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I am submitting herewith a dissertation written by Rikki Noel Waterhouse entitled "The Synthesis of Radioiodinated Carbohydrates and Butyrothienones as Potential Imaging Agents for Computed Tomography". 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.

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Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor
and Dean of The Graduate School

THE SYNTHESIS OF RADIOIODINATED CARBOHYDRATES AND BUTYROTHIENONES AS POTENTIAL IMAGING AGENTS FOR COMPUTED TOMOGRAPHY

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Rikki Noel Waterhouse

May 1993

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DEDICATION

The author wished to dedicate this work to her wonderful parents, Richard G. Waterhouse, Jr., Lt./Col. USAF (ret.) and Jacqueline A. Waterhouse, R.N., whose patient love, support and guidance have made completion of this degree possible.

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ABSTRACT

Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) are two relatively new imaging techniques which allow for the non-invasive evaluation of biochemical processes in living subjects. Currently, SPECT is more widely accessible than PET, however, only a limited number of radiotracers have been successfully developed for imaging by SPECT. Two classes of radioiodinated compounds were developed as potential imaging agents for SPECT: 1) Radioiodinated carbohydrates for the assessment of glucose metabolism and 2) Radioiodinated butyrothienones for the evaluation of dopamine D_2 receptors in the brain. In both classes of compounds, the radioiodine was attached to an sp^2 hybridized carbon atom to provide radiotracers that were chemically and metabolically stable. Radioiodine incorporation was easily accomplished by radioiodo-destannylation of vinyl- and aryl-trialkylstannanes in the presence of an oxidizing agent. The incorporation of radioiodine into small molecules can have a significant effect on the biological activity of the resulting radiotracer because of the relatively large size and lipophilicity of the iodine atom. Preliminary evaluations of the effectiveness of the radioiodinated carbohydrates and butyrothienones as imaging agents are presented.

TABLE OF CONTENTS

CHAPTER	Page
1. BACKGROUND	1
A. Introduction	1
B. General Considerations	2
1. Historical Background	2
2. Common Radionuclides in PET and SPECT	3
3. Synthetic Challenges in the Production of Radiotracers	5
4. Labeling with Radioiodine	7
5. Methods of Radioiodine Incorporation	9
a. Isotopic Substitution	10
b. Direct Iodination	10
c. Direct Substitution	11
d. Iododemetallation	12
C. Research Goals	18
2. Synthesis and Preliminary Evaluation of 3-C-((E)-2-[^{125}I]Iodoethenyl)-D-allose and 3-O-(E)-3-[^{123}I]Iodopropen-2-yl-D-glucose as Possible AgentsFor the Assesment of Disturbances of Glucose Metabolism by SPECT	20
A. Introduction	20
B. Rational	21
1. Project	21
2. Synthesis	26
C. Results and Discussion	30
1. Synthesis of the Alkynyl Precursors	33
2. Attempted Hydroboration of the Alkynyl Precursors	42
3. Addition of Tributyltinhydride to the Alkynyl Precursors	43
4. Iododestannylation of the Vinylstannanes	54
5. Hydrolysis of the Isopropylidene Groups	56
6. Preparation of the Radioiodinated Agents	61
7. Animal Distribution Studies	65
8. Computer Modeling Studies	67
D. Conclusions	69
E. Experimental	76
1. General	76
2. The Synthesis of 3-O-(E)-[123I]Iodopropen-2-yl-D-glucose	78
3. The Synthesis of 3-C-((E)-Iodoethenyl)-D-allose	88

CHAPTER	PAGE
3. The Synthesis of Iodinated Butyrothienones for SPECT Evaluation of Dopamine D_2 Receptor in the Brain	99
A. Introduction	99
B. Rational	99
1. Project	99
2. Synthesis	104
C. Results and Discussion	118
1. General	118
2. Synthesis of the Precusors	119
3. Synthetic Method for the Formation of Butyrothienones	127
a. Method A	127
b. Method B	143
4. Formation of the Radioiodinated Butyrothienones	145
5. Preliminary Evaluation of Butyrothienones	152
a. *In Vitro* Binding Studies	152
b. Lipophilicity	155
c. Structure-Activity Analysis	157
D. Conclusions	168
E. Experimental	172
1. General	172
2. Synthethic Procedures	176
3. Evaluation of the Lipophilicity of the Butyrothienones	217
References	220
Appendix	235

LIST OF TABLES

TABLE		PAGE
1-1.	Common Radionuclides Used in PET	4
1-2.	Common Radionuclides Used in SPECT	4
1-3.	Radioisotopes of Iodine and Their Properties of Emission	8
2-1.	Some Inhibition Constants (K_i) of D-Glucose Analogs Modified at C_3 and Tested as Inhibitors of L-Sorbose Entry into Human Erythrocytes	27
2-2.	Reaction Conditions for Attempted Addition of Catecholborane to 3-C-((E)-2-Iodoethenyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose, **4**	44
2-3.	Addition of Tributyltin Hydride to 3-C-((E)-2-Iodoethenyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose, **4**	49
2-4.	The Distribution of Radioactivity in Rat Tissues at Various Times After Intravenous Administration of 3-C-((E)-2-[^{125}I]Iodoethenyl)-D-allose	66
2-5.	The Distribution of Radioactivity in Rats Tissues at Various Times After Intravenous Administration of 3-O-((E)-3-[^{123}I]Iodopropen-2-yl)-D-glucose	68
2-6.	Commercially Available Chemicals and Their Manufacturers (Part A)	79
3-1.	Results of *In Vitro* Binding Studies of Butyrothienones at 10^{-5} M	153
3-2.	Results of *In Vitro* Binding Studies of Butyrothienones at 10^{-8} M	154
3-3.	Experimental Log P Values for Butyrothienones	158
3-4.	The Effect of Various Aromatic Rings on the S_2 Receptor Affinity of Ketanserin Analogs	161

Table | Page

3-5. Manipulation of the 4-Fluorobenzoyl Group of Ketanserin and the Corresponding Changes in Binding Affinity for the S_2 Receptor 163

3-6. Commercially Available Chemicals and Their Manufacturers (Part B) 175

3-7. Retention Times and Log P Values of Standards Used for the Log P Experiment 219

LIST OF FIGURES

FIGURE
PAGE

1-1. Common Methods of Radioiodination 9

2-1. Structures and Numbering of the α and β Forms of D-Glucose 22

2-2. Model for the Glucose-Carrier Complex in the Human Erythrocyte 28

2-3. Synthetic Scheme for the Synthesis of 3-C-((E)-2-Iodoethenyl)-D-allose, **7** 31

2-4. Synthetic Scheme for the Synthesis of 3-O-((E)-Iodopropen-2-yl-D-glucose, **1 2** 32

2-5. 90 MHz Carbon-13 Spectrum of 3-C-(2-Trimethylsilylethynyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose in $CDCl_3$ plus 1.0% TMS. 36

2-6. A View From Above the Lowest Energy Conformation of 1,2:5,6-Di-O-Isopropylidene-α-D-Ribohexafuranose-3-ulose, **3** 38

2-7. Diagram Displaying Van der Waals Radii of the Lowest Energy Conformation of 1,2:5,6-Di-O-Isopropylidene-α-D-Ribohexa-furanose-3-ulose 39

2-8. ^1H NMR Analysis of *Cis* and *Trans* Trialkyltin Hydride Adducts of Propargyl Alcohols 51

2-9. 250 MHz Proton NMR Spectrum of 3-C-((E)-2-Tri-n-butylstannyl-ethenyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose in $CDCl_3$ plus 1.0% TMS 52

2-10. ^{13}C NMR Analysis of Cis and TransTrialkyltin Hydride Adducts of Propargy Alcohols 53

2-11. ^1H NMR Analysis of *Cis* and *Trans* Trialkyltin Hydride Adducts of Propargyl Ethers 55

FIGURE		PAGE
2-12.	^{1}H NMR Analysis of Vinyl Iodides Derived From Propargyl Alcohols	57
2-13.	^{1}H NMR Analysis of Vinyl Iodides Derived From Propargyl Ethers	58
2-14.	250 MHz NMR Spectrum of 3-((E)-2-Iodoethenyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose in $CDCl_3$ plus 0.3% TMS	59
2-15.	Ion Chromatogram of 3-O-((E)-2-Iodoethenyl)-D-allose, **7**	62
2-16.	Ion Chromatogram of 3-O-((E)-3-Iodopropen-2-yl)-D-glucose, **12**	63
2-17.	Radio-Thin Layer Chromatogram fo the Crude Reaction Mixture of the Formation of 3-O-((E)-3[123I]Iodopropen-2-yl)-1,2:5,6-di-O-isopropylidene-α-D-glucose	64
2-18.	Diagram Displaying the Van der Waals Radii of β-D-Glucose	70
2-19.	Diagram Displaying Van der Waals Radii of 3-O-Propen-2-yl-β-D-glucose	71
2-20.	Diagram Displaying Van der Waals Radii of 3-O-((Z)-Iodopropen-2-yl)-β-D-glucose	72
2-21.	Diagram Displaying Van der Waals Radii of 3-O-((E)-Iodopropen-2-yl-β-D-glucose	73
2-22.	Diagram Displaying the Van der Waals Radii of 3-C-((E)-2-Iodo-ethenyl)-β-D-allose	74
3-1.	The Structures of Some Dopamine D_2 Antagonists	101
3-2.	D_2 Receptor Interactions of Select Neuroleptics	106
3-3.	Model for a Parabolic Relationship Between Lipophilicity and Biological Response	108

FIGURE		PAGE
3-4.	Spiperone Analogs with Radioiodine Attached to Different Positions on the Aromatic Rings	110
3-5.	Log P Values and Ratio of D_2 and 5-HT_2 Receptor Binding Affinities of Selected N-Alkylated Spiperone Derivatives	112
3-6.	Iodinated Butyrothienone Analogs of Spiperone	113
3-7.	Scheme I: Synthesis of Butyrothienones	115
3-8.	Scheme II: Synthesis of Butyrothienones	116
3-9.	Synthesis of Radioiodinated Butyrothienones	117
3-10.	Iodination of 4-Chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane	122
3-11.	250 MHz Proton NMR Spectrum of 4-Chloro-1,1-(ethylene-dioxy)-1-{2-(5-iodothienyl)]butane in $CDCl_3$ plus 0.3% TMS	123
3-12.	Expected Chemical Shifts and Coupling Constants for Iodinated Thiophenes	124
3-13.	90 MHZ Carbon-13 NMR Spectrum of 4-Chloro-1,1-(ethylene-dioxy)-1-[2-(5-iodothienyl)]butane in $CDCl_3$ plus 1.0% TMS	126
3-14.	90 MHz Proton Spectrum of 3-Ethyl-8-[4,4-ethylendioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 1.0% TMS	131
3-15	90 MHz Carbon-13 Spectrum of 3-Ethyl-8-[4,4-ethylendioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 1.0% TMS	132
3-16.	FTIR Spectrum of 3-Butyl-8-[4,4-ethylenedioxy-4-(2-thienyl)]-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one on a Sodium Chloride Plate	133

FIGURE PAGE

3-17. 250 MHz Proton Spectrum of 3-Ethyl-8-[4-(2-thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 0.3% TMS 135

3-18. 90 MHz Carbon-13 Spectrum of 3-Ethyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 1.0% TMS. 136

3-19. 90 MHz Carbon-13 Spectrum of 3-Benzyl-8-[4-[2(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 1.0% TMS 137

3-20. FTIR Spectrum of 3-Butyl-8-[4-(2-thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one on a Sodium Chloride Plate 138

3.21. Mass Spectrum (FAB, M + 1) of 8-[4-[2-(5-Iodothienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one 139

3-22. High Resolution Mass Spectrum (FAB, M + H) of 8-[4-[2-(5-Iodo-thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one 140

3-23. Mass Spectrum (FAB, M + 1) of 3-Benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one 141

3-24. High Resolution Mass Spectrum (FAB, M + H)of 3-Benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one 142

3-25. Radio-Thin Layer Chromatogram of the Crude Reaction Mixture From the Formation of 8-[4,4-Ethylenedioxy-4-[2-(5-[^{125}I]iodo-thienyl)]-butane]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one 149

3-26. Radio-Thin Layer Chromatogram of the Crude Reaction Mixture From the Acid Hydrolysis of 8-[4,4-Ethylenedioxy-4-[2-(5-[^{125}I]-iodothienyl)]-butane]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5] decan-4-one 150

Figure		Page
3-27.	Radio-Thin Layer Chromatography of purified 8-[4-[2-(5-[^{125}I]-Iodo-thienyl)]-4-oxo-butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one	151
3-28.	Log P of Standards Versus Retention Time Relative to Catechol	156
3-29.	A Hypothetical Model for the Binding of Compounds Derived from 1-Phenyl-1,3,8-triazaspiro[4,5]decan-4-one to Serotonin S_2 Receptors	164
3-30.	3-Dimentional Plot of the Conformational Energy of **10f** Versus the Angle of Rotation of the Benzyl and Phenyl Groups	167
3-31.	Compounds that may be Selective for Dopamine D_2 Receptors Versus Serotonin S_2 Receptors	170
A-1.	250 MHz Proton NMR Spectrum of 1-Phenyl-1,3,8-triazaspiro-[4.5]decan-4-one, 5a, in $CDCl_3$ plus 0.3% TMS	237
A-2.	^{1}H-NMR Assignments for the Piperidine Ring Protons of 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5 a**	238

Chapter 1

BACKGROUND

A. Introduction

The strategies involved in the design and synthesis of radioiodinated, biologically active compounds as biomedical imaging agents for single photon emission computed tomography (SPECT) are presented in this thesis. The synthetic routes described in this thesis were designed to introduce radioiodine at a specific site in a molecule so as to produce radiotracers that are both chemically and biologically stable. Because of the relatively short half-lives of the nuclides involved in SPECT, the radiolabeling must take place in the final steps of the syntheses. The radiolabel should be incorporated into the substrate stereospecifically and in high yield. There should also be minimal byproduct formation in order to simplify purification and to obtain a high specific activity of the radiolabeled product. Usually, femtomolar quantities of the radionuclide are introduced in the labeling step and, therefore, one must take great care in the preparation of all reagents and glassware since any side reactions caused by impurities can severely reduce radiochemical yields. The incorporation of radioiodine into biologically active molecules can cause large structural, electronic and lipophilicity changes and the possible consequences of these changes on biological activity must be considered while formulating the synthetic strategy of a specific radiopharmaceutical.

The constraints noted above create considerable challenges to the synthetic chemists who, to be successful, must have a knowledge of synthetic

chemistry, radiochemistry, analytical chemistry, biochemistry, pharmacology, drug design, metabolism and computer science. In this chapter, background material is presented which will set the foundation for subsequent chapters.

B. General Considerations

1. Historical Background

The first artificially produced radioisotopes were positron emitting nuclides generated in 1934 by F. Joliot and I. Curie (daughter of Mme. Curie), who found that boron and aluminum could be made radioactive by bombarding them with the α rays produced by polonium.[1] Positrons were discovered as components of cosmic radiation just two years earlier by C. D. Anderson.[2] At the time of the Curie-Joliot discovery, several laboratories had developed instruments for the acceleration of hydrogen and helium ions to energies great enough to cause nuclear transmutations in bombarded atoms. One of these devices, the cyclotron, was developed by Lawrence in 1930. With the discovery of the neutron in 1932 and deuterium in 1933, two other bombarding particles became available and, by 1963, over 1300 artificially produced radioisotopes were known.[3] Every known element has at least one radioactive isotope and many of these have found applications in chemistry, physics, biology, medicine and engineering.

The half-lives of the known isotopes range from fractions of seconds to millions of years and many modes of decay are possible, including emission of positrons, photons, α particles, β particles and neutrons. In this discussion, the focus will be on radioisotopes that emit positrons or photons and that have half-lives ranging from a few minutes to several hours.

The development of nuclear medicine began in the 1940s when scientists in the United States and England tried to quantitate the percentage of ^{131}I taken up by the thyroid gland. At that time, the only detector that could measure emission from ^{131}I was the Geiger-Müller tube and with an efficiency of only 1%. Many advancements have since been made in the detection of radionuclides and procedures for biomedical imaging purposes have been developed, including single photon emission tomography (SPECT) by Kuhl and Edwards[4] in 1963 and positron emission tomography (PET) by Ter-Pogossian[5] in 1974. Several books and articles have been published which outline the development of the techniques and instrumentation of radionuclide imaging by PET and SPECT.[6] Today, both PET and SPECT are powerful tools for the non-invasive imaging of internal organs and evaluation of biological processes in humans.[7] The use of PET and SPECT to perform clinical examinations has become the major motivation to develop radiotracers that mimic the biological properties of compounds that are normally involved in biochemical processes.

2. Common Radionuclides in PET and SPECT

The radionuclides commonly used for PET and SPECT are listed in Table 1-1 and Table 1-2, respectively. Radionuclides used for these imaging techniques are required to have a half-life long enough to allow incorporation onto the substrate, including any further synthetic steps and/or purification, but short enough to minimize the time of exposure to the patient. The decay energy of the emission produced must be sufficient to penetrate tissues and reach the detectors; typically, isotopes with decay energies between 0.02 and 0.50 MeV should be used for best results.

Table 1-1. Common Radionuclides Used in PET

Isotope	Half-life (min.)	Emax
Oxygen-15	2.04	1.70 MeV
Nitrogen-13	9.96	1.19 MeV
Carbon-11	20.40	0.98 MeV
Gallium-68	68.00	1.90 MeV
Bromine-75	95.50	1.70 Mev
Fluorine-18	110.0	0.64 Mev

Table 1-2. Common Radionuclides Used in SPECT

Isotope	Half-life	Emax
Iodine-123	13.3 hr.	0.159 MeV
Bromine-82	35.3 hr.	0.44 MeV
Thallium-201	73 hr.	0.41 MeV
Technecium-99*m*	6.0 hr.	0.143 MeV
Indium-111	2.8 d.	0.247 MeV

Advantages of the PET isotopes include the fact that they can be produced in high specific activities (between 2,000 and 10,000 Ci/mmol),[8b] allowing the production of radiolabeled agents that are essentially free of unlabeled material. In addition, many of the radionuclides used in PET, such as ^{11}C and ^{13}N, are isotopes of elements that are native to many biologically active compounds. This allows the chemist to synthesize radiolabeled agents without changing the chemical and biological properties of the original compound.

The radionuclides used for SPECT imaging are generally longer-lived than those for PET and they can often be produced by an on-site reactor (or generator) or purchased commercially. The longer half-lives of the SPECT isotopes allow more time for synthesis and purification of radiolabeled agents as opposed to the isotopes used in PET. The positron emitting isotopes usually are produced by a cyclotron and are so short-lived that it is generally not feasible to produce these nuclides commercially. Therefore, in order to utilize PET imaging techniques, a cyclotron capable of producing the desired radionuclides must be available at the site of synthesis and application.

3. Synthetic Challenges in the Production of Radiotracers.

The development of short-lived radiotracers for PET and SPECT presents many challenges to the synthetic chemist. In general, the total time for the synthesis, purification and delivery of the radiolabeled product to the patient should be no more than 3 half-lives. This often leaves little time for subsequent chemical manipulations and purification. Also, radiolabeling in the final steps of the synthesis helps limit the exposure of the chemist to radiation. To be

successful, the chemist must be acquainted with the synthetic methods available for the incorporation of the radionuclide of interest and be able to incorporate the radiolabel near the end of the synthesis. Finally, the radiotracer produced must be both chemically and biologically stable.

Most of the radiolabeled compounds used in PET and SPECT are derived from compounds of known biological activity. If the radioisotope used is of an element not native to the parent substrate, the physical and electronic changes imposed on the molecule will most likely effect its biological activity. This effect can be either positive or negative. Therefore, when creating a synthetic strategy for a specific radiopharmaceutical, the chemist must consider the structural, electronic, and lipophilic changes that arise upon incorporation of the radiolabel and the possible consequences of these changes on biological activity.[8] In recent years, computer programs have been developed to aid the chemist in estimating the consequences of these physical changes by exploring structure-activity relationships and conformational analysis.[9] Methods for determining lipophilicities by HPLC, TLC, computer analysis and chemical partitioning have also been developed.[10] Utilization of these aids can provide valuable insight into the design of a radiotracer for a specific biological activity and/or can provide an explanation of observed biological activity.

In general, there are two types of radiolabeled imaging agents; these are defined as receptor binding agents or non-receptor binding agents. The non-receptor binding agents may be separated into two categories; those with high affinity, where the binding depends on structural and electronic effects, and those with low affinity, where binding is independent of these effects. Examples of high affinity, non-receptor binding usually involve carrier proteins

such as those found in conjunction with the human sex steroids and glucose transport. Because of the large numbers of these carrier proteins in the body, a certain amount of unlabeled agent is usually tolerated. However, radiotracers that are designed as receptor binding agents must be produced "carrier free" (in high specific activity) because of the relatively low numbers of receptors found in the body. With respect to radioiodinations, the term "carrier" is used to refer to the presence of substrate labeled with stable ^{127}I and "carrier free" refers to a radioiodinated agent that is free of all ^{127}I labeled substrate. In order to accomplish the synthesis of a radioiodinated receptor binding agent in a carrier free manner, it is necessary to start with radioiodine with a high specific activity. This is important because unlabeled materials will swamp the receptors and inhibit the radiolabeled analog from binding.

4. Labeling with Radioiodine

Iodine-123 possesses very favorable radionuclidic properties for SPECT imaging; these include a 13.3 hour half-life, ideal emission energy and high attainable specific activity (4×10^3 mCi/μmol).[11] In addition, many more medical centers are equipped to perform SPECT than PET, therefore radioiodinated imaging agents can be utilized by a larger patient population than agents labeled with PET isotopes. Although somewhat expensive, ^{123}I is commercially available, usually in the form of sodium iodide in aqueous sodium hydroxide solution. There is also the possibility of producing the ^{124}I labeled analogs for PET imaging,[12] however, few medical centers have cyclotrons capable of producing this isotope. As an added advantage, Exploratory synthesis and evaluation can be carried out with the longer-lived isotope, ^{125}I,

Table 1-3. Radioisotopes of Iodine and Their Properties of Emission

Nuclide	Half-life	Decay Mode	Energy (Kev)
Iodine-122	3.62 min.	β+	1363 (β+, 76%)
Iodine-123	13.13 hr.	EC	159 (γ, 83.4%)
Iodine-124	4.18 d.	β+	830(β+, 23.3%)
Iodine-125	60.14 d.	EC	27 (X-ray, 73%)
Iodine-131	8.04 d.	β-	364 (γ, 8.9%)
			191 (β-, 89%)

which has a half-life of 60.14 days. The radioactive isotopes of iodine are outlined in Table 1-3.

The incorporation of iodine isotopes into a substrate often has a significant effect on its biological properties. This is due to the relatively large increases in size, polarizability, and lipophilicity of radiotracers labeled with iodine isotopes. Therefore, one must consider that a radioiodinated analog is not a true tracer for the parent compounds for which they are derived, especially when the parent compound is relatively small in size.[13]

A second consideration in radiolabeling with isotopes of iodine is the susceptibility of the carbon-iodine bond to metabolic cleavage. This is especially true when the iodine is attached to a sp^3 hybridized carbon atom. It has been shown that an iodine atom, when attached to an sp^2 hybridized

carbon atom, is significantly more stable *in vivo*. Many laboratories have incorporated this strategy into the design of their radioiodinated agents.

5. Methods of Radioiodine Incorporation

Free iodine in the bloodstream would do little except concentrate in the thyroid, therefore, a radioiodine atom must be attached to a substrate molecule capable of exhibiting the desired biological properties needed for the experiment. Any synthetic method used for the incorporation of radioiodine should take place quickly, stereospecifically and in high yield. One must also consider the fact that the radionuclide will be present in very small quantities, often in femtomolar concentrations. Therefore, any chemical impurities or side reactions that interfere with incorporation of radioiodine could severely reduce radiochemical yields. An outline of some of the common methods for radioiodination is given in Figure 1-1.

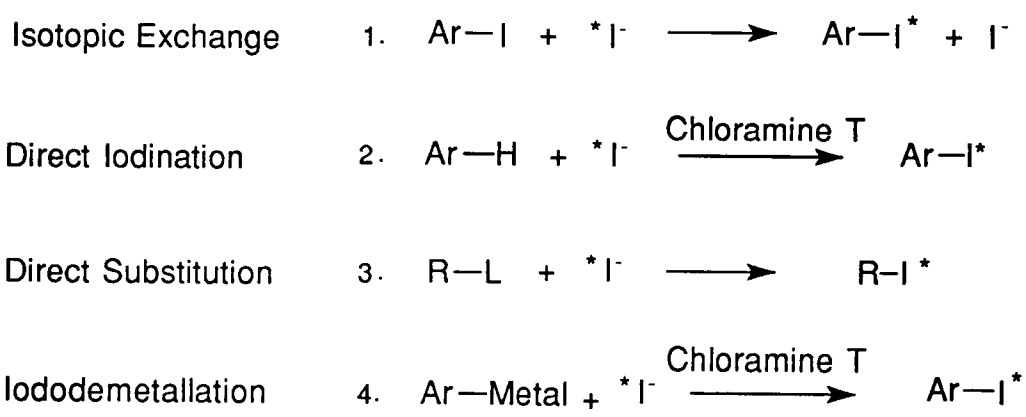
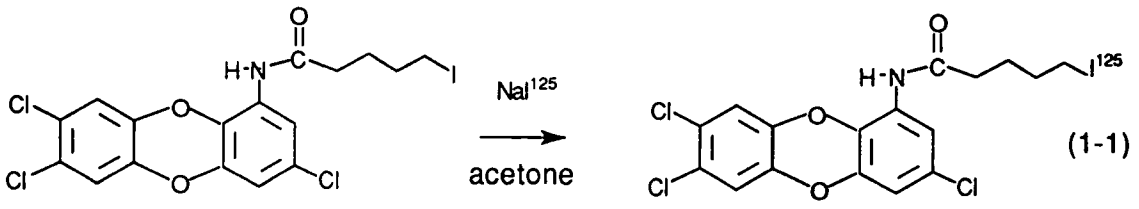


Figure 1-1. Common Methods of Radioiodination

a. Isotopic Exchange

Isotopic exchange has been used to incorporate radioiodine onto a variety of substrates.[14] For example, a tryptomine hapten was labeled with ^{125}I by the reaction of the ^{127}I labeled parent substrate with $Na^{125}I$ in acetone for 66 hours (equation 1-1).[15] One disadvantage of this method is that long reaction times are required, often resulting in lower radiochemical yields and degradation of the targeted material. Also, the presence of the unlabeled substrate, which is inseparable from the radiolabeled product, lowers the specific activity of the radiotracer and, therefore, this technique would not be suitable whenever high specific activities of the product are needed.

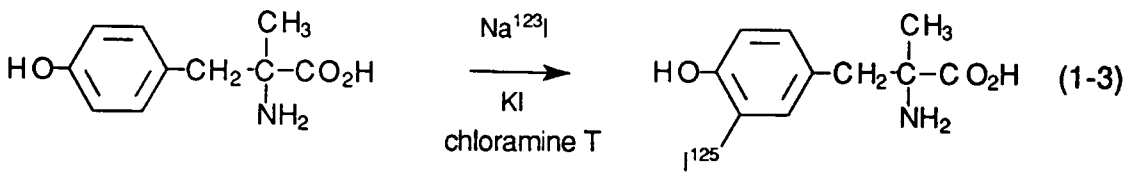


(1-1)

b. Direct Iodination

Direct iodination has proven effective when the substrate contains activated aromatic rings.[17] In general, the substrate is exposed to ICl, which is formed *in situ* by the reaction of sodium or potassium iodide and an oxidizing agent, such as N-chlorosuccinamide or chloramine-T. The reaction takes place in an aqueous buffered solution, limiting this method to water-soluble compounds unless two-phase reactions are employed. Because iodination might occur at various positions on the aromatic ring, direct iodination may not

be suitable for the synthesis of radiotracers whose function is sensitive to the position of the iodine atom on the aromatic ring. An example of direct radioiodination is the formation of L-3-[^{123}I]iodomethyltyrosine for imaging of brain tumors by SPECT. Exposure of L-α-methyltyrosine to potassium[^{123}I]iodide and chloramine-T for 2 minutes at room temperature resulted in the formation of the desired radiotracer in 65% radiochemical yield (equation 1-3).[18]



(1-3)

c. Direct Substitution

Direct substitution has some advantages over isotopic substitution, including the fact that high specific activities can be obtained provided that the unlabeled precursors can be separated from the radiolabeled product. The reaction usually takes place by refluxing the brominated precursor in acetone in the presence of sodium radioiodide or by exchange in a melt. For example, radioiodination by exchange for bromine has been used to label long-chain fatty acids for heart imaging (equation 1-2).[16]

$$Br(CH_2)_{16}CO_2H + NaI^* \xrightarrow[\text{reflux}]{\text{acetone}} {}^*I(CH_2)_{16}CO_2H + NaBr \quad (1\text{-}2)$$

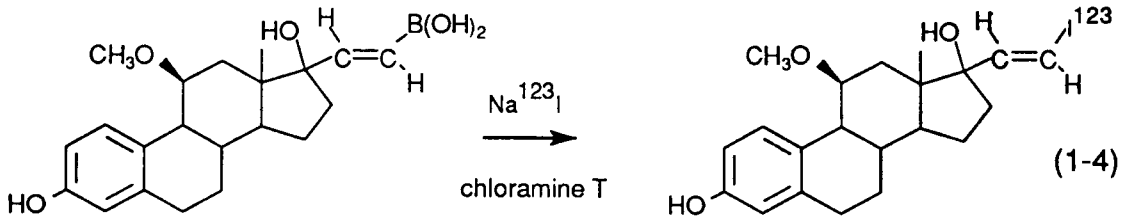
d. Iododemetallation

One of the most successful methods for stereospecific radiohalogen incorporation into a substrate molecule is iododemetallation in the presence of an oxidizing agent.[19] The most common metals utilized in these transformations are boron, tin, mercury, thallium, silicon, and germanium. Iododemetallation is very useful for the radioiodine incorporation of highly deactivated aromatic rings because iodination sometimes cannot be accomplished by the methods previously described. Examples of radioiododemetallation reactions of organic substrates containing boron, tin, thallium and silicon are provided in the following discussion.

Some of the earliest radioiodinated vinyl iodides were formed by reaction of vinylboronic acids with ICl formed *in situ* by the reaction of sodium radioiodide with chloramine-T.[20] This methodology can also be applied to obtain radioiodinated aromatic rings by treatment of the corresponding arylboronic acids with sodium radioiodide in the presence of an oxidizing agent.[20] This method is limited because of the relatively small number of boronic acids that can easily be prepared; however, transmetallation reactions have been developed which allow a wider variety of boronic acids to be obtained.[21] One example of incorporation of radioiodine by iododeboronation is the synthesis of ^{125}I labeled steroids by the treatment of the corresponding

vinyl boronic acid precursors with $Na^{125}I$ in the presence of an oxidizing agent (equation 1-4).[22]

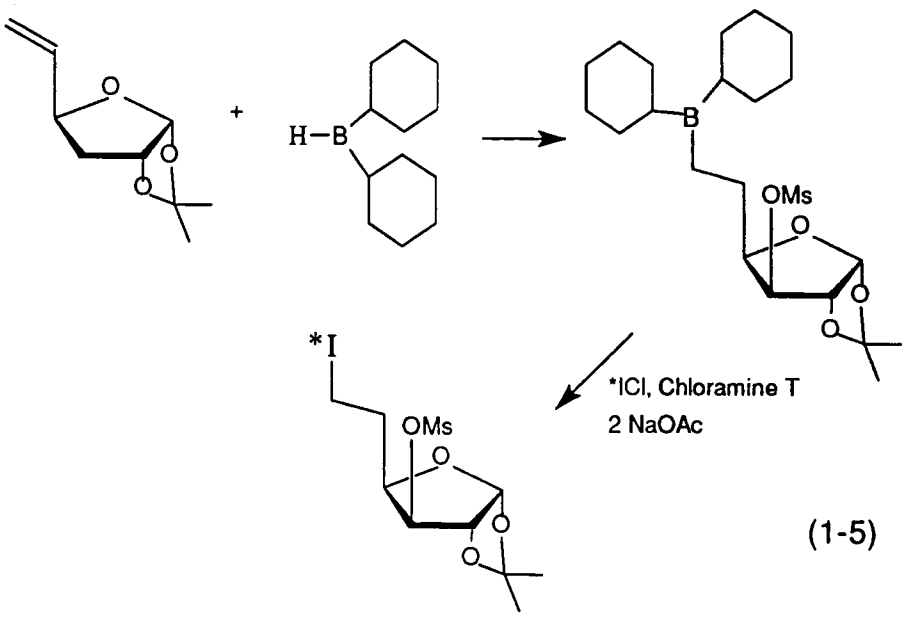
Other methods of iododeboronation have been used to attach radioiodine to sp^3 hybridized carbon atoms of substrate molecules. One method for the radioiodination of carbohydrate derivatives was developed by



(1-4)

Hall and Nesser[23] (equation 1-5). This reaction was based on the observation that trialkylboranes, derived from the addition of dicyclohexylborane to alkenes, could be reacted with iodine under oxidative conditions, resulting in the attachment of iodine to the primary alkyl group of the trialkyboranes.[24]

Moerlein and Coenen have compared group IVb organometallic derivatives as candidates for radioiodination and radiobromination. A series of *para*-substituted aryltrimethyl-silicon, -germanium, and -tin compounds were radiohalogenated with "no carrier added" radiobromide and radioiodide oxidized *in situ* by dichloroamine-T.[25] With respect to the radioiodination experiments, the rate of iododemetallation decreased in the order Sn > Ge > Si under a variety of reaction conditions and different ring substituents. It was concluded that organostannanes were the most versatile of the reagents for radioiodine incorporation, however, the lability of the carbon-tin bonds caused several problems, including protodestannylation and the formation of



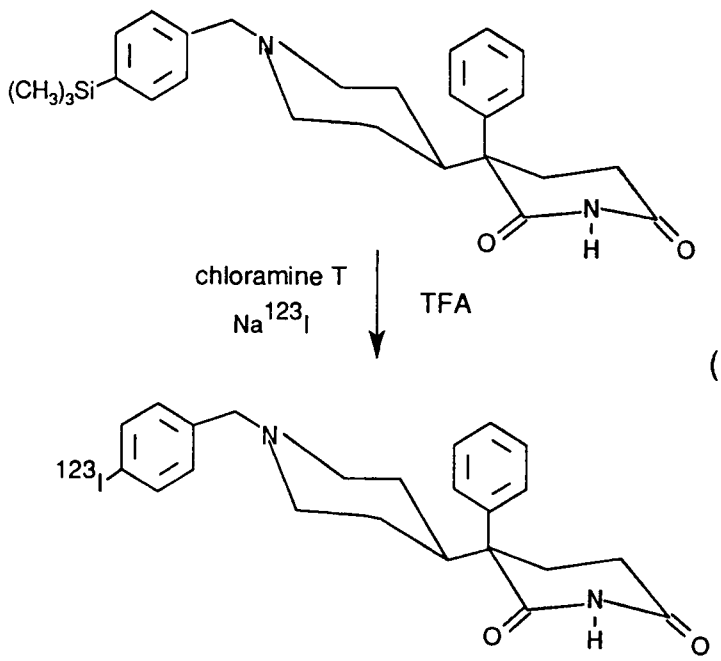
(1-5)

chlorinated byproducts when N-chlorinated oxidizing agents were used. Organogermanes were less likely to form chlorinated by-products but were more susceptible to the acidity and polarity of the solvent and the electronic effects of substituents on the aromatic ring. Organosilanes were found to be generally less reactive to iododemetallation reactions and require more forcing conditions to obtain good radiochemical yields. However, like the organogermanes, they have greater chemical stability and are not as likely to degrade as the organostannanes and are also not nearly as toxic. These observations held true for radiobrominations as well as for the radioiodinations.

Organosilanes have been widely used as intermediates for radioiodinations. As previously stated, the advantages of organosilanes are that they are inexpensive, relatively non-toxic, and have good chemical stability allowing easy storage.[26] Arylsilanes are easily prepared[27] and allow

incorporation of halogens even when the aromatic ring is strongly deactivated.[28] A recent example of radioiododesilylation is the preparation of a muscarinic cholinergic receptor imaging agent, ^{123}I labeled dexetimide, from the arylsilane precursor (equation 1-6).[29]

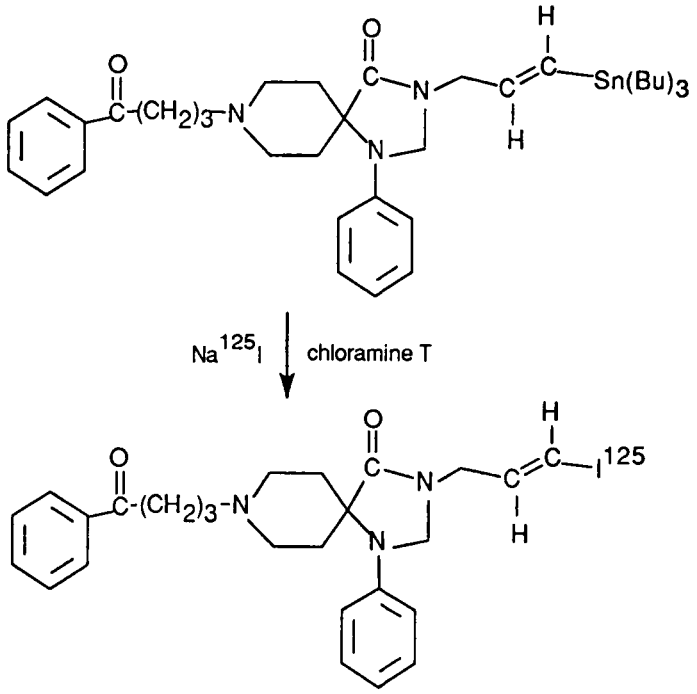
Because of the ease of the cleavage of the tin-carbon bond and because this cleavage is more selective for aromatic and vinyl groups than alkyl groups,[30] iododestannylation of arylstannanes and vinylstannanes has become one of the most popular and versatile reactions for attachment of an iodine atom to an sp^2 hybridized carbon. The addition of trialkyltinhydrides to alkynes to form the corresponding vinylstannanes has been extensively studied and will be discussed in detail in Chapter 2. Incorporation of radioiodine takes place stereospecifically and in high yield when vinylstannanes are treated with



(1-6)

sodium iodide in the presence of an oxidizing agent. Because the addition of trialkyltinhydrides to alkynes usually takes place by radical processes, most functional groups are well tolerated and this approach has been successfully applied to compounds containing a variety of functional groups. One example of this type of reaction is the radioiodination of the N-iodoallyl spiperone derivative shown below (equation 1-7).[31]

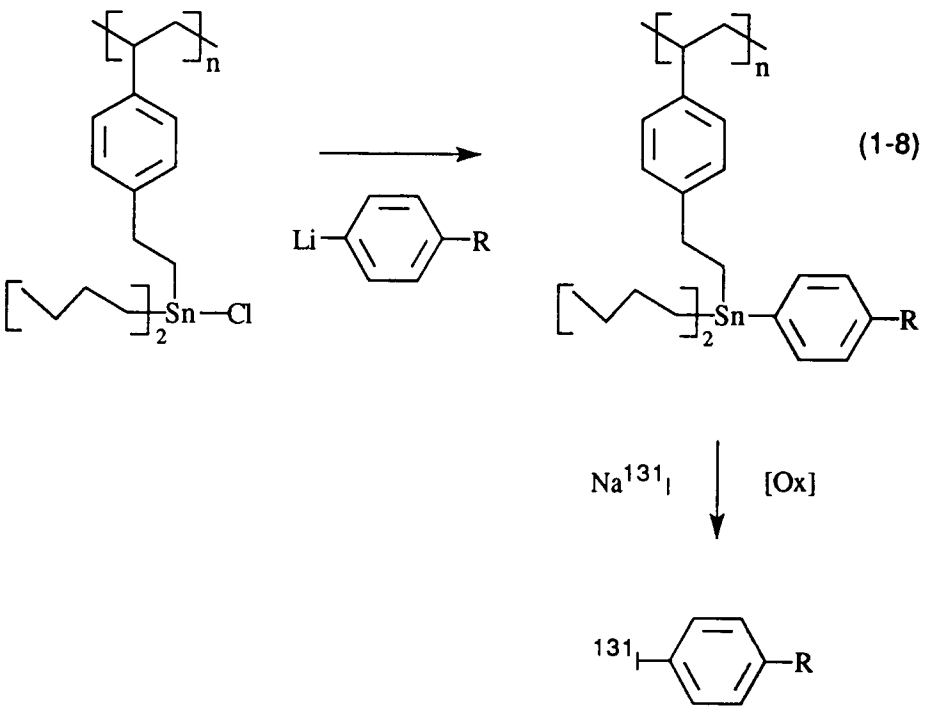
Aryltin compounds are usually prepared by the reaction of an aromatic substrate with butyllithium followed by the addition of a trialkyltin chloride.[32] The resulting aryltin compounds can be reacted with iodine or iodide and an oxidizing agent to give the corresponding radiolabeled compounds in high yield. One problem with this method has been the separation of the radioiodin-



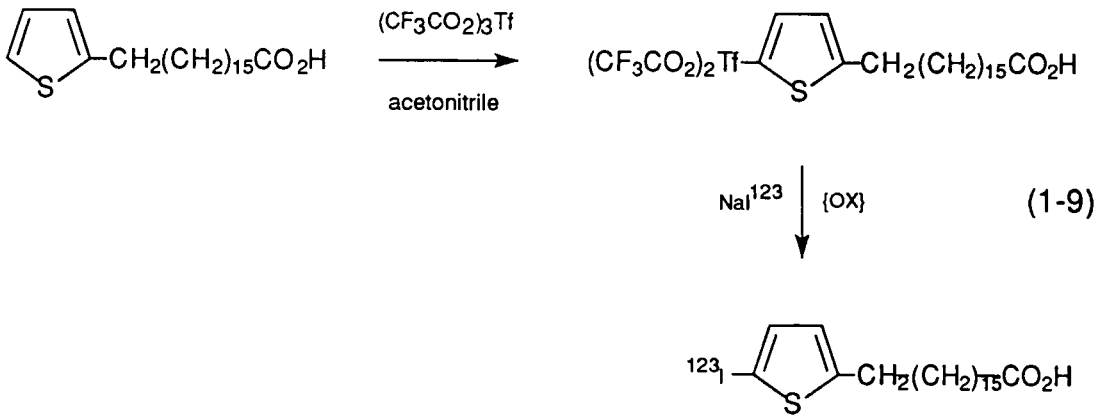
(1-7)

ated compounds from tin-containing by-products. In order to circumvent this problem, Culbert and Hunter have developed polymer-supported tin chloride reagent that can be used to bind the aromatic substrate to be labeled. They have obtained radioiodinated compounds that were free of tin-containing byproducts in good yield (equation 1-8).[33]

Iododethallation has proven to be an effective method for radioiodine incorporation onto substrates containing aromatic groups. Thallium(III)trifluoroacetate (TTFA) electrophilically attacks aromatic rings, usually at the para position, except when strong electron-withdrawing groups are present, in which case substitution at the meta position predominates.[34] Thallation is usually carried out by first reacting thallium(III) oxide with 80-90% trifluoroacetic acid to



form TTFA *in situ* and then using the resulting solution for the thallation reaction.[35] However, when acid sensitive groups are present, the trifluoroacetic acid can be evaporated to leave TTFA as a salt which is then collected and subsequently used in inert solvents such as acetonitrile. One example of iododethallation of a substrate containing an acid-sensitive group is shown in the synthesis of the radioiodinated 5-iodo-2-thienyl fatty acids for heart imaging by SPECT (equation 1-9).[36]



(1-9)

The disadvantages of thallium-based reagents are their high toxicity and reduced product yield due to the formation of polythallated byproducts.

Other methods of radioiodination include iododediazonization, electrolytic methods, enzyme catalyzed radioiodination and recoil labeling.[14]

C. Research Goals

Because the number of medical centers capable of performing PET imaging is limited, we set out to develop new radioiodinated imaging agents

for SPECT. Radioiodine was chosen for the labeling because of its excellent radionuclidic properties and availability of the SPECT isotope, ^{123}I. Two different types of iodinated compounds were synthesized: carbohydrate derivatives for determining abnormalities in glucose metabolism and spiperone derivatives for evaluating dopamine D_2 receptor densities in the brain. Preliminary evaluations of the biological activity of these agents were used to help determine their potential for biomedical imaging applications.

Chapter 2

Synthesis and Preliminary Evaluation of 3-C-((E)-2-[^{125}I]Iodoethenyl)-D-allose and 3-O-(E)-3-[^{123}I]Iodopropen-2-yl-D-glucose as Possible Agents for the Assessment of Disturbances of Glucose Metabolism by SPECT

A. Introduction

The design, synthesis and preliminary evaluation of radioiodinated carbohydrates as potential single photon emission computed tomography (SPECT) imaging agents for the evaluation of glucose metabolism in humans is presented in this chapter. Glucose is the primary source of energy for the brain and for oxygen-deficient heart muscle. Therefore, a number of radiolabeled glucose analogs labeled with the positron emitters, ^{11}C and ^{18}F, have been developed to measure rates of glucose utilization in the brain and heart by positron emission tomography (PET).[37] 2-Deoxy-2-[18F]fluoro-D-glucose has become a widely used and reliable biochemical marker for the evaluation of abnormalities of glucose metabolism by PET.[38] There is, to date, no radiolabeled carbohydrate suitable for the evaluation of glucose metabolism by SPECT and, because many more medical centers are equipped for SPECT than for PET, we sought to design radioiodinated carbohydrates for SPECT evaluation of glucose metabolism in humans.

