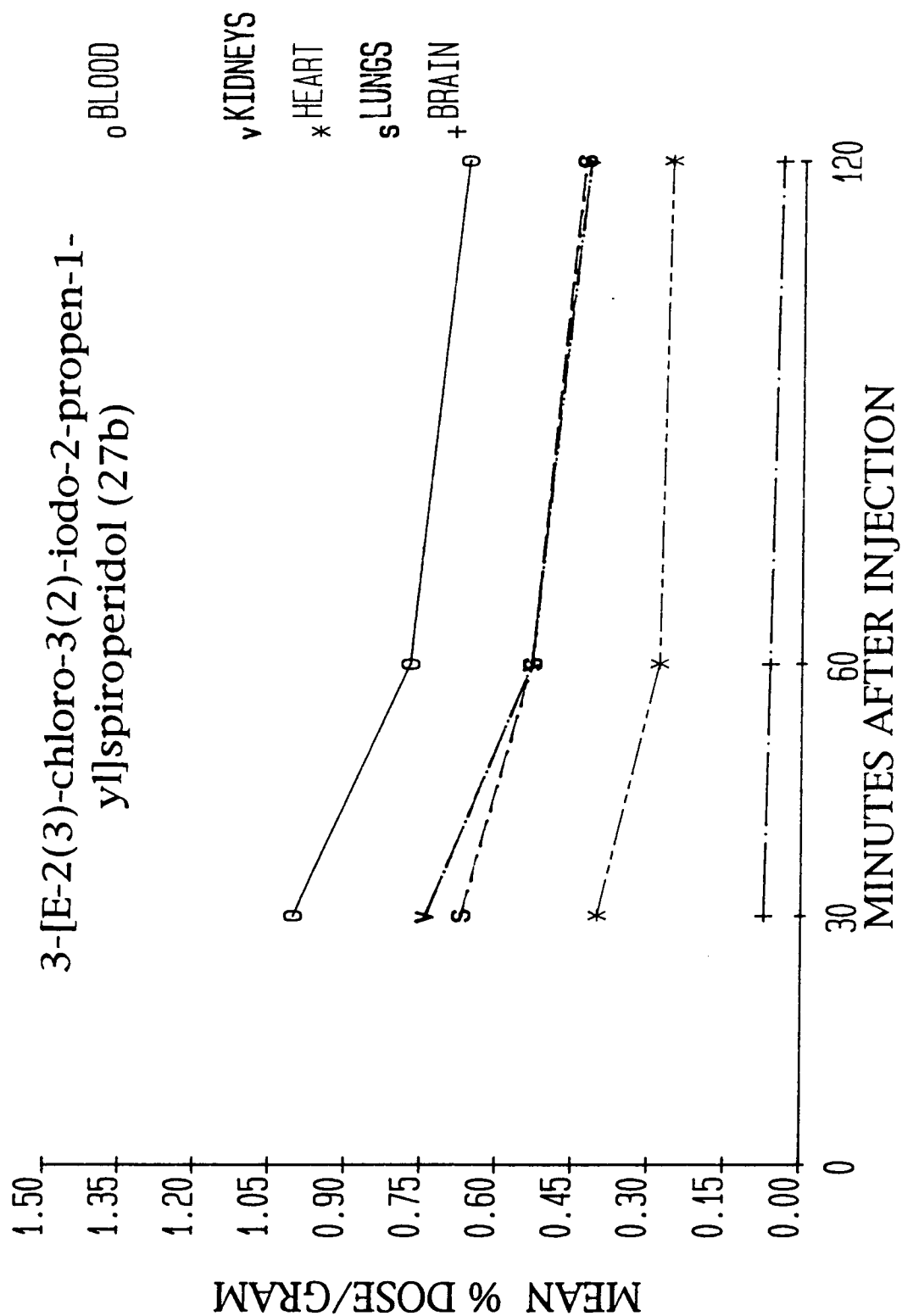


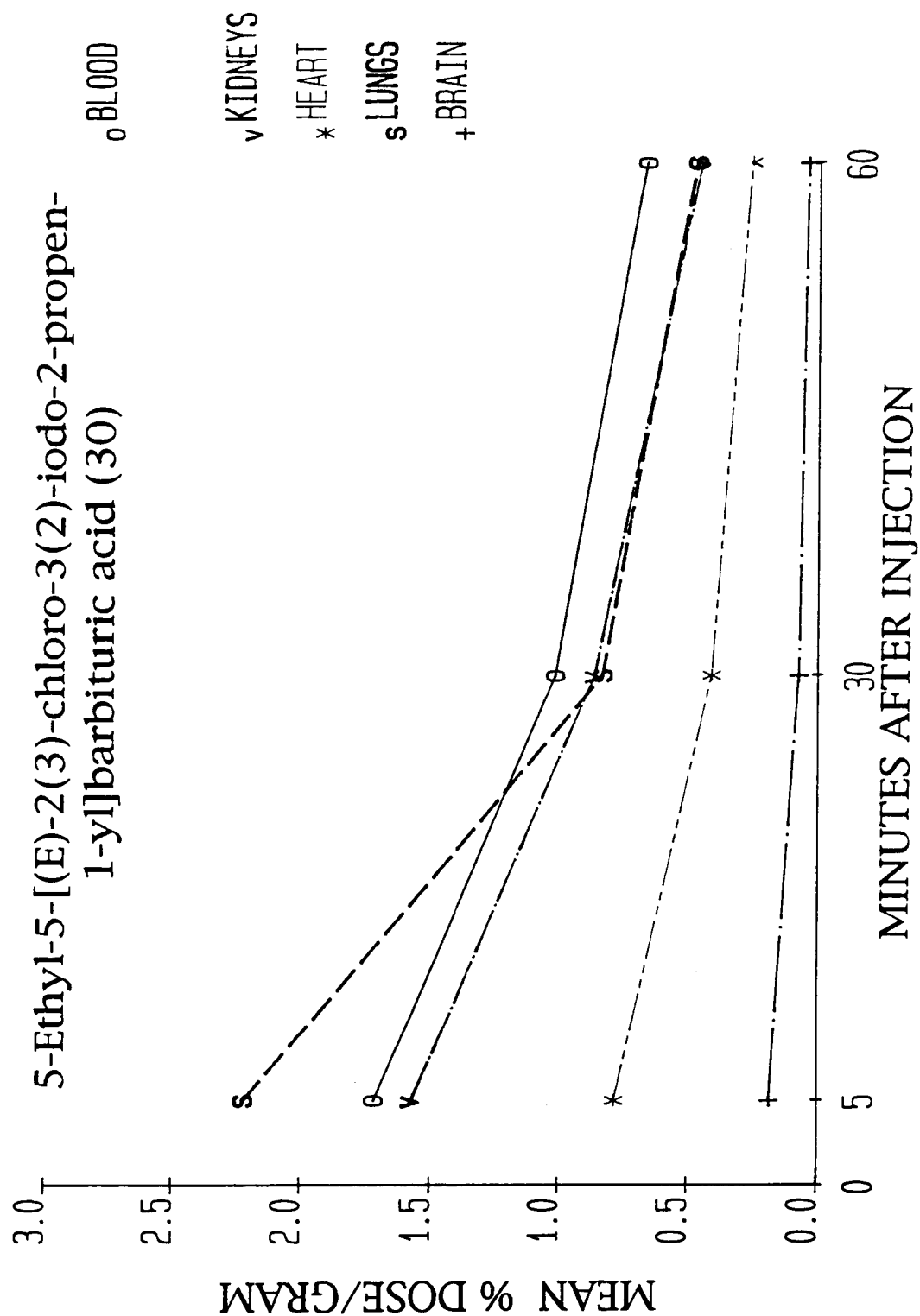


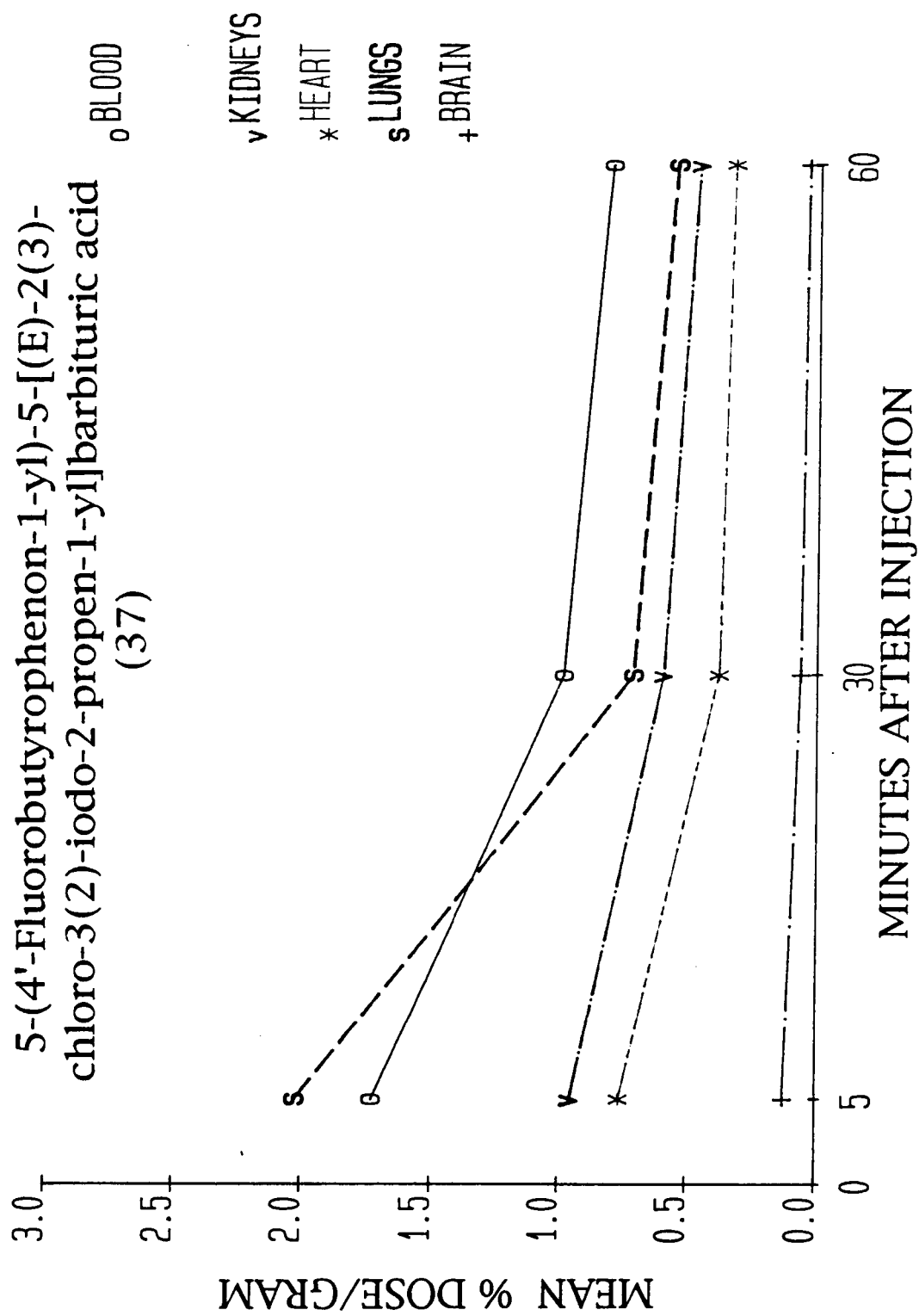


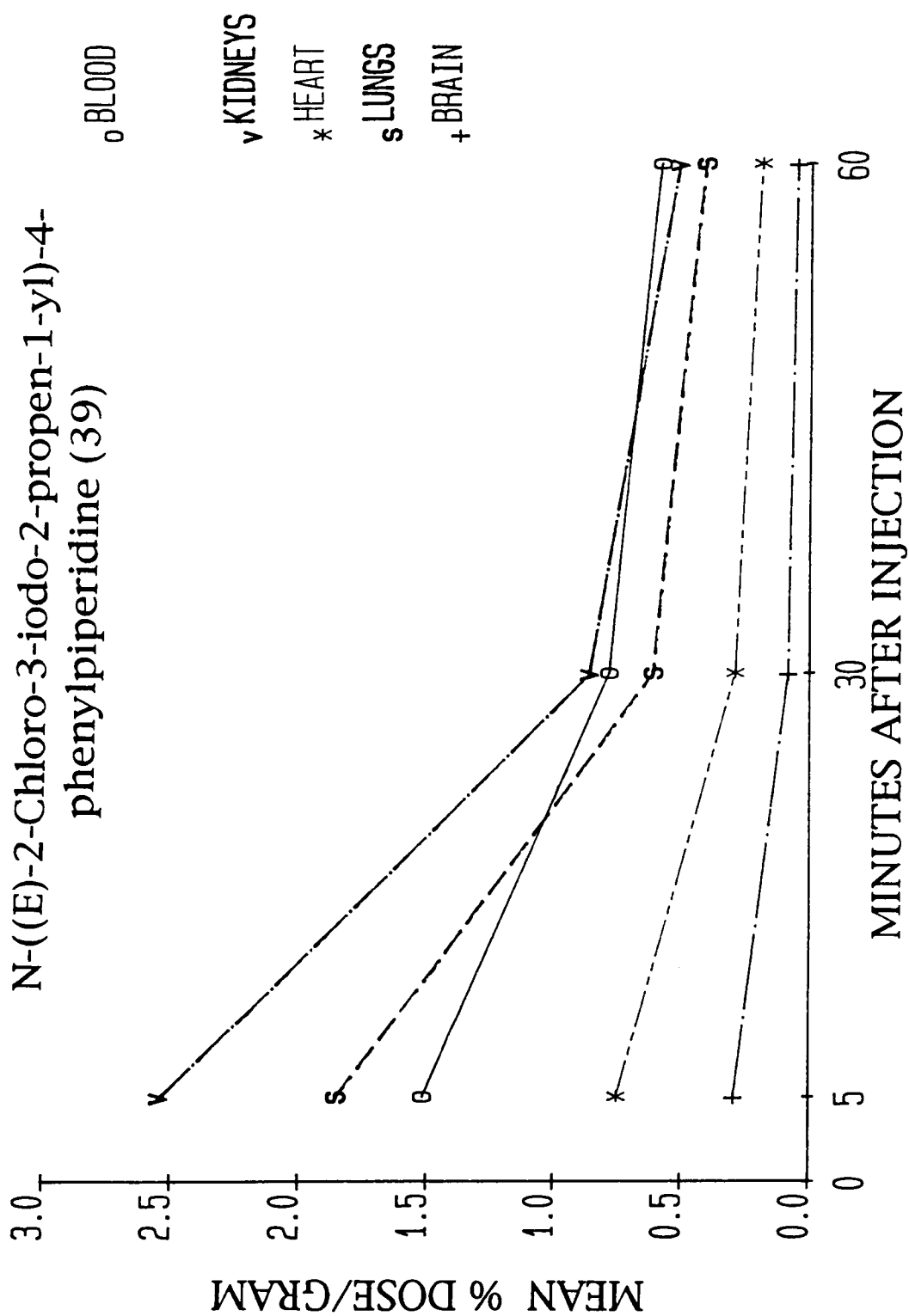


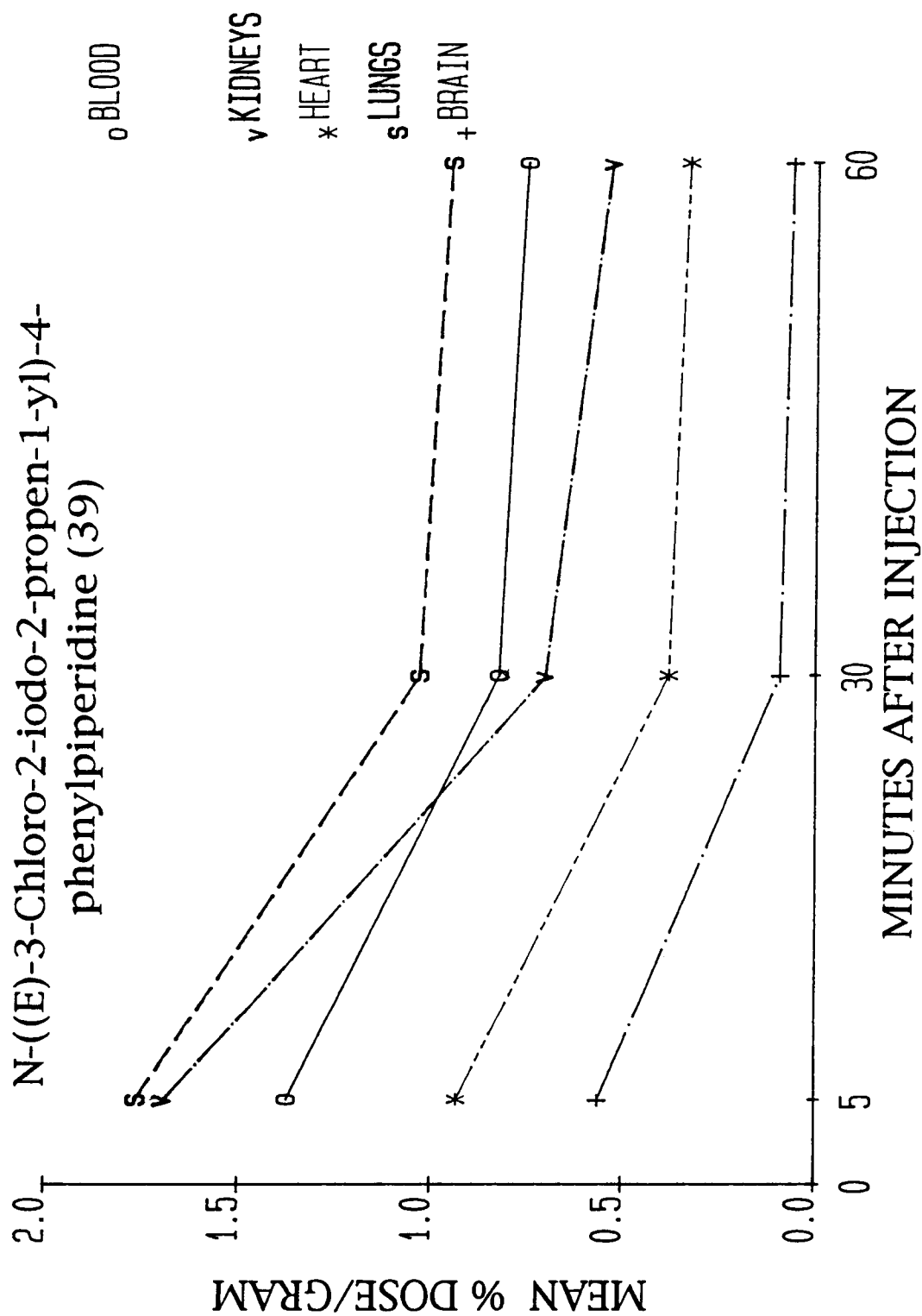