In the first part of this chapter, the rational for the design of radioiodinated carbohydrates is discussed. Included are certain aspects of the mechanism of glucose distribution and metabolism in healthy versus diseased animals as well as the structural requirements of glucose necessary to maintain biological

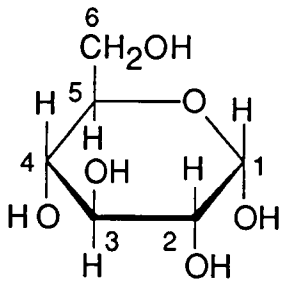
activity. The second part of this chapter describes the synthesis of two radioiodinated carbohydrates, including a detailed discussion of the synthetic methods used to attach the radioiodine. Finally, conclusions are drawn based on the results of animal studies and computer analysis of the structural features of the radioiodinated carbohydrates as compared to D-glucose and other carbohydrates of known biological activities.

B. Rational

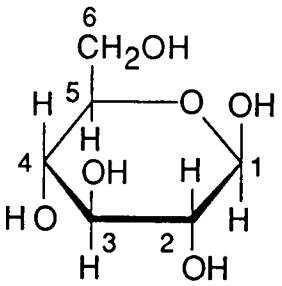
1. Project

Radiolabeled carbohydrate derivatives have been used to noninvasively diagnose and evaluate a wide variety of medical abnormalities in which there is a disturbance in the normal uptake and metabolism of glucose.[39] These diseases include schizophrenia, Parkinson's disease, Huntington's chorea, epilepsy, stroke, depression, and cancer. In order to understand how these radiolabeled carbohydrates can be effective aids in the diagnosis of these diseases and to interpret the animal biodistribution studies, it is important to be familiar with certain aspects of glucose metabolism in healthy and diseased animals.

The structures of the α and β anomers of D-glucose are given in Figure 2-1 as is the proper numbering of each according to the IUPAC rules. Glucose is not an essential dietary nutrient for humans and other highly developed animals because of the ability of the liver and kidneys to provide glucose via gluconeogenesis and glyconeolysis. Once in the bloodstream, the metabolic fate of glucose depends on several factors, including the dietary state, insulin levels, hormonal status and the general health of the animal.[40] In the fed state,



α-D-(+)-Glucose



β-D-(+)-Glucose

Figure 2-1. Structures and Numbering of the α and β Forms of D-Glucose

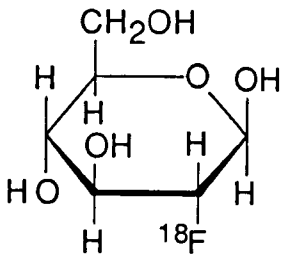
glucose is taken up to a great extent by the liver, where it is phosphorylated by hexokinase and trapped within the liver cells. The amount of glucose that is trapped within the liver depends on the concentration of glucose and insulin in the bloodstream and on the hormonal status of the animal.

Because glucose is not stored or produced in the heart or the brain, it must be supplied to these tissues from the blood by transport through cell membranes. Uptake in muscle tissues is dependant on the presence of insulin, insuring that muscle tissues will use glucose only when both glucose and insulin levels are high. When glucose levels are low, muscle cells metabolize other substrates, leaving the available glucose for the central nervous system, which depends heavily on it. Normal heart muscle, especially at rest, utilizes long-chain fatty acids for approximataly 70% of its energy needs with glucose providing most of the remaining 30%.[40] Other energy sources of the heart include lactate, pyruvate, ketone bodies, and amino acids. When the presence of glucose in the bloodstream is high, the concentration of fatty acids is lowered and the heart metabolizes glucose for most of its energy needs, however, when the supply of oxygen becomes short, as is the case with coronary artery disease, glucose becomes the main fuel for ischemic heart muscle no matter what the blood levels are.

Glucose is the major fuel for the central nervous system except in cases of prolonged starvation, when the brain adapts to the use of ketone bodies for energy. Glucose is taken directly across the blood/brain barrier by a carrier mediated process which includes facilitated diffusion and active transport.[41] It has been shown that this transport is very similar to the transport process in the human erythrocyte, as both processes are independant of insulin and Na^+ and

the structural requirements for transport are very similiar in both systems.[42] Uptake of glucose in muscle takes place in a similar manner, however, it is very sensitive to other factors such as insulin levels, hormonal status and the general health of the animal. For this reason, it is important that the dietary status of the animal be considered when testing radiolabeled glucose analogs, especially when uptake in the heart is to be determined. This will be discussed further in the conclusion section of this chapter.

As noted, the most commonly used agent for evaluation of glucose metobolism in the radiotracer 2-deoxy-2-[^{18}F]fluoro-D-glucose I (2-FDG). Physicians with access to PET facilities routinely use 2-FDG in combination with a blood flow agent, such as $^{13}NH_3$ or $H_2{}^{15}O$, to determine ischemic regions of the heart.[43] Any area of the heart which shows reduced uptake of the blood flow agent along with an increased uptake of 2-FDG is considered to be ischemic but viable tissue. With the aid of 2-FDG, patients with angina can safely be evaluated for risk of heart attack and can undergo the proper therapy needed to prevent permanent damage to the heart muscle. Patients who have



I

2-FDG

suffered a heart attack can be evaluated to determine if they are a candidate for bypass surgery or angioplasty. Certain neurological disorders in which there is a characteristic change in normal pattern of glucose metabolism, such as epilepsy, stroke and Alzheimer's disease can be evaluated with 2-FDG.[44] This agent has found a place in oncology because it pinpoints the location of tumors throughout the body. Also, the intensity of the radiation emitted from the tumor site is proportional to the rate of glucose utilization by the tumor and gives the physician a good indication of how fast the tumor is growing.[45]

While 2-FDG has proven to be an effective marker for the rate of glucose metabolism in a variety of diseases, a major drawback of this agent is that it can only reach a limited patient population due to the relatively low numbers of sites that have cyclotrons to make positron emitters such as ^{18}F. Because of the short half-life of most positron emitters, it is not feasible to commercially produce and distribute radiolabeled agents for PET and it would therefore be beneficial to create carbohydrates labeled with single photon emitting radionuclides to be used as SPECT agents. With this in mind, we set out to synthesize radioiodinated carbohydrates for this purpose.

In order to achieve our goal, the following criteria had to be met: 1) The radioiodine had to be attached to the carbohydrate in a way such that the resulting radiotracer would be stable to *in vivo* deiodination. 2) Any structural changes should not interfere with the biological activity of the molecule. 3) The radioiodination had to take place quickly, in high yields and near the end of the synthesis. 4) Subsequent synthetic steps and purification had to take place quickly and with minimal loss of activity.

2. Synthesis

The structural requirements for binding of a carbohydrate to the sugar-transport system of the human erythrocyte has been investigated by Barnett et al.[46] who looked at the inhibition constants, K_i, of different specifically substituted glucose analogs using L-sorbose as the penetrating sugar. For this study, the sites on the carrier protein were saturated with L-sorbose and a particular glucose derivative introduced which would then compete with the L-sorbose for the sites. The K_i was defined as the concentration of the glucose analog needed to displace 50 % of the L-sorbose from the binding sites on the carrier protein. By substituting different groups onto the various positions of D-glucose and determining corresponding values of K_i, Barnett et al. deduced which hydroxyl groups of glucose are involved in hydrogen bonding in the sugar-carrier protein complex.[46] For example, the hydroxyl group at C_3 of D-glucose is probably involved in this hydrogen bonding because of the higher K_i (71.5 mM) of 3-deoxy-D-glucose as opposed to the K_i (6.3 mM) determined for D-glucose (see Table 2-1). In support of this hypothesis, when a group capable of hydrogen bonding is used to replace the hydroxyl group at C_3, the K_i becomes comparable to that of D-glucose. This effect can be seen by examining 3-deoxy-3-fluoro-D-glucose, which has a Ki value of 6.9 nM. By systematically preparing and examining analogs of deoxyglucose and halogen-substituted glucose derivatives with modifications occuring at all posible positions, Barnett et al. determined which hydroxyl groups of D-glucose were most likely involved in binding to the carrier protein. Based on the results, they proposed a model for the glucose-carrier system, which is shown in Figure 2-2.

The binding of glucose to the carrier system appears to be by hydrogen

Table 2-1. Some Inhibition Constants (K_i) of D-Glucose analogs modified at C_3 and Tested as Inhibitors of L-Sorbose Entry into Human Erythrocytes

Sugar	Conc. tested (mM)	Ki (mM)
D-glucose	10	6.3
3-deoxy-D-glucose	10	71.5
3-deoxy-3-fluoro-D-glucose	10	6.9
3-chloro-3-deoxy-D-glucose	10	33
3-bromo-3-deoxy-D-glucose	10	30
3-O-methyl-D-glucose	10	12
3-O-ethyl-D-glucose	14.65	48
3-O-propyl-D-glucose	10	59
3-O-propenyl-D-glucose	10	25
3-O-(2',3'-epoxypropyl)-D-glucose	10	25

Source: Barnett, J. E. G.; G. D. Holman and K. A. Munday. "Structural Requirements of Binding to the Sugar-Transport System of the Human Erythrocyte". *Biochemical Journal*, Volume 131, pages 211-221, 1973.

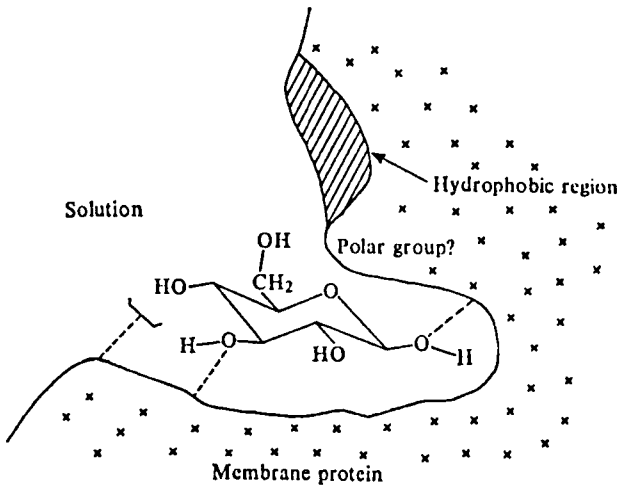


Figure 2-2. Model for the Glucose-Carrier Complex in the Human Erythrocyte

Source: Barnett, J. E. G.; G. D. Holman and K. A. Munday. "Structural Requirements of Binding to the Sugar-Transport System of the Human Erythrocyte". *Biochemical Journal*, Volume 131, page 220, 1973.

bonding of the hydroxyl groups in the β configuration at C_1 and in the D-gluco configuration at C_3, probably C_4 and possibly C_6. The C_2 postion was determined not to be involved in binding to the carrier, however, because of the reduced ability of 2-O-methyl-D-glucose to displace L-sorbose (Ki = 199 mM), it was proposed that this site comes close to the carrier protein and that larger groups at C_2 would cause substantial steric interferences. Of the positions which might be involved in binding to the carrier protein, substituents at C_3 and C_4 were tolerated fairly well and it was proposed that there was some space around these positions in the sugar-carrier complex which might allow modification without loss of binding ablility. Because of the relatively low K_i values for analogs with alkylated hydroxyl groups at the 3 or 4 position, it is probable that hydrogen bonding is still ocurring between between the carrier protein and the ether oxygen of these agents. Based on this knowledge and on the availability of 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranoside, we set out to synthesize glucose derivatives with the radiolabel attached at the 3 position.

Radioiodinated carbohydrates in which the radiolabel is attached to a sp^3 hybridized carbon have been synthesized and evaluated as potential SPECT imaging agents. Two examples are 2-deoxy-2-iodo-D-glucose[47] and 3-deoxy-3-iodo-D-glucose,[48] both of which readily undergo deiodination *in vivo* .

It has been established that the attachment of iodine to a sp^2 hybridized carbon atom results in a bond which is more stable to *in vivo* deiodination. One such example is 2-O-(p-iodobenzyl)-D-glucose[49], in which the radioiodine was attached to an sp^2 hybridized carbon of the benzyl group, resulting in a compound that showed low *in-vivo* deiodination. This agent had a significantly

lower brain and heart uptake as compared to 2-FDG, however, and this can probably be attributed to the size of the structural modification at the C_2 position of the carbohydrate. Other examples of the stability of the iodovinyl group to *in vivo* deiodination are structurally modified, radioiodinated fatty acids[50] and steroids[22] in which the radioiodine is attached to an sp^2 hybridized carbon atom of the vinyl group. Because of its smaller size as compared to a benzene ring, we chose the iodovinyl group to introduce the radiolabel into the carbohydrates. It was felt that the smaller size of the iodovinyl group would help to reduce any structural interferences which might inhibit binding of the labeled carbohydrate to the glucose/sorbose carrier protein.

C. Results and Discussion

The radioiodinated carbohydrates 3-C((E)-2-[^{125}I]iodoethenyl)-D-allose[51], **7**, and 3-O-((E)-[^{123}I]iodopropen-2-yl)-D-glucose[52], **12**, were synthesized and evaluated and the synthetic scheme followed for each of these compounds is given in Figures 2-3 and 2-4, respectively. As discussed earlier, the iodovinyl was chosen as a way to introduce the radiolabel because of its relatively small size and established metabolic stability. The basic theme of both syntheses was to create alkynyl precursors to the desired compounds and to introduce iodine into these molecules by hydrometallation followed by iododemetallation. The final step in the synthesis involved the removal of the isopropylidene protecting groups to give the corresponding free sugars. The synthetic procedures were first optimized using stable iodine, providing adequate material for analysis and for standards. Finally, the radioiodinated analogs of **7** and **12** were synthesized and their biological distrubution in rats

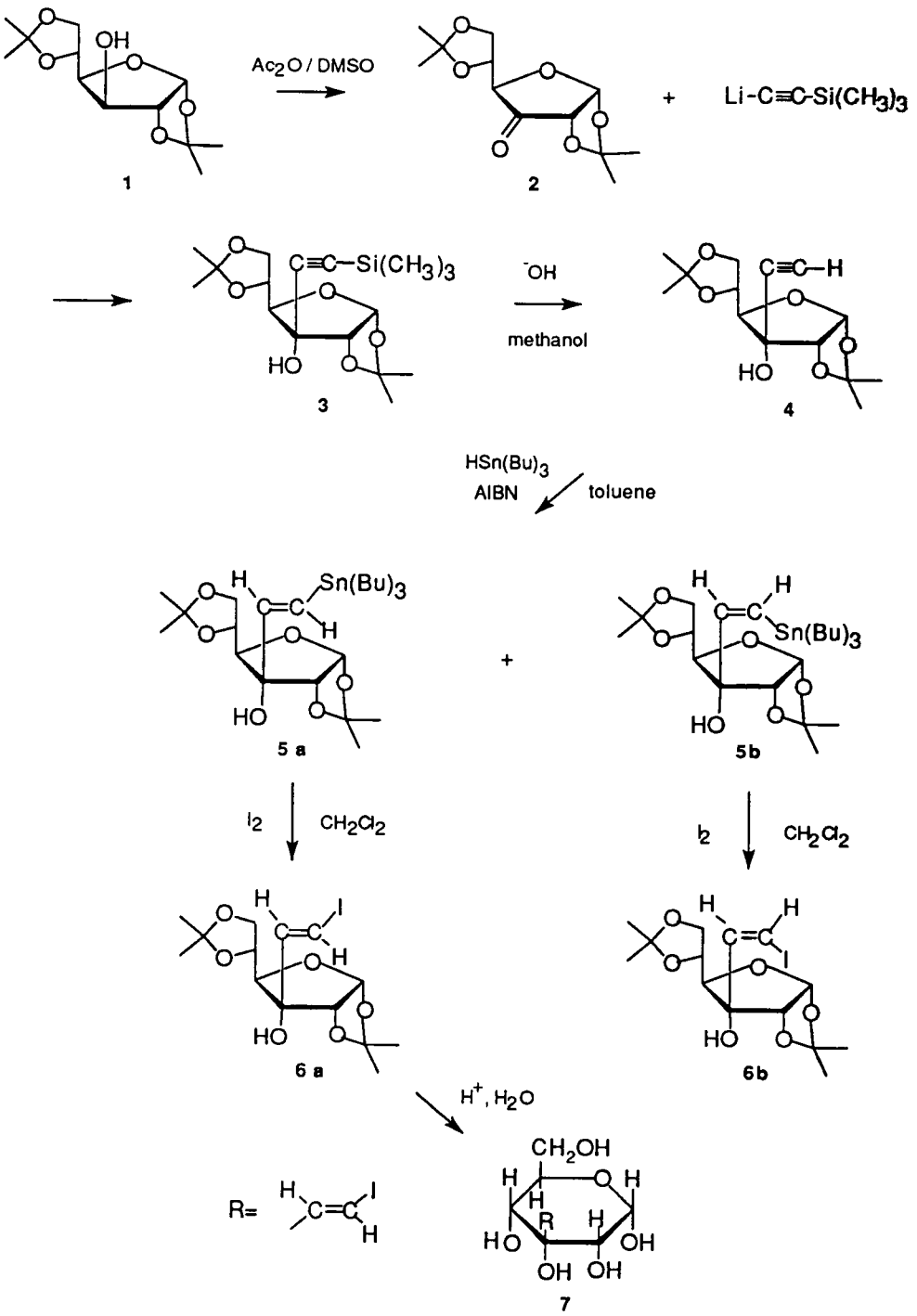


Figure 2-3. Synthetic Scheme for the Synthesis of 3-C-((E)-2-Iodoethenyl)-D-allose, **7**

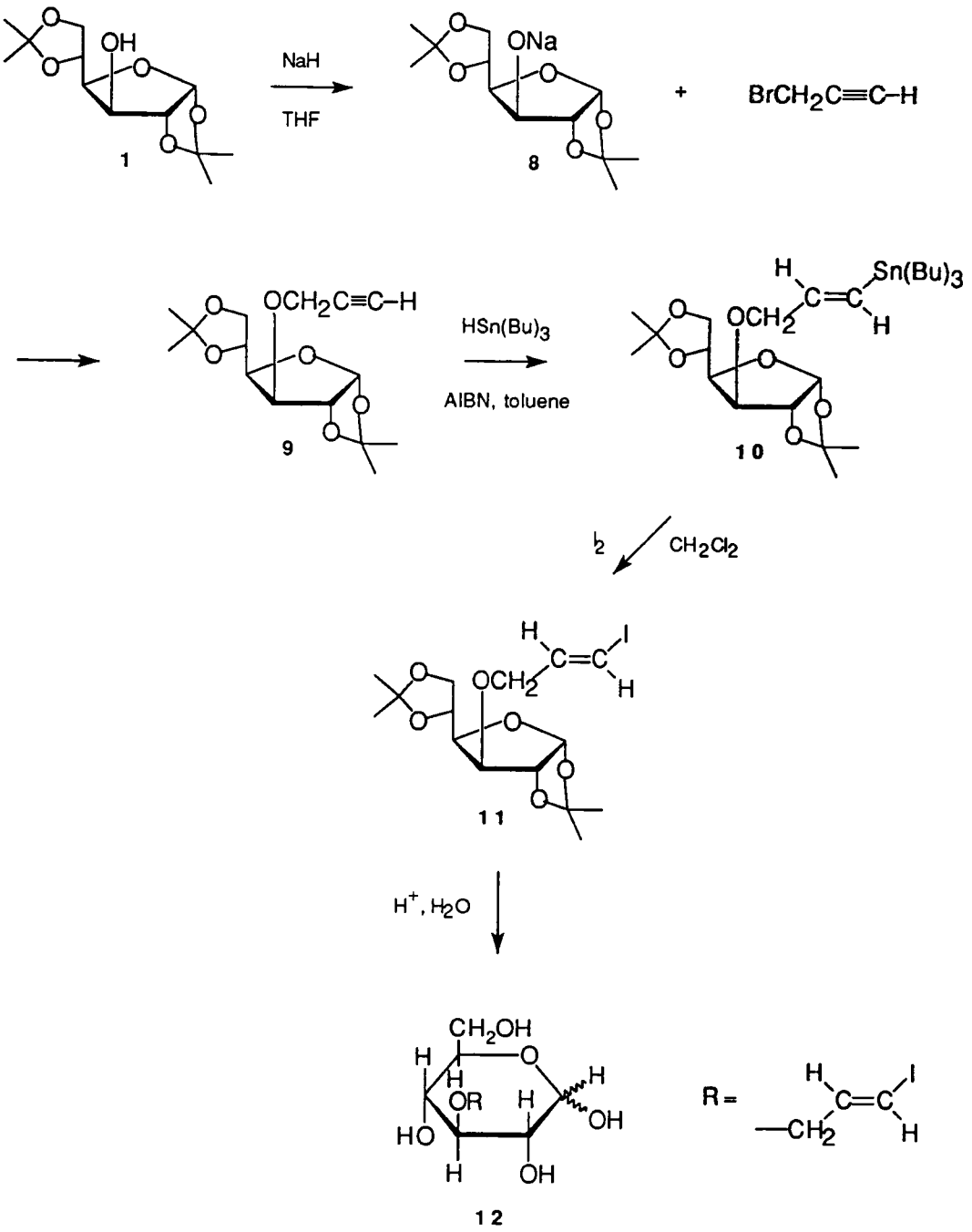


Figure 2-4. Synthetic Scheme for the Synthesis of 3-O-((E)-Iodopropen-2-yl)-D-glucose, **12**

evaluated to determine their possible effectiveness as SPECT imaging agents for gucose uptake and metabolism in humans.

1. Synthesis of the Alkynyl Precursors

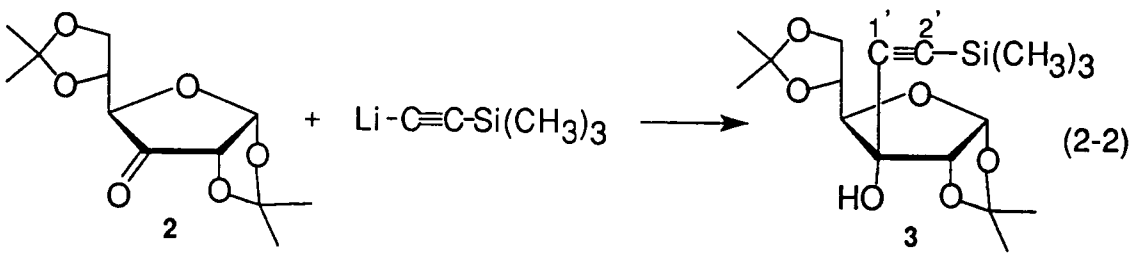
The synthetic strategy for the preparation of the iodoethenyl-branched carbohydrate **7** involved the attachment of an ethynyl group at the 3-position of the furanose ring by direct alkynylation of keto-sugar **2**. The first step in the synthesis of **7** was the oxidation of the alcohol group of commercially available 1,2:5,6-di-O-isopropylidene-α–D-glucose, **1**, under Albright-Goldman[53] conditions to give 1,2:5,6-di-O-isopropylidine-α–D-ribohexafuranose-3-ulose, **2** (equation 2-1). The procedure was followed as previously described[54] and involved the treatment of **1** with a mixture of dimethyl sulfoxide and acetic anhydride for 24 hours at room temperature. The dimethyl sulfoxide, acetic anhydride and other moderately volatile components were then removed by vacuum distillation, leaving behind a yellow, foul-smelling syrup which contained the desired keto-sugar, the expected thiomethoxymethyl byproduct[55] (approx. 20%), and small amount of unreacted starting material. The presence of these compounds was determined by thin layer chromatography (TLC)



1 2 (2-1)

(mobile phase = benzene/ethyl acetate (17 : 3); stationary phase = silica, detection = acid char) and by ^{13}C and ^{1}H NMR spectroscopy. The three components were separated by column chromatography under the same conditions as used for the TLC and the desired product **2** confirmed by melting point (77-78°C) and by ^{1}H and ^{13}C NMR spectroscopy. The chemical properties were found to correspond to those reported for this compound in the literature.[54] Evidence that the hydroxyl group at C_3 was oxidized demonstrated best by the appearance of a resonance at 208.1 ppm (C_3) in the ^{13}C spectrum and dissapearance of the doublet at 2.65 ppm (C_3-O<u>H</u>) in the ^{1}H spectrum. Also observed was a characteristic shift in the anomeric proton resonance from 5.81 ppm (J_{12} = 3.42 Hz) to 6.14 ppm (J_{12} = 4.88 Hz). The identification of the starting material and the byproduct were deduced by ^{1}H NMR spectroscopy.

The ethynylation of 1,2:5,6-di-O-isopropylidine-α-D-ribohexafuranose was accomplished by a method developed by Mukaiyama *et al.* (equation 2-2).[56] The usual route involves the initial preparation of the ethynyl magnesium bromide reagent from acetylene gas and an alkylmagnesium bromide; however, using the approach described by Mukaiyama *et al.*, one can avoid the



(2-2)

sometimes tedious preparation of the Grignard reagent. Lithium trimethylsilylacetylide was prepared *in situ* by the addition of 1.0 equivalent of *n*-butyllithium to trimethylsilylacetylide in anhydrous tetrahydrofuran at -78°C. The resulting solution was stirred for 20 minutes and then 1,2:5,6-di-O-isopropylidene-α-D-ribohexafuranose-3-ulose dissolved in tetrahydrofuran was added dropwise over a period of 2 minutes. Stirring was continued for 90 minutes at -78°C and then the mixture was allowed to warm to room temperature. Work-up included neutralization of the reaction mixture with 1.0 N hydrochloric acid and extraction of the crude product into dichloromethane. Thin layer chromatography (mobile phase = benzene/ethyl acetate (17:3), stationary phase = silica, detection by acid char) revealed the presence of only one product along with a small amount of the keto sugar. Purification by column chromatography, under the same conditions as given for the TLC, gave the major compound as clear, colorless crystals with a chemical yield of 85%. The remaining material eluted from the column consisted of the ketosugar and a small amount of unidentified material that remained at the origin and was later washed off the column with ethanol. The product was identified by ^{1}H and ^{13}C NMR spectroscopy and the disappearance of the carbon resonance at 208.1 ppm (C_3) and appearance of the relatively small peaks corresponding to the alkynyl carbons at 92.43 ppm ($C_{2'}$) and 102.14 ppm ($C_{1'}$) were key factors in the ^{13}C NMR. The ^{13}C NMR spectrum of **3** has been reproduced on the following page in Figure 2-5. The fact that the alkynyl carbon resonances are more deshielded than expected can be attributed to the resonance contributor II.[74]

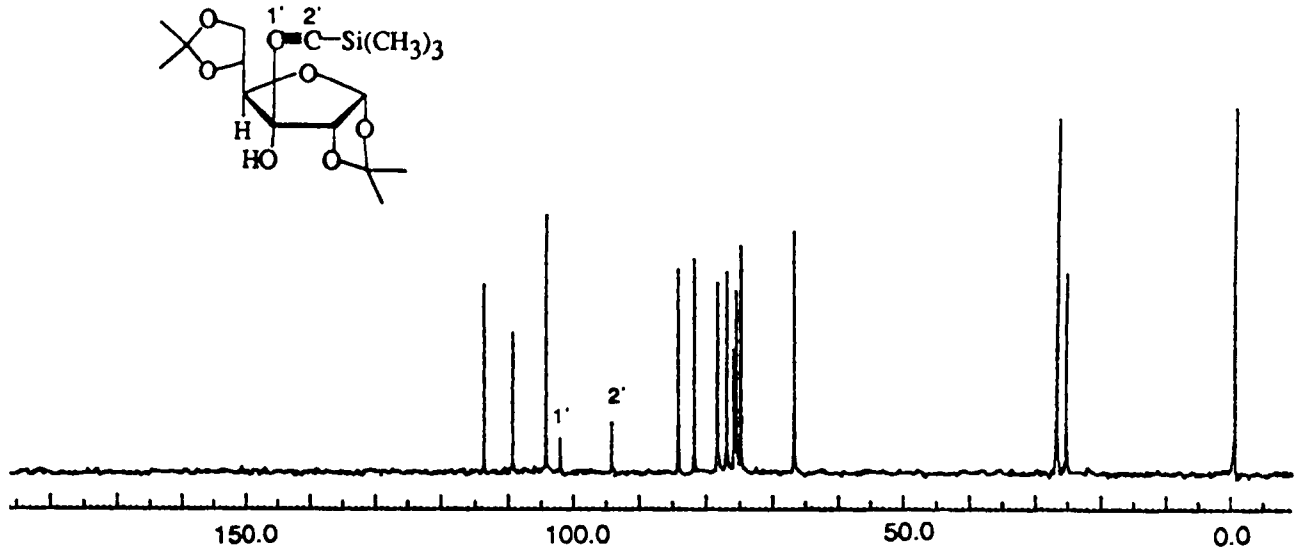
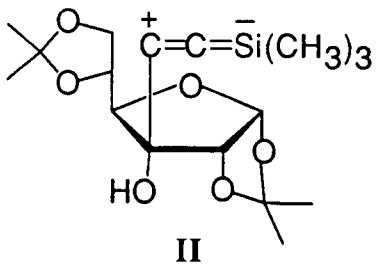


Figure 2-5. 90 MHz Carbon-13 Spectrum of 3-C-(2-Trimethylsilylethynyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose in $CDCl_3$ plus 1.0% TMS.



II

The ^{1}H NMR spectrum of **3** revealed 3 new singlets (12H, 1.37 ppm, 1.45 ppm, 1.59 ppm) of the trimethylsilyl group as well as a singlet at 3.03 ppm, corresponding to C_3-O<u>H</u>. The allose configuration was confirmed by ^{1}H and ^{13}C NMR as well as by a melting point determination of the ethynylcarbinol formed in the next step as will be discussed. The allose derivative was the expected product based on the previous observation that the addition of hydride and other nucleophiles under a variety of conditions gave the allose analogs as the only detectable products.[57]

The reason for the apparent preferential attack of nucleophiles from the *beta* face to give the corresponding allose derivatives was explored by doing a random search for all possible low energy conformations of **2** using SYBYL.[58] In the lowest energy conformation of **2** (18.86 Kcal/mol), one of the methyl groups lies directly below the carbonyl carbon, shielding it at the *alpha* face from nucleophilic attack. This can be seen by looking down at the carbonyl group from above the lowest energy conformation of **2** as shown in Figure 2-6. Figure 2-7 displays a side view of the same structure and includes an outline of the Van der Waals radii to show the extent of the shielding caused by the methyl group. This may account for the fact that nucleophilic attack of **2** occurs only from the *beta* face to provide the corresponding allose derivatives.

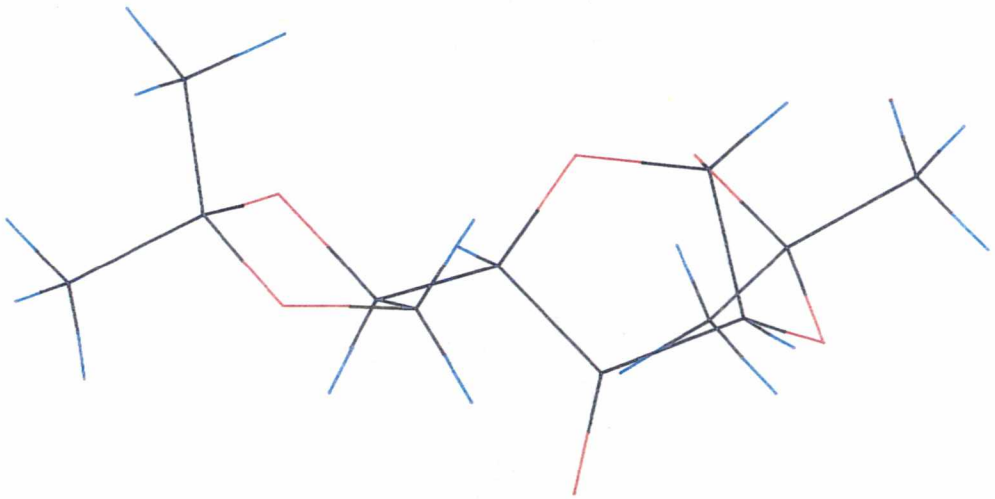


Figure 2-6. A View From Above the Lowest Energy Conformation of 1,2:5,6-Di-O-Isopropylidene-α-D-Ribohexafuranose-3-ulose, **2**

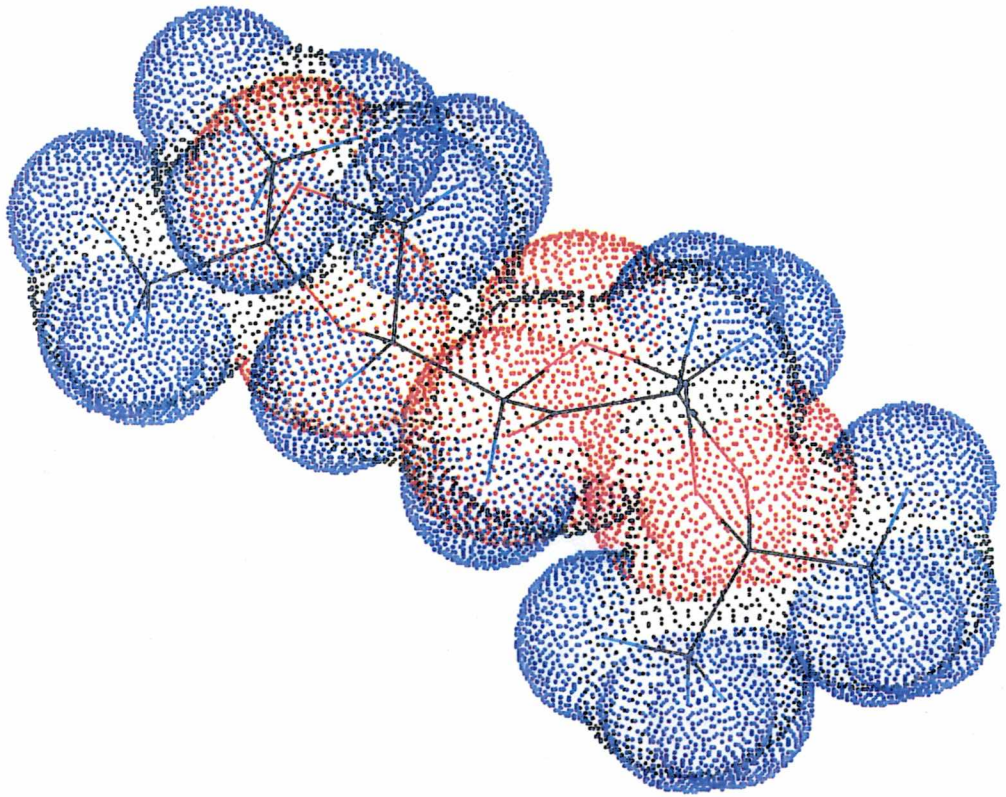
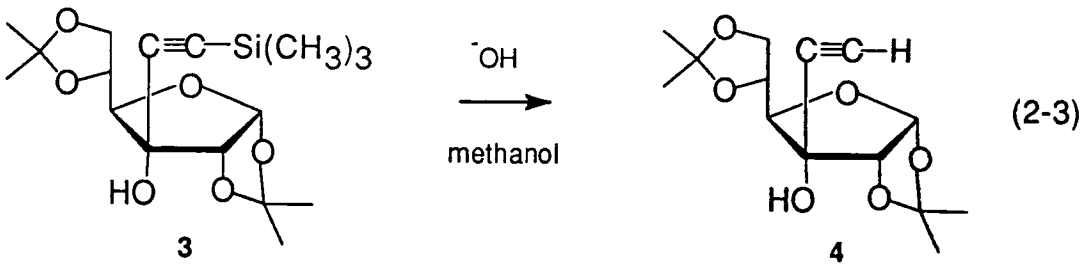


Figure 2-7. Diagram Displaying Van der Waals Radii of the Lowest Energy Conformation of 1,2:5,6-Di-O-Isopropylidene-α-D-Ribohexafuranose-3-ulose, **2**

The formation of alkynes from trimethylsilylacetylenes was described in 1978 by Mori, et al.[59] Cleavage of the trimethylsilyl group of 3-C-(2-trimethylsilylethynyl)-1,2:5,6-Di-O-isopropylidene-α-D-allofuranose, **3**, was accomplished by treatment of this compound for ten minutes at room temperature with 3.0 N aqueous sodium hydroxide in methanol (equation 2-3). The resulting solution was then poured into a beaker containing 100 ml of ice water and neutralized by the careful addition of 1.0 N hydrochloric acid dropwise until a pH of 7 was obtained. The product was extracted into dichloromethane and the organic layers combined, dried, and the solvent evaporated to give a yellow solid. Purification was accomplished via column chromatography to give 93 % of the desired product **4** as a white solid. It was determined by ^{1}H and ^{13}C NMR, as well as by melting point, that the isolated ethynylcarbinol had the allose configuration by direct comparison of the chemical and spectral data obtained with that reported in literature for **4** prepared by a convergent synthetic route.[60] The characteristic ^{1}H NMR spectral features included loss of the singlet at 0.20 ppm from the trimethylsilyl group and the presence of a singlet at 2.17 ppm for the alkynyl proton. In the ^{13}C NMR, the two peaks corresponding to the alkynyl carbons were identified as expected at 77.0 ppm ($C_{2'}$) and 80.4 ppm ($C_{1'}$).

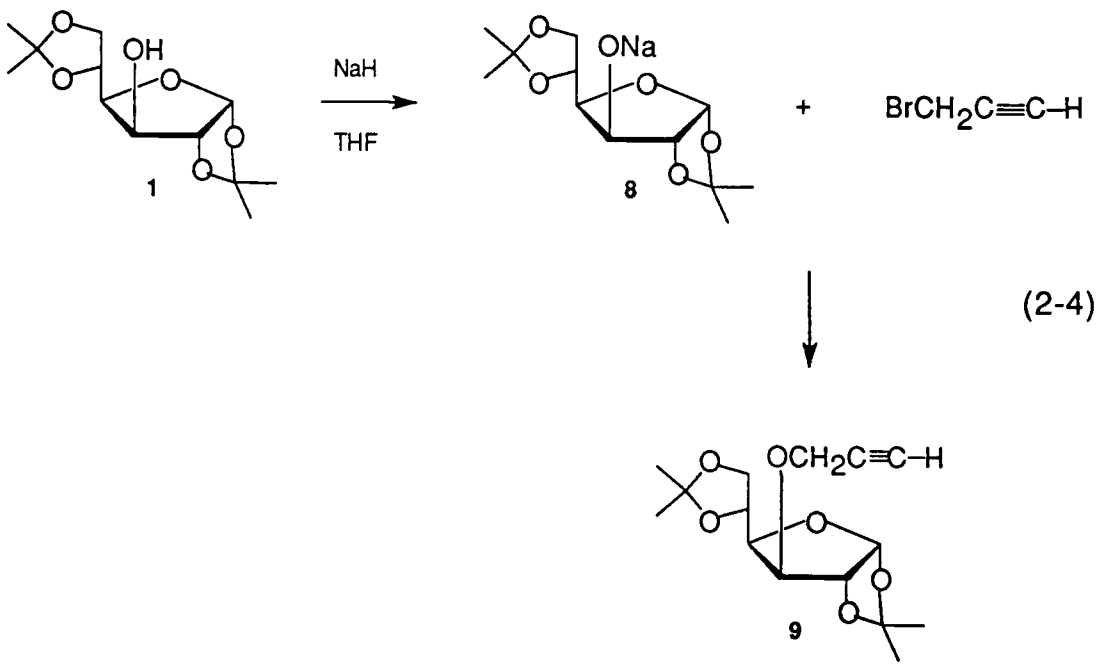
It was noted that if traces of solvent remained and this compound was left on the bench as an oil, decomposition occurred and a material formed that was insoluble in dichloromethane or other solvents. The ethynylcarbinol is best stored as a solvent-free solid and in a cool, dark place.

The synthesis of the second alkynyl precusor, 3-O-propargyl-1,2,:5,6-di-O-isopropylidene-α-D-glucofuranoside, **9**, was accomplished by treatment of



(2-3)

1,2:5,6-di-O-isopropylidene-α-D-glucose, **1**, with sodium hydride in anhydrous tetrahydrofuran at room temperature for 3 minutes, followed by the addition of propargyl bromide and heating of the resulting solution to 40°C (equation 2-4). The reaction progress was monitored by thin layer chromatography, and the alkylation was determined to be complete in 4.0 hours. The work-up included addition of water to destroy any remaining sodium hydride and extraction of the product into dichloromethane. The organic layers, were combined, dried and the solvent removed by evaporation to yield the crude product as a dark-brown oil. Purification by column chromatography provided **9** in 82% yield as a clear, colorless oil. The product was characterized by ^{1}H and ^{13}C NMR spectroscopy and by IR spectroscopy. The determining features in the ^{1}H spectrum included the presence of a triplet at 2.48 ppm, corresponding to the terminal hydrogen of the alkyne, and the disappearance of the doublet from the hydroxyl group of **1** at 2.50 ppm. The ^{13}C NMR spectrum revealed the alkynyl carbon resonances at 74.98 ppm and 79.23 ppm and the IR sprectrum shows the alkyne C-H stretch at 3270 cm^{-1} and the alkyne C-C stretch at 2118 cm^{-1}.



(2-4)

2. Attempted Hydroboration of the Alkynyl Precursors

Because of the high stereoselectivity of the addition of catecholborane to terminal alkynes[61] and the ability of vinylboronic acids to undergo iododeboronation in high yield with retention of stereochemistry,[62] hydroboration of **4** was attempted by the addition of catecholborane to the alkyne under a variety of conditions without success. This route was investigated based on the knowledge that catecholborane adds to terminal alkynes to afford the corresponding *trans* alkenes in high yields, as opposed to trialkyltin hydride addition which can provide a mixture of the two possible *cis* and *trans* isomers as well as the α adduct. The failure to produce the boronic acid of **3** is not unexpected due to the presence of the ketals and acetals which

are likely to react with catecholborane at elevated temperatures. The hydroxyl group will also react with catecholborane, even at room temperature, but it was hoped that under the right conditions addition of catecholborane to **4** would proceed to give the corresponding vinylboronic ester. After numerous attempts under a variety of conditions, none of the desired product was obtained and it became apparent that, if we were to prepare the boronic acid of **4**, the isopropylidene groups would have to be removed and another protecting group resistant to hydroborating agents used instead of the isopropylidene groups. Boronic esters have been employed to protect the hydroxyl groups of carbohydrates during reactions where hydroborating agents were used[63] . Therefore, it should be possible to prepare the corresponding analog of **1** and react this with catecholborane. However, it was decided that, instead of adding these extra synthetic steps, the tributyltin hydride method would be employed. A summary of the reaction conditions used in an effort to obtain the vinylboronic acid of **4** is given in Table 2-2.

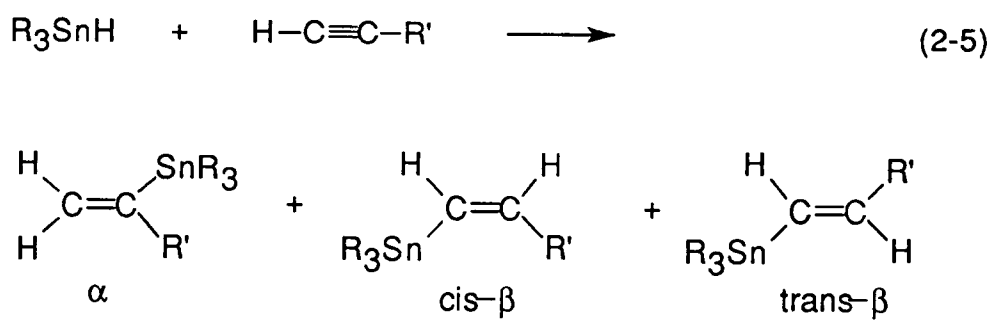
3. Addition of Tributyltinhidride to the Alkynyl Precursors

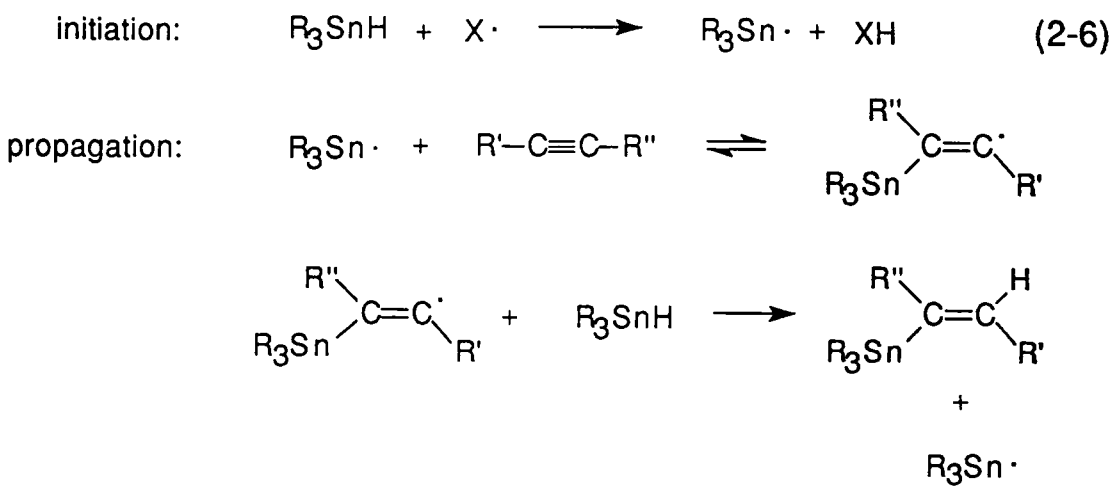
A second method for the synthesis of vinyl iodides involves the oxidative iododestannylation of the corresponding vinylstannanes with I_2 or ICl formed *in situ* by the reaction of sodium iodide and N-chlorosuccinimide or chloramine-T. The iododemetallation of vinylstannanes has been shown to occur with retention of configuration at the vinylic carbon, except in a few special cases. This method has been used to prepare several radioiodinated compounds as well as some bromovinyl carbohydrates.[64] The mechanism of the addition of trialkyltin hydrides to terminal alkynes has been examined and is dependent on

Table 2-2. Reaction Conditions for Attempted Addition of Catecholborane to 3-C-((E)-2-Iodoethenyl)-1,2:5,6-Di-O-isopropylidene-α-D-allofuranose, **4**

Alkyne (equiv.)	Catecholborane (equiv.)	RXN Time	RXN Temp.	Solvent
1.0	2.0	30 min.	70°C	none
1.0	2.2	1.0 hr.	70°C	none
1.0	3.0	4.0 hrs.	50°C	none
1.0	3.0	6.0 hrs.	r.t.	none
1.0	1.5	12.0 hrs.	70°C	THF
1.0	1.1	5.0 hrs.	70°C	THF
1.0	1.0	5.0 hrs.	70°C	THF

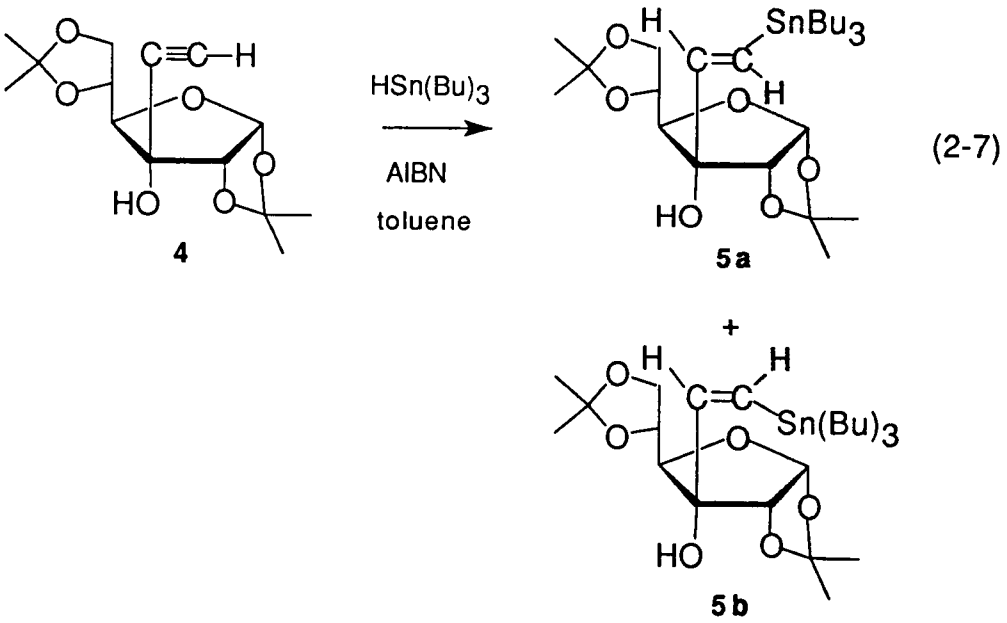
the effect of electron withdrawing or donating substituents attached to the alkyne.[65, 66, 67] In addition, the solvent used in the reaction and the temperature of the reaction play a role because the product stereochemistry is determined by whether the reaction takes place by a polar mechanism or by a radical mechanism. The distribution of α, *cis*–β and *trans*–β products formed are influenced by all of these factors and, with a knowledge of how these factors influence the product distribution, one can alter reaction conditions to obtain a high yield of the desired isomer. Radical initiators and radical inhibitors have also been used to influence the product distribution. A general reaction scheme for the addition of trialkyltin hydrides to monosubstituted alkynes is shown in equation 2-5. The hydrostannylation of terminal alkynes containing strongly electron-withdrawing substituents proceeds mainly (and sometimes exclusively) by an ionic process leading to α-adducts, or by a free radical reaction to provide the corresponding β adducts.[65] Monosubstituted alkynes containing other groups, including those that are electron-donating, undergo hydrostannylation





mainly by a radical mechanisms to give the β adducts in good yield (equation 2-6).[66] When an excess of alkyne is used, the formation of the *cis*-β isomer predominates, however, if an excess of the trialkyltin hydride is used, the *trans*-β somer is usually obtained in high yield. The β adducts are formed preferentially when radical reactions are employed because of the increased stability of secondary radicals relative to primary radicals. It has been shown that the *cis*-β isomer is formed initially by anti-addition, and that the *trans*-β isomer is formed by isomerization of the initial *cis*-β product in the presence of an organotin radical (R3Sn·) or by photolysis.[67] When β products are desired, the use of non-polar solvents and radical initiators will retard the formation of α adducts. An excess of trialkyltin hydride will usually ensure that sufficient quantities of the *trans*-β isomer are formed. If the *cis*-β adduct is desired, then an excess of the alkyne should be used. These strategies were used to obtain the desired trialkyltin adducts required for the targeted iododestannylation

reactions. Studies have been published on the hydrostannylation of propargylic ethers and alcohols with excess trialkyltin hydride in the presence of a radical initiator.[68] The reaction of trialkyltin hydrides with propargylic ethers under these conditions produced mainly the *trans*-β adduct, while the reaction of the propargylic alcohols was less selective, yielding mixtures of the three possible isomers. The observed product distribution of the hydrostannylation of the alkynes **4** and **9** corresponded well to these models. The hydrostannylation of **4** was accomplished by the addition of tributyltin hydride to **4** in the presence of a radical initiator (equation 2-7). Toluene was used as the solvent because it is relatively non-polar and should inhibit the formation of polar intermediates. The reaction took place under anhydrous conditions and in an atmosphere of argon gas. As expected, the reaction provided the three possible isomers and



(2-7)

the quantity of each depended on the amount of tributyltin hydride used and on the reaction time. This expectation was based on the observations of the product distribution when other propargyl alcohols were reacted with tributyltin hydride. When the *trans* isomer was desired, an excess of tributyltin hydride was added and longer reaction times and higher temperatures were used. In order to obtain the *cis* isomer, less than one equivalent of tributyltin hydride was used and milder reaction conditions employed. A summary of the reaction conditions and the percent yields of **5a** and **5b** is given in Table 2-3. Thin layer chromatography of the reaction mixture usually showed the presence of three compounds which were easily separated by column chromatography [silica; benzene/ethyl acetate (17:3); R_f (**5a**) = 0.66, R_f (**5b**) = 0.77, R_f (α adduct) = 0.59] and identified by the coupling constants from ^1H NMR as well as from the chemical shifts of the olefinic carbons by ^{13}C NMR spectoscopy. A small amount of the third product (Rf = 0.59) was obtained and was not fully characterized but was determined by ^1H NMR to be the α adduct. Because of the tendency for **5b** to isomerize to **5a** when exposed to light, **5b** was stored in a cool, dark place in order to prevent this from occurring.

The stereochemistry of the vinylstannanes **5a** and **5b** was assigned based on the coupling constants of the vinylic protons in the ^1H NMR spectra and on the chemical shifts of the vinylic carbons in the ^{13}C NMR spectra as compared to the assignments of vinylstannanes derived from propargyl alcohols reported in literature.[68] Typical ^1H coupling constants for the vinylic protons of vinylstannanes are 12-14 Hz for *cis* isomers, 19-20 Hz for *trans* isomers and 2-3 Hz for the α adduct. Although the vinylic protons of *cis* and *trans* vinylstannanes can overlap, the protons of the α adduct are usually more

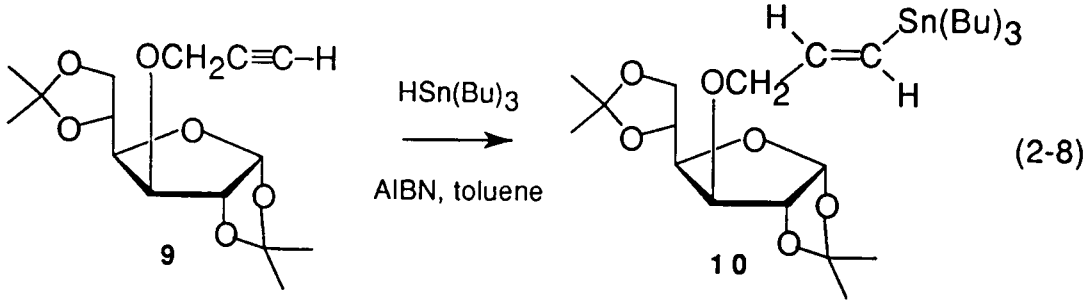
Table 2-3. Addition of Tributyltin Hydride to 3-C-((E)-2-Iodoethenyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose, 4

Alkyne (equiv.)	$HSn(Bu)_3$(equiv.)	Temp.	Time (hrs.)	cis-β	trans-β
1.00	1.30	110°C	11.0	12%	47%
1.00	1.05	110°C	9.0	22%	74%
1.00	1.00	110°C	10.0	21%	75%
1.00	0.90	85 °C	10.0	59%	22%
1.00	0.90	85°C	8.0	46%	18%
1.00	0.91	70°C	8.0	62%	19%

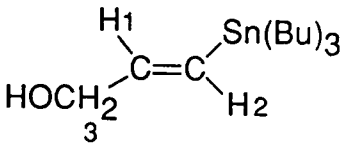
upfield and can be readily identified. Therefore, assignment of stereochemistry was easily accomplished and the ratio of the isomers correlated well with the products expected under the reaction conditions applied (see Figure 2-8 for ^{1}H NMR assignments for **5a** and **5b**). A 250 MHz ^{1}H NMR spectrum of **5a** has been reproduced in Figure 2-9.

The ^{13}C data obtained for the **5a** and **5b** also support the stereochemical assignments. In general, the vinylic carbon resonances of *cis* isomers are closer together than those of the *trans* isomer (see Figure 2-10 for ^{13}C NMR assignments). As can be shown for **5a** and **5b**, $C_{1'}$ is shifted further downfield and $C_{2'}$ is shifted further upfield for the *trans* isomer as compared to the corresponding carbons of the *cis* isomer.

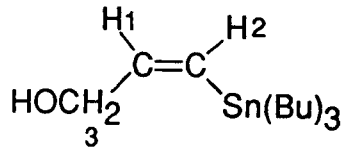
It has been observed that hydrostannylation of propargyl ethers provides mainly the *trans* isomer and sometimes small amounts of the α adduct[68] and this was found to correlate well with the results of the addition of tributyltin hydride to **9**. Hydrostannylation of **9** was accomplished in the same manner asdescribed for **4** with excess tributyltin hydride in the presence of AIBN (equation 2-8), however, only the *trans*-β isomer was detected and isolated. As



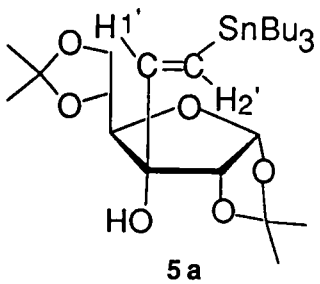
(2-8)



H_1: 6.19 ppm J_{12}: 19.2 Hz
H_2: 6.39 ppm J_{13}: 4.2 Hz
H_3: 4.17 ppm J_{23}: ——

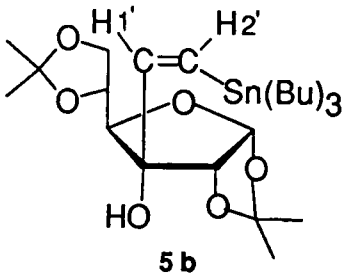


H_1: 6.07 ppm J_{12}: 12.8 Hz
H_2: 6.68 ppm J_{13}: 5.8 Hz
H_3: 4.11 ppm J_{23}: 1.1 Hz



5a

H_1': 5.94 ppm $J_{1'2'}$: 19.4 Hz
H_2': 6.51 ppm



5b

H_1': 6.24 ppm $J_{1'2'}$: 13.6 Hz
H_2': 6.45 ppm

Figure 2-8. ^{1}H NMR of Analylsis of *Cis* and *Trans* Trialkyltin Hydride Adducts of Propargyl Alcohols.

Source: Young, Michael E. and Lynn A. Light. "Preparation of Iodoallylic Alcohols Via Hydrostannylation: Spectroscopic Proof of Structures". *Tetrahedron Letters*. Great Britain: Pergamon Press Ltd., Vol. 213, No. 38, pp 3851-3854, 1982.

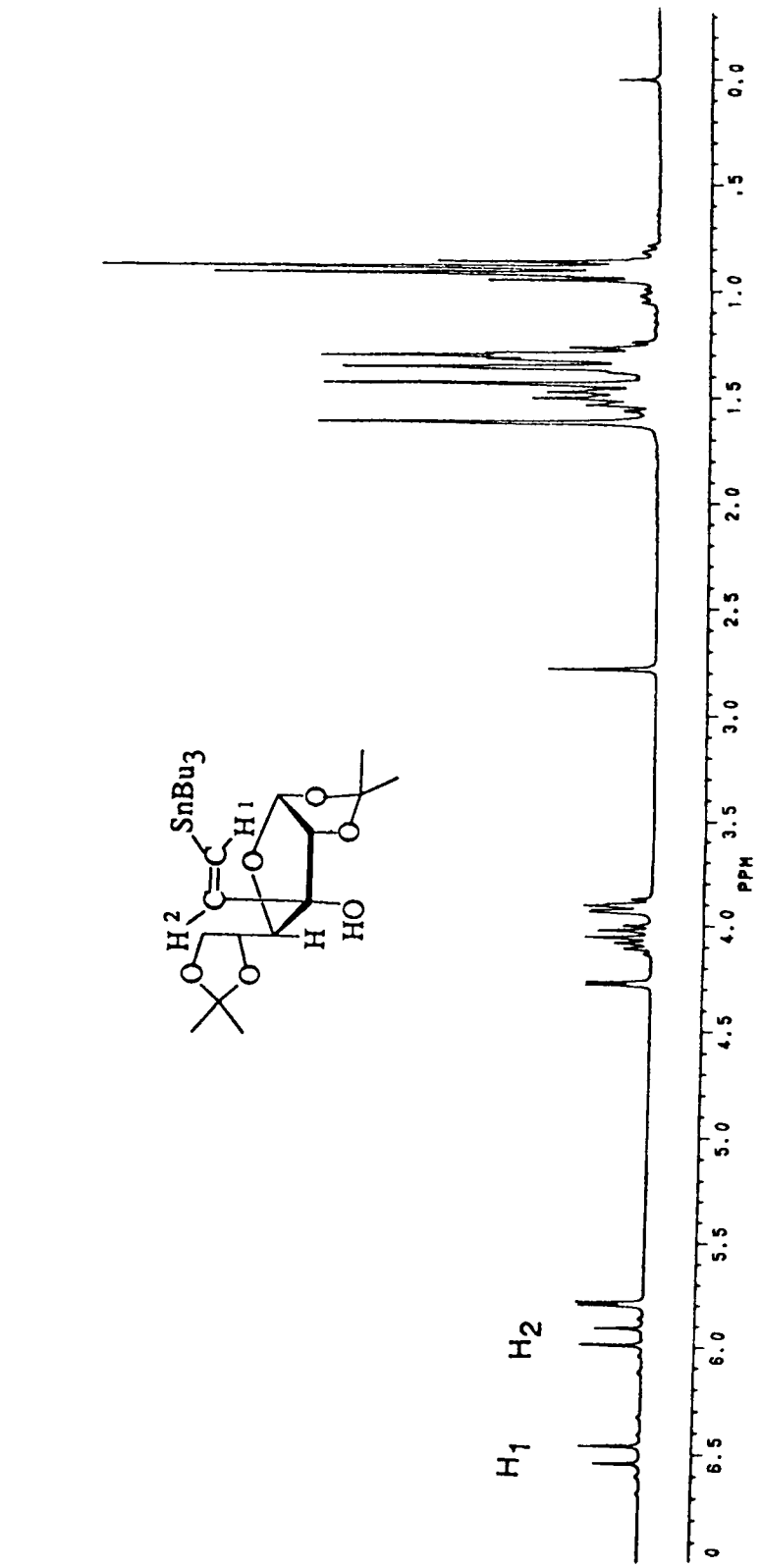
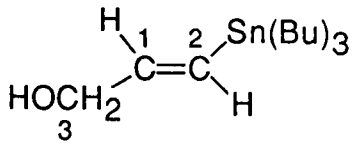


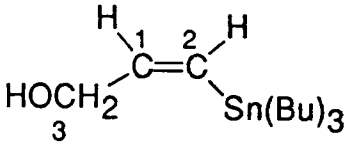
Figure 2-9. 250 MHz Proton NMR Spectrum of 3-C-((E)-2-Tri-n-butylstannylethenyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose in $CDCl_3$ plus 1.0% TMS.



C_1: 128.2 ppm

C_2: 147.3 ppm

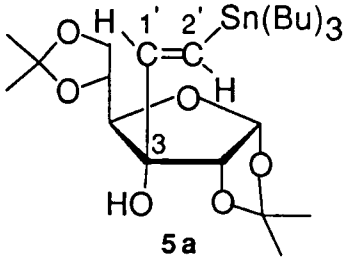
C_3: 66.2 ppm



C_1: 131.2 ppm

C_2: 146.6 ppm

C_3: 66.0 ppm

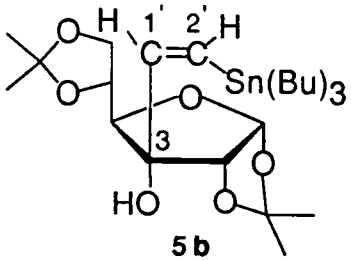


5a

C_1': 130.7 ppm

C_2': 144.1 ppm

C_3: 82.2 ppm



5b

C_1': 133.8 ppm

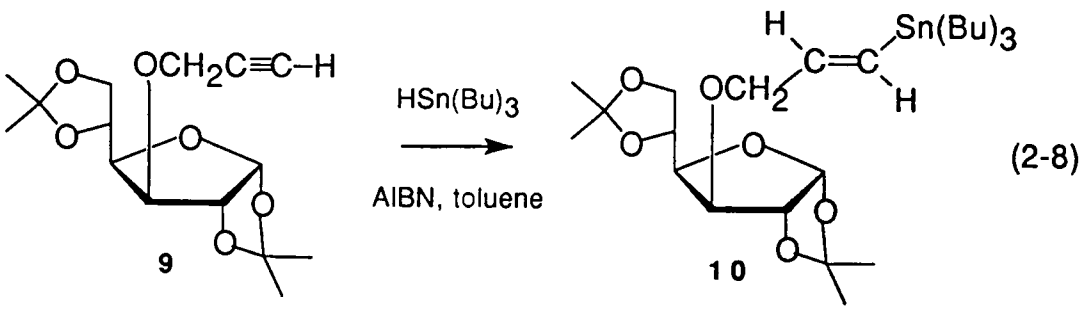
C_2': 141.7 ppm

C_3: 81.9 ppm

Figure 2-10. ^{13}C NMR Analysis of Trialkyltin Hydride Adducts of Propargyl Alcohols

Source: Young, Michael E. and Lynn A. Light. "Preparation of Iodoallylic Alcohols Via Hydrostannylation: Spectroscopic Proof of Structures". *Tetrahedron Letters*. Great Britain: Pergamon Press Ltd., Vol. 213, No. 38, pp 3851-3854, 1982.

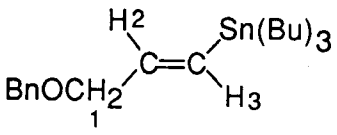
before, the product was purified by column chromatography (stationary phase = silica, mobile phase = benzene/ethyl acetate (17:3); detection by acid char; R_f (**10**) = 0.86) and the desired compound obtained in 91% yield as a clear, colorless oil. The stereochemistry of **10** was confirmed by comparison of the coupling constants of the vinylic protons in the ^1H NMR spectrum and the carbon resonances in the ^{13}C NMR spectrum to model vinylstannanes derived from propargyl ethers reported in literature. The ^1H assignments of **10** are given in Figure 2-11.



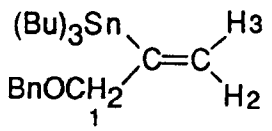
(2-8)

4. <u>The Iododestannylation Reactions of Vinylstannanes</u>

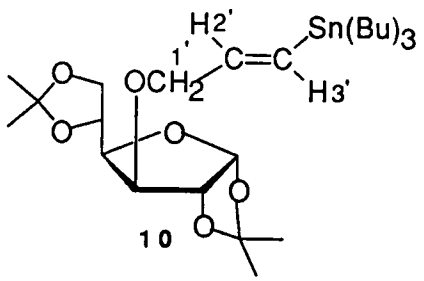
The *cis* and *trans* isomers of 3-C-(2-iodoethenyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose, **6b** and **6a**, respectively, and 3-O-((E)-iodopropen-2-yl)-1,2;5,6-di-O-isopropylidene-α-D-glucose, **10**, were prepared by the addition of iodine to the appropriate trialkyltin derivative in dichloromethane in the absence of light. The reactions proceeded in high yields with complete retention of stereochemistry and the desired products were easily purified by column chromatography [silica; benzene/ethyl acetate (17:3)]. The spectroscopic properties for **6a** and **6b** corresponded well with values for



H_1: 4.05 ppm J_{23}: 19.1 Hz
H_2: 6.10 ppm J_{12}: 4.6 Hz
H_3: 6.23 ppm J_{13}: 0.8 Hz



H_1: 4.05 ppm J_{12}: n.r.
H_2: 5.3 ppm J_{23}: n.r.
H_3: 5.9 ppm J_{13}: n.r.



H_1': ------ $J_{2'3'}$: 19.16 Hz
H_2': 6.01 ppm $J_{1'2'}$: 4.95 Hz
H_3': 6.24 ppm $J_{1'3'}$: ------

n.r. = not reported

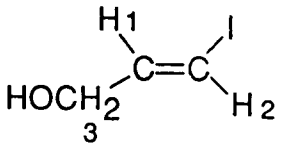
Figure 2-11. ^1H NMR of Analylsis of *Cis* and *Trans* Trialkyltin Hydride Adducts of Propargyl Ethers.

Source: Young, Michael E. and Lynn A. Light. "Preparation of Iodoallylic Alcohols Via Hydrostannylation: Spectroscopic Proof of Structures". *Tetrahedron Letters*. Great Britain: Pergamon Press Ltd., Vol. 213, No. 38, pp 3851-3854, 1982.

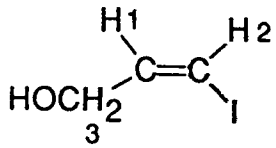
cis and *trans* vinyliodides derived from propargyl alcohols reported in literature[68] as is shown in Figure 2-12. Likewise, the spectroscopic properties of **10** were consistent with those reported for *trans* vinyl iodides derived from propargyl ethers (see Figure 2-13). As with the vinylstannanes, the assignment of stereochemistry was not difficult because the coupling constants of the vinylic protons of the *cis* and *trans* isomers are very different. For example, the coupling constant of the vinylic protons in the *cis* vinyl iodide **6b** was 8.78 Hz and was much smaller than that for the *trans* isomer **6a** (14.50 Hz). The ^{1}H NMR spectrum of **6b** has been reproduced in Figure 2-14.

5. Hydrolysis of the Isopropylidene Groups

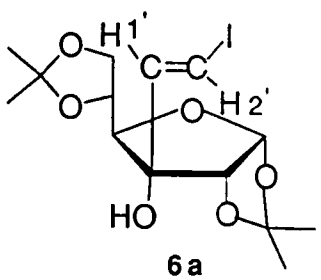
Hydrolysis of the isopropylidene groups of **6a** and **11** to give the corresponding carbohydrates **7** and **12**, respectively, was accomplished by treating the protected sugars with 1 N aqueous sulfuric acid at reflux for one hour under an inert atmosphere (equation 2-9). The resulting mixture was cooled to room temperature and carefully neutralized with solid potassium hydrogen carbonate. The water was evaporated *in vacuo* with careful heating to leave copious amounts of a white solid. The solid was extracted with warm ethanol, the ethanol extracts were combined and the volatile components removed to provide the crude product as a white solid. Thin layer chromatography of the ethanol extracts containing **7** or **12** revealed only one component after acid charring. Purification of both **7** and **12** was accomplished by column chromatography [stationary phase = silica; mobile phase = acetonitrile / water (95:5); Rf (**7**) = 0.61, Rf (**12**) = 0.77]. Both compounds obtained as white solids which were characterized by ^{1}H NMR and ^{13}C NMR



H_1: 6.71 ppm J_{12}: 14.5Hz
H_2: 6.40 ppm J_{13}: 5.4 Hz
H_3: 4.10 ppm J_{23}: 1.5 Hz

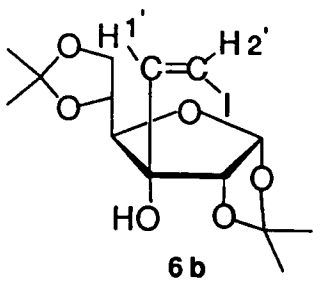


H_1: 6.49 ppm J_{12}: 7.6 Hz
H_2: 6.36 ppm J_{13}: 5.6 Hz
H_3: 4.24 ppm J_{23}: 1.3 Hz



6a

H_1':6.74 ppm $J_{1'2'}$: 14.16 Hz
H_2':6.48 ppm

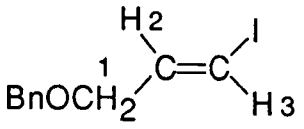


6b

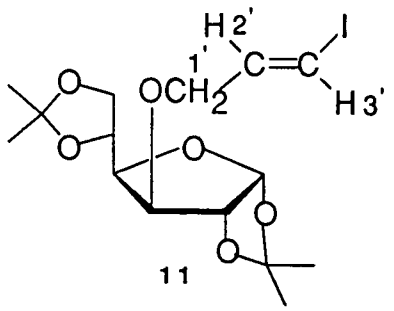
H_1': 6.77 ppm $J_{1'2'}$: 8.78H
H_2':6.64 ppm

Figure 2-12. ^{1}H NMR Analysis of Vinyl Iodides Derived From Propargyl Alcohols

Source: Young, Michael E. and Lynn A. Light. "Preparation of Iodoallylic Alcohols Via Hydrostannylation: Spectroscopic Proof of Structures". *Tetrahedron Letters*. Great Britain: Pergamon Press Ltd., Vol. 213, No. 38, pp 3851-3854, 1982.



H_1: 3.95 ppm	J_{23}: 14.5 Hz
H_2: 6.66 ppm	J_{12}: 5.6 Hz
H_3: 6.40 ppm	J_{13}: 1.0 Hz



H_1': 6.48 ppm	$J_{2'3'}$: 14.55 Hz
H_2': 6.60 ppm	$J_{1'3'}$: 4.90 Hz
H_3': -------	$J_{1'2'}$: -------

Figure 2-13. ^1H NMR Analysis of Vinyl Iodides Derived From Propargyl Ethers

Source: Young, Michael E. and Lynn A. Light. "Preparation of Iodoallylic Alcohols Via Hydrostannylation: Spectroscopic Proof of Structures". *Tetrahedron Letters*. Great Britain: Pergamon Press Ltd., Vol. 213, No. 38, pp 3851-3854, 1982.

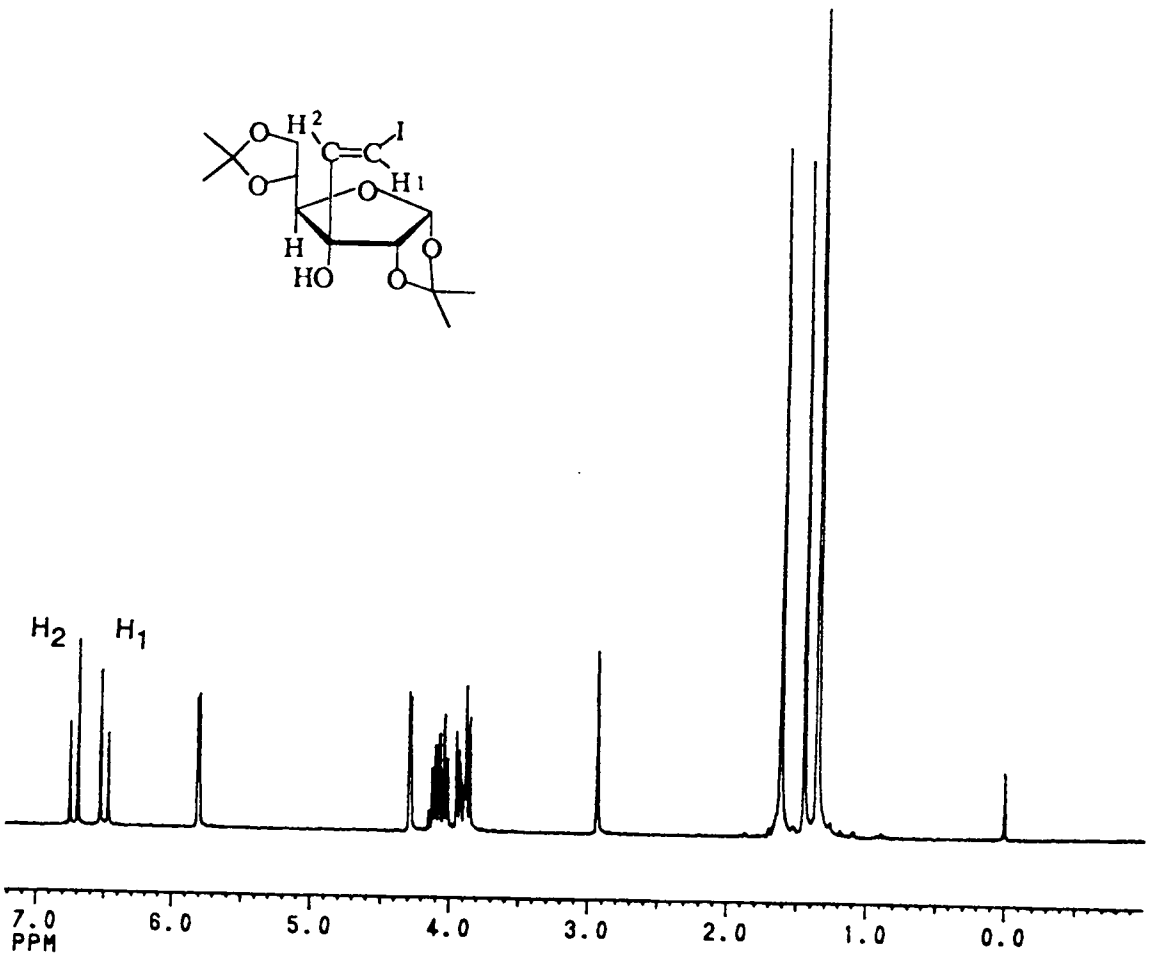
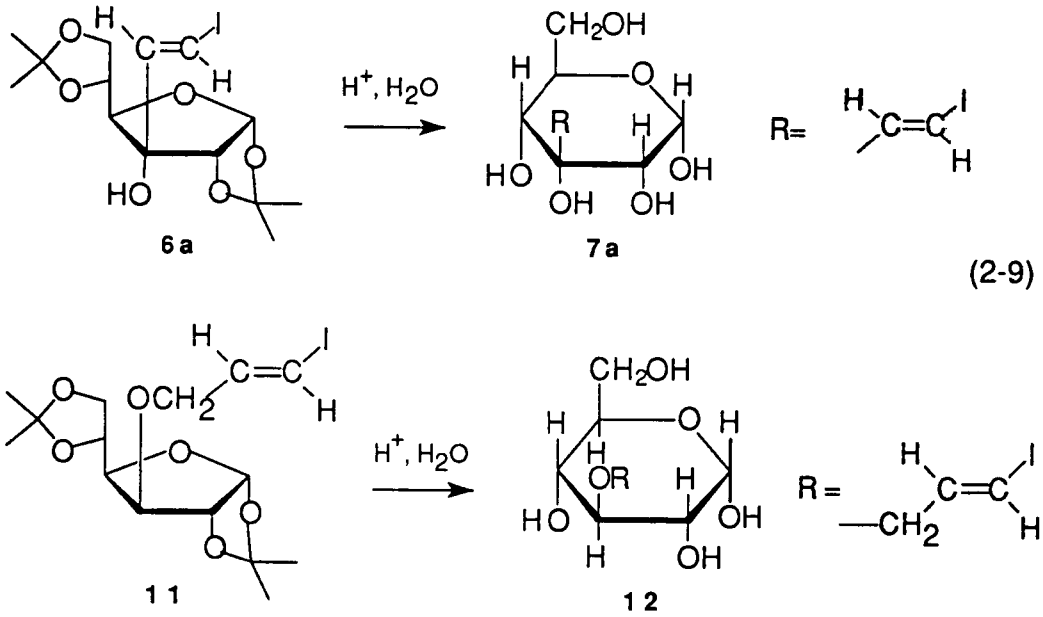


Figure 2-14. 250 MHz NMR Spectrum of 3-C-((E)-2-Iodoethenyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose in $CDCl_3$ plus 0.3% TMS.



spectroscopy. The ^{1}H NMR spectra of **7** and **12** were complicated because both the α and β forms were present in each case, however, the ^{13}C spectra provided more information and the resonances for each anomer were identified by the use of model compounds and the relative peak intensities. The reported assignments for α and β forms of D-glucose and 3-O-methyl-D-glucose were used for the spectral assignments of **7** and **12**,[73] along with the fact that there was approximately three times more of the β anomer than the α anomer in both cases (as determined by integration of the anomeric proton resonances in the ^{1}H NMR spectrum). The disappearance of the resonances corresponding to the methyl groups of the isopropylidenes between 1.2 ppm and 1.5 ppm in the ^{1}H NMR spectra and 25.0 ppm and 27.0 ppm in the ^{13}C spectra provided further evidence that the hydrolysis was successful.

Both **7** and **12** were analyzed for purity by ion chromatography (Dionex

system, mobile phase = 200 mM NaOH, flow rate = 1ml/min, column = CarboPac PA1 Analytical 4 x 250 mm) and the ion chromatograms have been reproduced in Figure 2-15 and Figure 2-16, respectively. The mass spectrum (chemical ionization) of **7** displayed peaks corresponding to the loss of 1, 2 and 3 molecules of water, however, the molecular ion was not observed. This is not an unexpected result for branched carbohydrates.[69] Elemental analysis of **12** (C, H and I) proved that the molecular formula was $C_9H_{15}O_6I$ and the reported values were within +/- 0.30% of the theoretical values.

6. Preparation of the Radioiodinated Agents

The radioiodinated anologs of **7** and **12** were prepared by reacting **6a** or **11**, respectively, with $Na^{123}I$ or $Na^{125}I$ in a mixture of dichloromethane and water in the presence chloramine-T. The radioiodinations took place quickly at 0-5°C and the initial red color of the reaction mixture disappeared as the reaction progressed. The solution was washed with 10% sodium hydrogen sulfite solution and the product was extracted into diethyl ether. The ether washes were combined, dried over anhydrous sodium sulfate, and the reaction progress monitored by radio-thin layer chromatography [stationary phase = silica; mobile phase = benzene/ethyl acetate (18:2)]. A typical radiochromatogram is shown in Figure 2-17 which reveals the presence of one major radiolabeled product along with iodide salts which remained at the origin. The radiolabeled products were purified by column chromatography [stationary phase = silica; mobile phase = benzene/ethyl acetate (18:2)] and the isopropylidene groups removed by hydrolysis in 1.0 N aqueous sulfuric acid at reflux for two hours. The solution was then cooled, neutralized with 1.0 N

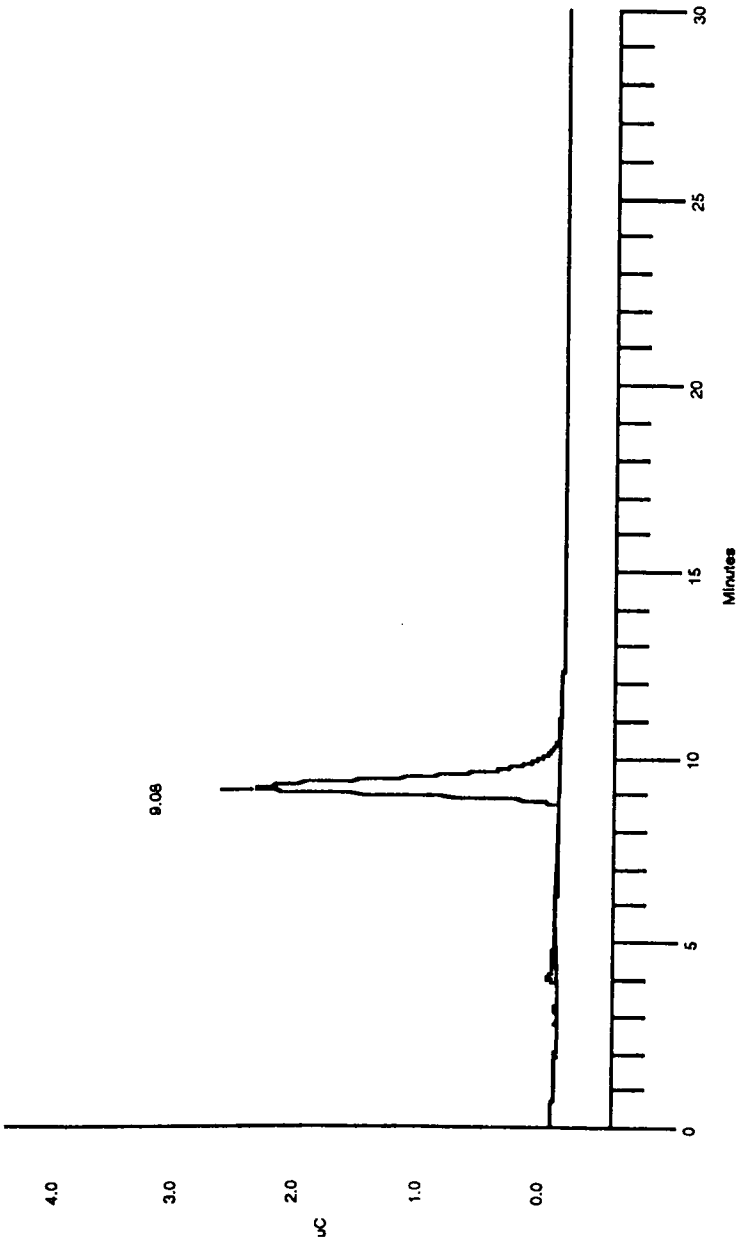


Figure 2-15. Ion Chromatogram of 3-O-[(E)-2-IodoEthenyl]-D-Allose **7**.

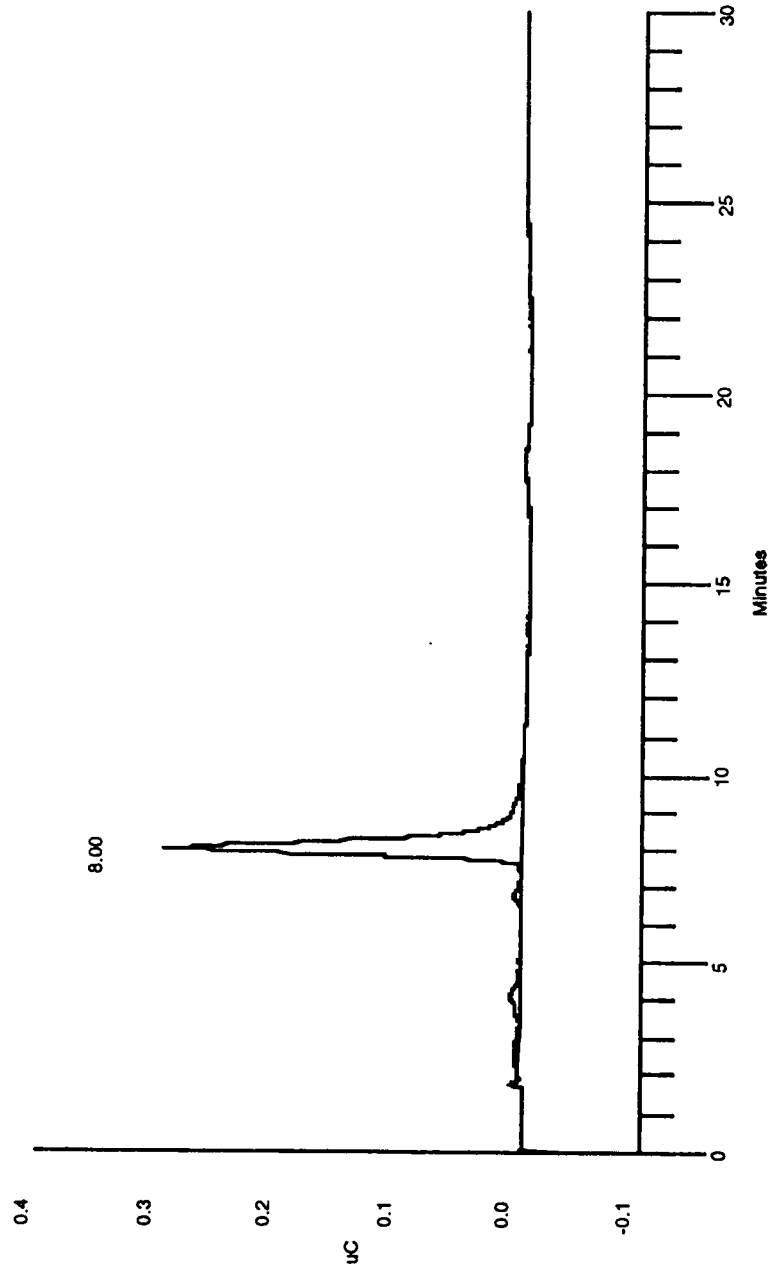


Figure 2-16. Ion Chromatogram of 3-O-[(E)-3-Iodopropen-2-yl]-D-Glucose **12**.

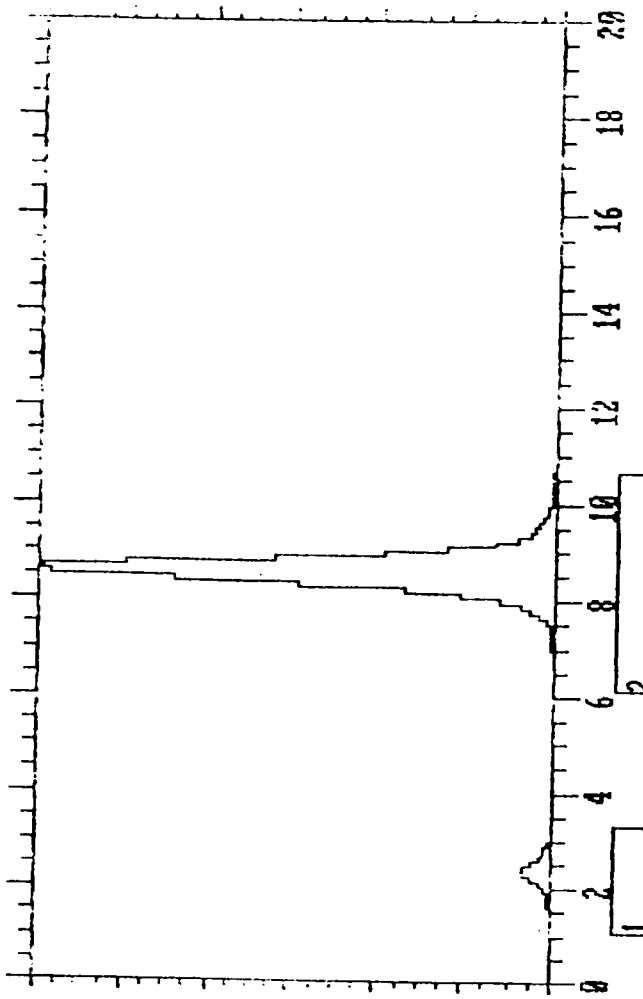


Figure 2-17. Radio-Thin Layer Chromatogram of the Crude Reaction Mixture of the Formation of 3-O-((E)-3-[^{123}I]Iodopropen-2-yl)-1,2:5,6-di-O-isopropylidene-α-D-glucose.

aqueous sodium hydroxide, and the water removed with heat under a stream of argon. The product was extracted from the resulting residue into ethanol and was purified by column chromatography [stationary phase = silica; mobile phase = acetonitrile/water (95:5)] to provide radiolabeled compounds with R_f values identical to the non-radioactive analogs of **7** and **12**. The radiochemical purities of the final products were confirmed by radio-thin layer chromatography. The overall radiochemical yields of the labeled analogs of **7** and **12**, after correcting back to the time of the addition of the sodium radioiodide (time = 0), were 31% and 40%, respectively.