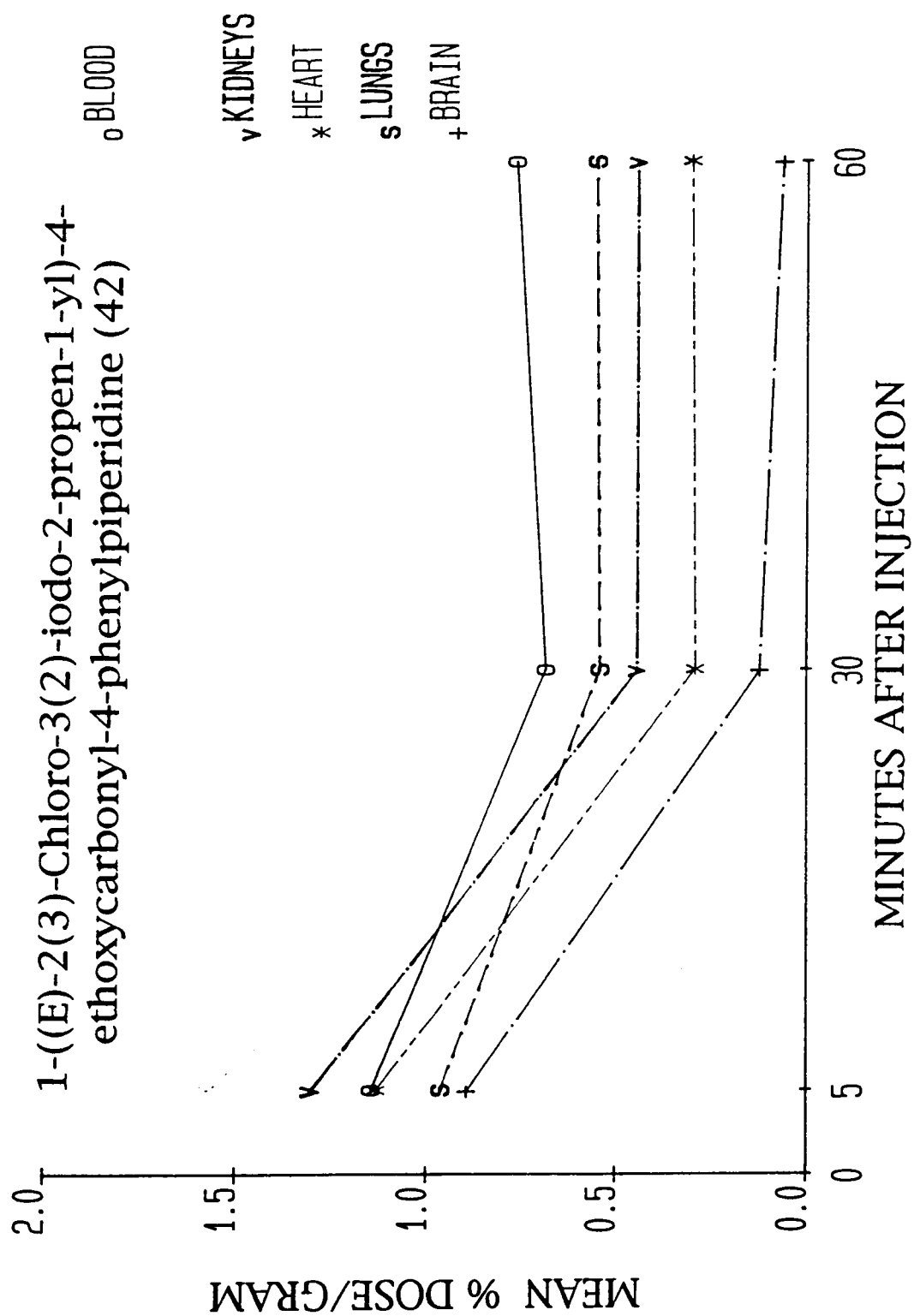












VITA

Steven James Lambert was born in Columbus, Ohio on July 10, 1954. He attended various elementary schools in Michigan, Iowa, and West Virginia. He attended Morgantown High School, in Morgantown, West Virginia and graduated in June 1972. The following September, he enrolled in West Virginia University for one year then transferred to Memphis State University. After two years in a pre-pharmacy program, he was accepted to the University of Tennessee Pharmacy School where he graduated three years later in June 1977 with a B. S. in Pharmacy. After practicing pharmacy for several years, he decided to further his education by enrolling in the graduate school of Memphis State University and earned a Master of Science degree in Chemistry. He then applied and was accepted to the University of Tennessee where he earned the Ph. D. Degree in 1994. His academic interests include mechanistic organic chemistry and organic medicinal chemistry.