7. Animal Distribution Studies

The first agent to be evaluated was 3-C-((E)-2-[^{125}I]iodoethenyl)-D-allose. This agent was dissolved in saline and the resulting solution filtered through a 0.22 mm Millipore filter. The filtered solution was then administered by injection into the lateral tail vein of ether-anesthetized animals. The distribution of radioactivity was determined in the tissues of the liver, kidneys, heart, lungs, thyroid and brain of 10-12 week old female Fischer 344 rats (170-200g) after intravenous administration of the radioiodinated agent (see Table 2-4). The animals were allowed food and water prior to, and during, the course of the experiment. At the times indicated in Table 2-4, the animals were killed by cervical fracture and blood samples obtained by cardiac puncture. Three animals were sacrificed at each timepoint and the organs were then removed, rinsed with saline solution and blotted dry to remove residual blood. The organs were weighed and the radioactivity contained in each was determined using a NaI auto-gamma counter (Packard Instruments). The weights of the

Table 2-4. The Distribution of Radioactivity in Rat Tissues at Various Times After Intravenous Administration of 3-C-((E)-[^{125}I]-2-Iodoethenyl)-D-allose

% Injected Dose Per Gram*

Tissue	5 Min	15 Min	60 Min
Blood	1.46	0.98	0.33
Heart	0.64	0.50	0.31
Brain	0.08	0.05	0.03
Liver	1.94	0.99	0.28
Kidneys	5.72	2.09	0.65
Thyriod	17.4	10.7	6.07

*Average values for three female Fischer rats at each time indicated.

thyroid glands were obtained in the usual manner by assuming each animal has 7.5 mg of thyroid tissue for every 100 g that the animal weighs. Samples of the injected radioactive solutions were assayed as standards to calculate the percent injected dose per gram of tissue. As is revealed in Table 2-4, there was very little uptake of the radioiodinated agent in the heart or brain.

Evaluation of 3-O-((E)-[^{123}I]iodopropen-2-yl)-D-glucose was accomplished in a similar manner and the tissue distribution was determined in Fisher rats at time intervals of 2, 4, 6, 16 and 31 minutes after injection. The results are presented in Table 2-5 and reveal that there was little uptake of the radiolabeled agent in the heart or brain.

8. Computer Modeling Studies:

Two factors that influence the binding of a carbohydrate to the glucose/sorbose carrier protein are steric bulk and the electronic environment at points that are critical to binding. The study by Barnett et al. (see pages 26-29) explored both of these parameters by analyzing the effect of modifications at different positions on D-glucose and other carbohydrates on their ability to bind to the glucose/sorbose carrier protein. In order to assess the steric bulk generated at C_3 of **7** and **12** by the addition of the iodovinyl and iodopropen-2-yl groups, energy minimized structures of the β form of **7** and **12** were obtained using SYBYL. From the minimized structures of **7** and **12**, diagrams were produced that display the Van der Walls radii of every atom in each structure. These diagrams reveal the relative size of the iodovinyl and iodopropen-2-yl groups with respect to β-D-glucose. For comparison, a series of carbohydrates with known biological activity were evaluated in the same manner for

Table 2-5. The Distribution of Radioactivity in Rats Tissues at Various Times After Intravenous Administration of 3-O-((E)-3-[^{123}I]Iodopropen-2-yl-D-glucose

	% Injected Dose Per Gram*				
Tissue	2 Min	4 Min	6 Min	16 Min	31 Min
Blood	1.23	1.02	0.72	0.47	0.30
Heart	0.96	0.77	0.62	0.42	0.30
Brain	0.07	0.06	0.06	0.07	0.07

*Average time for female Fischer rats at the times indicated.

comparison, including β-D-glucose and 3-O-propen-2-yl-D-glucose, both of which are biologically active.

Figures 2-18 through 2-22 display the Van der Walls surfaces of β-D-glucose, 3-O-propen-2-yl-β-D-glucose, *cis* and *trans* 3-O-iodopropen-2-yl-β-D-glucose and *trans*-C-iodoethenyl-β-D-glucose, respectively. The plots are oriented such that C_3 and any groups attached to it are clearly shown on the left side of each figure. The structures presented for β–D-glucose and 3-O-propen-2-yl-D-glucose give one an idea of the bulk which is allowed at C_3 without preventing the binding of the carbohydrate to the carrier protein. As noted previously, both β-D-glucose and 3-O-propen-2-yl-D-glucose bind to the carrier protein and have relatively low K_i values. Upon examination of *cis* and *trans* 3-O-(iodopropen-2-yl)-D-glucose, it becomes apparent that the addition of iodine caused a relatively large increase in steric bulk and that, in order for these two compounds to bind to the carrier protein, there would have to be a space large enough in the complex formed to accommodate the iodine atom. The overall bulk of the iodoethylenyl group is not as large as the bulk of the iodopropen-2-yl group. However, the bulk at C_3 is distributed differently in *trans* 3-C-(2-iodoethenyl)-β-D-glucose than in 3-O-allyl-β-D-glucose and there would have to be space in the bound complex with adequate dimensions to accommodate the iodoethenyl group.

D. Conclusions

Both of the radioiodinated carbohydrate analogs of **7** and **12** demonstrated little *in vivo* deiodination during the course of the animal experiments, reaffirming that the iodovinyl group provides a reliable method for

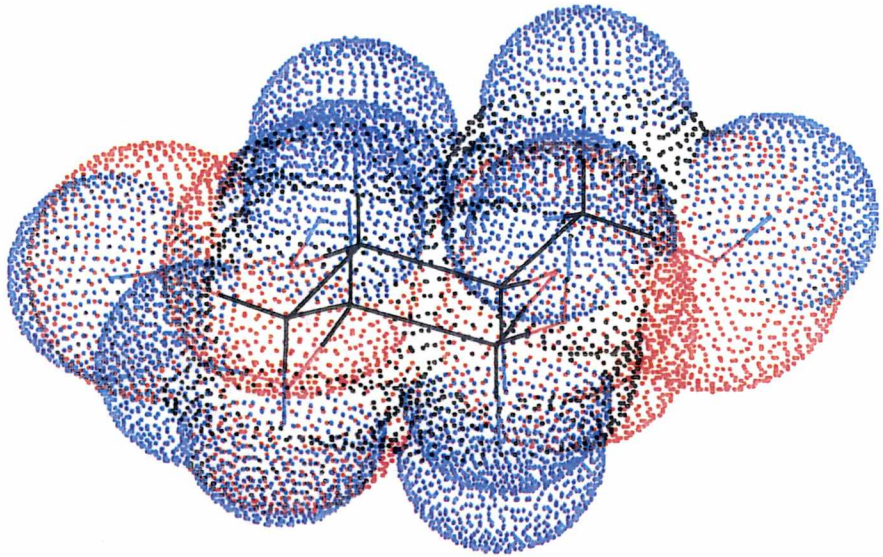


Figure 2-17. Diagram Displaying the Van der Waals Radii of β-D-glucose

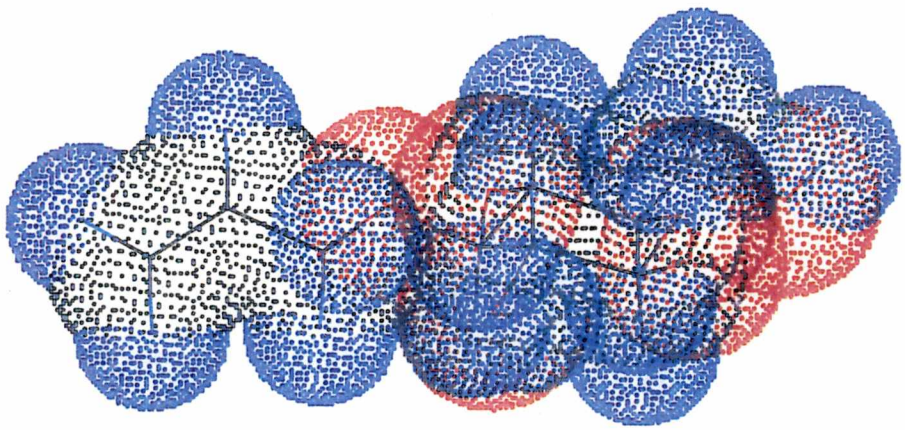


Figure 2-19. Diagram Dispaying Van der Waals Radii of 3-O-Propen-2-yl-β-D-glucose

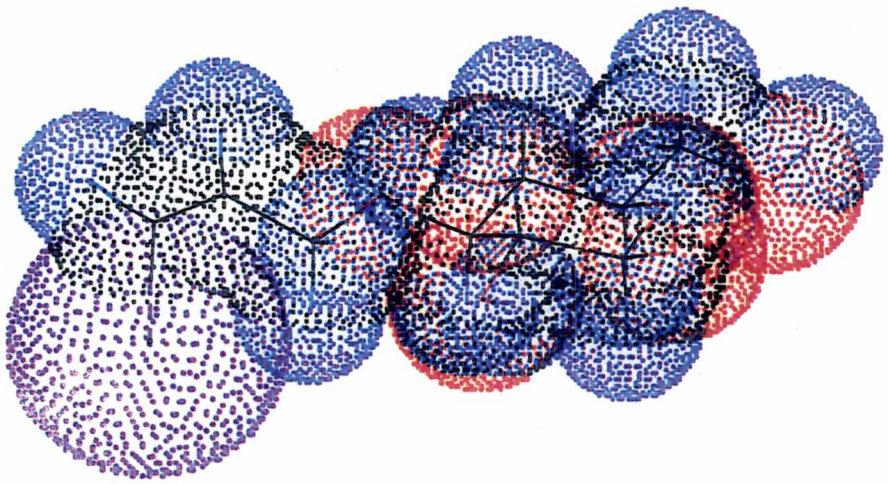


Figure 2-20. Diagram Dispaying Van der Waals Radii of 3-O-((Z)-Iodopropen-2-yl-β-D-glucose

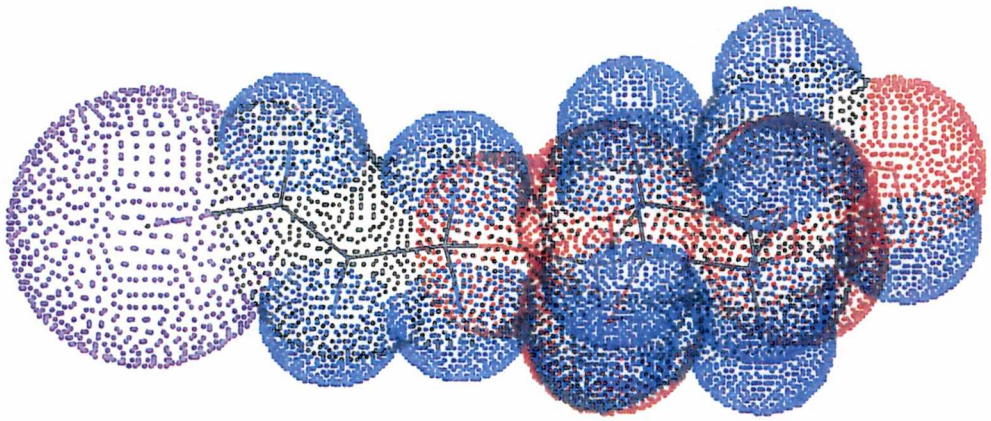


Figure 2-21. Diagram Dispaying Van der Waals Radii of 3-O-((E)-Iodopropen-2-yl-β-D-glucose

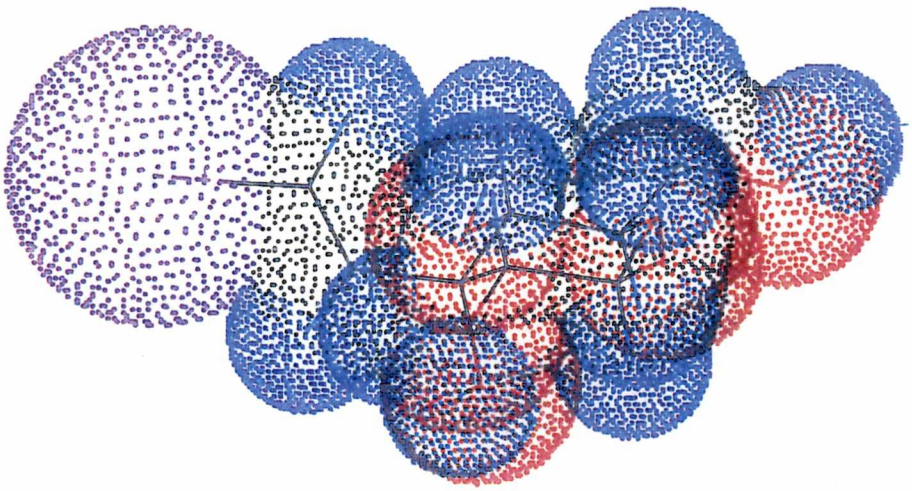


Figure 2-22. Diagram Dispaying Van der Waals Radii of 3-C-((E)-2-Iodoethenyl)-β-D-allose

stabilizing the carbon-iodine bond. However, neither agent showed significant brain or heart uptake. The result for 3-C-((E)-2-[125I]iodoethenyl)-D-allose was not unexpected; Barnett et al. reported earlier that allose did not bind to the glucose/sorbose carrier protein in the human erythrocyte, presumably due to steric interferences of the α hydroxyl group at C_3 with the approach to the carrier protein and/or the lack of a group capable of hydrogen bonding in the β position at C_3. Our observations support their conclusions. The steric and electronic differences between allose and glucose at C_3 are great enough to prevent allose from binding to the carrier protein and, therefore, the extra bulk added by the iodoethenyl group is probable irrelevant. It is unlikely that the corresponding glucose analog would bind to the carrier protein because there would still be a relatively large group in the α position at C_3.

3-O-(E)-3-Iodopropen-2-yl-D-glucose also demonstrated little biological activity in spite of the fact that 3-O-propenyl-D-glucose has a K_i value similar to that of glucose. This probably is due to the added steric bulk of the iodine atom and not electronic factors. It is possible that the *cis* isomer would have been a better candidate in view of the fact that the iodine atom is oriented differently and occupies a space which might be available in the bound complex. The are several examples in which the *cis* isomer displays different biological activity than the trans isomer for this reason.[70]

It can be concluded from the biological distribution data presented here and on the results obtained from carbohydrates containing radioiodine at C_4,[71] that the incorporation of an iodine atom into a relatively small molecule, such as glucose, can cause steric interferences in binding to target proteins. Therefore, it seems unlikely that the incorporation of radioiodine into simple carbohydrates

will result in suitable imaging agents for evaluation of glucose metabolism. It is also doubtful that bromine isotopes could be used because the Van der Waals Radius of bromine (1.95 Å) is much closer to that of iodine (2.15 Å) than that of hydrogen (1.2 Å),[72] Therefore, there would be a relatively small reduction in bulk by replacing an iodovinyl group with a bromovinyl group.

It is noteworthy that the dietary state of the animals was not determined before the start of each experiment. While this should not effect the saturable process of glucose uptake in the brain, the dietary state of the animal has a profound effect in the glucose uptake in the heart. For example, before 2-FDG is given to human patients for PET studies, a large amount of a glucose drink is given to ensure that insulin levels are high to facilitate glucose uptake. It is possible that the animals had not eaten and were in a fasting state, even though food was available, resulting in low insulin levels at the time of the experiment.

E. Experimental

1. General

All glassware, syringes and syringe needles were oven-dried for 24 hours prior to use when required. All NMR spectra were recorded on a JEOL FX-90Q FT-NMR spectrometer or a Brücker AC-250 FT-NMR spectrometer. Deuterochloroform ($CDCl_3$) or deuteroacetone ($(CD_3)_2C=O$) were used as solvents. The ^{1}H-NMR spectra were referenced internally using either tetramethylsilane (TMS) at 0.0 ppm or the methyl resonance of deuteroacetone, which was a pentet centered at 2.04 ppm. The integration parameters acquired on the JEOL FX-90Q were plotted directly onto the ^{1}H-NMR specta and the integration parameters acquired on the Brücker AC-250 were obtained in

numerical form on a separate print-out and do not appear on the spectra. The ^{13}C-NMR spectra were referenced internally using either $CDCl_3$ (center of triplet at 77.1 ppm) or deuteroacetone (carbonyl resonance at 206.5 ppm). Infrared spectra were obtained on a Bio-Rad FTS-7 IR Spectrometer controlled by a Bio-Rad SPC 3200 computer and were referenced using polystyrene (1601 cm^{-1}). Melting points were obtained using an Electrothermal Digital Melting Point Apparatus F0290 and are uncorrected. Thin layer chromatography was performed using Whatman Silica Gel 60A MK6F plates (250 μm thickness) with a fluorescent indicator (254 nm) and detection was by acid char using phosphomolybdic acid. Radio-thin layer chromatography was performed using Analtech silica gel G PF-254 glass plates (250 μm) which were analyzed by a Bioscan System 200 Imaging Scanner controlled by an IBM Personal Computer. Column chromatographic separations were accomplished using Baxter C4582-86 60A (230-400 mesh) silica gel. Ion chromatography was accomplished using a Dionex Model DX-300 IC system with a 20 μL sample loop controlled by a Dionex AI-450 Automated Workstation. Detection was accomplished with a Dionex Pulsed Electrochemical Detector (PED) P/N 42039 with a Gold Electrode PED Cell (P/N 42300) and the separations were performed using a Dionex CarboPac PA1 Guard (4 x 250 mm guard column) and a Dionex CarboPac PA1 Analytical Ion Chromatography Column (4 x 250 mm). Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee and Atlantic Microlabs, Norcross, Georgia and all experimental values are within +/-0.40% of the theoretical values. Mass spectra were obtained on either a Kratos MS-25 instrument (70 eV) or a VG Analytical ZAB-EQ instrament (8 KV). All reagents were analytical grade and were used

without further purification. The commercially available chemicals and solvents used and their manufacturers are listed in Table 2-6.

2. The Synthesis of 3-O-(E)-3-[^{123}I]Iodopropen-2-yl-D-glucose

a. 3-O-Propargyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (9)

An oven dried 100 ml round-bottomed flask, equipped with a magnetic stirring bar, was flushed with argon and into the flask was placed 1,2:5,6-di-O-isopropropylidine-α-D-glucofuranose (2.40 g, 9.22 mmol). The flask was then sealed with a rubber septum, vented (oil bubbler), and 12 ml of anhydrous tetrahydrofuran added via syringe to dissolve the glucofuranose, resulting in a clear, colorless solution. Sodium hydride (460 mg, 11.5 mmol, 60% in mineral oil) was added and evolution of hydrogen gas was observed. The mixture was stirred at room temperature for 30 minutes and then propargyl bromide (0.928 ml, 11.1 mmol) was added using an Eppendorf pipet. The resulting dark brown mixture was heated to 40°C and the reaction monitored by thin layer chromatography [stationary phase = silica; mobile phase = benzene/ethyl acetate (17:3); R_f (product) = 0.61, R_f (starting material) = 0.13]. After 4.0 hours, the reaction was complete and the mixture was cooled to room temperature. A 50 ml portion of distilled water was added to destroy excess sodium hydride and the product extracted into dichloromethane (3 X 15 ml). The organic layers were combined, dried over anhydrous sodium sulfate, and the solvent evaporated under reduced pressure to give 2.50 g of a dark brown oil. The product was purified by column chromatography [stationary phase = silica; mobile phase = benzene / ethyl acetate (17:3)] to give 2.25 g (7.56 mmol, 82.5 %) of **9** as a clear, colorless oil; ^{1}H-NMR ($CDCl_3$) : δ1.22, 1.33, 1.40, 1.48 (4s,

Table 2-6. Commerially Available Chemicals and Their Manufacturers (Part A).

Reagent	Manufacturer
Acetic Anhydride	A
Acetone	A
Acetone-d_6	A
Acetonitrile	A, B
Benzene	A
Butyllithium (1.6 M in hexanes)	A
Catecholborane	A
Dichloromethane	A
Diethyl Ether, anhydrous	B
Ethanol	A
Ethyl Acetate	A
Tetrahydrofuran (anhydrous)	A
Trimethylsilylacetylene	A
Tributyltin Hydride	A
Iodine	A
Propargyl Bromide	A
Sodium Hydrogen Sulfite	B
Chloroform-d_3 (1% TMS)	A
Sodium Hydroxide (50% w/w)	M

Table 2-6 (continued)

Reagent	Manufacturer
Water	A
Sodium Iodide	B
Chloramine-T	A
Phosphomolybdic Acid	A
Dimethyl Sulfoxide	A
Diacetone-D-glucose	A
Methanol	A
Sodium Bicarbonate	A
Sodium hydroxide	A
Silica Gel	B
Silica Gel Plates	W
Sodium Carbonate	A
Sodium Hydride	A
Sulfuric Acid (concentrated)	B
Sodium Sulfate	A
Sodium ^{125}I	N
Sodium ^{123}I	N

*Manufacturers

A = Aldrich Chemical Company

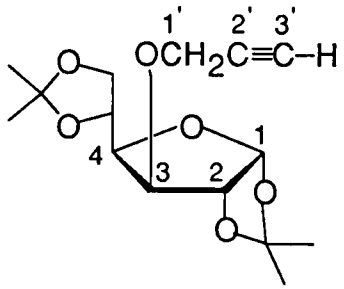
B = Baxter Chemical Company

N = New England Nuclear, Inc.

M = Mallinckrodt Chemical Company

W = Whatman Chemical Company

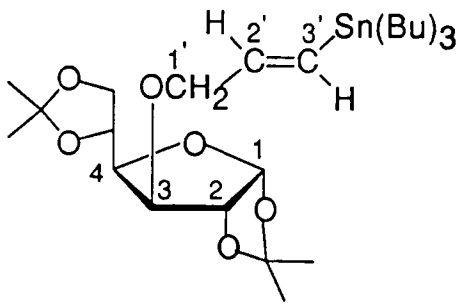
12H, -C-(C<u>H</u>$_3$)$_3$), 2.48, (t, 1H, J=2.20 Hz, $H_{3'}$), 3.40-4.20 (m, 4H, H_4 and -OC<u>H</u>C<u>H</u>$_2$O-), 4.28 (d, 2H, J=2.20 Hz, $H_{1'}$), 4.61 (d, 1H, J=3.52 Hz, H_2), 5.84 (d, 1H, J= 3.52 Hz, H_1); ^{13}C-NMR ($CDCl_3$): δ 25.42, 26.28, 26.88, (C_4, -C(<u>C</u>H$_3$)$_2$), 58.19 ($C_{1'}$), 67.24 (-OCH<u>C</u>H$_2$O-), 72.58 (C5), 74.98 ($C_{2'}$), 79.23 ($C_{3'}$), 81.59 (C_3), 82.90 (C_2), 105.27 (C_1), 109.06, 111.93 (-<u>C</u>(CH$_3$)$_2$), 81.05 (C_4); IR (film, cm^{-1}): 3270 (alkyne C-H stretch), 2989, 2938, 2896 (C-H stretch), 2118 (alkyne stretch), 1456, 1382 (C-H bend), 1165, 1075, 1022 (C-O-C stretch), 638 (alkyne C-H bend); elemental analysis: (calculated for $C_{15}H_{22}O_6$) C, 60.39%; H, 7.43% (found) C, 60.42; H, 7.19%



b. <u>3-O-[(E)-3-Tributylstannylpropen-2-yl]-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (10)</u>

A 100 ml, two-necked, round-bottomed flask was equipped with a magnetic stirring bar, a water cooled reflux condenser, a rubber septum and a gas outlet (oil bubbler). The flask was purged with argon gas and then anhydrous toluene (20 ml), AIBN (50 mg) and 3-O-propargyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (400 mg, 1.34 mmol) were added. The

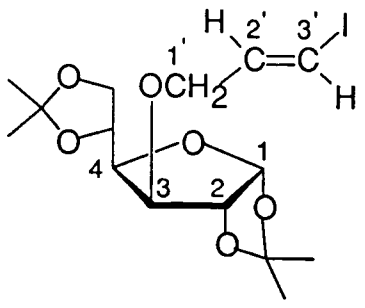
resulting solution was heated to 60°C and tributyltin hydride (0.50 ml, 1.86 mmol) was added via syringe. Heating continued for 16 hours, after which time the reaction mixture was checked by thin layer chromatography [stationary phase = silica; mobile phase = benzene / ethyl acetate (17:3)] to confirm formation of the product. Four components were detected and the most prominent corresponded to the desired product (Rf = 0.86). No starting material was detected. The clear, colorless reaction mixture was then cooled to room temperature and concentrated by removal of the solvent under reduced pressure until a volume of 5 ml was attained. The remaining liquid was applied directly to a silica gel column and the product was eluted with a mixture of benzene and ethyl acetate (17:3). All fractions were checked by thin layer chromatography and those containing the desired product combined and the solvent removed. The product was obtained in this manner as a clear, colorless oil (712 mg, 1.21 mmol, 91.3 %); ^{1}H-NMR ($CDCl_3$) δ 0.82, 0.89, 0.96, (t, 9H, J=3.45 Hz, -$(CH_2)_2C\underline{H}_3$), 1.19-1.74 (m, 30H, -$(C\underline{H}_2)_2CH_3$, -$C(C\underline{H}_3)_2$), 3.90-4.3 (m, 6H, H_4, -$C\underline{H}C\underline{H}_2O$-, and $H_{1'}$), 4.55 (d, 1H, J=3.42Hz, H_2), 5.89, (d, 1H, J=3.42 Hz, H_1), 6.01 (dt, $J_{2'3'}$ =19.16 Hz and $J_{1'2'}$ =4.95 Hz, $H_{2'}$), 6.24 (d, 1H, $J_{2'3'}$ =19.16 Hz, $H_{3'}$); ^{13}C-NMR ($CDCl_3$): 9.43 (-$(CH_2)_2C\underline{H}_3$), 10.33, 27.29, 29.10 (3C, -$(\underline{C}H_2)_2CH_3$), 25.41, 26.23, 26.83, (4C, -$C(CH_3)_2$), 67.20 (-$OCHC\underline{H}_2O$-), 72.58 (-$O\underline{C}HCH_2O$-), 81.16 (C_4), 82.71 (C_2), 84.14 (2C, C_3 and $C_{1'}$), 105.27 (C_1), 108.90, 111.69 (2C, -$\underline{C}(CH_3)_2$), 131.76 ($C_{2'}$), 143.90 ($C_{3'}$); IR (film, cm^{-1}): 2985, 2942, 2929, 2888, 2852 (C-H stretch), 1603 (alkene stretch) 1165, 1076, 1062 (C-O-C stretch), 849 (alkene C-H deformation); elemental analysis: (calculated for $C_{27}H_{48}O_6Sn$) C, 55.02%; H, 8.55%; O, 16.29% (found) C, 54.82%; H, 8.68%; O, 16.46%.



c. 3-O-[(E)-3-Iodopropen-2-yl]-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (11)

A 100 ml round-bottomed flask was equipped with a magnetic stirring bar, a rubber septum and a gas outlet (oil bubbler). The flask was flushed with argon and 3-O-[(E)-Iodopropen-2-yl]-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (805 mg portion, 1.37 mmol), dissolved in dichloromethane (10 ml), was added via syringe. A solution of iodine (420 mg, 1.65 mmol) in dichloromethane (15 ml) was added in one portion via syringe and the mixture stirred at 0°C in the absence of light for 2 hours. The resulting clear red solution was then poured into a 150 ml separatory funnel and washed with saturated sodium hydrogen sulfite solution (1 X 10 ml). The layers were separated and the aqueous layer was backwashed with dichloromethane (2 X 10 ml). The organic layers were combined, dried over anhydrous sodium sulfate, filtered by gravity filtration, and the solvent evaporated under reduced pressure to give 750 mg of a clear yellow oil. The product was purified by column

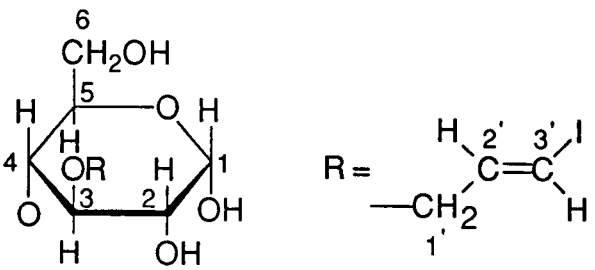
chromatography [mobile phase = benzene/ethyl acetate (17:3) ; stationary phase = silica; detection = acid char; R_f(product) = 0.70] to give 158 mg (0.371 mmol, 27.1 %) of the desired product as a clear, colorless oil, ^{1}H-NMR ($CDCl_3$) δ1.23, 1.37, 1.43, 1.46 (4s, 12H, (-C(H_3)$_2$), 3.92-4.53 (m, 6H, H_4, -OC<u>H</u>C<u>H$_2$</u>O- and $H_{1'}$), 4.51 (d, 1H J=3.74 Hz, H_2), 5.87 (d, 1H, J=3.74, H_1), 6.48 (d, 1H, $J_{2'3'}$ =14.55 Hz, $H_{3'}$), 6.60 (td, 1H, $J_{2'3'}$ = 14.55 Hz, $J_{1'2'}$ = 4.90 Hz, $H_{2'}$); ^{13}C-NMR ($CDCl_3$) δ 25.46, 26.28, 26.85, 26.91 (4C, (-C(CH_3)$_2$), 67.58 (-OCH<u>C</u>H$_2$O-), 71.88 ($C_{1'}$), 72.54 (-O<u>C</u>HCH$_2$O-), 79.04 ($C_{3'}$), 81.19 (C_3), 81.53 (C_4), 82.80 (C_2), 105.27 (C_1), 109.15, 111.88 (2C, (-C(CH_3)$_2$), 141.60 ($C_{2'}$); IR (film, cm^{-1}): 2985, 2932, 2892 (C-H stretch), 1620 (alkene stretch), 1165, 1095, 1075, 1022 (C-O-C-stretch), 848 (alkene C-H deformation); elemental analysis: (calculated for $C_{15}H_{21}O_6I$) C, 42.47%; H, 5.42%; I, 29.67% (found) C, 42.38%; H, 5.45%; I, 29.71%.



d. 3-O-[(E)-3-Iodopropen-2-yl]-D-glucose (12)

3-O-[(E)-3-iodopropen-2-yl]-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (100 mg, 0.235 mmol) was placed into a 100 ml round-bottomed flask equipped with a magnetic stirring bar, a water cooled reflux condenser and a gas outlet (oil bubbler). The flask was flushed with argon, 12 ml of 1.0 N aqueous sulfuric acid solution added in one portion via syringe and the resulting mixture heated at 60°C for 2.0 hours. At the start of the reaction, a colorless oil could be seen in the reaction mixture because the glucofuranoside was not soluble in the sulfuric acid solution. However, after two hours of heating at 60°C, the oil had disappeared and the reaction mixture became clear and colorless. The reaction mixture was then cooled to 0°C using an ice-water bath and neutralized by the careful addition of solid sodium hydrogen carbonate until gas evolution ceased. The water was then removed under reduced pressure with heating, leaving behind a large amount of a white solid. The product was extracted into warm ethanol (3 x 10 ml). The ethanol extracts were combined and the volatile components removed under reduced pressure to give 89 mg of a clear, colorless oil. Thin layer chromatography [mobile phase = acetonitrile/water (95:5); stationary phase = silica] revealed two components, the major one (R_f = 0.70) corresponding to that of the desired product. The other component (R_f = 0.06) was easily separated from the product by column chromatography under the same conditions as used for the thin layer chromatography. A 70 mg (0.20 mmol, 83%) portion of the product was obtained after purification by column chromatography (same conditions as described for the TLC) as a white solid; ion chromatography (Dionex, mobile phase = 200 mM aqueous NaOH, rate = 1.0 ml/min) retention time = 8.00 min;

mp, 107-108°C; ^{1}H-NMR (acetone-d_6): δ3.14-3.78 (m, 7.5H, αH_4, αH_2, βH_3, βH_2, βH_5, αH_5, αH_3, αH_6, βH_6 and βH_4), 4.18-4.47 (m, 5H, α-OH_6, α-OH_2, βH_1, α-OH_4, β-OH_2, β-OH_6 and $H_{1'}$), 5.04 (m, 0.3H, αH_1), 5.50 (d, 0.2H, J= 1.1 Hz, α-OH_1), 5.74 (d, 0.8H, J= 3.0 Hz, β-OH_1), 6.43 (d, 1H, $J_{2'3'}$ = 14.47 Hz, $H_{3'}$), 6.64 (dt, 1H, $J_{2'3'}$ = 14.47 Hz, $J_{1'2'}$ = 5.19 Hz, $H_{2'}$). ^{13}C-NMR (acetone-d_6): δ62.71 (2C, αC_6 and βC_6), 71.15 (βC_4), 71.31 (αC_4), 72.44 (αC_5), 73.49 (αC_2), 74.68 (β$C_{3'}$), 74.74 (βC_2), 76.34 (α$C_{3'}$), 77.00 (βC_5), 79.86 (β$C_{1'}$), 80.79 (α$C_{1'}$), 83.25 (αC_3), 85.69 (βC_3), 93.52 (αC_1), 97.87 (βC_1), 144.82 (β$C_{2'}$), 144.91 (α$C_{2'}$); elemental analysis: (calculated for $C_9H_{14}O_6I$) C, 31.32%; H, 4.09%; I, 36.77%; (found) C, 31.29%; H, 4.38%; I, 36.65%.



e. <u>3-O-(E)-3-[^{123}I]Iodopropen-2-yl-D-glucose (14)</u>

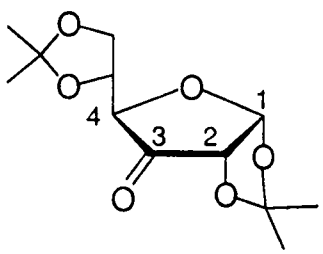
N-Chlorosuccinamide (1.8 mg, 0.013 mmol) was dissolved in methylene chloride (3 ml) and this solution was added to a 10 ml glass vial containing Na^{123}I (7.42 mCi, 1.5 mg, 0.01 mmol) dissolved in 1 ml of water. A light pink solution resulted due to the formation of ICl *in situ*. This mixture was cooled to 0°C and a solution of 3-O-(E)-3-tributylstannylpropen-2-yl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside (5.9 mg, 0.01 mmol) in dichloromethane

(1.0 ml) was added. The radioiodination took place under dark room conditions. Decolorization of the pink solution occured instantly upon addition of the stannylated glucofuranoside. Stirring was continued for 30 minutes, after which time aqueous sodium bisulfite (1 ml, 0.1 M) was added to destroy the remaining ICl. The product was extracted into diethyl ether (2 X 1 ml). The ether extracts were combined, washed with water (1 X 1 ml) and dried over anhydrous sodium sulfate. The ether solution was removed from the sodium sulfate with a pipet and then evaporated with a stream of argon to afford an oil which contained 5.25 mCi (70 % EOB) of the desired compound. Radio-thin layer chromatography [stationary phase = silica; mobile phase = benzene / ethyl acetate (17:3)] revealed one major radiochemical peak with a R_f= 0.66, which corresponded with the R_f value of the unlabeled standard. The oil was then subjected to 1 N sulfuric acid solution (1 ml) and the mixture heated, with stirring, at 70°C for two hours. The resulting solution was cooled to room temperature and passed through a 11.0 cm X 0.7 cm column filled with an ion retardation resin (BioRad AG 11 A8). The product was purified by passing the resulting solution through an alumina Seppak and then through a C-18 Seppak affording 3.26 mCi (63 % EOB) of I-123 labeled 3-O-(E)-3-iodopropen-2-yl-D-glucose. Radio-thin layer chromatography [stationary phase = silica; mobile phase = acetonitrile / water (95:5)] revealed one radiochemical peak with a R_f = 0.65, which corresponded well with the known R_f value of the unlabeled analog.

3. The Synthesis of 3-C-((E)-Iodoethenyl)-D-allose

a. 1,2:5,6-Di-O-Isopropylidene-α-Ribohexafuranose-3-ulose (2)

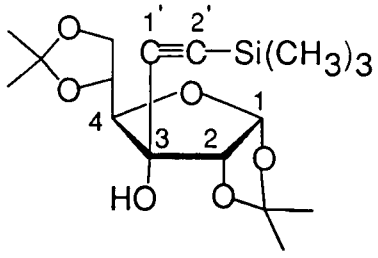
A 250 ml round-bottomed flask was equipped with a magnetic stirring bar and to this flask was added dimethyl sulfoxide (30 ml), anhydrous acetic anhydride (20 ml) and 1,2:5,6-di-O-isoproprylidene-α-D-glucofuranoside (5.00 gram, 19.2 mmol). The flask was then sealed with a ground glass stopper and the contents stirred at room temperature for 24 hours. The reaction mixture remained clear and colorless throughout the reaction. The volatile components were then removed by vacuum distillation and the white solid which remained in the distillation flask was dissolved in a minimal amount of benzene and analyzed by thin layer chromatography (mobile phase = benzene/ethyl acetate (17:3); stationary phase = silica) to reveal one major component with a R_f = 0.42. The desired ketone was purified by column chromatography to give 4.2 g (16.2 mmol, 85.5%) of a white solid. The spectroscopic properties obtained for this compound correlated well with those reported in the literature[54]; mp = 77-78°C; ^{1}H-NMR ($CDCl_3$): δ 1.34 (s, 6H, -C(CH_3)$_2$), 1.44 (s, 6H, -C(CH_3)$_2$), 3.99-4.44 (m, 5H, H_2, H_4 and -OC<u>H</u>C<u>H$_2$</u>O-), 6.14 (d, J= 4.88 Hz, 12H, H_1); ^{13}C-NMR ($CDCl_3$): δ25.32, 25.97, 27.11, 27.46 (4s, -C(<u>C</u>H$_3$)$_2$), 64.58 (-OCH<u>C</u>H$_2$O-), 77.33 (-O<u>C</u>HCHO-), 76.43 (C_4), 78.95 (C_2), 103.20 (C_1), 108.90, 114,27 (2C, -<u>C</u>(CH$_3$)$_2$), 208.10 (C_3).



b. 3-C-(2-Trimethylsilylethynyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (3)

A 100 ml, oven-dried, round-bottomed flask was flushed with argon and equipped with a magnetic stirring bar and a rubber septum with a gas outlet (oil bubbler). To this flask was added, via syringe, tetrahydrofuran (50 ml, anhydrous) followed by trimethylsilylacetylene (0.421 ml, 2.91 mmol). The resulting solution was stirred and cooled to -78°C (dry ice-acetone bath). Over the course of one minute, a 1.6 M solution of *n*-butyllithium in hexane (1.80 ml, 2.90 mmol) was added and the reaction mixture was clear and slowly turned from colorless to pink as the *n*-butyllithium was added. Stirring continued for 10 minutes, after which time 1,2:5,6-di-O-isopropylidene-α-ribohexafuranose-3-ulose (500 mg, 1.94 mmol) in anhydrous tetrahydrofuran (5.0 ml) was added dropwise over a period of 2 minutes via syringe. After stirring for 90 minutes at -78°C, the reaction mixture was allowed to warm to room temperature. Work-up included neutralization of the reaction mixture by the careful addition of aqueous 1.0 N hydrochloric acid and extraction of the crude product into dichloromethane (5 X 50 ml). The organic layers were combined, dried over sodium sulfate and the solvent removed under reduced pressure to give 640 mg of a light red solid. The product was purified by column chromatography [mobile phase = benzene/ethyl acetate (17:3); stationary phase = silica] to give 590 mg (1.65 mmol, 85.4%) of clear, colorless crystals. Thin layer chromatography under the same conditions revealed one spot after purification with a R_f = 0.60, mp= 117-118 °C; ^{1}H-NMR ($CDCl_3$): δ0.20 (s, 9H, -Si($C\underline{H}_3$)$_3$), 1.37, 1.59, 1.45 (3s, 12H, -C-($C\underline{H}_3$)$_2$), 3.03 (s, 1H, -O$\underline{H}$), 3.80-4.50 (m, 4H, H_4 and -O$C\underline{H}C\underline{H}_2$O-), 4.75 (d, J= 3.42 Hz, 1H, H_2), 5.82 (d, J= 3.42 Hz, 1H, H_1);

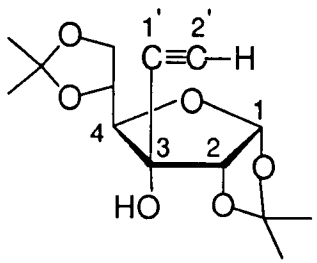
^{13}C-NMR ($CDCl_3$): δ O.33 (-Si($\underline{C}H_3)_3$), 25.16, 26.62, 26.67 (4C, [C-($\underline{C}H_3)_3$]), 66.84 (-OCH$\underline{C}H_2$O-), 74.89 (-O$\underline{C}$HCH$_2$O-), 75.99 (C_3), 81.82 (C_4), 84.26 (C_2), 94.23, ($C_{2'}$), 102.14 ($C_{1'}$), 104.33 (C_1), 109.37, 113.65 (2C, -C(CH3)2); elemental analysis: (calculated) C, 57.30%; H, 7.87% (found) C, 57.73%; H, 7.98%.



c. <u>3-C-Ethynyl-1,2:5,6-Di-O-isopropylidene-α-D-allofuranose (4)</u>

3-C-(2-Trimethylsilylethynyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (400 mg, 1.12 mmol) was placed into a 100 ml round-bottomed flask equipped with a magnetic stirring bar. Methanol (10 ml) was then added followed by 2 ml of an aqueous 3.0 N sodium hydroxide solution. The resulting clear yellow mixture was stirred at room temperature for 10 minutes and was then poured into a 500 ml beaker containing 100 ml of ice water and then neutralized slowly by the dropwise addition of an aqueous 1.0 N hydrochloric acid solution until a pH of 7 was attained. The neutralized solution was then poured into a separatory funnel and the product extracted into dichloromethane (5 X 30 ml). The organic layers were combined, dried over anhydrous sodium sulfate, filtered by gravity filtration, and the solvent evaporated to give a yellow

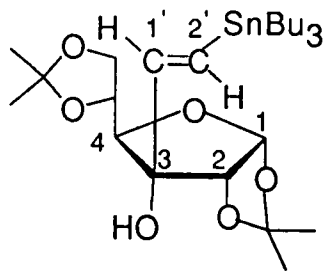
solid. Purification was accomplished by column chromatography (mobile phase = benzene / ethyl acetate (17:3); stationary phase = silica) to give 299 mg (1.05 mmol, 93.7%) of the desired product as a white solid. Thin layer chromatography of the purified compound revealed one component with an R_f = 0.50 using the same conditions given above and the spectroscopic properties were identical to values reported in literature;[60] mp 107-108°C (lit. mp 108-108.5°C) ; ^{1}H-NMR ($CDCl_3$): δ1.38, 1.46, 1.60, (3s, 12H, C-(CH_3)$_2$), 2.17 ($H_{2'}$), 2.96 (s, 1H, -O<u>H</u>), 3.8-4.57 (m, 4H, H_4 and -OC<u>H</u>$_2$C<u>H</u>O-), 4.62 (d, J= 2.93 Hz, 1H, H_2), 5.83 (d, J= 2.93 Hz, 1H, H_1); ^{13}C-NMR ($CDCl_3$): δ24.99, 26.48, 26.59, (4C, [-C-(<u>C</u>H$_3$)], 66.98 (-OCH<u>C</u>H$_2$O-), 74.72 (-O<u>C</u>HCH$_2$O-), 75.73 (C_3), 77.0 ($C_{2'}$), 80.41 ($C_{1'}$), 80.60 (C_4), 83.66 (C_2),103.98 (C_1), 110.26, 113.70 (2C, -<u>C</u>(CH$_3$)$_2$).



d. <u>3-C-[(E)-2-Tri-*n*-butylstannylethenyl]-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (5a)</u>

A 100 ml oven-dried, three-necked, round-bottomed flask was assembled with a magnetic stirring bar, a water cooled condenser, and a gas outlet (oil bubbler). To this flask was added 3-C-ethynyl-1,2:5-6-di-O-

isopropylidene-α-D-allofuranose (517 mg, 1.82 mmol) followed by 4 ml of toluene, 30 mg of AIBN , and tributyltin hydride (0.570 ml, 2.11 mmol). This mixture was quickly heated to 70°C and the temperature maintained for 10 hours, after which time no discoloration had occurred. The reaction mixture was then cooled to room temperature and thin layer chromatography carried out to determine the number of components [mobile phase = benzene / ethyl acetate; stationary phase = silica; detection = acid char; R_f (**5b**) = 0.77, R_f (**5a**) = 0.66, R_f (β) = 0.59]. The product **5a** was purified by column chromatography to give 798 mg (1.36 mmol, 75%) of a white solid, mp 52-53°C; ^{1}H-NMR ($CDCl_3$) δ 0.93 (t, J= 6.0 Hz, 9H, -Sn(CH_2)$_3$C<u>H</u>$_3$), 1.32 (m, 18H, Sn(C<u>H</u>$_2$)$_3$$CH_3$), 1.40, 1.47, 1.67 (3s, 12H, -C(C<u>H</u>$_3$)$_2$), 2.92 (s, 1H, -O<u>H</u>), 3.9-4.2 (m, 4H, H_4 and -OC<u>H</u>C<u>H</u>$_2$O-), 4.33 (d, J= 3.52 Hz, 1H, H_2), 5.78 (d, J= 3.52, 1H, H_1), 5.94 (d, J=19.40 Hz, 1H, <u>H</u>-C=C-Sn), 6.51 (d, J=19.40 Hz, 1H, -C=C-<u>H</u>(Sn)); ^{13}C-NMR ($CDCl_3$) δ11.80 (Sn(CH_2)$_3$<u>C</u>H$_3$), 13.48, 27,12, 29.01 (-Sn(<u>C</u>H$_2$)$_3$$CH_3$), 25.21, 26.46, 26.56 (4C, -C(<u>C</u>H$_3$)$_2$), 67.221 (-OCH<u>C</u>H$_2$O-), 75.56 (-O<u>C</u>HCH$_2$O-), 81.33 (C_3), 82.23 (C_4), 83.96 (C_2), 104.09 (C_1), 109.21, 112.89 (2C, -C(<u>C</u>H$_3$)$_2$), 130.69 (-<u>C</u>=C-Sn), 144.12 (-C=<u>C</u>-Sn); elemental analysis: (calculated for $C_{26}H_{48}SnO_6$) C, 54.26%; H, 8.35% (found) C, 53.98%; H, 8.47%.

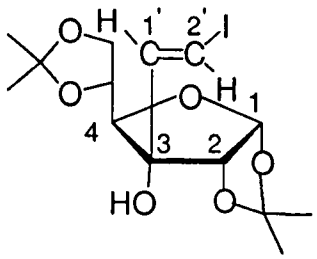


e. 3-C-[(Z)-2-Tri-*n*-butylstannylethenyl]-1,2:5,6-di-O-isopropyl idene-α-D-allofuranose (5b)

An oven-dried, three-necked, round-bottomed flask was equipped with a magnetic stirring bar, a water cooled reflux condenser, a gas outlet (oil bubbler) and then flushed with argon. To this flask was added 3-C-ethynyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (100 mg, 0.35 mmol.) in anhydrous tetrahydrofuran (3 ml). Tributyltin hydride (0.085 ml, 0.32 mmol) and AIBN (10 mg) were then added and the reaction mixture quickly heated to 70°C. Heating continued for 8.0 hours, after which time the reaction was allowed to cool to room temperature. Thin layer chromatography (mobile phase = benzene/ethyl acetate (17:3); stationary phase = silica) was performed which revealed the *cis* isomer as the major component. It also appeared that the *trans* isomer and some unreacted starting material were present. The products were purified by column chromatography (same conditions as the TLC) to yield 115 mg (62.7%) of the *cis* isomer **5b** as a clear colorless oil. Also, 36 mg (19%) of the *trans* isomer **5a** was isolated; ^{1}H-NMR ($CDCl_3$) δ 0.93 (t, J= 6.2 Hz, 9H, $Sn(CH_2)_3C\underline{H}_3$), 1.12-1.4 (4s, 24H, $Sn(C\underline{H}_2)_3CH_3$ and $-C(C\underline{H}_3)_2$), 1.47, 1.60 (2s, 6H, $-C(C\underline{H}_3)_2$), 2.90 (s, 1H, -O<u>H</u>), 3.9-4.2 (m, 4H, H_4 and $-OC\underline{H}C\underline{H}_2O-$), 4.23 (d, J= 3.56 Hz, 1H, H_2) 5.80 (d, J= 3.56, 1H, H_1); ^{13}C-NMR ($CDCl_3$) δ 11.9 ($-Sn(CH_2)\underline{C}H_3$), 13.65, 27.38, 29.27 ($-Sn(\underline{C}H_3)_2CH_3$), 25.21,26.51,26.65 (4C, $-C(\underline{C}H_3)_2$), 66.68 ($-OCHC\underline{H}_2O-$), 75.59 ($-O\underline{C}HCH_2O-$), 81.63 (C_3), 81.90 (C_4), 84.30 (C_2), 103.90 (C_1), 133.83 (-<u>C</u>=C-Sn), 141.77 (-C=<u>C</u>-Sn); elemental analysis: (calculated for $C_{26}H_{48}SnO_6$) C, 54.26%; H, 8.35% (found) C, 54.21%; H, 8.71%.

f. 3-C-[(E)-2-Iodoethenyl]-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (6a)

A 100 ml round-bottomed flask equipped with a magnetic stirring bar and a ground glass stopper was placed in an ice-water bath. To this flask was added 3-C-[(E)-2-tri-n-butylstannylethenyl]-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (203 mg, 0.350 mmol), dichloromethane (30 ml) and iodine (100 mg, 0.394 mmol). This mixture was stirred for 30 minutes at 0°C in the absence of light. The resulting clear, colorless reaction mixture was poured into a separatory funnel and washed with 10% aqueous sodium hydrogen sulfite solution (1X20 ml). The layers were separated, the organic layer dried over anhydrous magnesium sulfate, filtered by gravity filtration, and the solvent removed under reduced pressure to give a colorless syrup which solidified upon standing. Recrystallization from petroleum ether (30-60°C) gave 134 mg (0.32 mmol, 92%) of the desired product as white rosettes; mp 138-139°C; ms: m/z, 412 (M+, 5%); ^{1}H-NMR ($CDCl_3$) δ 1.33, 1.43, 1.60 (3s, 12H, -C(C$\underline{H}_3$)$_3$), 2.93 (s, 1H, -O$\underline{H}$), 3.80-4.10 (m, 4H, H_4 and -OC$\underline{H}$C$\underline{H}_2$O-), 4.54 (d, J= 3.90 Hz, 1H, C2-H), 5.75 (d, J= 3.90 Hz, 1H, H_1), 6.48 (d, J= 14.16 Hz, 1H, $\underline{H}$-C=C-I(H)), 6.74 (d, J=14.16 Hz, 1H, H-C=C-I($\underline{H}$)); ^{13}C-NMR ($CDCl_3$): δ 13.60, 25.21 (4C, -C($\underline{C}H_3$)$_3$), 66.71 (-OCH$\underline{C}H_2$O-), 73.61 (-O$\underline{C}$HCH$_2$O-), 79.84, 80.77 (C_3, C_4), 82.61 (H-C=$\underline{C}$-I(H)), 103.55 (C_1), 109.53, 113.27 (2C, -$\underline{C}$(CH$_3$)$_2$), 141.90 (H-$\underline{C}$=C-I(H)); elemental analysis: (calculated for $C_{14}H_{21}O_6I$) C, 40.78%; H, 5.10% (found) C, 40.93%; H, 4.97%.



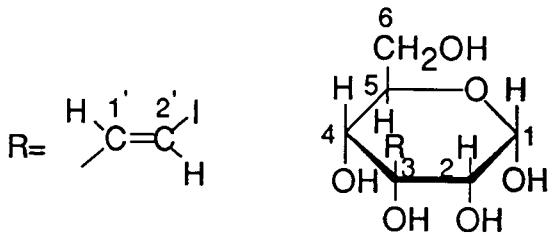
g. 3-C-((Z)-2-Iodoethenyl)-1,2-5,6-di-O-isopropyliden-α-D-allofuranose (6b)

The *cis* vinyl iodide **6b** (101 mg, 0.17 mmol) was prepared exactly as described for the *trans* isomer **6a** from the *trans* vinylstannane **5a**. The product was purified by column chromatography (stationary phase = silica; mobile phase = benzene / ethyl acetate (18.5:1.5); R_f = 0.66, R_f of the *trans* isomer = 0.50.) to give 64 mg (0.15 mmol, 89%) of a clear, colorless oil; ^{1}H-NMR ($CDCl_3$): δ1.35, 1.45, 1.38, 1.62 (4s, 12H, -C(CH_3)$_2$), 2.95-415 (m, 4H, H_4 and -OC<u>H</u>C<u>H$_2$</u>O-), 4.54 (d, J= 3.52 Hz, 1H, H_2), 5.75 (d, J= 3.52 Hz, 1H, H_1), 6.64 (d, J= 8.78 Hz, 1H, H-C=C(<u>H</u>)I), 6.77 (d, J= 8.78 Hz, 1H, H-C=C(<u>H</u>)I); ^{13}C-NMR ($CDCl_3$): δ 13.46, 25.32, 26.67 (4C, -C(CH_3)$_2$), 67.55 (-OCH<u>C</u>H$_2$O-), 73.88 (-O<u>C</u>HCH$_2$O-), 81.08 (C_4), 81.58, 81.98 (C_3 and H-C=<u>C</u>-I), 82.93 (C_2), 104.11 (C_1), 109.83, 113.51 (2C, -C(CH_3)$_2$), 136.10 (H-<u>C</u>=C-I).

h. 3-C-[(E)-2-Iodoethenyl]-D-allose (7)

3-C-[(E)-2-iodoethenyl]-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (100 mg, 0.242 mmol) was treated with 1 N sulfuric acid (10 ml) in a round-bottomed flask equipped with a water-cooled reflux condenser and a magnetic stirring bar. The allofuranose did not dissolve in the acid solution, as round

droplets of oil could be seen in the reaction mixture. The mixture was heated at reflux for 2 hours under a blanket of argon and was cooled to room temperature. The resulting solution was clear and colorless and no oil droplets were observed. The solution was neutralized by the careful addition of solid sodium hydrogen carbonate to the reaction mixture at 0°C until no further gas evolution was observed. The water was then evaporated under reduced pressure with heating (rotary evaporation) to yield a white solid. The product was extracted from the white solid with hot ethanol (3 X 20 ml). The ethanol extracts were combined and the volatile components removed under reduced pressure to give 80 mg of a clear colorless oil. The product was purified by column chromatography [mobile phase = acetonitrile/water (95:5); stationary phase = silica] to give 58 mg (0.18 mmol, 72%) of the desired compound as a white solid. Thin layer chromatography of the purified compound under the same conditions given above showed one component with an R_f = 0.78 and the purity of the isolated material was confirmed by ion chromatography (Dionex, mobile phase = 200 mM aqueous NaOH, flow rate = 1.0 ml/min) ret. time = 9.08 min; ^{1}H-NMR ($CDCl_3$): δ3.2-4.2 (complex multiplet, 11H), 4.81 (d, 0.8H, βC_1-OH), 5.04 (s, 0.2H, αC_1-OH), 6.49 (d, J= 14.46 Hz, $\alpha H_{2'}$) 6.51 ppm (d, J= 14.16 Hz, $\beta H_{2'}$), 6.58 (d, J=14.46 Hz, $\alpha H_{1'}$), 6.65 (d, J= 14.16 Hz, $\beta H_{1'}$); ^{13}C-NMR ($CDCl_3$): δ62.70 (αC_6), 64.88 (βC_6), 68.99 (αC_5), 70.64 (αC_4), 70.82 (βC_4), 71.25 (αC_2), 75.47 (βC_2), 76.37 (βC_5), 79.22 (βC_3), 79.79 (αC_3), 80.22 ($\beta C_{2'}$), 81.90 ($\alpha C_{2'}$), 92.45 (αC_1), 95.38 (βC_1), 147.75 ($\beta C_{1'}$), 150.111 ($\alpha C_{1'}$); MS (CI) m/e (rel intensity) 315 (5%, $M^+ - H_2O$), 297 (25%, $M^+ - 2H_2O$), 279 (10%, $M^+ - 3H_2O$).



i. 3-C-((E)-2-[I-125]Iodoethenyl)-D-allose (13)

A mixture of 3-C-((E)-2-tri-*n*-butylstannyletheny)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (0.01 mmol), iodine (2.5 mg, 0.01 mmol), and sodium [I-125] iodide (7.0 mCi) in a mixture of dichloromethane (3 ml) and water (0.5 ml) was stirred under dark room conditions at 0°C for 30 minutes. The reaction mixture was then poured into 30 ml diethyl ether and the resulting solution washed with 10% aqueous sodium hydrogen sulfate, dried over anhydrous sodium sulfate, and the ether removed by a stream of argon to afford the crude radioiodinated product. This crude material was applied to a column slurried in benzene. Fractions (20 ml) were eluted with benzene/ethyl acetate (18:2). Fractions numbered 10-15 were combined, and the solvent removed by heating under a stream of argon to afford 3.18 mCi (46%) of the desired product. This material was next suspended in 2 ml 1.0 N aqueous sulfuric acid solution and refluxed for 2 hours. The mixture was cooled, neutralized with 1.0 N aqueous sodium hydroxide, and the water removed under a stream of argon. The residue was extracted into absolute ethanol 1 ml and applied to a column of silica gel slurried in ethyl acetate. Fractions (20 ml) were eluted with ethyl acetate/ethanol (4:1). Fractions numbered 4-6 were combined and the solvent

removed by heating under a stream of argon to afford 2.11 mCi (31% overall yield) of the desired compound. The radiochemical purity was confirmed by radio-thin layer chromatography [mobile phase = ethyl acetate / ethanol (4:1); stationary phase = silica]) to show one peak at R_f = 0.70, corresponding to that of the unlabelled standard **7**.

Chapter 3

The Synthesis of Iodinated Butyrothienones for SPECT Evaluation of Dopamine D_2 Receptors in the Brain

A. Introduction

With development of biomedical imaging techniques, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), came the possibility to extend the principles of biochemistry to the study of the chemistry of the brain. This has been accomplished, for the most part, by the design and synthesis of ^{123}I, ^{11}C and ^{18}F labeled radiotracers for the evaluation of the effect of drugs on the brain and for the study of neuroreceptor distributions in healthy and diseased subjects.[75] This technology has been applied to the study of various neurotransmitters and neuroreceptors, including those involved in serotonin, acetylcholine, histamine, opiate, muscarinic and dopamine systems.[76] The focus of this chapter is the design, synthesis and preliminary evaluation of novel radioiodinated agents for SPECT imaging of dopamine D_2 receptors in the brain.

Part B: Rational

1. Project

The study and evaluation of dopamine D_2 receptors is important because of their involvement in a number of neuropsychiatric diseases, such as schizophrenia, Parkinson's disease, Huntington's chorea and depression.[77] The role of dopamine in certain neurological disorders was first suggested in

the 1960's when it was determined that dopamine deficiency was common in patients with Parkinson's disease and that the use of L-DOPA for treatment of this disease alleviated symptoms involving muscular movement.[78] It was also discovered that patients with schizophrenia were helped by the administration of drugs that block D_2 dopamine receptors. A common factor in Parkinson's disease and schizophrenia was suggested when it was found that signs of Parkinson's disease often occurred in psychotic schizophrenic patients after they were treated with large doses of dopamine antagonists. These findings stimulated numerous investigations into the role of dopamine in diseases which involved disturbances in muscular movement and behavior.

The common drugs used to treat schizophrenic patients are dopamine antagonists and are termed "neuroleptics". Common classes of neuroleptics include the phenothiazines, such as reserpin and chlorpromazine, the benzamides, such as sulpiride, and the butyrophenones, including spiperone and haloperidol. The structures of some dopamine D_2 antagonists are given in Figure 3-1. It has been established, by autoradiographic studies of post-mortem tissues using [^{3}H]spiperone and other tritiated neuroleptics, that the distribution of dopamine D_2 receptors in the brain are abnormal and show characteristic patterns for diseases, such as schizophrenia and Parkinson's disease, which are linked to abnormalities in the dopaminergic system.[79] In 1978, Kuhar *et al.*[80] demonstrated the feasibility of using autoradiography to image D_2 receptors in rats and, in 1983, Wagner *et al.*[81] used 3-N-[^{11}C]methylspiperone to successfully image D_2 receptors in the brains of baboons and humans by PET. With the development PET and SPECT, it has become possible to evaluate the density and distribution of dopamine receptors in living patients

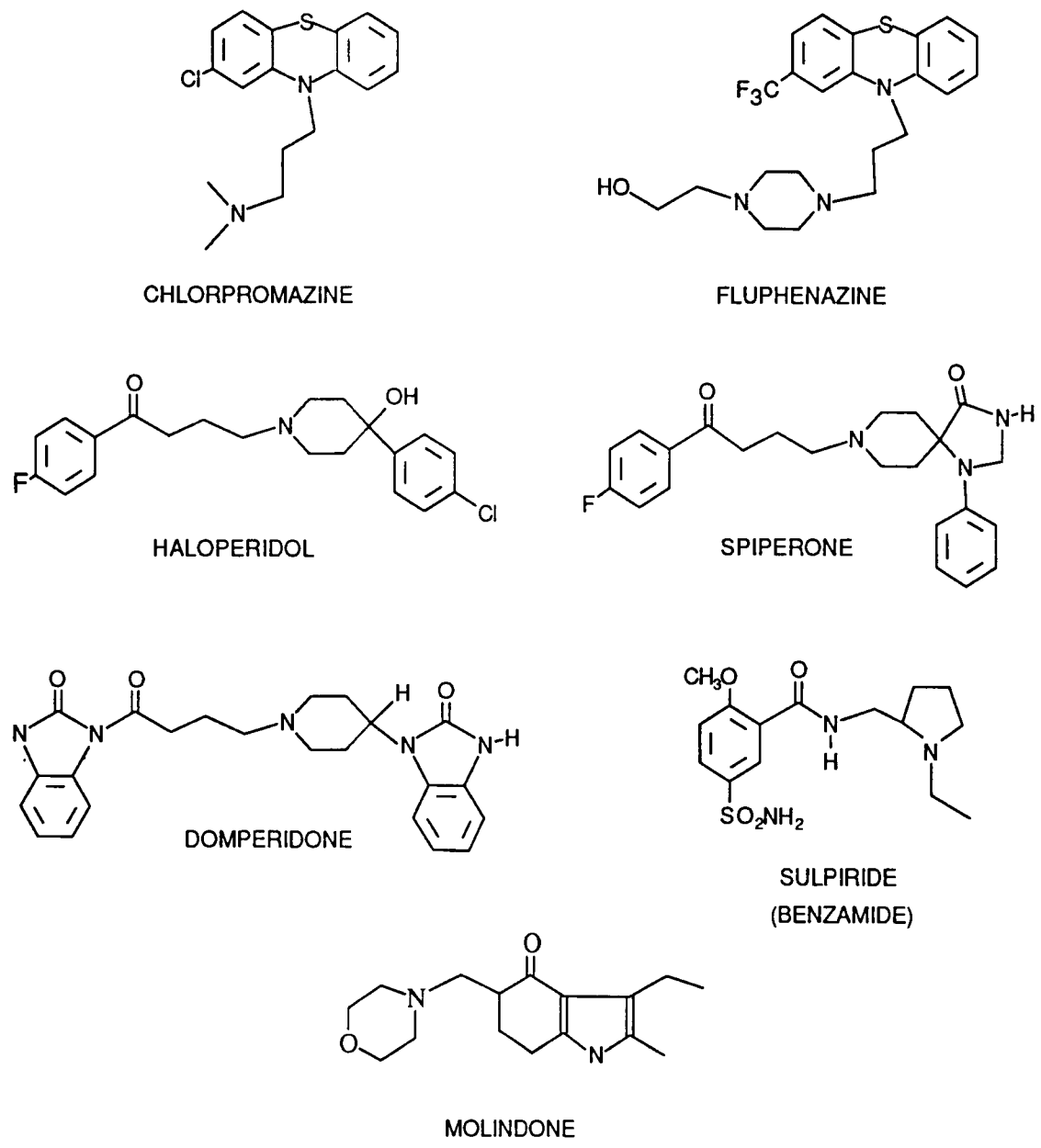


Figure 3-1. The Structures of Some Dopamine D_2 Antagonists

and, in recent years, much research has been directed to develop radiolabeled agents capable of binding to D_2 receptors to aid in the diagnosis and treatment of neurological disorders as well as to gain insight into the biochemistry involved. Various dopamine agonists and antagonists have been radiolabeled with PET isotopes and studied in laboratory animals and in humans to access the state of dopamine D_2 receptors. Some examples are ^{11}C, ^{18}F and ^{75}Br derivatives of spiperone, [^{75}Br]bromosulpiride, N-[^{11}C]methylclorpromazine, [^{18}F]fluoroDOPA, [^{18}F]haloperidol, ^{18}F and ^{13}C benzamide derivatives and [^{11}C] raclopride.[82] In an attempt to create D_2 imaging agents for SPECT, several radioiodinated analogs of these agents have been synthesized, however, most showed less than ideal characteristics as imaging agents when tested in laboratory animals because of significant non-specific binding due to the increase in lipophilicity caused by the iodine atom.[83] Non-specific binding is determined by the ratio of the amount radiotracer in the brain that accumulates in the striatum, a region of high dopamine receptor density, versus the amount taken up in the cerebral cortex and cerebellum, regions of lower dopamine receptor density.[84] A high degree of non-specific binding will complicate the imaging process because of the amount of activity which originates from areas which are not of interest and one must wait until all non-specifically bound agent dissipates from the brain before imaging can begin. The radioiodinated spiperone derivatives tested *in vivo* did not show a high ratio of activity in the striatum versus the other regions of the brain until several hours after injection. Iodinated benzamide derivatives show promise,[85] however, it is not yet clear if the radiolabeled benzamides prepared to date satisfy all of the criteria necessary for use in quantitative imaging studies.[86]

The characterization of dopamine receptors has proven extremely difficult. To date, there are 5 known subtypes of the two major classes of dopamine receptors (D_1 and D_2) and that at least 2 of these subtypes belong to the D_2 class.[87] The D_1 and D_2 receptors both have distinct mechanisms of action. For example, the D_1 receptor binds via the guanine nucleotide regulatory protein to stimulate adenylate cyclase, while the D_2 receptor binds via the adenylate cyclase regulatory protein to inhibit adenylate cyclase synthesis.[88] Stimulation of D_2 receptors decreases the rate of γ-aminobutyric acid (GABA) synthesis in the striatum, cerebellum, frontal cortex and hippocampus. In contrast, stimulation of D_1 receptors either increases GABA synthesis in the striatum or does not alter it.[89] Both receptor classes are distributed differently within the brain and D_2 receptors are three times more abundant than D_1 receptors.[88] The distribution patterns of the D_2 receptors in the brain change in a characteristic way in patients with certain neurological diseases, however, the D_1 receptor density and distribution is usually not disturbed. Different classes of dopamine agonists and antagonists bind to D_2 and D_1 receptors. The D_1 receptors are receptive to antagonists of the thioxanthene and phenothiazine classes and agonists of the benzazepines. The D_2 receptors recognize antagonists of the butyrophenone and benzamide classes but also recognized some of the compounds that bind to D_1 receptors, such as the thioxanthenes. To further complicate the matter, most of the compounds that bind to D_2 receptors will bind to other receptor types as well. For example, spiperone, a butyrophenone, binds to serotonin 5-HT_2 receptors as well as dopamine D_2 receptors.[90] Of course, this lack of selectivity will cause interferences when one is trying perform an imaging experiment on one specific

receptor type, however, as more insight is gained on how each radiolabeled agent interacts with each type of receptor, it may become possible to chemically modify radiotracers to produce more selective imaging agents.

While many dopamine D_2 agonists and antagonists radiolabeled with ^{11}C and ^{18}F have been developed for PET imaging studies, very few such agents show promise as imaging agents for SPECT studies of dopamine receptors. We therefore set out to synthesize radioiodinated dopamine antagonists in hopes of developing radiotracers for the assessment of D_2 receptors in the brain by SPECT. Because spiperone derivatives were well characterized and were selective for D_2 receptors over D_1 receptors, we focused on the development of compounds similar in structure to the butyrophenones.

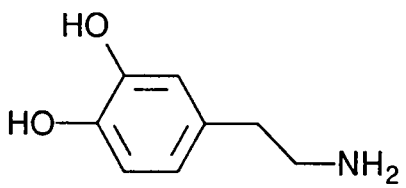
2. Synthesis

When creating a radiolabeled agent to image neuroreceptors in the brain, certain desired properties should be maintained. The radiolabeled agent must have a high affinity for the receptor of interest and should be completely selective for that receptor. The agent must also be able to cross the blood/brain barrier and should not bind non-specifically to proteins and membranes encountered in the body. Finally, the agent should be chemically and metabolically stable and relatively easy to synthesize.

One way to predict whether or not an agent possesses the essential structural and electronic features needed to bind to the receptor of interest is to perform a structure/activity analysis of the compound by comparing it to other compounds that exhibit the desired biological activity. Olsen *et al.* proposed a

hypothetical dopamine receptor model based on structure activity relationships of several classes of compounds that are known to bind to dopamine receptors (see Figure 3-2).[91] They determined, in agreement with previous work,[92] that the two essential pharmacophoric groups are a basic nitrogen and an aromatic ring; these must be separated from each other by a two to four carbon (5-7 Å) chain or framework. These two pharmacophores are found in dopamine, I, and all known dopamine agonists and antagonists and are assumed to bind to a complimentary functionality on the receptor protein. For example, the basic nitrogen could hydrogen bond to an acidic amino acid residue, such as tyrosine, and the aromatic ring might form a π-π stacking interaction with another aromatic ring of a side chain on the protein.

When all dopamine antagonists are taken as a whole, a third pharmacophore can be identified.[91] This consists of a carbonyl group or a second aromatic ring, in close proximity to the first aromatic ring, which could interact with a polar amino acid residue of the receptor. In the butyrophenones, the presence of a carbonyl group next to an aromatic ring is comparable to



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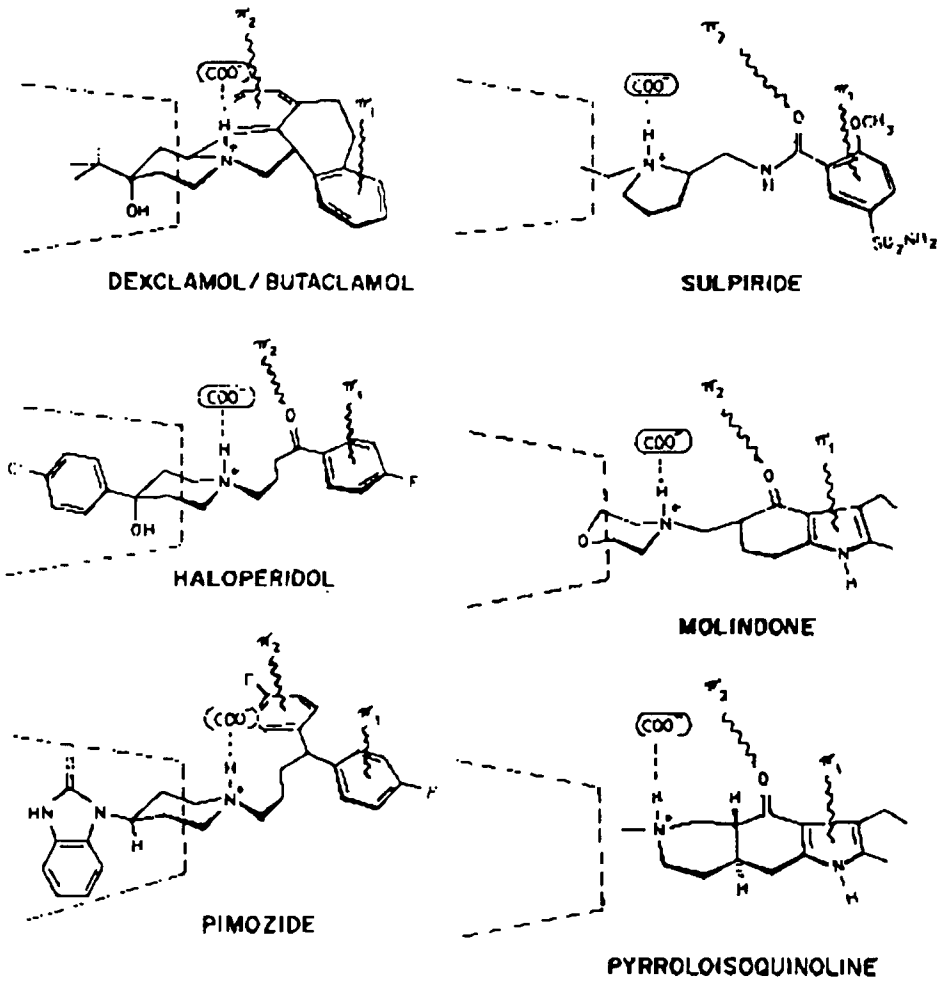


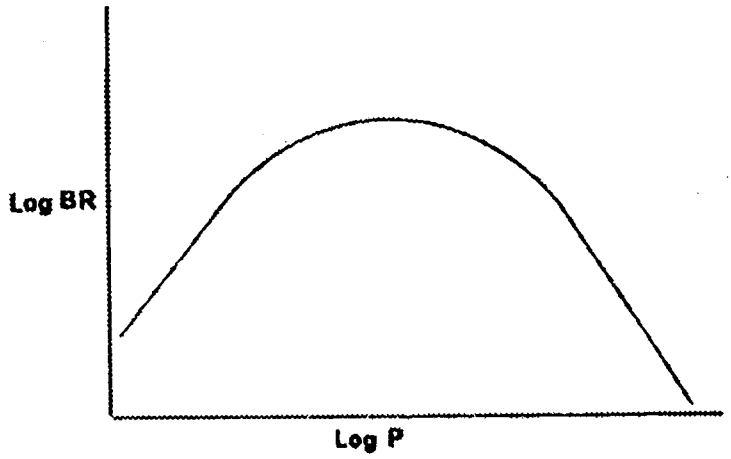
Figure 3-2. Receptor Interactions of Select Neuroleptics. Key: π_1, π_2, aromatic groups and -------, auxillary binding site.

Source: Olsen, G. L., H. C. Cheung, E. Chiang and L. Berger. "Confomationally Defined Pyrroloisoquinoline Antipsychotics" In Carl Kaiser and John W. Kebabian, eds., *Dopamine Receptors*; In M. Joan Comstock, series ed., *ACS Synposium Series*, vol. 224. Washington D. C.: American Chemical Society, 1983.. p. 259.

those of the atypical neurocleptics, molindone and the benzamides. This third binding site would not be occupied by dopamine or other agonists, which might explain the mechanism of antagonism by suggesting that some polar, electronegative group at this third site somehow leads to antagonistic activity.

A fourth binding region was also identified which would require a lipophilic cavity in the receptor. Structure activity relationships suggest that this binding site discriminates D_1 and D_2 dopamine receptor antagonists. This site may bind the bulky alkyl or aryl groups which are found on some of the dopamine antagonists.

The ability of a radiolabeled agent to cross the blood/brain barrier and to exhibit low non-specific binding is determined by its lipophilicity.[93] The common parameter used to represent lipophilicity is the log of the octanol/water partition coefficient (log P value) of a compound. It has been shown that the lipophilicity of a compound can have a major influence on the biological activity of receptor imaging agents and that the relationship is often a parabolic one (see Figure 3-3) as it is in the case of ^{76}Br and ^{125}I labeled spiperone analogs with alkyl groups of various lengths at the amide nitrogen.[94] The parabolic relationship has been described mathematically by equation 3-1 in Figure 3-3, where log BR is the log of the biological response, a and b are regression coefficients and c is a constant.[93] The desired log P range is 2.67 - 4.20 for high initial brain uptake for N-alkylated spiperones and the optimum log P has been reported to be 3.27.[86,94] Log P values higher than 4.20 result in decreased uptake in the brain and a significant amount of non-specific binding. Log P parameters can be calculated experimentally by the shake-flask[95] method or by techniques developed for HPLC or TLC.[96] Computer analysis is also possible



$$\log BR = a \log P - b (\log P)^2 + c \qquad (3\text{-}1)$$

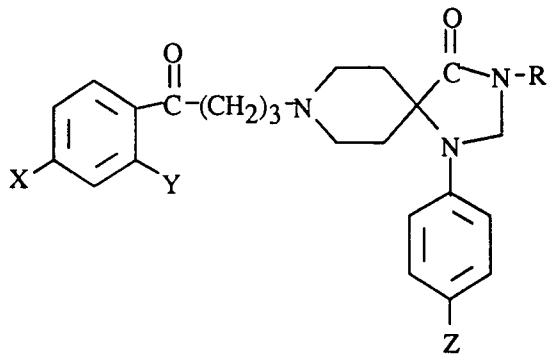
Figure 3-3. Model for a Parabolic Relationship Between Lipophilicity and Biological Response.

Source: Coats, Eugene A. "Quantitative Structure-Activity Relationships" In Richard P. Spencer, M.D. ed.; *Radiopharmaceuticals: Structure-Activity Relationships.* New York: Grune and Stratton, 1981. p. 11.

with programs such as CHEMICALC II or CLOGP,[97] however, these programs have a limited database and can sometimes give inaccurate values of log P.

Increased lipophilicity becomes a problem especially when compounds are labeled with radioiodine. As previously mentioned, radioiodine has been incorporated into various positions of spiperone; however, the resulting compounds were found to exhibit a high degree of non-specific binding. It was postulated that the use of iodine resulted in compounds that exhibited an affinity for the lipophilic areas of proteins and membranes found throughout the brain and body. There was also the possibility of steric interference cause by the presence of the large iodine atom on the radiotracer and it has been shown that the position of radioiodine on spiperone analogs has an effect on the biological activity of the resulting compounds. One study measured the changes in *in vivo* binding of spiperone derivatives in the striatum, cortex and cerebellum when an iodine atom is attached at different positions of the butyrophenone aromatic ring or the phenyl ring of spiperone (see Figure 3-4).[98] The most effective analog was [125I]-2'-iodospiperone, in which 1.3% injected dose per gram was detected in the dopamine receptor containing regions of the brain 30 minutes after injection, as compared to 0.5% for [125]-4-iodospiperone and 0.01% for [125I]-4'-iodospiperone under the same conditions. These agents show a high degree of non-specific binding during the early part of *in vivo* experiments, presumably due to their high lipophilicities, and optimal conditions for imaging are not reached until 3-4 hours post-injection.

The *in vitro* and *in vivo* binding of (*E*)- and (*Z*)-N-(iodoallyl)-spiperone to D_2 receptors has been evaluated by Lever et al.[99] *In vitro*, the *Z* isomer proved to be slightly more potent than the *E* isomer as is shown by their binding



	X	Y	Z	%injected dose/gram (striatum)
2'-iodospiperone	F	*I	H	1.3
4-iodospiperone	F	H	*I	0.5
4'-iodospiperone	*I	H	H	0.01

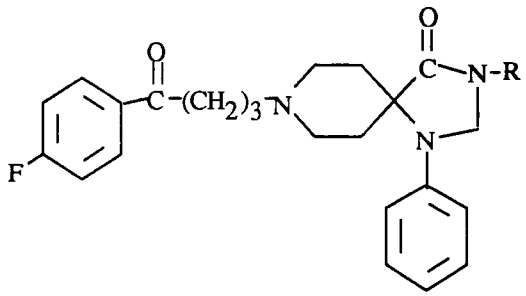
Figure 3-4. Spiperone Analogs with Radioiodine Attached to Different Positions on the Aromatic Rings.

Source: Saji, H., T. Tokui, A. Saiga, Y. Kuge, I. Nakatasuka, M. Okuno, A. Yoshitake, K. Torizuka, and A. Yokoyama "In Vivo Evaluation of Radioiodinated Spiroperidol Derivatives as Radioparmaceutical for Dopamine Receptor Study" In *Journal of Labeled Compounds and Radiopharmaceuticals*, vol. 26, 1986, pp. 298-300.

affinities of 0.35 and 0.72, respectively. The *Z* isomer also has a higher selectivity for D_2 receptors over 5-HT_2 serotonin receptors than the *E* isomer. It was concluded from this study that there is some sensitivity to the substituent on the lactam nitrogen which might be due to increased lipophilicity caused by the addition on the iodopropen-2-yl group. However, *in vivo* studies of the *E* isomer revealed that non-specific binding occurs and, therefore, more studies are needed to determine whether or not these analogs will be good candidates for D_2 imaging.

The effect of N-alkylation on the affinities of spiperone analogs was evaluated by Mach et al. who looked closely at the ratio of binding to D_2 versus serotonin receptors when different alkyl groups are placed on the lactam nitrogen.[100] It was determined that a certain amount of steric bulk was allowed at the lactam nitrogen when spiperone binds to both receptors. However, when groups with increased steric demand are placed on the lactam nitrogen, the ratio of binding to D_2 receptors versus serotonin $5HT_2$ receptors is increased significantly (see Figure 3-5). This is due almost solely to an increase in the K_i value for binding to the 5-HT_2 receptor and the K_i values for binding to D_2 receptors remained unchanged for the most part.[100] The N-benzyl compounds would be candidates for imaging agents of D_2 receptors, however, the log P values of these compounds are out of the useful range because of the increase in lipophilicity caused by the introduction of the benzyl group.

As mentioned earlier, we chose the butyrophenone class of compounds to develop because they have been widely studied and were known to bind selectively to D_2 receptors over D_1 receptors. Janssen Pharmaceuticals Research Laboratory had already developed a series of 1,3,8-triazaspiro[4.5]-

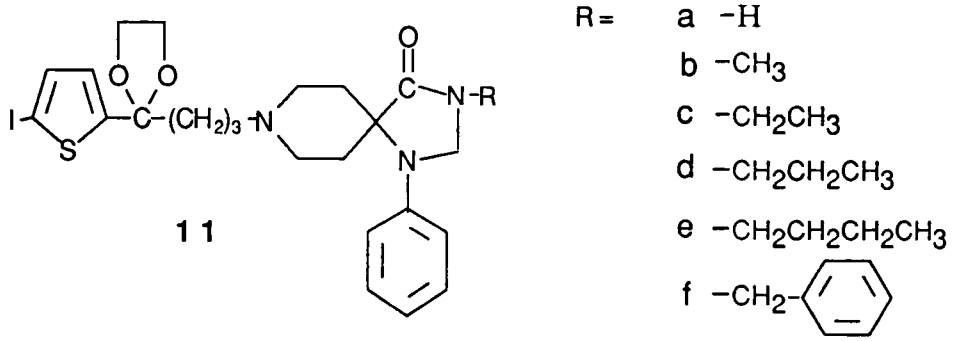


R	Ki (5-HT_2) / Ki (D_2)	Log P
-H	7.8	2.67
-CH_3	4.7	3.23
-CH_2CH_3	5.4	3.69
-$(CH_2)_2CH_3$	3.3	4.22
-$(CH_2)_3CH_3$	11.0	4.72
-CH_2Ph	140	4.68
-CH_2(4'-iodo)Ph	420	5.80
-CH_2(4'-CH_3)Ph	550	5.24

Figure 3-5. Log P Values and Ratio of D_2 and 5-HT_2 Receptor Binding Affinities of Selected N-Alkylated Spiperone Derivatives.

Source: Mach, Robert H., Joseph R. Jackson, Robert R. Luedtke, Kathryn J. Ivins, Perry B. Molinoff, and Richard L. Ehrenkaufer. "Effect of N-Alkylation on the Affinities of Analogues of Spiperone for Dopamine D2 and Serotonin 5-HT2 Receptors". Philip S. Portoghese, ed., *Journal of Medicinal Chemistry*, vol. 35. Washington D. C.: American Chemical Society, 1992. pp. 423- 430.

decanes, including spiperone and a butyrothienone analog of spiperone in which the 4-phenyl-4-oxo-butyl group of spiperone had been replaced by a 4-(2-thienyl)-4-oxo-butyl group.[101] The butyrothienone possessed the necessary pharmacophores for dopamine D_2 antagonistic activity and the thiophene ring would allow easy incorporation of an iodine atom to provide a radiolabeled agent that is metabolically stable. It had been previously reported that 5-radioiodothiophene derivatives were easily prepared and were resistant to chemical and *in vivo* deiodination.[102] It had also been shown that the placement of different alkyl groups on the lactam nitrogen was an effective method to manipulate lipophilicity without changing the *in vitro* binding affinities for D_2 receptors. Therefore, it was decided that the N-methyl, -ethyl, -propyl and -butyl derivatives of the butyrothienone **11** would be synthesized to provide a series of compounds with a range of log P values (see Figure 3-6). Also, the N-benzyl derivative was to be prepared in the hopes of obtaining a radioiodinated



11

Figure 3-6. Iodinated Butyrothienone Analogs of Spiperone

agent which exhibits selectivity for D_2 over serotonin receptors. The uniodinated analogs were also prepared as potential antipsychotic agents.

In an attempt to obtain high yields of desired compounds, two different synthetic routes were explored. The first route correlates with the common method used to prepare spiperone and its derivatives and is outlined in Scheme I (Figure 3-7). The amide precursors **7a** and **9a** were prepared by the alkylation of 1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, **5a**, with either 4-chloro-1,1-(ethylenedioxy)-2'-butyrothienone, **2**, or its iodinated derivative **4**. Alkylation of the amide nitrogen of the resulting compounds was accomplished using the appropriate alkyl iodide and was followed by acid hydrolysis of the ethylenedioxy protecting group to afford the desired N-alkyl analogs in an overall yield of 30-40%. Alternatively, the desired compounds were synthesized as outlined in Scheme II (Figure 3-8). Alkylation of **6a** with the appropriate alkyl iodide followed by removal of the BOC group with acid afforded the N-alkylated derivatives of 4-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, **5b-f**. Coupling of **5b-f** with **2** or **4** followed by acid hydrolysis of the ethylenedioxy protecting group afforded the desired butyrothienones with no improvement in overall yield. Because radioiodine must be incorporated in the last steps of the synthesis, a third synthetic scheme was devised and is shown in Figure 3-9. The formation of stannylated thiophenes had been well developed, and radioiododestannylation of thiophenes derivatives had been accomplished with good results.

The utility of these butyrothienones as D_2 imaging agents was explored by *in vitro* binding studies, lipophilicity (log P) determinations, and structure/activity analysis.

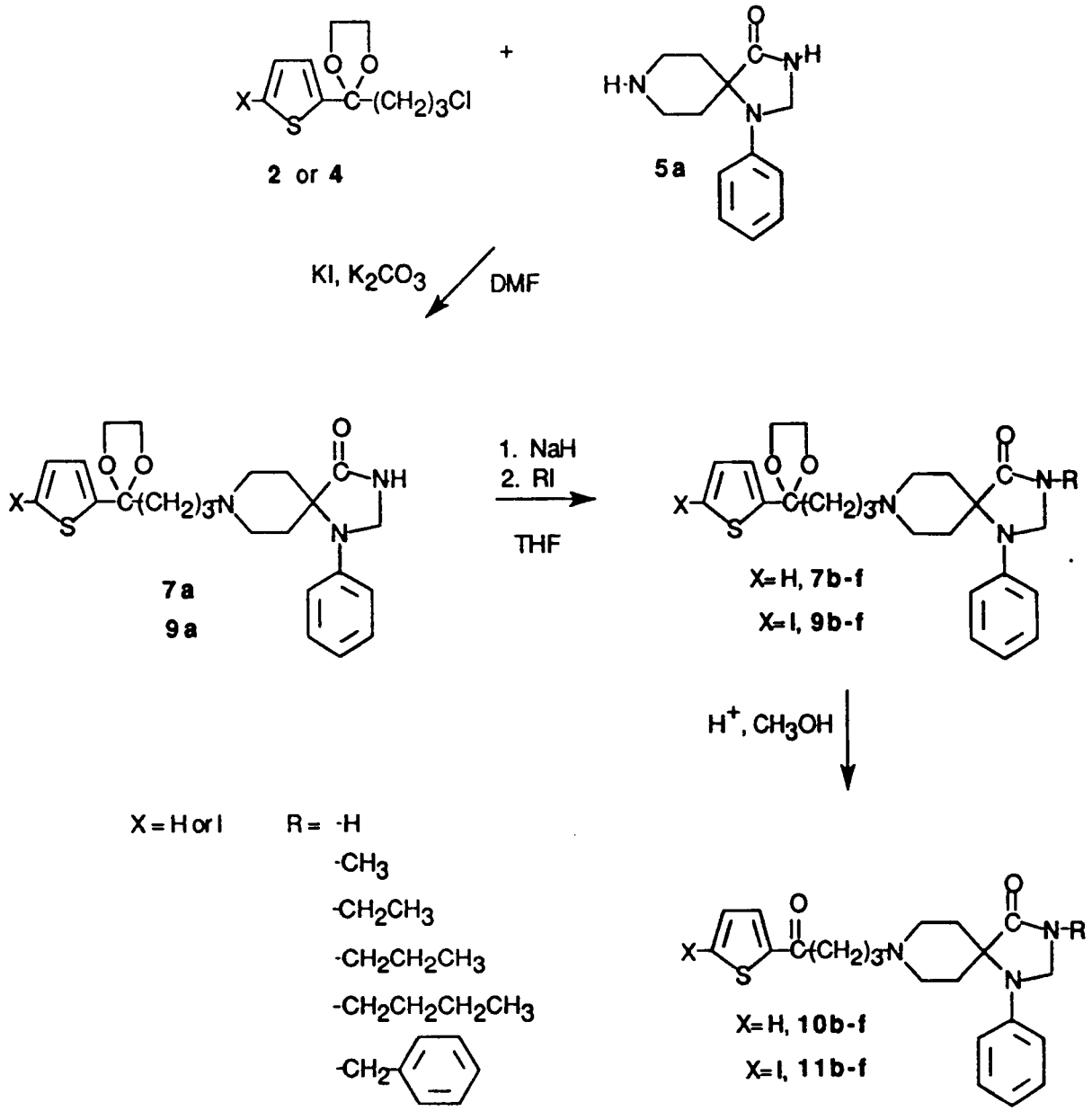


Figure 3-7. Scheme I: Synthesis of Butyrothienones

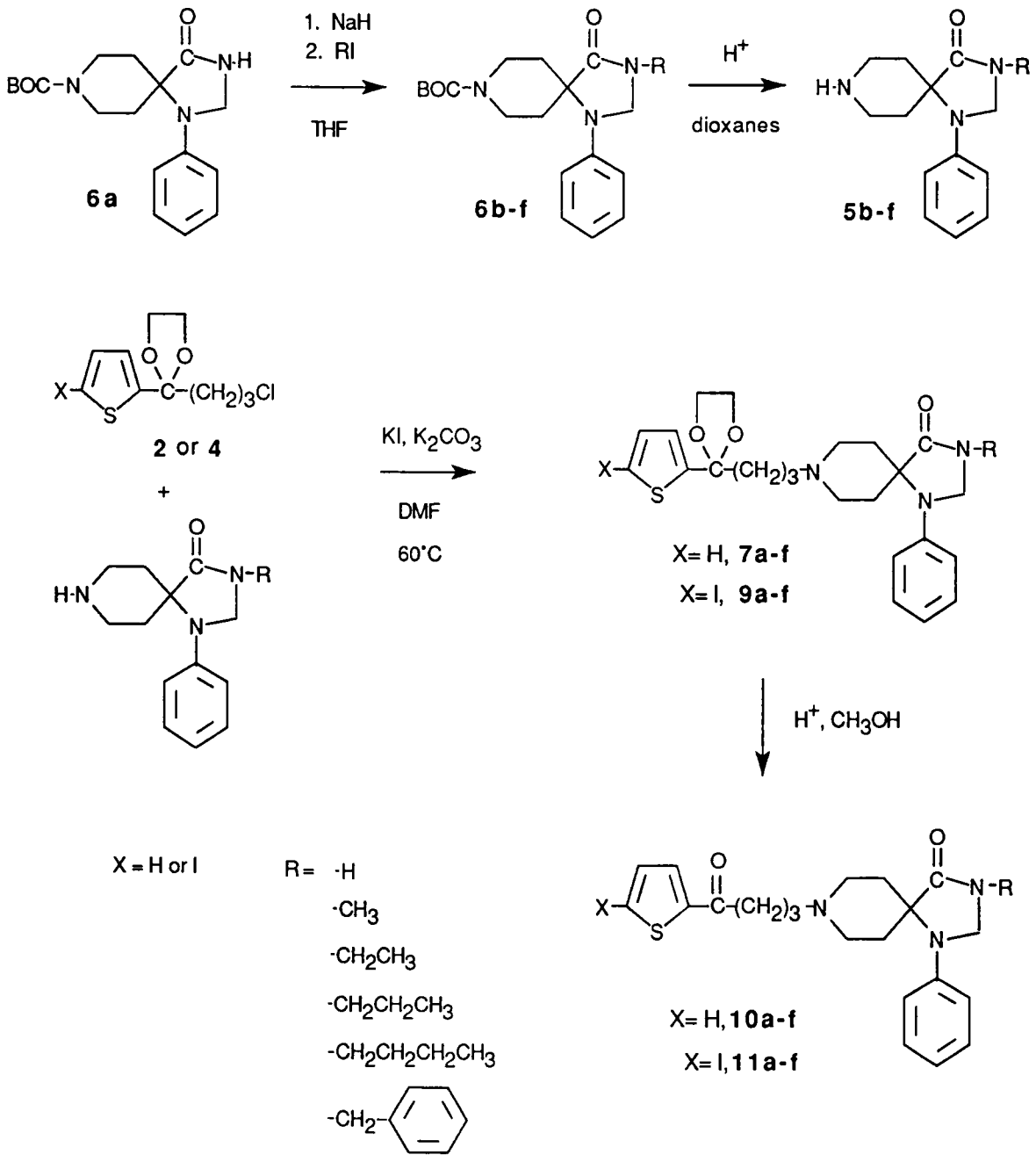


Figure 3-8. Scheme II: Synthesis of Butyrothienones

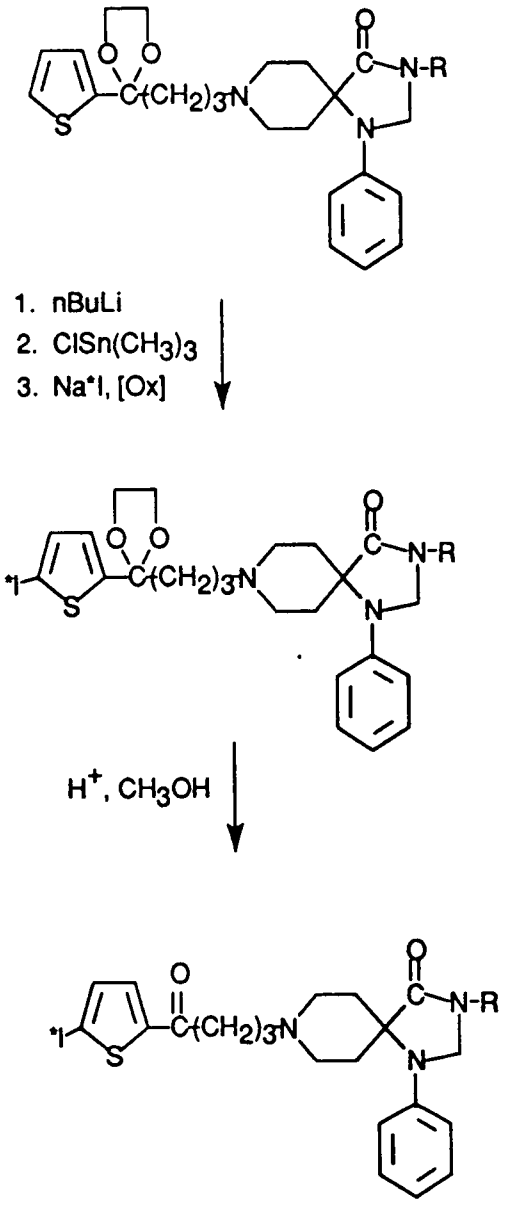


Figure 3-9. Synthesis of Radioiodinated Butyrothienones

C. Results and Discussion

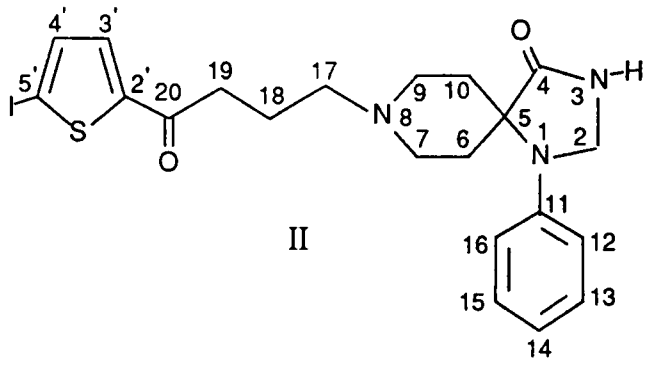
1. General

The goal of this study was to synthesize novel radioiodinated agents for SPECT analysis of dopamine D_2 receptors in the brain. Also, preliminary work was done to establish the biological characteristics of these agents. The synthetic approach chosen involved alkylation of the amide nitrogen of the butyrothienones with groups possessing increased lipophilicity and steric bulk. It had been previously shown that one can manipulate transport across the blood/brain barrier and reduce non-specific binding by preparing compounds of optimum lipophilicities.[83,93] It had also been shown that the presence of a benzyl group on the amide nitrogen of spiperone increased the ratio of radiotracer binding to dopamine D_2 receptors versus serotonin 5-HT_2 receptors.[100]

The two synthetic pathways utilized in the formation of the unlabeled butyrothienones are outlined in Figure 3-7 and Figure 3-8. These pathways are analogous to those published for the synthesis of spiperone and its derivatives.[100,101] However, because of the sensitivity of the thiophene group to strong acids, the formation of HCl salts could not be used as a method of purification. Also, the butyrothienones degraded to a large extent when silica was used for column chromatography, regardless of other chromatographic conditions. Column chromatography using neutral alumina was more successful, however, degradation was observed on alumina TLC plates in the presence of strong light or after irradiation with the UV lamp. Attempted sublimation resulted in significant degradation and recrystallization was inadequate for the removal of the O-alkylated material or parent amide from the

desired compounds. In short, the synthesis of the desired iodinated butyrothienones was straightforward, however, subsequent purification proved to be quite a challenge.

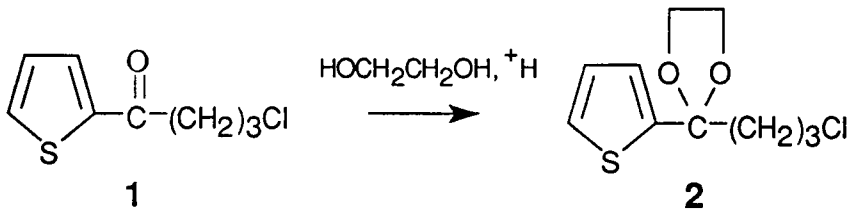
In order to simplify the reporting of spectral assignments, a numbering system for the basic structure of the butyrothienones was defined and is shown in structure II. Structures of this type appear throughout the text and the experimental section to aid the reader in interpretation of the spectral data.



II

2. Synthesis of the Precursors

Treatment of commercially available 4-chloro-1-(2-thienyl)-1-oxo-butane, **1,** with ethylene glycol and p-toluenesulfonic acid in benzene afforded 4-chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane, **2**, in 65% yield (equation 3-2). Removal of water using a Dean-Stark trap facilitated the reaction. The product was easily purified by column chromatography to give a clear, colorless oil which solidified when cooled to 0°C. The product was identified by ^{1}H and ^{13}C



1 2 (3-2)

NMR. The characteristic feature in the ^{1}H NMR spectrum was the presence of a multiplet between 3.8 ppm and 4.1 ppm corresponding to 4 hydrogen atoms of the ketal. In the ^{13}C NMR spectrum, the peak corresponding to the carbonyl carbon at 191.74 ppm had vanished and two new peaks were observed. The first, at 65.1 ppm, corresponded to the two carbons of the ethylenedioxy group and the second at 108.30 ppm, corresponded the carbon atom that was formerly the carbonyl carbon.

The next step involved the iodination of the thiophene ring to provide 4-chloro-1,1-(ethylenedioxy)-1-[2-(5-iodothienyl)]butane, **4**. Iododemetallation was determined to be the best route for the formation of the desired compound and thallation, mercuration and stannylation were utilized in an attempt to obtain a precursor suitable for iododemetallation reactions. Both the mercuration and thallation took place under reaction conditions where water and acid were present in appreciable amounts and hydrolysis of the ketal occurred. Several products were formed during the thallation reaction which might have been the result of di- and tri- thallation. Compound **4** was finally obtained via the formation of 4-chloro-1,1-(ethylenedioxy)-1-[2-(5-trimethylstannyl-thienyl)]butane followed by iododestannylation using iodine

(see Figure 3-10). A one pot procedure was developed which circumvented the isolation of the stannylated intermediate in order to limit the chance of exposure to highly toxic trimethyltin chloride and other tin-containing compounds. The trimethyltin derivative of **2** was synthesized by a previously reported method for addition of a trialkyltin group to a thiophene ring[103]. Treatment of **2** with *n*-butyllithium in anhydrous diethyl ether at 0°C followed by the addition of trimethyltin chloride. After the trimethyltin derivative formed *in situ*, water was added followed by 1.1 equivalents of iodine. The iododestannylation reaction was followed by thin layer chromatography and was determined to be complete within a matter of minutes [stationary phase = silica; mobile phase = dichloromethane/hexane (1:1); R_f(2) = 0.63, R_f(trimethyltin adduct) = 0.79 (seemed to decompose, probably protodestannylation), R_f(4) = 0.72]. The iodinated product **4** was easily separated from the uniodinated material by liquid chromatography and was obtained in an overall yield of 80% as a clear, colorless oil. The product was identified by the presence of an AB pattern in the aromatic region of the ^{1}H NMR spectrum (see Figure 3-11). The resonances were centered at 6.69 ppm and 7.14 ppm and presence of iodine at the 5 position of the thiophene ring was confirmed by evaluation of the coupling constant between the aromatic protons ($J_{1'2'}$ = 3.74 Hz). The typical coupling constants between protons in various positions of a thiophene ring are presented at the bottom of Figure 3-12 and the value of 3.74 Hz is typical for coupling between protons at the 3 and 4 positions of thiophene.[104] To further support the assignments, the differences in chemical shift ($\Delta\delta$, Figure 3-12) on the protons of thiophene caused by the addition of iodine onto the ring at $C_{5'}$ was estimated and these values added to

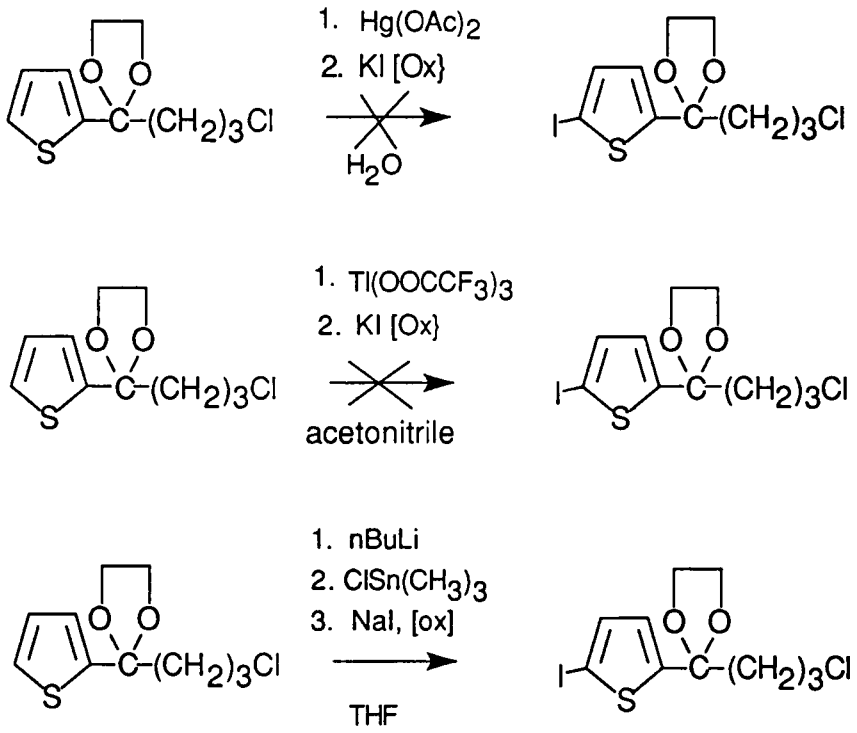


Figure 3-10. Iodination of 4-Chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane

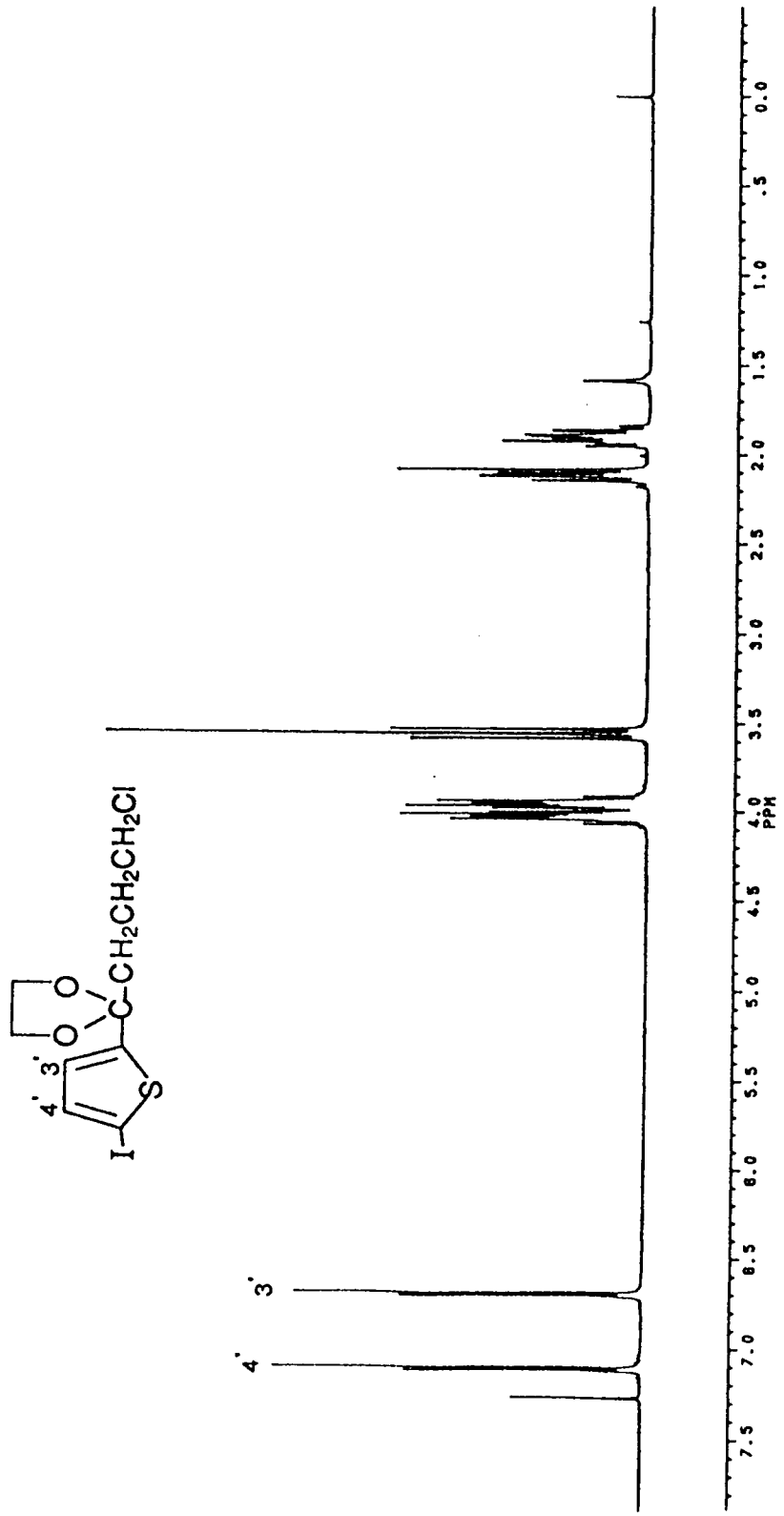
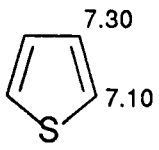
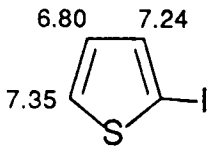


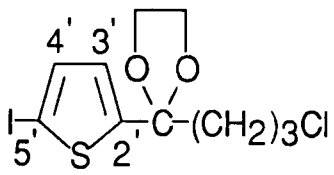
Figure 3-11. 250 MHz Proton NMR Spectrum of 4-Chloro-1,1-(ethylendioxy)-1-[2-(5-iodothienyl)]butane in $CDCl_3$ plus 0.3% TMS.



A



B



4

	H5'	H4'	H3'
$\Delta\delta$	+.25	−.06	−.50

Typical coupling constants for protons on thiophene:

$J_{2'3'}$ = 4.9-6.2 Hz

$J_{3'4'}$ = 3.4-5.0 Hz

$J_{2'4'}$ = 1.2-1.7 Hz

$J_{2'5'}$ = 3.2-3.7 Hz

Figure 3-12. Expected Chemical Shifts and Coupling Constants for Iodinated Thiophenes.

Source: Silverstein, R. M., G. C. Bassler and Terence C. Morrill. "Spectroscopic Identification of Organic Compounds", 5th edition. New York: John Wiley & Sons, 1991. pp. 219 and 221.

the chemical shifts of the protons of the thiophene ring of **2**. For example, if iodination occurred at $C_{5'}$ of the thiophene ring of **2**, the expected chemical shift at $H_{4'}$ would be -0.06 ppm and the chemical shift of $H_{4'}$ of **4** should be approximately 7.23 ppm - 0.06 ppm = 7.17 ppm. This is in good agreement with the value assigned (7.14 ppm). Likewise, the chemical shift assigned to $H_{3'}$ of **4** is 6.69 ppm and is in agreement with the estimated value of 6.55 ppm (see Figure 3-12).

The ^{13}C NMR of **4** has been reproduced in Figure 3-13 and reveals the movement of $C_{5'}$ from 124.61 ppm to 73.25 ppm, which was expected when compared to model compounds. For example, the chemical shift of C_2 for 2-iodothiophene is 73.6 ppm.[105] The other carbon assignments were made in a similar manner using thiophene and 2-iodothiophene and estimating the chemical shifts of the carbon atoms of **4** based on those assigned for **2**.

The final precursor needed was 1-phenyl-8-(*tert*-butyloxycarbonyl)-1,3,8-triazaspiro[4.5]dec an-4-one, **6a**, which was prepared as described[106] by the reaction of **5a** with di-*tert*-butyl dicarbonate (BOC-ON) in dichloromethane in the presence of triethylamine (equation 3-3). Product **6a** was purified by recrystallization from a mixture of dichloromethane and ethyl acetate (1:1) to give a white solid in 91% yield. The product was identified by ^{1}H and ^{13}C NMR spectroscopy. The spectral data obtained were identical to that reported in literature.[106]

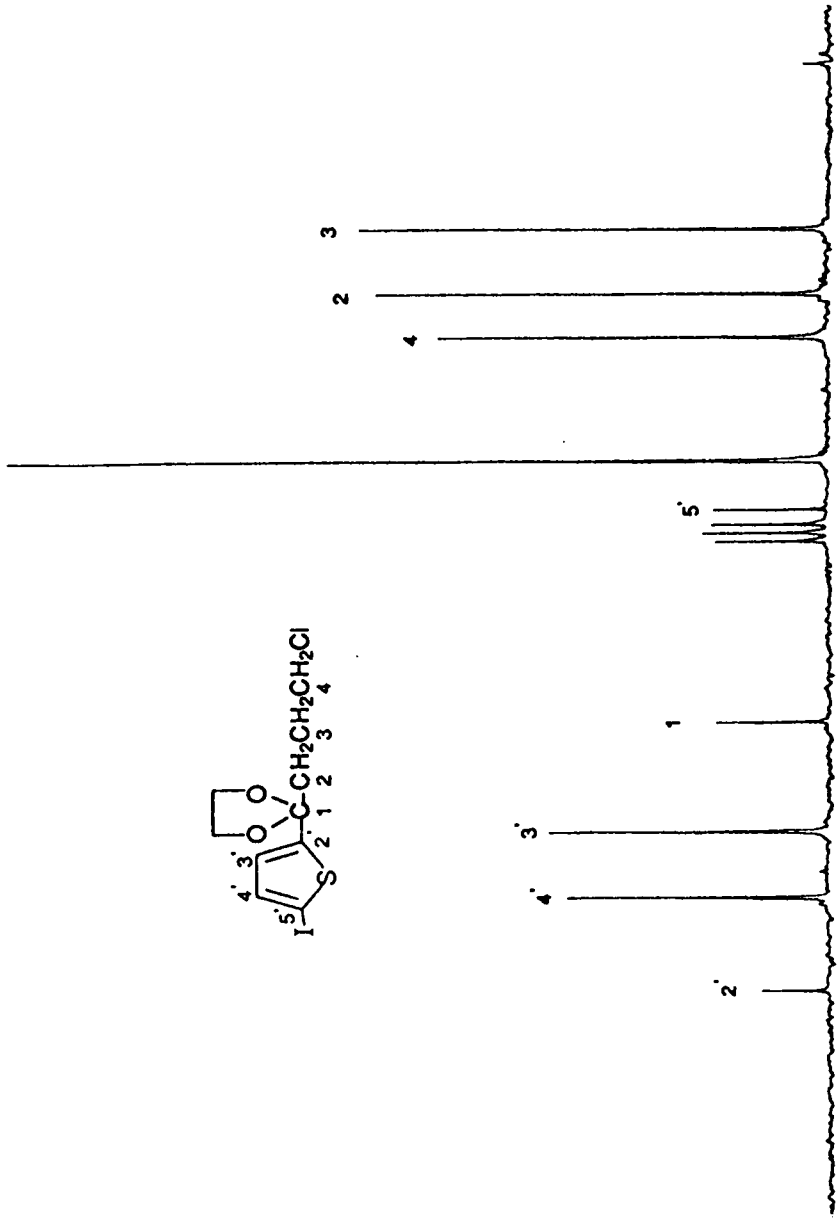
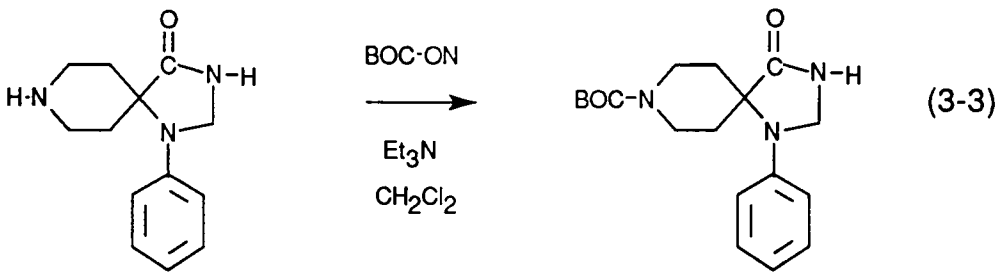


Figure 3-13.. 90 MHz Carbon-13 NMR Spectrum of 4-Chloro-1,1-(ethylenedioxy)-1-[2-(5-iodothienyl)]butane in $CDCl_3$ plus 1.0% TMS.

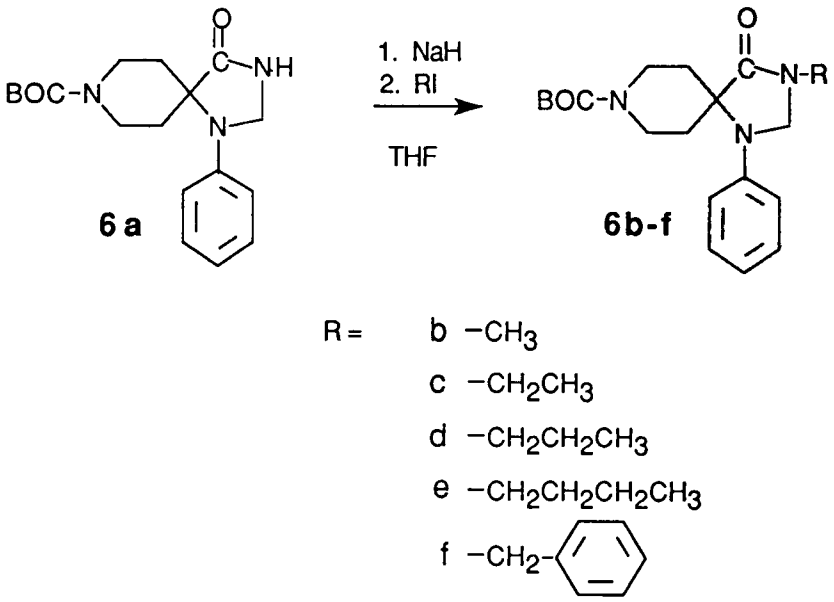


5 a **6a** (3-3)

3. Synthetic Methods for the Formation of Iodinated Butyrothienones

a. Method A:

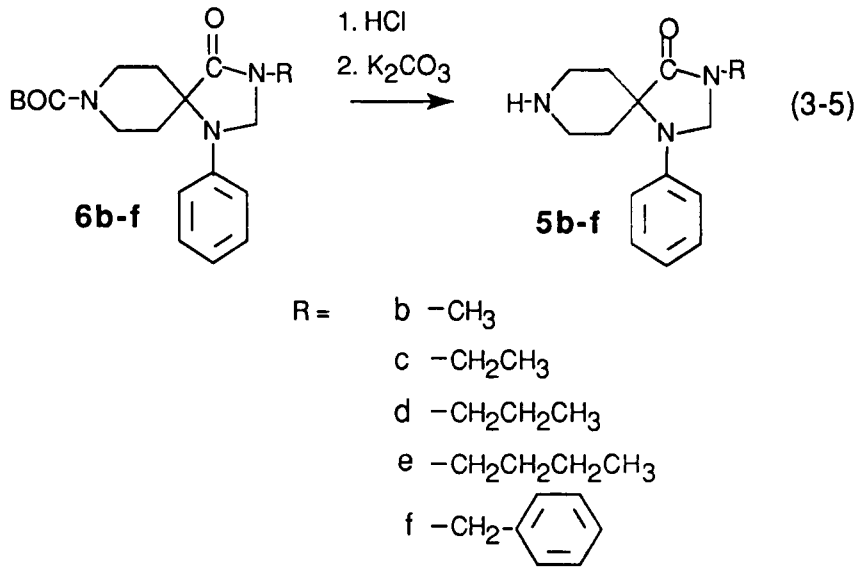
The first route employed in the synthesis of the butyrothienones required that the alkylations be carried out before the coupling reaction takes place. Treatment of **6a** with sodium hydride at room temperature in anhydrous tetrahydrofuran followed by the appropriate alkyl iodide provided the N-alkylated derivatives **6b-f** in high yield (equation 3-4). These compounds were easily purified by column chromatography and were characterized by ^{1}H-NMR and ^{13}C-NMR spectroscopy. All NMR assignments were based on model compounds and known chemical shift values of different N-alkylated amides. A characteristic shift was noted for the amide carbonyl carbon (C_4) from 178 ppm in **6a** to 174 ppm in **6b-f** as expected. Only one amide conformation was present as determined by the presence of only one peak for each carbon in the carbon NMR of **6b-f**. ^{1}H NMR spectroscopy confirmed this observation. No O-alkylated materials were detected unless the alkylations took place at



R = b $-CH_3$
c $-CH_2CH_3$
d $-CH_2CH_2CH_3$
e $-CH_2CH_2CH_2CH_3$
f $-CH_2-C_6H_5$

temperatures above 50°C. For example, O-alkylated materials were detected in the formation of the N-butyl derivative **6e** by TLC and by the presence of a triplet at approx. 3.8 ppm (8%) in the ^1H NMR spectrum. This material was easily separated from **6e** by column chromatography and was not characterized further.

Removal of the BOC group was accomplished by treatment of **6b-f** with an anhydrous solution of hydrochloric acid in dioxane (4.0 M) at room temperature (equation 3-5). All volatile components were then removed under vacuum and the remaining solid was mixed with an aqueous, saturated sodium carbonate solution and the product extracted into dichloromethane. The dichloromethane extracts were combined, dried over anhydrous sodium sulfate and the solvent evaporated to provide **5b-f**. Compounds **5b-f** were not characterized but were used directly in the next synthetic step. The coupling of



2 or **4** with **5b-f** to give the corresponding N-alkylated butyrothienone derivatives **7b-f** and **9b-f**, respectively, was accomplished by a variation of the reported method used to produce the analogous butyrophenone derivatives.[106] Compounds **2** or **4** were dissolved in anhydrous dimethyformamide and were treated with potassium iodide, sodium or potassium carbonate and **5a-f**. In general, the reaction mixture was heated to 60°C for 18 hours under an argon atmosphere. Next, the reaction mixture was diluted with distilled water and the product extracted into dichloromethane. Usually, thin layer chromatography [stationary phase = alumina (neutral); mobile phase = dichloromethane/ethanol (97:3)] of the dichloromethane extracts revealed three components; the first at R_f= 0, which was determined to be unreacted amine and degradation products, a second with an R_f value between 0.5 and 0.7, corresponding to the desired products, and a third that ran with the solvent front and was determined to be unreacted **2** or **4**. However, if the reaction mixture was not kept anhydrous or if

the temperature rose above 80-90°C, as many as seven components were detected by thin layer chromatography, and the desired products were difficult to isolate. Compounds **7b-f** and **9b-f** were obtained in moderate yields (60-75%) after purification by column chromatography [stationary phase = alumina, mobile phase - dichloromethane / ethanol (50:1), UV detection]. Purification could not be accomplished on silica and it appeared that degradation occurred during the separation.

Compounds **7b-f** and **9b-f** were characterized by ^{1}H and ^{13}C NMR spectroscopy and the chemical shifts were easily assigned based on those for the precursors **6b-f** and **2** or **4**. The ^{1}H and ^{13}C NMR spectrum for 3-ethyl-8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, **9c**, have been reproduced in Figure 3-14 and Figure 3-15, respectively. Note that protons $H_{3'}$ and $H_{4'}$ of the thiophene ring are clearly visible in the ^{1}H spectrum as are the proton resonances of the N-ethyl group. In the ^{13}C NMR spectrum, the amide carbonyl carbon (C_4) resonance is at 174.21 ppm and the carbon attached to the iodine atom resonates at 73.01 ppm. Several of these compounds were analyzed by IR spectroscopy. The IR spectrum of 3-butyl-8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **7e**, is shown in Figure 3-16 and clearly displays the amide C=O stretch at 1702 cm^{-1}. Elemental analysis (C, H, N, S, and I, if applicable) was obtained for all compounds and the values obtained were within 0.3% of the theoretical values.

The final step of the synthesis involved the hydrolysis of the ethylenedioxy protecting groups of **7b-f** and **9b-f** with methanolic HCl to provide the corresponding ketones **10b-f** and **11b-f**. As before, the chemical

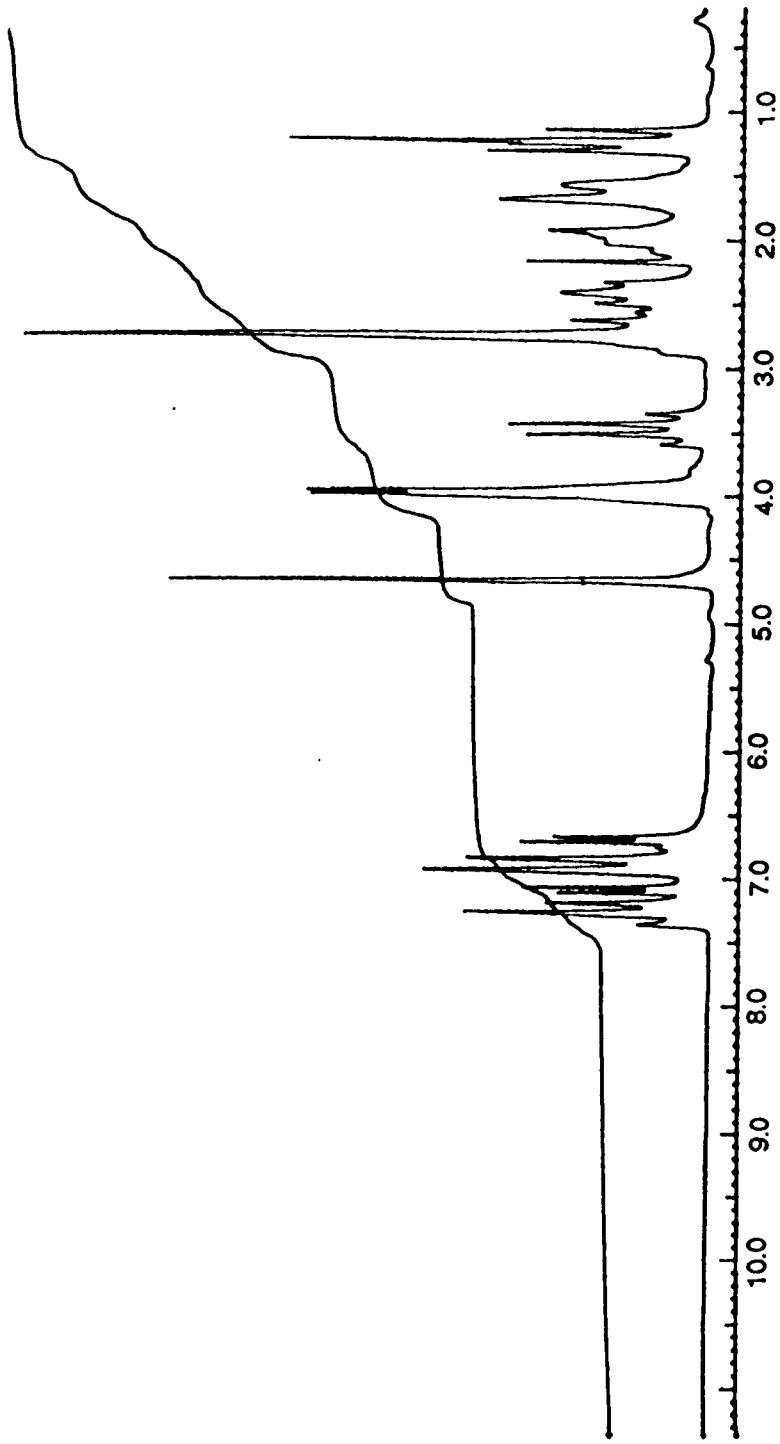


Figure 3-14. 90 MHz Proton Spectrum of 3-Ethyl-8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8,-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 1.0% TMS.

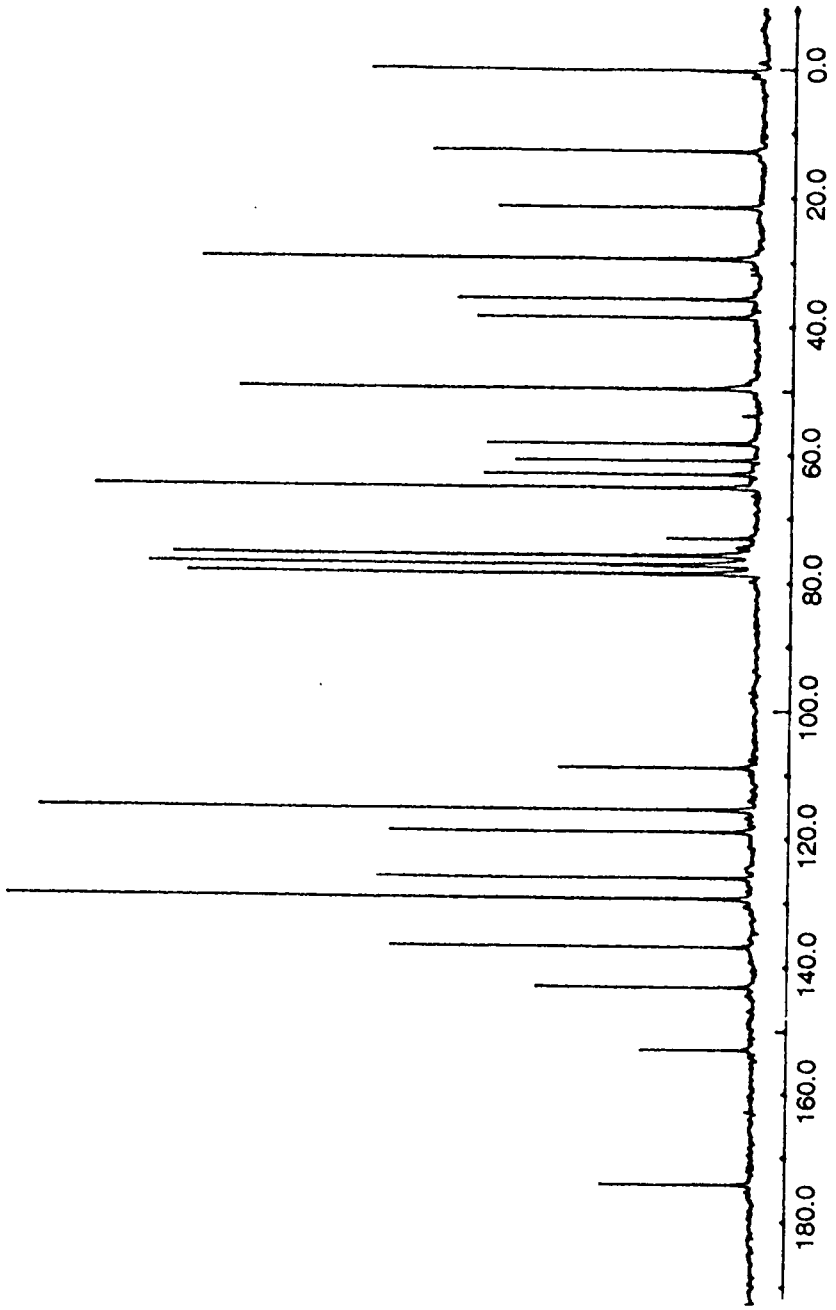


Figure 3-15. 90 MHz Carbon-13 Spectrum of 3-Ethyl-8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 1.0% TMS.

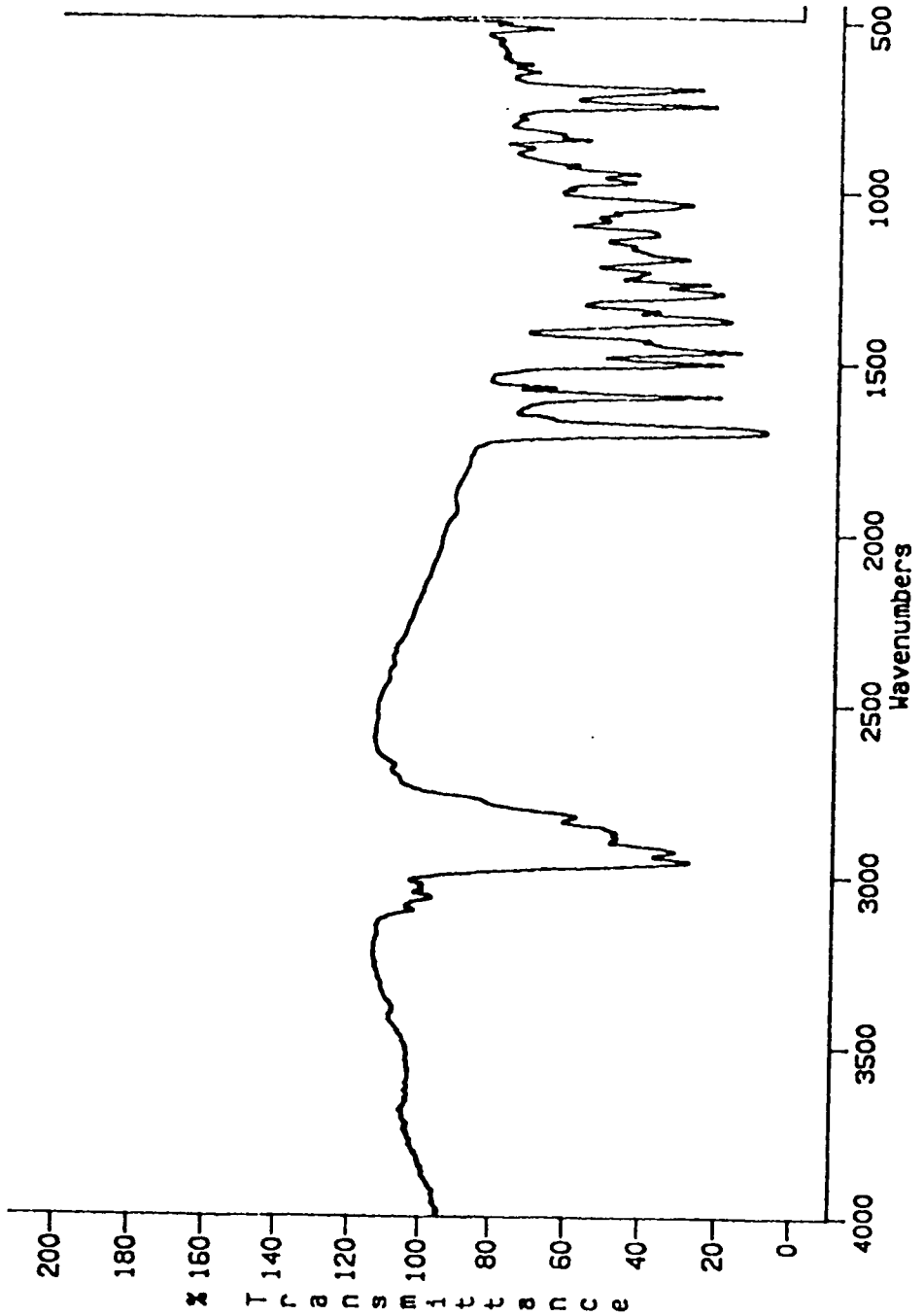


Figure 3-16. FTIR Spectrum of 3-Butyl-8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one on a Sodium Chloride Plate.

shifts in the ^{1}H and ^{13}C spectra were easily assigned based of the chemical shifts of the precursors, specifically **7b-e**, **9b-e**, **1** and **2**. The ^{13}C NMR spectra revealed the most information and the spectra for the uniodinated N-ethyl derivative **10c** and the iodinated N-ethyl and N-benzyl derivatives **11c** and **11f** are shown in Figures 3-17, 3-18 and 3-19, respectively. Of importance is the appearance of the resonance at approximately 192 ppm corresponding to the newly formed carbonyl carbon (C_{20}) as well as the disappearance of the resonance at 65 ppm, which corresponded to the carbons of the ethylenedioxy group . Notice the differences in the aromatic region between the iodinated and uniodinated materials and that the resonance of the carbon attached to the iodine atom has shifted from approximately 73.0 ppm to approximately 85.0 ppm. Some of the compounds were characterized by IR spectroscopy and the IR spectrum of 3-butyl-8-[4-(2-thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **10e,** has been reproduced in Figure 3-20. The amide C=O stretch is clearly visible at 1703 cm^{-1} as is the carbonyl C=O stretch at 1661 cm^{-1}. The composition and purity of all compounds were determined by either elemental analysis or HPLC and high resolution mass spectrometry. The mass spectra for the iodinated butyrothienones **11a** and **11f** are reproduced in Figures 3-21 through 3-24.

Even though this route requires extra steps, the overall chemical yield remains the same as for Scheme II and one can avoid the tedious situation found when following Scheme II in which the desired N-alkylated products must be separated from the parent amide and from any O-alkylated byproducts. It proved to be much easier to prepare and purify the N-alkyl derivatives **6b-f**, remove the BOC group and perform the coupling reactions to obtain the desired

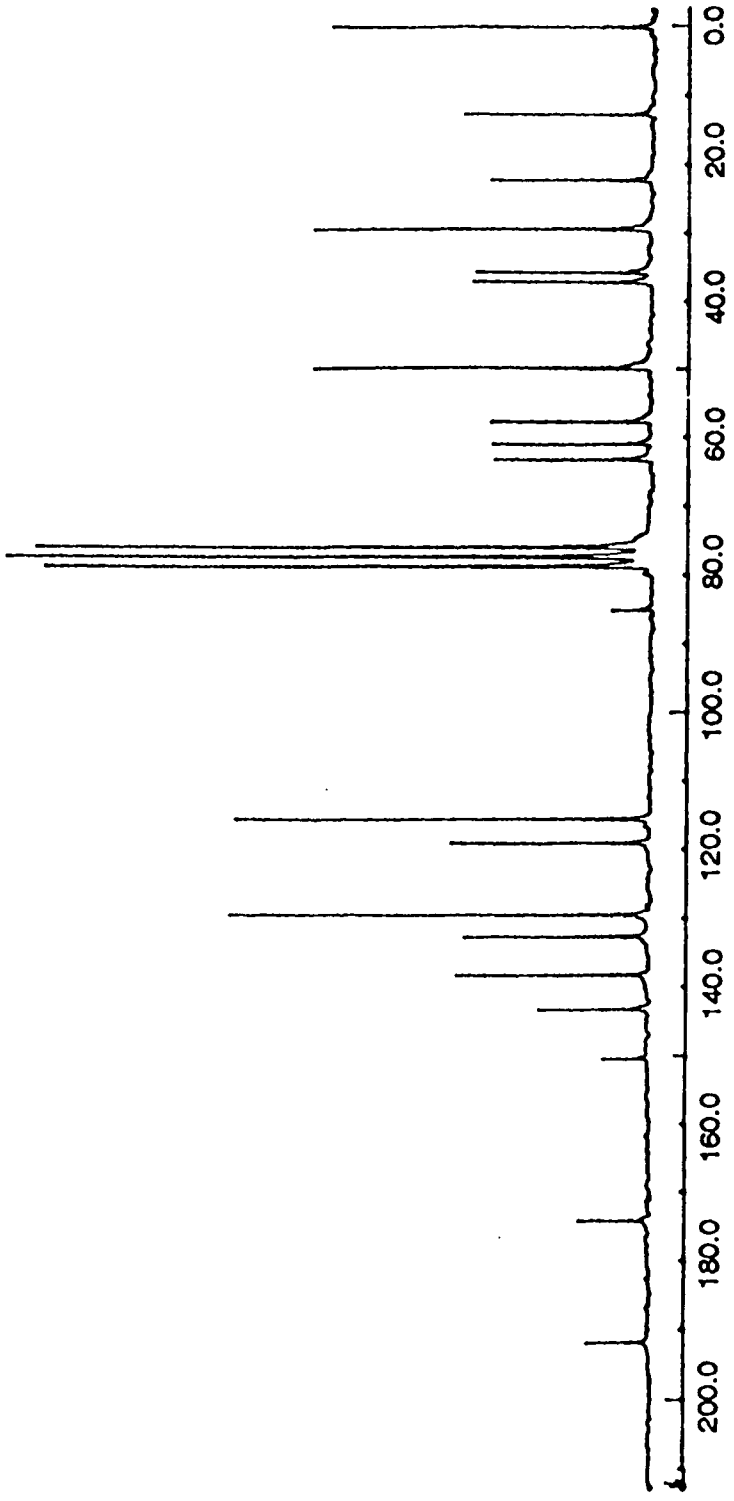


Figure 3-18. 90 MHz Carbon-13 Spectrum of 3-Ethyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 1.0% TMS.

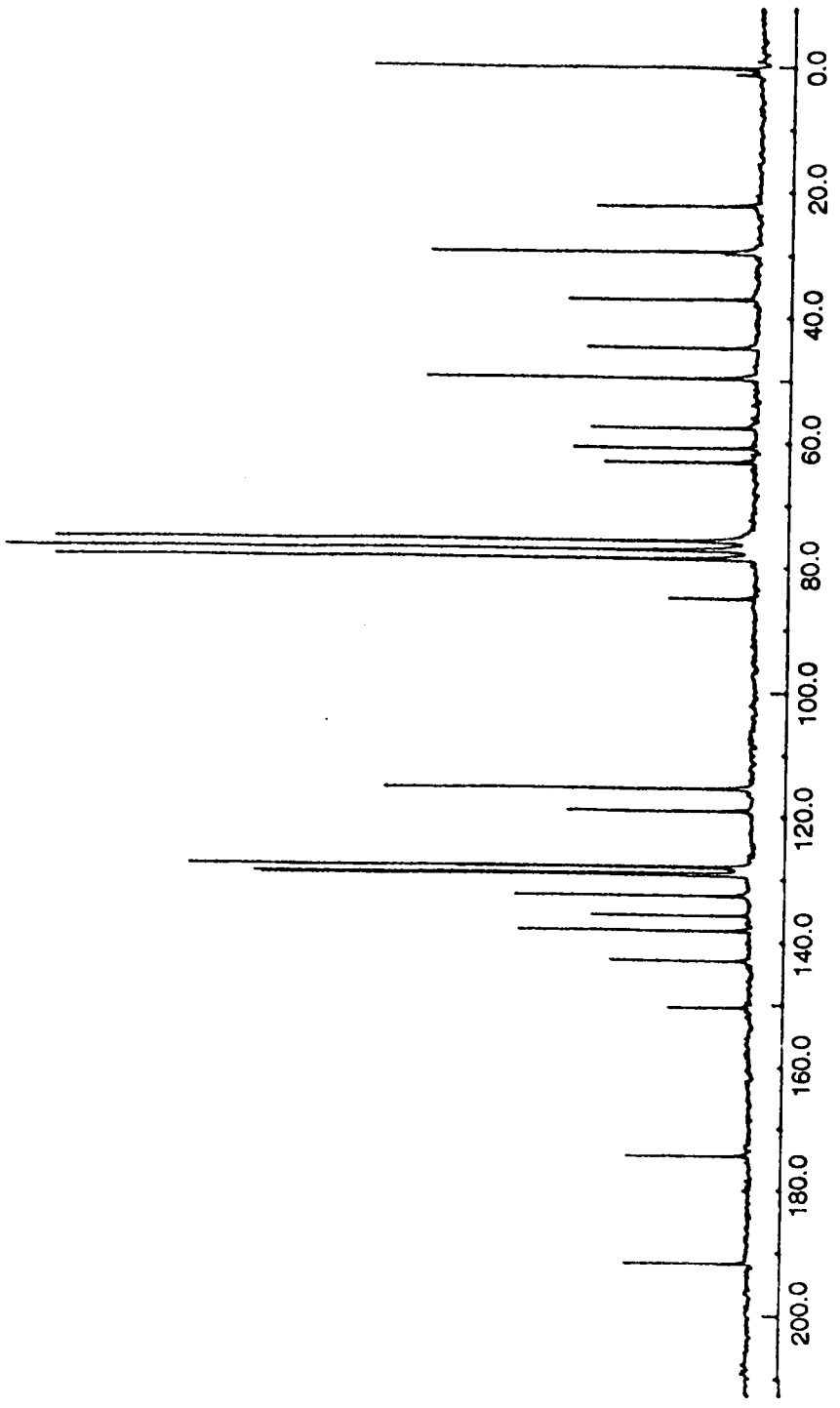


Figure 3-19. 90 MHz Carbon-13 Spectrum of 3-Benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 1.0% TMS.

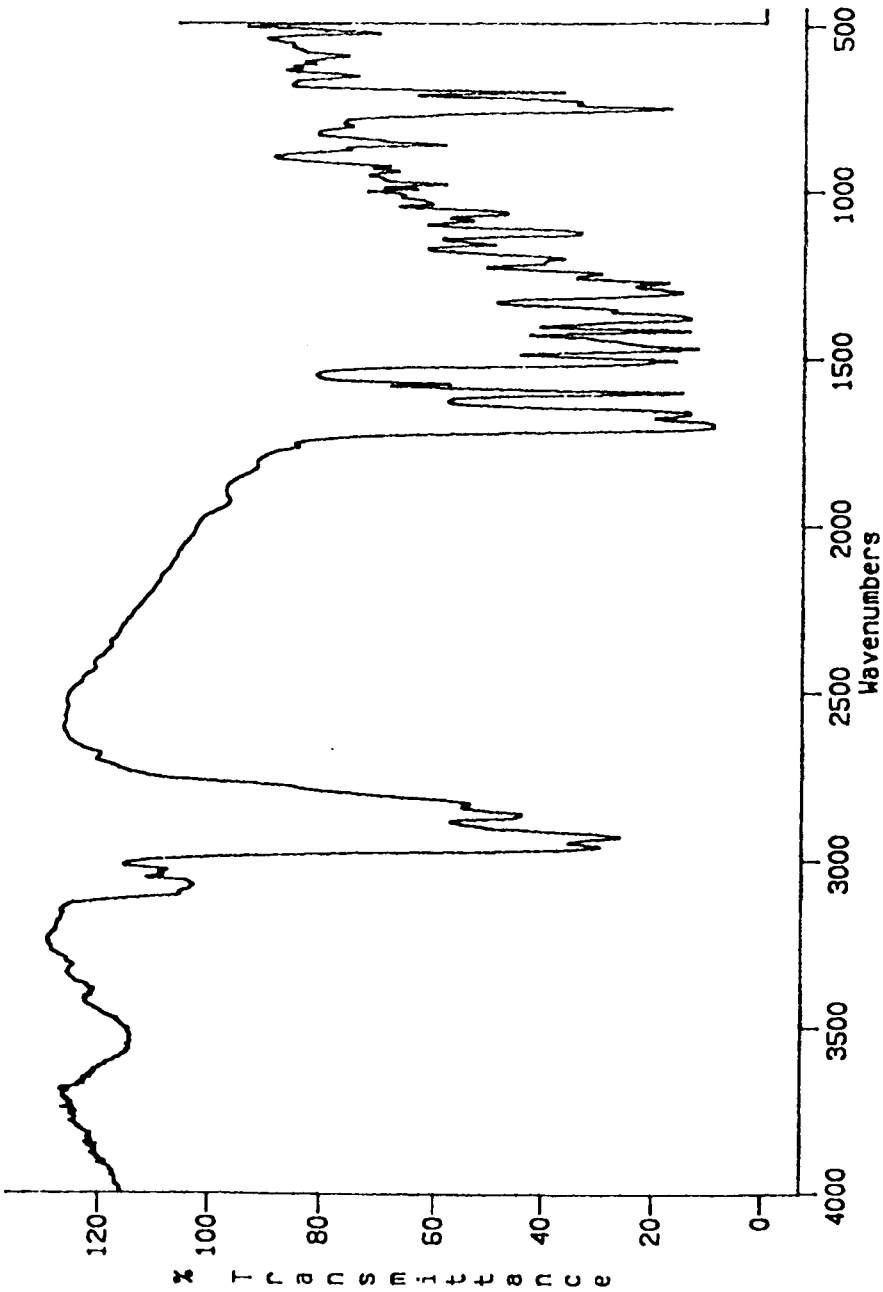


Figure 3-20. FTIR Spectrum of 3-Butyl-8-[4-(2-thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one on a Sodium Chloride Plate.

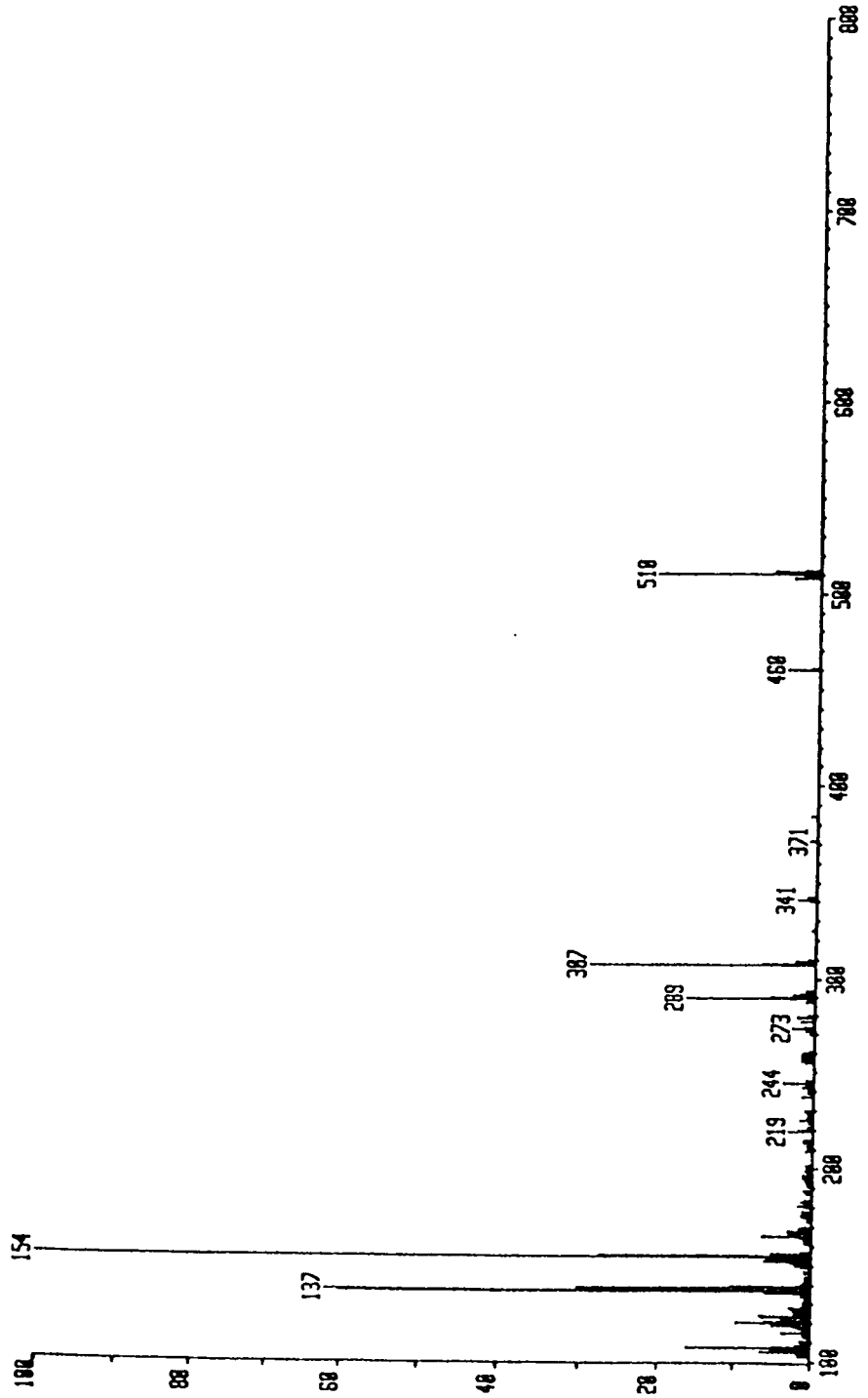


Figure 3-21. Mass Spectrum (FAB, M + 1) of 8-[4-[2-(5-Iodothienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

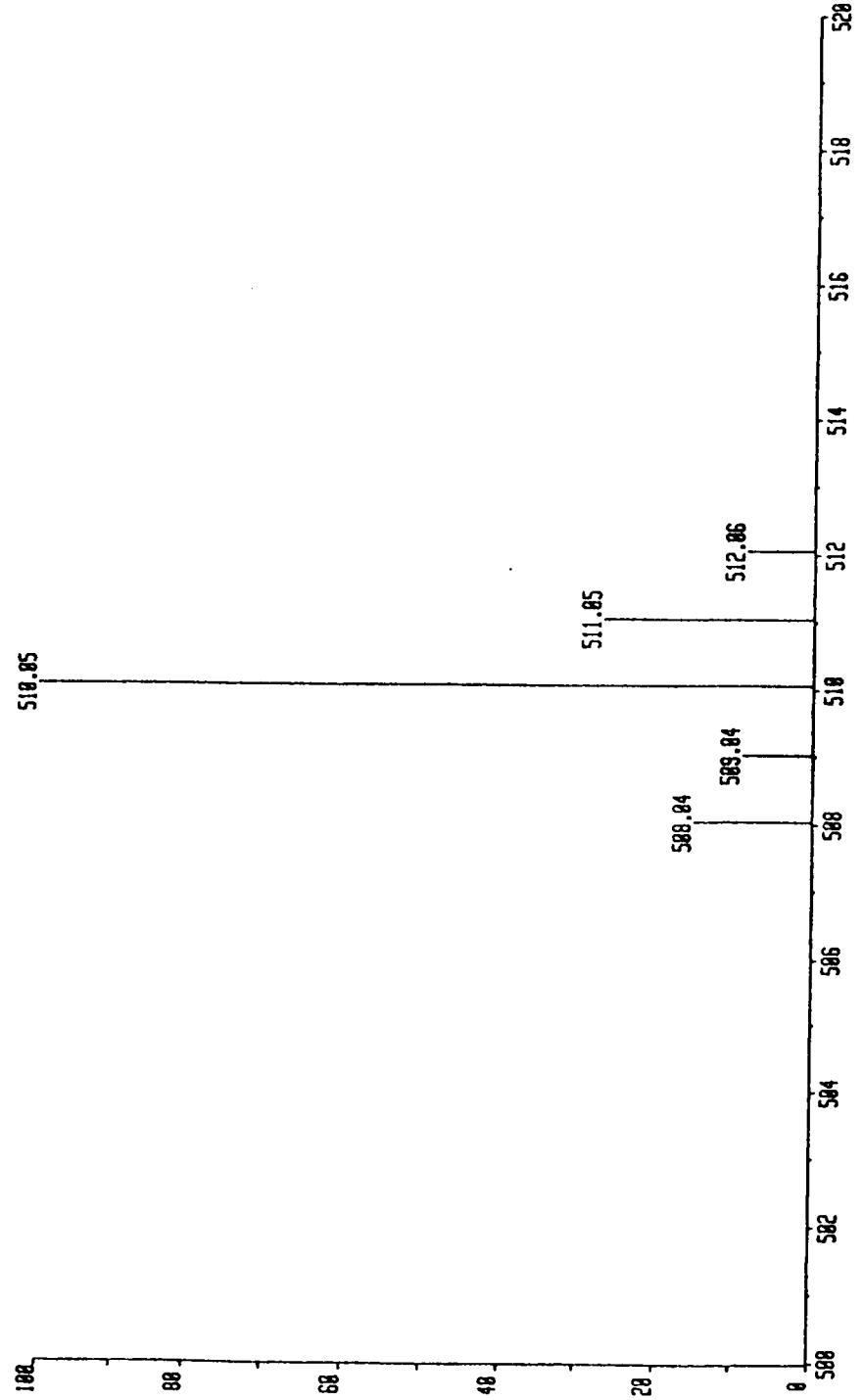


Figure 3-22. High Resolution Mass Spectrum (FAB, M + H) of 8-[4-[2-(5-Iodothienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

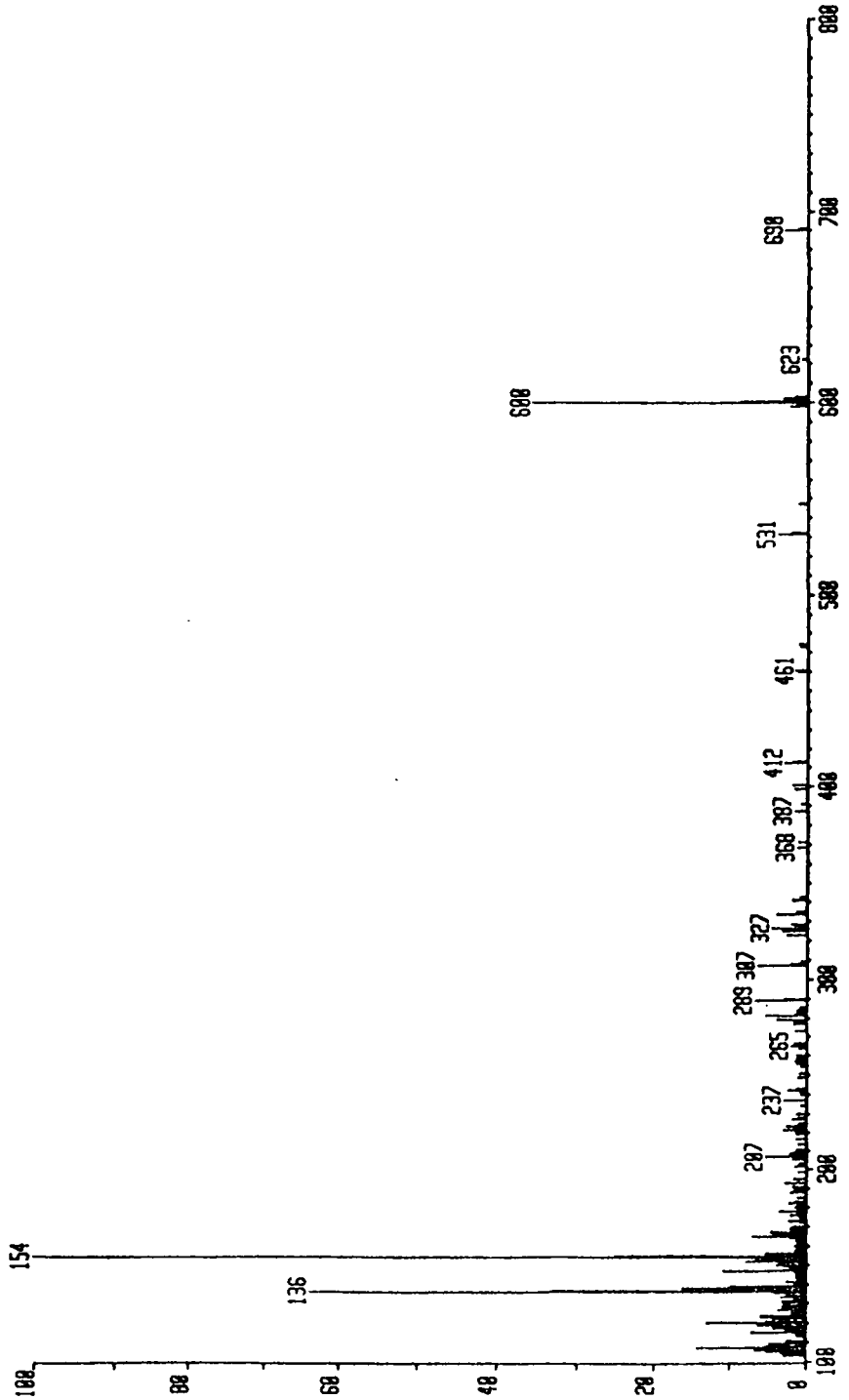


Figure 3-23. Mass Spectrum (FAB, M + 1) of 3-Benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

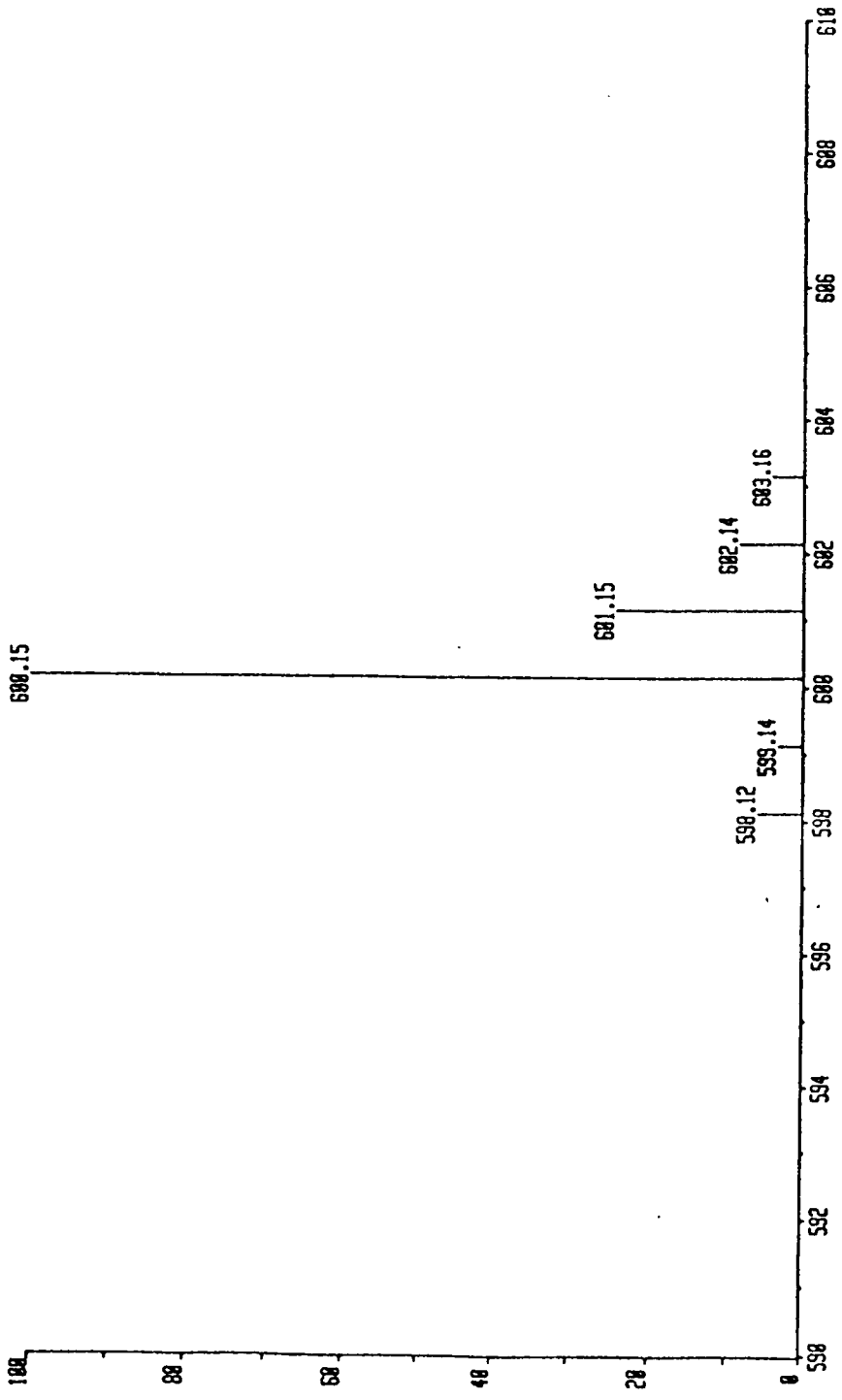


Figure 3-24. High Resolution Mass Spectrum (FAB, M + H) of 3-Benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

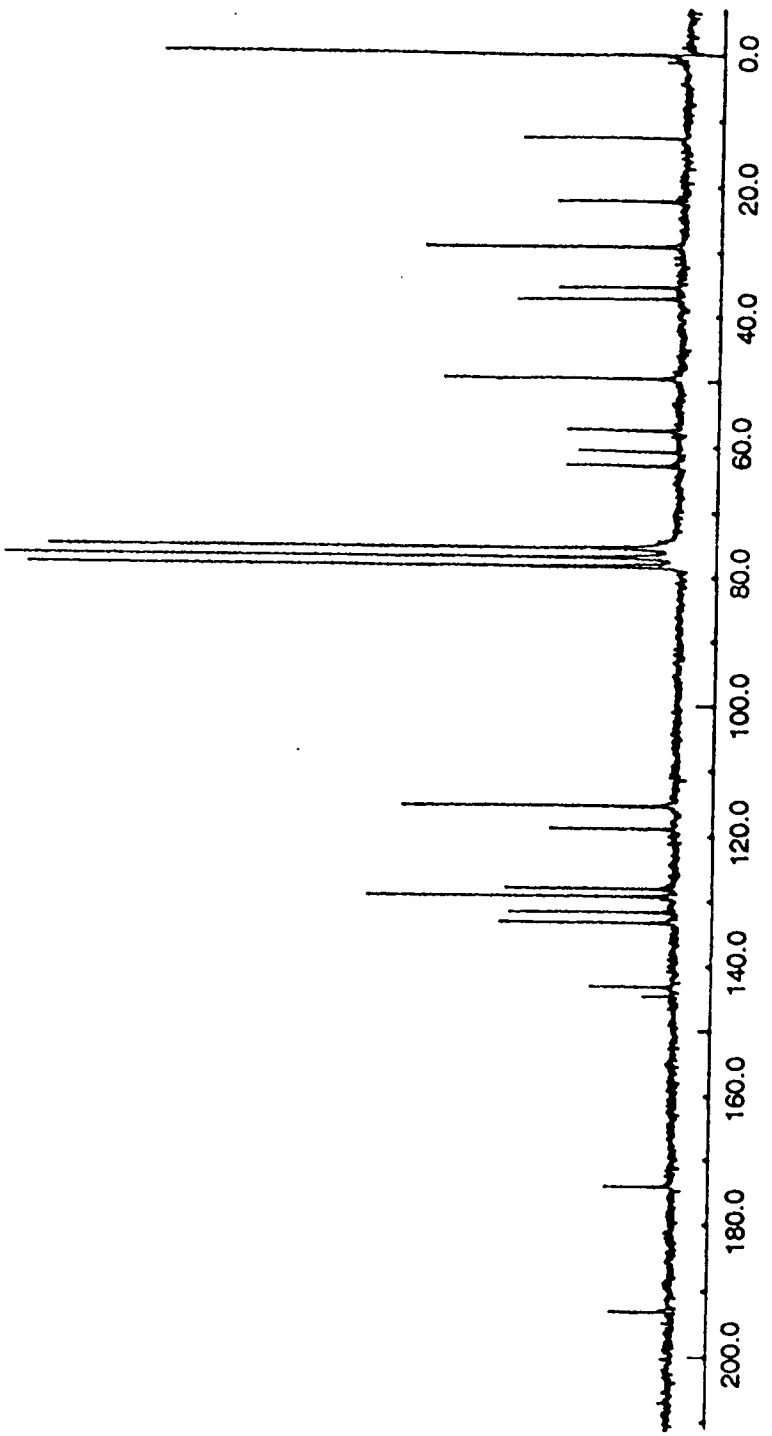
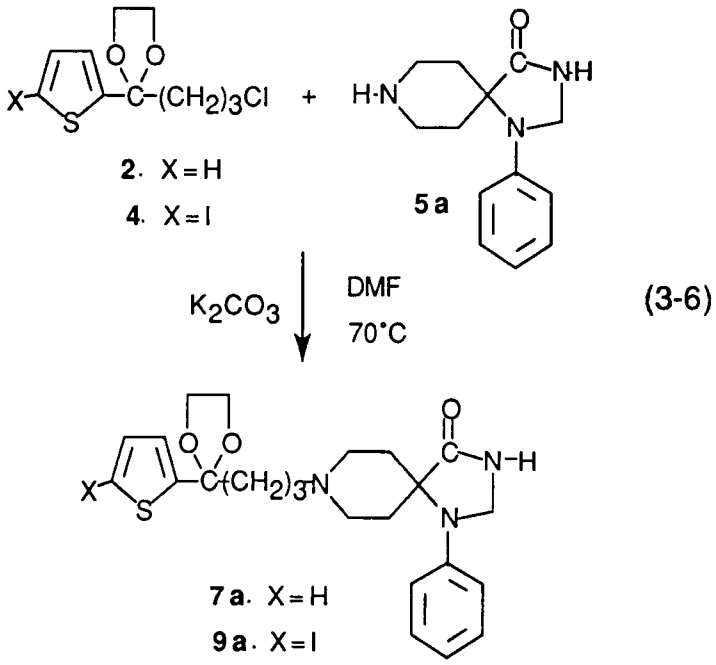


Figure 3-17. 250 MHz Proton Spectrum of 3-Ethyl-8-[4-(2-thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one in $CDCl_3$ plus 0.3% TMS.

N-alkylated ketals **7a-f** and **9a-f**.

b. Method B

The second route employed in the synthesis of the desired N-alkylated butyrophenones involved the formation of the amides **7a** and **9a** by the reaction of **5a** with the appropriate ketal, **2** or **4** (equation 3-6). This coupling reaction was performed in a variation of the method reported for the synthesis of the butyrophenones. It was observed that, if the reaction conditions were not kept anhydrous, byproducts formed which were difficult to separate from the desired product. The amide **7a** and **9a** were isolated by two different methods. The first method included dilution of the reaction mixture with water and extraction of the product into dichloromethane or ethyl acetate. It was, however, very difficult to remove all of the DMF by extraction while using solvents which would solubilize the amides **7a** and **9a** and any excess DMF had to be removed by vacuum with heat before the product could be purified by column chromatography. Usually the material would turn dark yellow during the removal of the DMF and yields of only 50-60% were obtained by this method. The reported yield for the formation of spiperone by the same method is 55-65%.[106] Since a white solid precipitated when the reaction mixture was diluted with water, a second method for purification was developed in which this white solid was isolated by vacuum filtration and dried. The solid was then dissolved in dichloromethane and applied to an alumina (neutral) column. The product was eluted from the column with a solution of dichloromethane and ethanol (97:3) and yields of 85% of the purified product were obtained in this manner. The products were identified by ^{1}H and ^{13}C NMR and elemental analysis.



Next, the parent amides **7a** and **9a** were alkylated to give the corresponding N-alkylated butyrothienone ketals **7b-f** and **9b-f** by the reaction of **7a** or **9a** with sodium hydride in anhydrous tetrahydrofuran followed by the addition of the appropriate alkyl iodide or bromide. The alkylations were carried out at temperatures which would allow completion of the reaction in relatively short periods of time. Alkylations with iodomethane, iodoethane and benzyl bromide took place at room temperature, however, the reaction of the sodium amide intermediate with 1-iodopropane and 1-iodobutane required temperatures between 40°C and 70°C to speed up the reaction. This sometimes resulted in the formation of small amounts of the O-alkylated byproducts, lowering the reaction yield and complicating purification. The

compounds obtained using this method were characterized in the same manner as those obtained by Method A and their chemical and spectral properties proved to be identical.

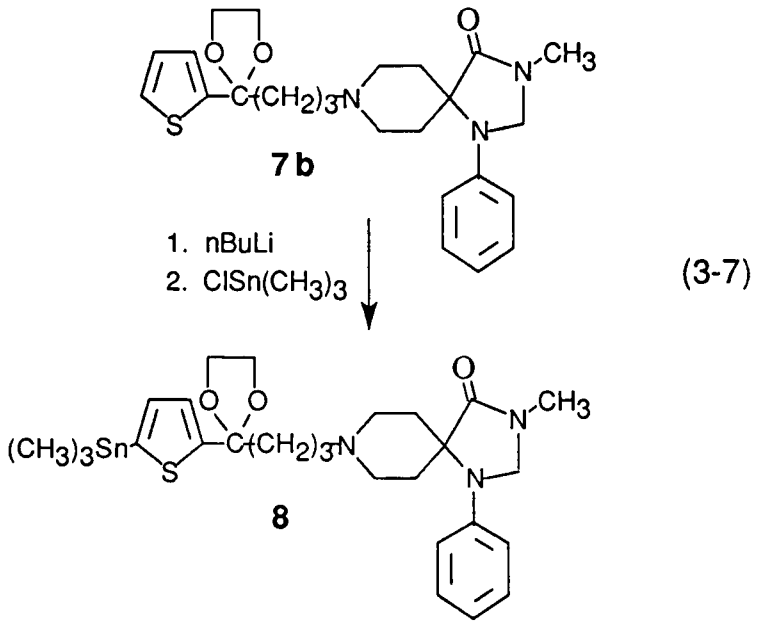
Hydrolysis of the ethylenedioxy protecting group was accomplished by treatment of **7a-f** and **9a-f** with a 40:1 mixture of methanol and hydrochloric acid at 50°C for 30 minutes. After the hydrolysis was complete, the reaction mixture was carefully neutralized with solid sodium hydrogen carbonate and the product extracted into dichloromethane. The organic layers were combined, dried over anhydrous sodium sulfate and the dichloromethane removed *in vacuo*. The products were purified by column chromatography and were usually obtained in yields between 70% and 85%. All compounds were characterized by ^{1}H and ^{13}C NMR and proved to have identical spectroscopic properties as **10a-f** and **11a-f** obtained by Method A.

4. Formation of the Radioiodinated Butyrothienones

A third synthetic route was used in the formation of the radiolabeled analog **13** to prove that radioiodination of the butyrothienones can occur by formation of a trimethylstannyl intermediate followed by radioiodestannylation. The iodine atom was introduced at an early stage in the synthesis of the "cold" compounds as a matter of convenience, however, when producing the radiolabeled version, the radioiodination must take place in the final steps. During HPLC analyses of the reaction mixtures and purified products, both a radiation detector and a UV detector (254 nm) were used. The method for the HPLC separation of **10b** from the iodinated analog, **11b**, was developed before the radioiodinations were attempted. An analytical grade silica column

was used for the separations and the mobile phase was dichloromethane/methanol (95:5). The flow rate was 1.0 ml/minute and the retention times for **10b** and **11b** were 6.66 minutes and 7.56 minutes, respectively. Note that **13** is the radiolabeled analog of **11b**.

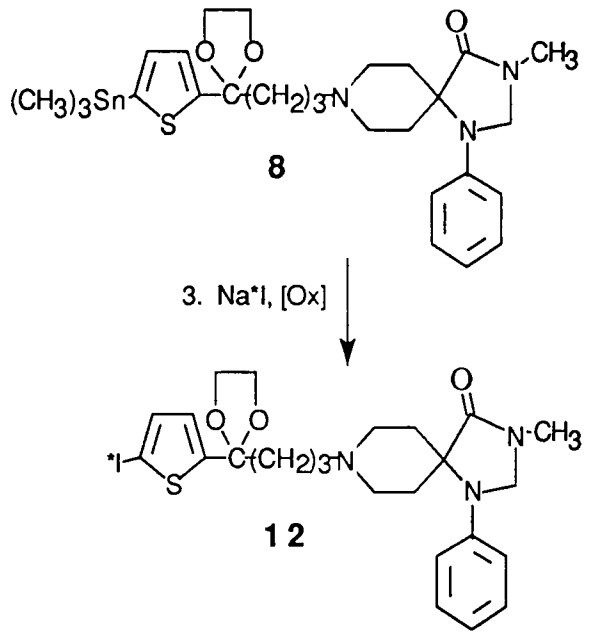
Treatment of the N-alkyl derivative **7b** with 1.2 equivalents of *n*-butyllithium in anhydrous tetrahydrofuran for 10 minutes at 0°C followed by the addition of 1.3 equivalents of trimethyltin chloride gave the corresponding trimethyltin derivative **8** in 55% yield (equation 3-7). No discoloration of the reaction mixture was observed. Intermediate **8** could not be isolated by chromatography using silica because decomposition (protodestannylation) occurred. Protodestannylation on silica has been reported for other trimethyltin substituted thiophene derivatives.[107] Better results were obtained using alumina as the stationary phase. The product was purified by column chromatography and was identified by ^{1}H NMR spectroscopy and by high resolution mass spectrometry. ^{1}H NMR revealed that **8** was 90% pure and that the major contaminant was the starting material **7b** which may have formed by protodestannylation. A primary feature of the ^{1}H NMR spectrum of **8** was a singlet at 0.34 ppm, which corresponded to the methyl groups of the trimethyltin substituent (9 H's; based on the integration of the ethylenedioxy group resonances = 4 protons). The aromatic region of the ^{1}H NMR spectrum was complicated; however, the integration indicated that there were 7 aromatic protons, as expected. High resolution mass spectroscopy revealed that a compound with the molecular formula of $C_{27}H_{39}O_3N_3SSn$ was present. The presence of **7b** should not create problems in the radioiodination step because relatively little radioiodine will be used (between 10^{-12} and 10^{-15} moles).



(3-7)

Furthermore, compound **7b** is formed during the radioiodination and subsequent workup by protodestannylation but can be removed from the radioiodinated product by HPLC. It was determined that **8** was sensitive to acid, light and heat and, therefore, the trimethyltin derivatives must be handled carefully and should be used soon after their preparation.

Treatment of **8** with $Na^{123}I$ in the presence of Chloramine-T generated the desired radioiodinated ketal **13** in 60% radiochemical yield after HPLC purification equation (3-8). The reaction was performed in ethanol at a pH of 7.0 (phosphate buffer) and the crude reaction mixture was analyzed by radio-TLC. A typical radiochromatogram of the reaction mixture has been reproduced in Figure 3-25. Hydrolysis of the ethylenedioxy group with methanolic HCl provided the desired radiotracer **13** with an overall radiochemical yield of 20%.



(3-8)

Compound **13** was purified by HPLC and the radio-thin layer chromatograms of the crude reaction mixture and of the purified product are shown in Figures 3-26 and 3-27, respectively. A portion of the purified product **13** was spiked with 1.0 mg of the "cold" standard **11b** and the resulting mixture was analyzed by HPLC. The peak corresponding to **11b** passed through the UV detector [retention time (**11b**) = 6.22 minutes] at the same time as the radiolabeled product passed through the radioation detector [retention time (**13**) = 6.25 minutes]. These preliminary results indicate that the radioiodinated analogs of **11b-f** can be prepared by the method presented in this section.

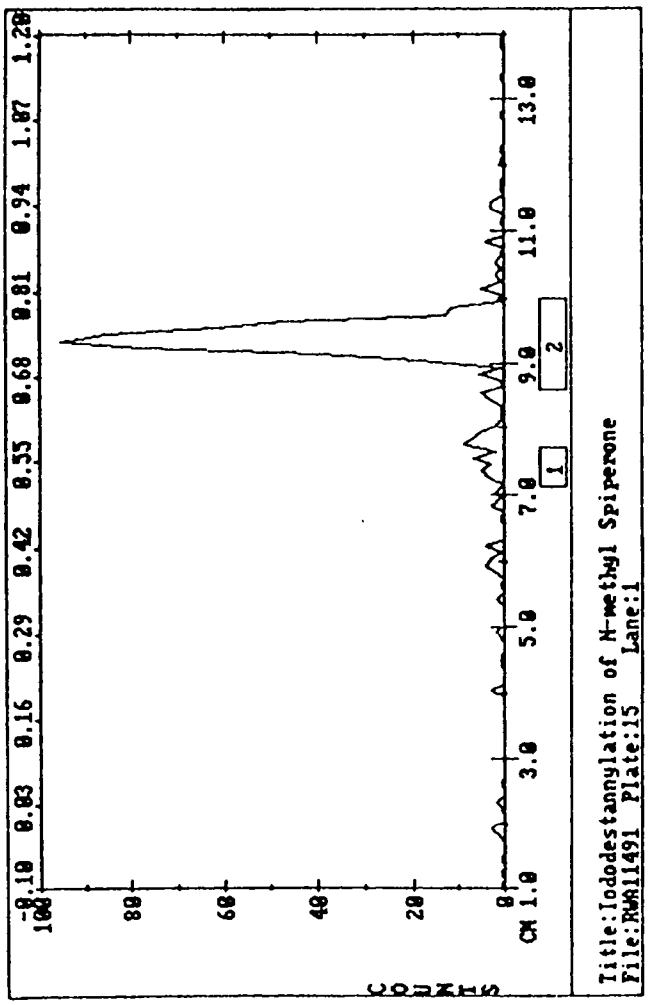


Figure 3-25. Radio-Thin Layer Chromatogram of the Crude Reaction Mixture From the fomation of 8-[4,4-Ethylenedioxy-4-[2-(5-[^{125}I]iodothienyl)]-butane]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

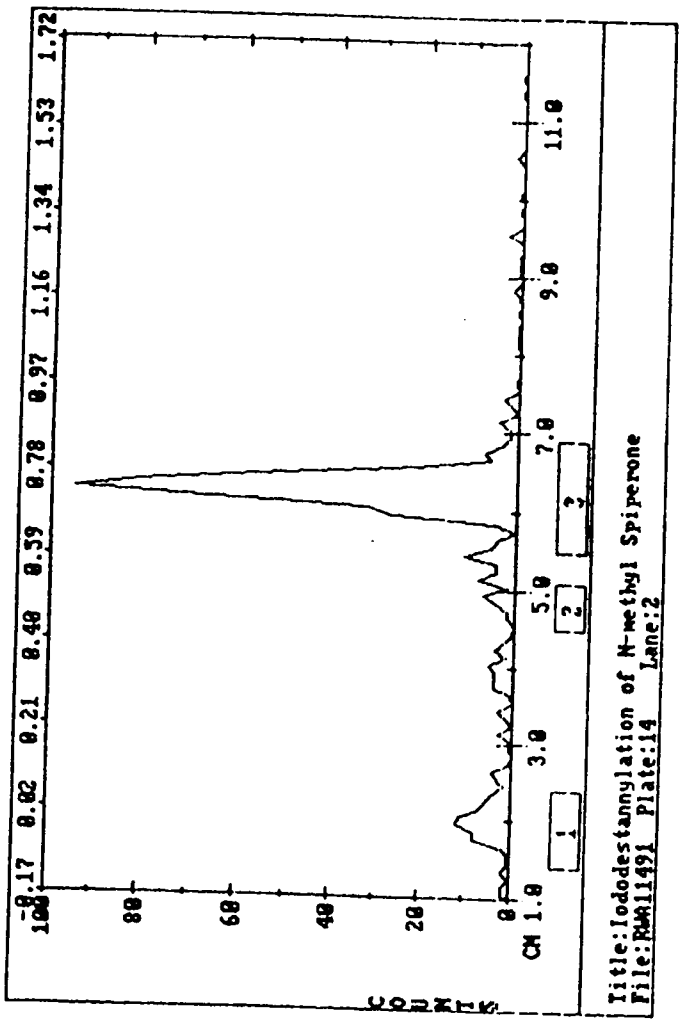


Figure 3-26. Radio-Thin Layer Chromatogram of the Crude Reaction Mixture From the Acid Hydrolysis of 8-[4,4-Ethylenedioxy-4-[2-(5-r[^{125}I]iodothienyl)]-butane]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

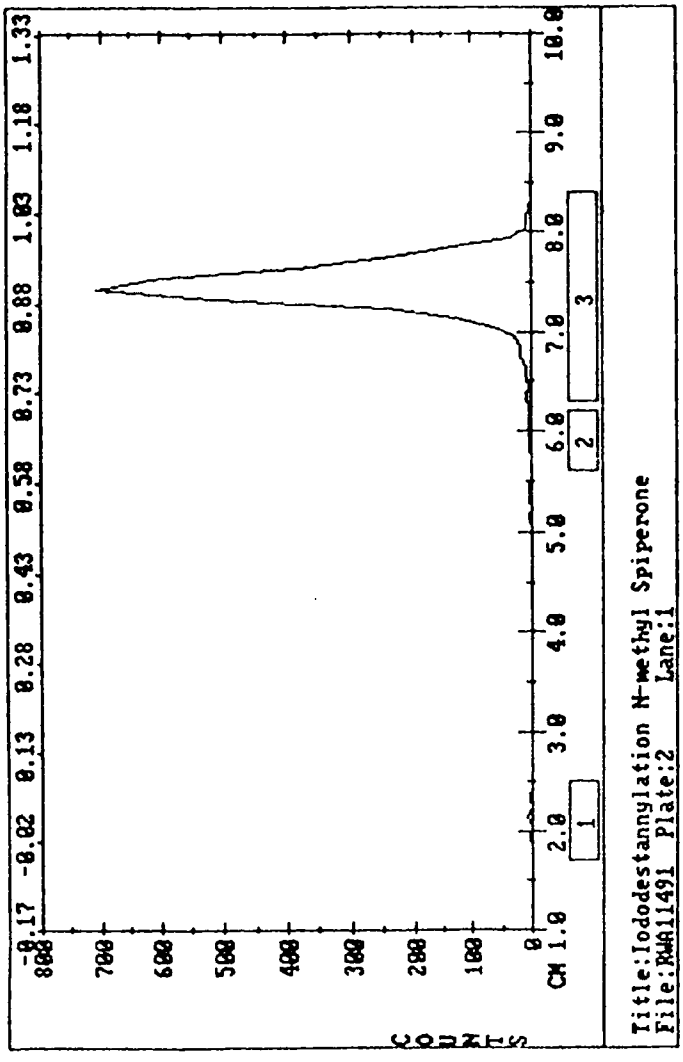


Figure 3-27. Radio-Thin Layer Chromatogram of purified 8-[4-[2-(5-[^{125}I]Iodothienyl)-4-oxo-butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

5. Preliminary Evaluation of the Butyrothienones

a. *In Vitro* Binding Studies

As noted earlier, the butyrothienone derivatives **10a-f** and **11a-f** have the necessary pharmacophores for binding to D_2 receptors. However, this observation reveals nothing about the affinity of these agents for D_2 receptors. *In vitro* binding studies, performed by Nova-Screen, confirm that all of the butyrothienones bind to both D_2 receptors and serotonin 5-HT_2 (S_2) receptors at concentrations of 10^{-5} M. In order to determine if these compounds have the high affinity needed to be effective D_2 receptor imaging agents, some of the butyrothienones were tested at 10^{-8} M. The percent inhibition values from binding studies at 10^{-5} M and 10^{-8} M are tabulated in Tables 3-1 and 3-2, respectively. The study was based on the ability of each compound to replace a standard compound [spiperone (D_2) and serotonin (S_2)] from the receptor at the concentrations indicated. A positive result is one in which at least 50% of the standard was displaced from the receptor and values of 85% and greater mean that the compound tested swamped the receptor. A value between -20% and 20% is interpreted as a negative result.

The information obtained from the study at 10^{-8} M is encouraging. The butyrothienones tested have a high affinity for D_2 receptors and exhibit some selectivity for D_2 receptors over S_2 receptors. However, the magnitude of the selectivity cannot be determined until the binding affinities (K_i values) for each compound are determined. Therefore, it is not known if the butyrothienones exhibit a difference in the magnitude of this selectivity as compared to the butyrophenones. Table 3-2 indicates that binding to serotonin S_1 receptors might be occurring, however, the percent inhibition values obtained at 10^{-8} M

Table 3-1. Results of *In Vitro* Binding Studies of Butyrothienones at 10^{-5} M.

*Compound X	R	**Percent Inhibition (Average; N=2) D_2	S_2	D_1	S_1
-H	-H	100.2	104.6	2.3	24.4
-H	$-CH_3$	100.5	104.2	5.5	34.0
-H	$-CH_2CH_3$	99.5	102.3	20.8	32.5
-H	$-(CH_2)_2CH_3$	100.5	103.6	26.1	30.7
-H	-(CH2)3CH3	100.5	83.1	-4.2	20.2
-H	-Bn	99.7	80.4	-5.6	2.2
-I	-H	100.9	92.7	2.4	30.8
-I	-CH3	100.7	77.0	-2.9	28.0
-I	$-CH_2CH_3$	100.0	93.9	14.3	42.5
-I	$-(CH_2)_3CH_3$	100.8	95.8	13.2	38.5

Reference compounds: Spiperone (D_2); Methylsergide (S_2); Butaclamol (D_1); Serotonin (S_2).

*Bn= Benzyl

**The data presented here were reproduced directly from a report of the results of *in vitro* binding studies performed by NovaScreen.

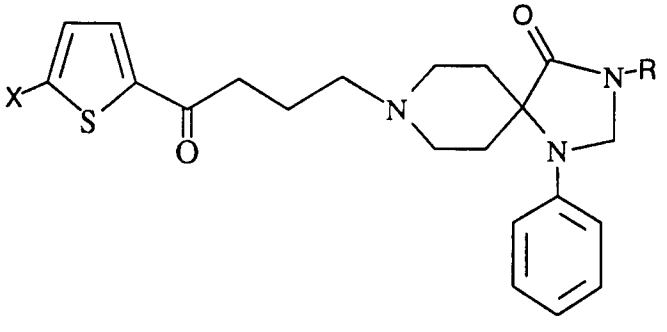


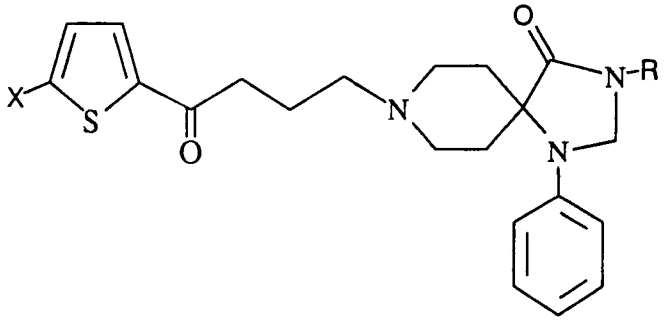
Table 3-2. Results of *In Vitro* Binding Studies of Butyrothienones at 10^{-8} M.

*Compound		**Percent Inhibition (Average; N=2)			
X	R	D_2	S_2	D_1	S_1
-H	-(CH2)2CH3	100.6	18.4	4.9	7.4
-H	-Bn	101.0	-7.4	2.8	12.6
-I	-CH3	87.2	-17.8	0.1	37.3
-I	-(CH2)3CH3	97.6	-7.0	-0.8	32.5
-I	-Bn	100.6	-15.1	1.4	20.7

Reference compounds: Spiperone (D2), K_i= 4.82 nM; Methylsergide (S2), K_i= 4.79 nM; Butaclamol (D1), K_i= 8.03 nM; Serotonin (S1), K_i= 2.05 nM.

* Bn = Benzyl

**The data presented here were reproduced directly from a report of the results of *in vitro* binding studies performed by NovaScreen.



are similar to those obtained at 10^{-5} M and are probably within the experimental error. If the butyrothienones bind to the S_1 receptors at 10^{-8} M, then a very high percent inhibition value should have been obtained at 10^{-5} M. Futhermore, these compounds are very close in structure to spiperone derivatives which are known not to bind to S_1 receptors. It should be noted, however, that positive results for an *in vitro* binding study do not ensure that a compound will have the desired receptor interactions *in vivo*.

b. Lipophilicity:

In order to determine if the butyrothienones have reasonable lipophilicities for transport across the blood/brain barrier (BBB) and for low non-specific binding, log P values were calculated for each compound using the reversed phase HPLC method.[108] An octadecyl-bonded silica column and a mobile phase consisting of a water-methanol mixture buffered to blood pH and ionic strength were used for the determinations. Eight standards, with a wide range of log P values, [from catechol (log P = 0.98) to hexachlorobenzene (log P = 6.53)] were analyzed before and after the butyrothienones in order to ensure that the chromatographic conditions remained the same throughout the experiment. The log of the retention times of the standards relative to catechol (log t_{rel}) was plotted against the log P values of the standards and a least squares analysis was done to obtain a parabolic fit with a correlation factor of 0.995 (see Figure 3-28). The equation of the curve is given in equation 3-9. This equation was used to determine the log P values of the butyrothienones by substituting the retention time of a butyrothienone for **X** to obtain the log P

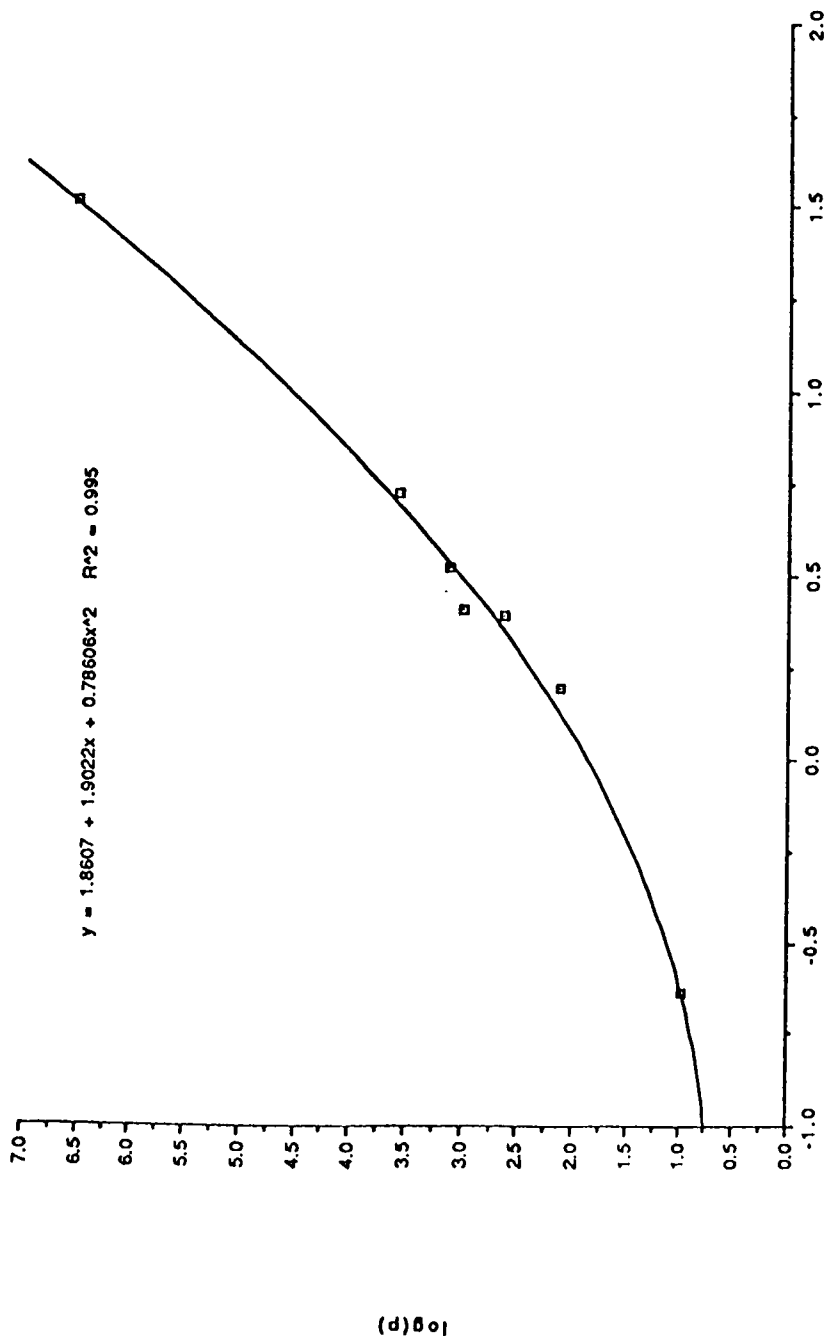


Figure 3-28. Log P of Standards Verses Retention Time Relative to Catechol.

value, **Y**. All compounds except **11e** were determined to have reasonable log P values. Notice that the iodinated N-benzyl derivative has a log P value of 4.11, whereas the analogous butyrophenone analog had a log P of 4.68 and is most likely too lipophilic to be an effective D_2 imaging agent (see Table 3-3). Compound **11f** may, therefore, be a good candidate for an imaging agent selective for D_2 receptors in the brain.

$$\mathbf{Y} = 1.8607 + 1.9022(\mathbf{X}) + 0.78606(\mathbf{X})^2 \qquad (3\text{-}9)$$

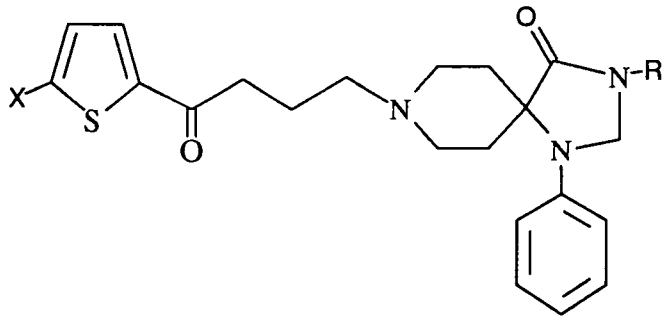
c. Structure-Activity Analysis

As discussed earlier (pages 105-107), the butyrothienones **10a-f** and **11a-f** have the two necessary pharmacophores for binding to D_2 receptors. These pharmacophores are 1) the basic nitrogen of the piperidine ring and 2) the aromatic group (thiophene ring). Both pharmacophores are separated from each other, as required, by a four carbon chain. In addition, the carbonyl group of the butyrothienone is thought to be necessary for the antagonistic activity of spiperone and its derivatives. The butyrothienones, like the butyrophenones, have a high affinity for serotonin S_2 receptors as well as for dopamine D_2 receptors. Only recently have detailed reports been published which explore structure/activity relationships for compounds which bind to serotonin receptors.[109] It is thought that a basic nitrogen and an aromatic ring are responsible for the ability of certain compounds, such as (+) lysergic acid diethylamide (LSD), to bind to S_2 receptors.[109a] It is also thought that the distance between the basic nitrogen and the center of the aromatic ring must be

Table 3-3. Experimental Log P Values for Butyrothienones.

Compounds		Retention Time (min.)	Log P
X	R		
H	-H	3.19	0.98
I	-H	4.75	2.57
H	$-CH_3$	5.10	2.56
I	$-CH_3$	6.83	3.14
H	$-CH_2CH_3$	5.72	2.84
I	$-CH_2CH_3$	8.24	3.62
H	$-(CH_2)_2CH_3$	6.66	3.14
I	$-(CH_2)_2CH_3$	9.56	4.00
H	$-(CH_2)_3CH_3$	8.01	3.67
I	$-(CH_2)_3CH_3$	11.58	4.50
H	-Bn	7.96	3.66
I	-Bn	10.46	4.11

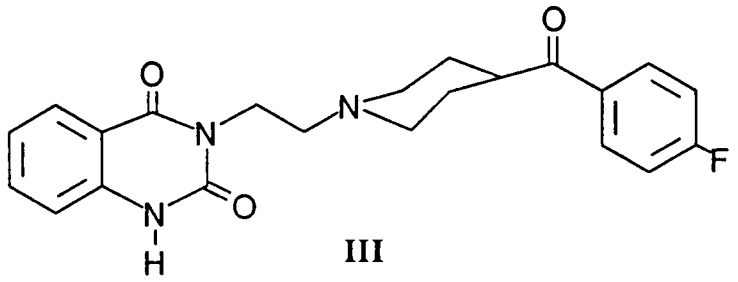
Desired log P = 2.67 - 4.20



approximately 4.8-5.7 Å. The exact conformation needed for binding to S_2 receptors has not yet been determined, however, there is strong evidence that at least two subtypes of the S_2 receptor family exist and that each has different structural requirements for binding. In addition, it has been shown that the S_2 receptor (or one of the S_2 subtypes) is sensitive to stereogenic centers.[109d]

To date, it has not been reported which structural features of spiperone (or spiperone derivatives) are responsible for its high affinity for serotonin S_2 receptors. The determination of these structural features is important because it might allow the manipulation of the basic structure of spiperone to create compounds that bind to D_2 receptors but have little or no affinity for S_2 receptors. Also, the information obtained might aid in the determination of the exact structural requirements necessary for binding to S_2 receptors. Finally, the S_2 receptor is important in cardiac function and in certain mental disorders, however, most of the serotonin agonists and antagonists that bind to the S_2 receptor also bind to the S_{1c} receptor.[109a] This lack of selectivity might prevent the preparation of ideal agents targeted to the S_2 receptor for imaging or therapeutic purposes. Because spiperone derivatives and the butyrothienones do not bind to the S_1 receptor family, information gained by the structural analysis of these compounds might allow for the design of agents that are selective for S_2 versus S_1 receptors.

Ketanserin, **III**, is a serotonin antagonist that exhibits a high affinity for both S_2 and S_1 receptors. In a study to determine which structural features of ketanserin are responsible for binding to the S_2 receptor family, it was determined that quinazolinone moiety is not essential for binding and that the benzoyl carbonyl group can be removed without causing a significant reduction

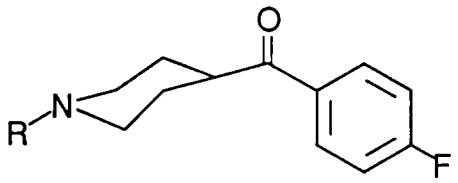


III

in the binding affinity.[109a] Of interest is the fact that compounds **IV-IX** have a high affinity for the S_2 receptor (Table 3-4). This observation suggests that the butyrophenone group of spiperone and the butyrothienone group of **10a-f** and **11a-f** are not involved in the binding of these agents to the S_2 receptor. In addition, compounds **X**, **XI** and **XII** (Table 3-5) have a high affinity for S_2 receptors, suggesting that the carbonyl group and the fluorine atom are not necessary for binding. This suggests that the S_2 receptor is receptive to both polar and non-polar groups on the carbon next to the benzene ring. In fact, it appears that a wide range of groups are allowed in the vicinity of the aromatic ring (originally the 4-fluorobenzoyl group of ketanserin). Two such examples are the serotonin antagonists **XIII** and **XIV**.

In view of the information reported thus far for the structural requirements of binding to the S_2 receptor, it is probable that the basic nitrogen and N-phenyl group of both spiperone and the butyrothienones are involved in the binding of these compounds to the S_2 receptor. One last piece of evidence to support this hypothesis is the butyrophenone, haloperidol **XV**. Haloperidol is a selective dopamine antagonist that has a high affinity for D_2 receptors and no affinity for S_2 receptors.[110] The phenyl group of haloperidol is positioned in front of the

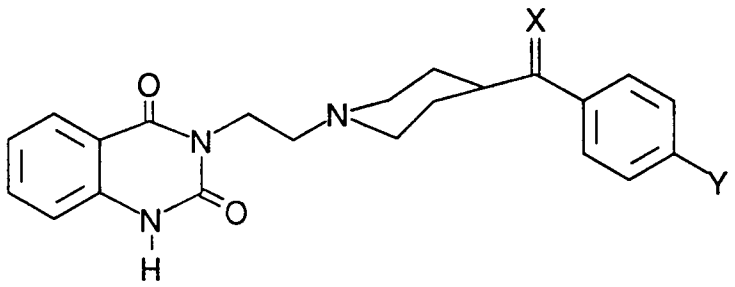
Table 3-4. The Effect Various Aromatic Substituents on the S_2 Receptor Affinity of Ketanserin Analogs.



Compound	R	Ki (nM)
IV	$-CH_2Ph$	25
V	$-(CH_2)_2Ph$	8.5
VI	$-(CH_2)_3Ph$	16
VII	$-(CH_2)_4Ph$	5.3
VIII	$-(CH_2)_5Ph$	10
VIIII	$-(CH_2)_3C(C=O)Ph$	6.5
IX	$-(CH_2)_2NHC(C=O)Ph$	5.3

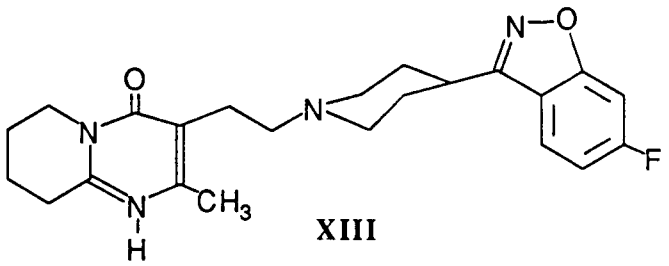
Source: Herndon, Jeff L., Abd Ismaiel, Stacy P. Ingher, M. Teitlerand Richard A. Glennon. 1992. "Ketanserin Analogs: Structure-Affinity Relationships for 5-HT_2 and 5-HT_{1C} Serotonin Receptor Binding." In Philip S. Portoghese, ed., Journal of Medicinal Chemistry. Washington, D. C.: American Chemical Society. pp. 4903-4910.

Table 3-5. Manipulation of the 4-Fluorobenzoyl Group of Ketanserin and the Corresponding Changes in Binding Affinity for the S_2 Receptor.

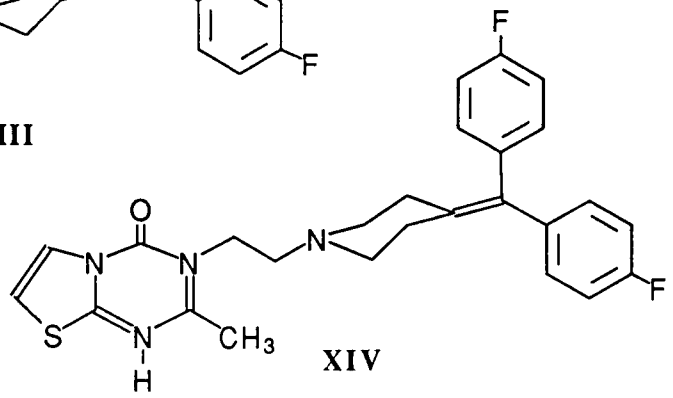


Compound	X	Y	K_i (nM)
X	O	F	3.5
XI	H, H	F	12.3
XII	H, H	H	32

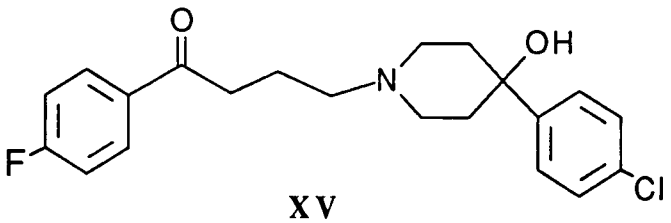
Source: Herndon, Jeff L., Abd Ismaiel, Stacy P. Ingher, M. Teitlerand Richard A. Glennon. 1992. "Ketanserin Analogs: Structure-Affinity Relationships for 5-HT_2 and 5-HT_{1C} Serotonin Receptor Binding." In Philip S. Portoghese, ed., Journal of Medicinal Chemistry. Washington, D. C.: American Chemical Society. pp. 4903-4910.



XIII



XIV



XV

ring and cannot move into the same positions that the phenyl group of spiperone or butyrothienone derivatives. The major drawback of haloperidol, however, is its relatively low solubility is aqueous media.[110]

Analysis of the ^{1}H and ^{13}C NMR of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5a**, suggests that only one conformation is present. It was determined the predominant structure is one in which the piperidine ring is in a chair conformation, however, further NMR studies must be done in order to determine which of the two possible chair conformations is predominant. The same conformation was also prevalent in all of the butyrothienone derivatives **10a-f** and **11a-f,** however, it is not imperative that the conformation of lowest energy

is defined because it may not be the biologically active conformation.[111] The analysis of the ^1H data for 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one is outlined in the Appendix.

A hypothetical model for the binding of the butyrothienones to serotonin S_2 receptors is illustrated in Figure 3-29. The basic nitrogen probably binds to a proton donating group of an amino acid, such as the hydroxyl group of tyrosine. The phenyl group would bind through π-π interactions with other aromatic systems in the receptor. It is likely that there is open space to the left of the basic nitrogen (see Figure 3-29) because both polar and non-polar groups are well tolerated, as are relatively large groups. The lipophilic cavity is

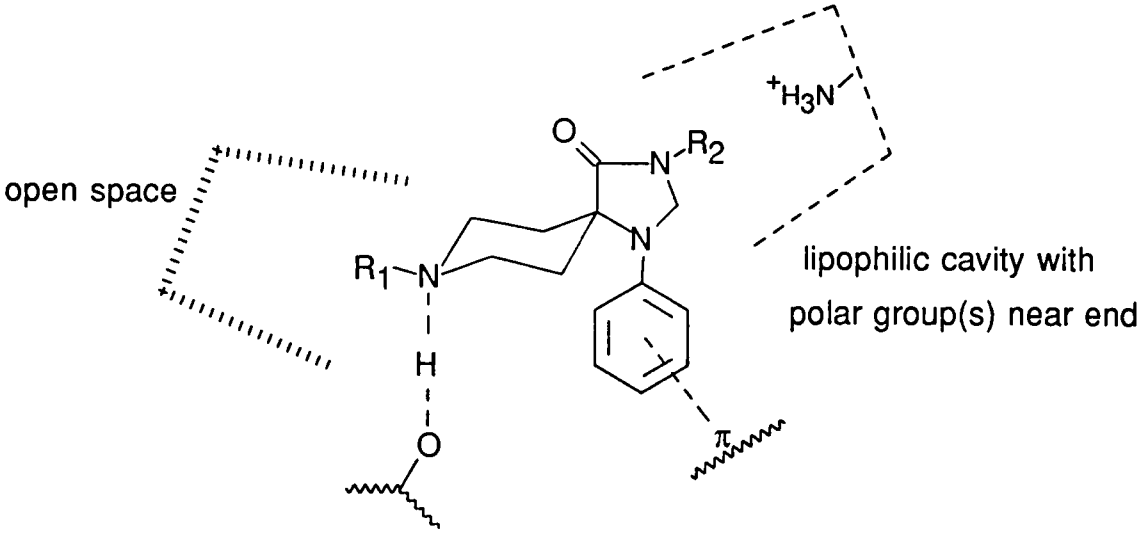
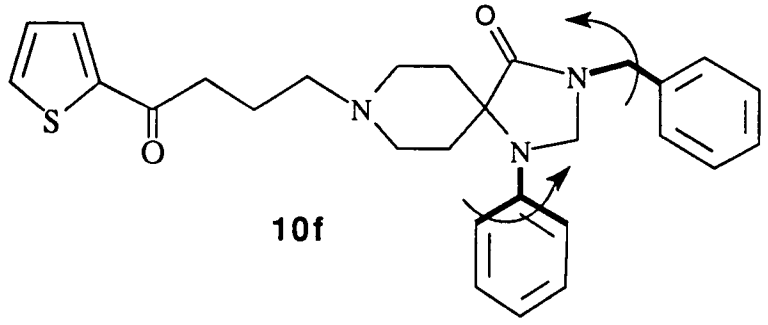


Figure 3-29. A Hypothetical Model for the Binding of Compounds Derived from 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-one to Serotonin S_2 Receptors.

proposed based on the report of Mach *et al.*[106] and on molecular modeling studies of the N-benzyl butyrothienone derivative **10f**. As discussed earlier (see pages 112 and 113), Mach *et al.* showed that alkylation of the amide nitrogen of spiperone with simple alkyl groups does not significantly effect S_2 receptor affinity. However, the N-benzyl derivative exhibited a large decrease in affinity for the S_2 receptor and placement of a lipophilic group, such as iodine or a methyl group, at the 3 or 4 position on the aromatic ring reduced the affinity even further. The conclusion given by the authors was that perhaps there were polar groups in the vicinity of the benzyl group of spiperone which resulted in the reduced affinity of these compounds.

A molecular modeling study, using SYBYL, was done on compound **10f** to determine if there was an energetically favorable position for the benzyl group. During the study, the phenyl group of **10f** was allowed to rotate in 15 degree increments until it had rotated a total of 180 degrees (equation 3-10). After each 15 degree rotation of the phenyl ring, the benzyl group was rotated through 360 degrees in 10 degree increments. After each 10 degree rotation of

rotated 360° by 10° increments

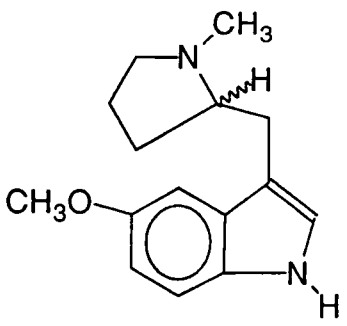


10f (3-10)

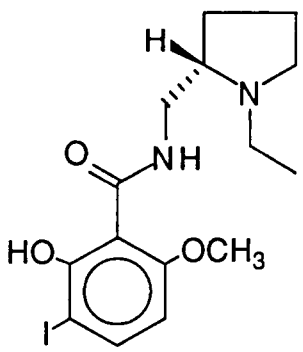
rotated 180° by 15° increments

the benzyl group, the energy of the resulting structures was minimized (the phenyl and benzyl groups were not allowed to move during the minimization) and the conformational energy obtained. A three dimensional plot of conformational energy versus the angle of rotation of the phenyl ring and benzyl group is given in Figure 3-30. The data obtained suggests that the benzyl group is apparently free to rotate about its point of attachment. Therefore, the benzyl group might somehow be restricted from rotating away from the polar group(s) when the compound is bound to the S_2 receptor, perhaps because it resides in a relatively small cavity. An alternative hypothesis is that there are several polar groups which lie in all directions surrounding the amide, but at a distance large enough that they do not to interact with simple alkyl groups attached to the amide nitrogen.

A recent study indicates that the S_2 receptor (and all four S_1 receptor subtypes) is sensitive to the presence of stereogenic centers and that compound **XVI**-(*R*) (K_i = 17 nM) has a significantly higher affinity for the S_2 receptor than its enantiomer, **XVI**-(*S*) (K_i = 680 nM).[109d] In view of this study,



XVI



XVII

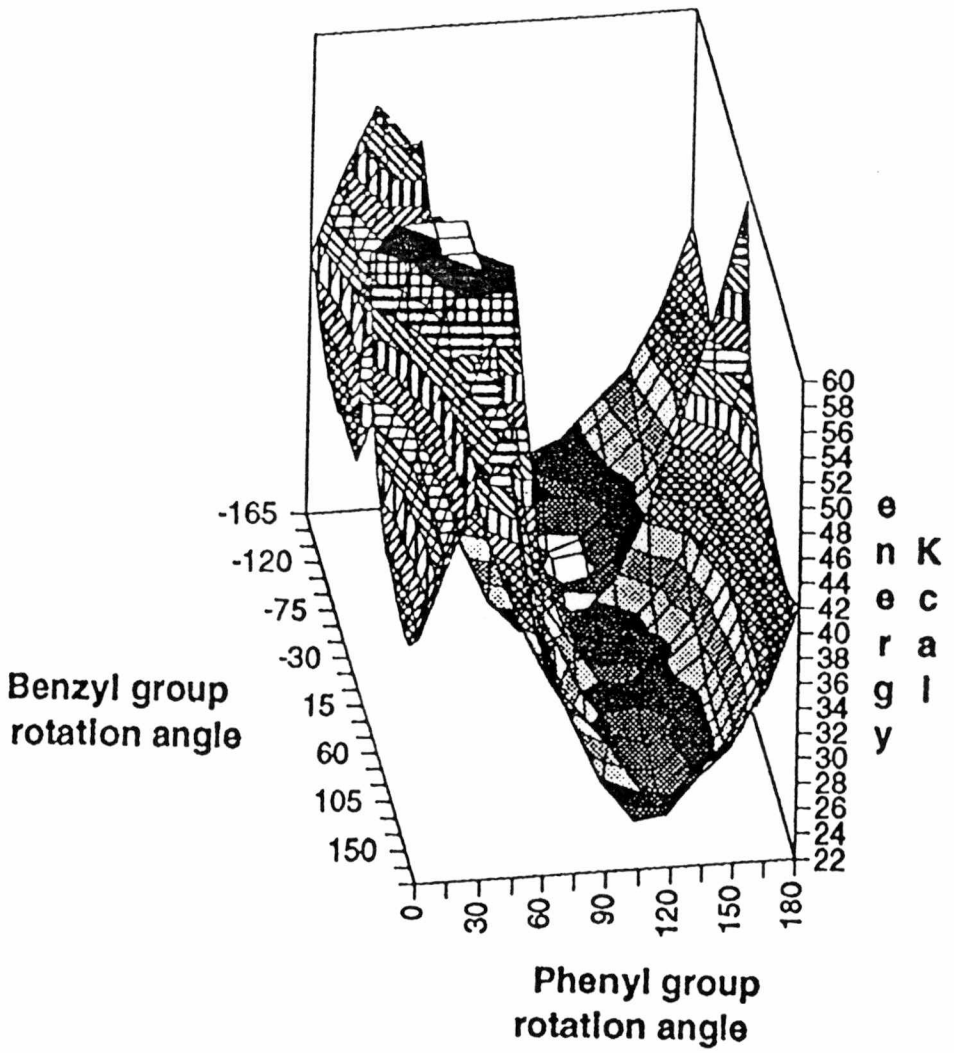


Figure 3-30. 3-Dimensional Plot of the Conformational Energy of **10f** Versus the Angle of Rotation of the Benzyl and Phenyl Groups.

the orientation of the phenyl ring with respect to the basic nitrogen might have an influence in the affinity of compounds derived from 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5a**, for S_2 receptors. The phenyl ring of **5a** can lie on either side of the molecule due to nitrogen inversion and, perhaps, only one of these two possible orientaions is required for binding to the S_2 receptor.

One final observation is that certain benzamides, which have a high selectivity for D2 receptors over S2 receptors, are of the *S* configuration. One such example is (S)-N-[(1-ethyl-2-pyrrolidinyl)]methyl-2-hydroxy-3-iodo-6-methoxy-benzamide, **XVII**.[112]

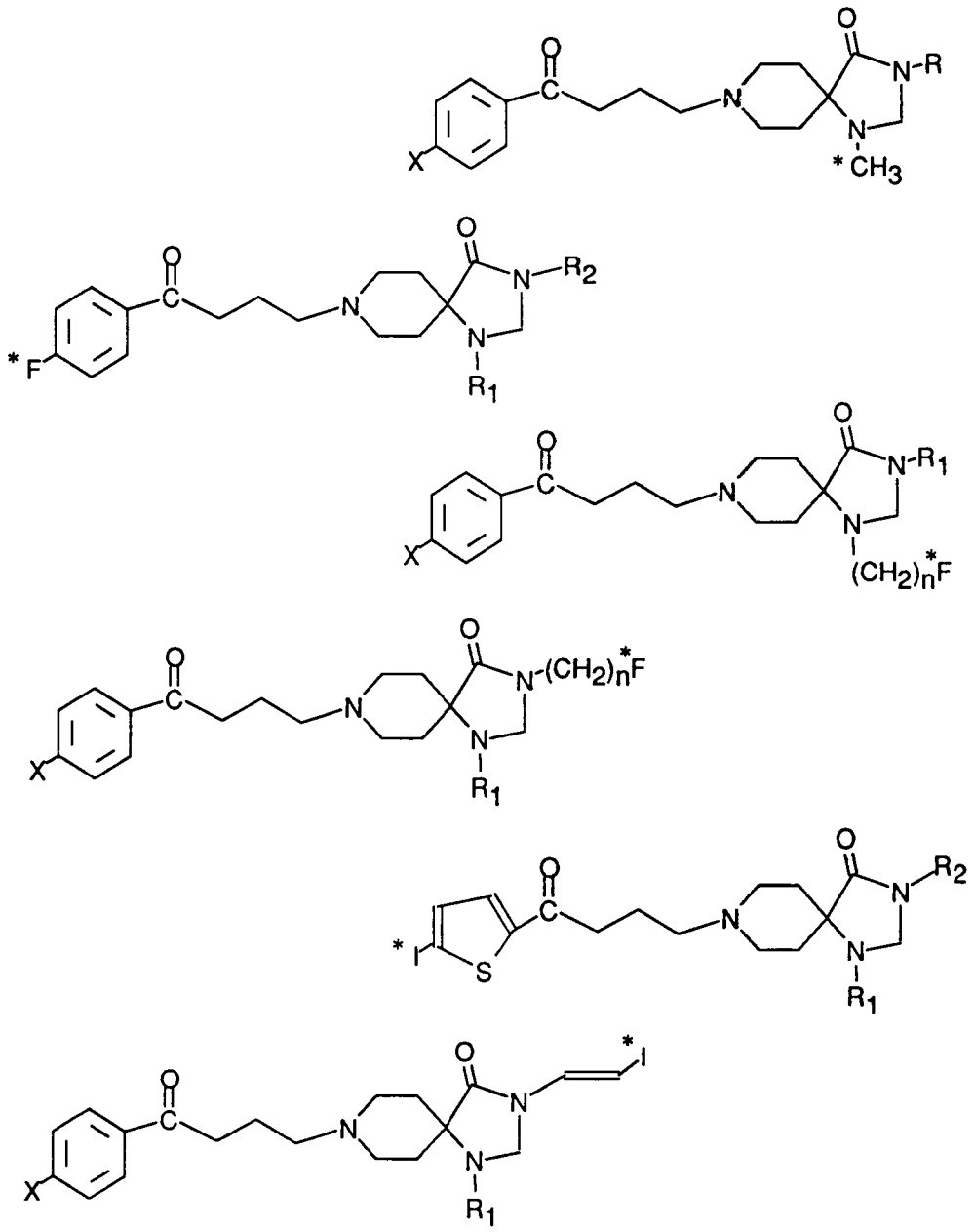
E. Conclusions

The butyrothienones **10a-f** and **11a-f** were successfully synthesized by two different synthetic routes; one in which the alkylation of the amide nitrogen was accomplished before the coupling reaction and one in which the alkylation took place after the parent amide **7a** or **9a** was formed. Neither synthetic method allowed for a significant increase in overall yield. A third synthetic route was developed to prepare the radioiodinated analog **13**. The third route involved the formation of a trimethyltin adduct of **7b** followed by radioiodestannylation by treatment of **7b** with $Na^{123}I$ in the presence of chloramine-T. The final step was the hydrolysis of the ethylenedioxy ketal to provide **13** in 20% overall radiochemical yield (from **7b**). The radioiodinated analogs of **11c-f** could be prepared in the same manner.

The experimental log P values obtained for all of the N-alkylated butyrothienones, except **11e**, are within the range needed for passage across the blood/brain barrier and for low non-specific binding. The *in vitro* binding

studies at 10^{-8} M reveal that the butyrothienones have a high affinity for dopamine D_2 receptors and exhibit selectivity for dopamine D_2 receptors over serotonin 5-HT_2 (S_2) receptors. In general, the iodinated butyrothienones are excellent candidates for SPECT imaging of D_2 receptors in the brain. The major advantage of these compounds, versus the iodinated spiperone analogs reported in literature, is their lower lipophilicity due to the substitution of the thiophene ring for the benzene ring of the butyrophenone group. The iodinated N-benzyl derivative **11f** holds the most promise because of the reported selectivity for D_2 receptors versus S_2 receptors of the corresponding analog of spiperone. However, the potential of these compounds as SPECT imaging agents cannot be fully evaluated until the K_i values of each compound are determined for D_2 and S_2 receptors and *in vivo* experiments are performed.

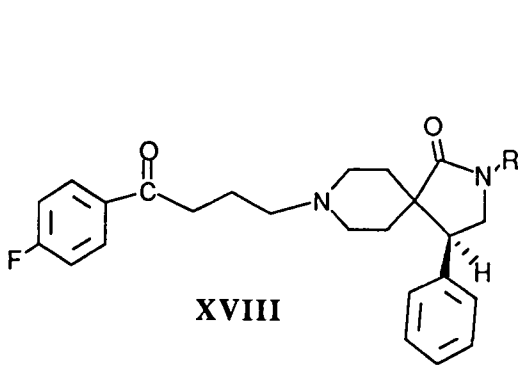
Comparison of the structure of the butyrothienones to the structure of several compounds that bind to serotonin S_2 receptors lead to the hypothesis that the phenyl group at position 1 and the basic nitrogen of the piperidine ring are involved in the binding of these compounds to S_2 receptors. A hypothetical model was proposed which shows how the binding might occur (see Figure 3-29). Because the phenyl group at position 1 is not involved in the binding of the butyrothienones (or spiperone derivatives) to D_2 receptors, removal of the phenyl group might result in compounds that bind selectively to D_2 receptors. The removal of the ring should not interfere with D_2 receptor binding and allows an alternative position at which to manipulate lipophilicity and/or attach a radioisotope. The nitrogen at position one could be replaced with a carbon atom, if needed. Compounds suitable to test this hypothesis would be ones such as those shown in Figure 3-31.



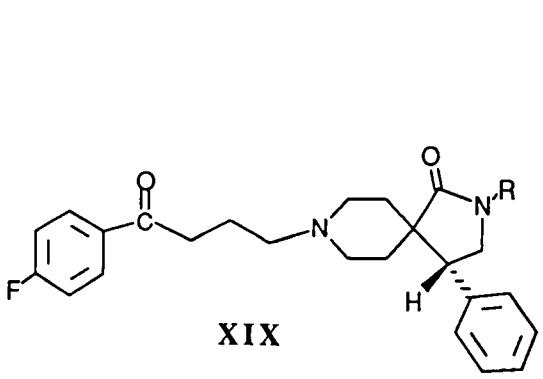
R_1 and R_2 = alkyl groups (can be aromatic if attached at the amide)
X = H or halogen

Figure 3-31. Compounds that may be Selective for Dopamine D_2 Receptors Versus Serotonin S_2 Receptors.

wo additional compounds which might be considered are **XIX**. The synthesis of **XVIII** and **XIX** (or similar compounds in which the butyrophenone group is replaced by other substituents) would serve two purposes: 1) Evaluation of the affinities of these compounds for the S_2 receptor could provide valuable information on the exact orientation between the phenyl group and the basic nitrogen that is required for binding to the receptor. 2) Compounds **XVIII** and **XIX** might prove useful in ^{1}H-NMR studies because the **XVIII** phenyl ring is held in a fixed position, destroying the symmetry that is displayed in compound **5a** and the butyrothienones. A comparison of the ^{1}H NMR spectra of these compounds with the ^{1}H NMR spectrum of **5a** and the butyrothienones might provide information on the extent of interaction between the piperidine ring protons and the π system of the phenyl group. This information could help to determine which chair conformation of the piperidine ring is predominant in compound **5a** and the butyrothienones.



XVIII



XIX

R = H or an alkyl group

F. Experimental

1. General

All glassware, syringes and syringe needles were oven-dried for 24 hours prior to use when required. All NMR spectra were recorded on a JEOL FX-90Q FT-NMR spectrometer or a Brücker AC-250 FT-NMR spectrometer in deuterochloroform ($CDCl_3$). The ^{1}H-NMR spectra were referenced internally using tetramethylsilane (TMS) at 0.0 ppm. The integration parameters acquired on the JEOL FX-90Q were plotted directly onto the ^{1}H-NMR spectra and the integration parameters acquired on the Brücker AC-250 were obtained in numerical form on a separate print-out and do not appear in the spectra. The ^{13}C-NMR spectra were referenced internally using $CDCl_3$ (center of triplet at 77.1 ppm). Infrared spectra were obtained on a Bio-Rad FTS-7 IR Spectrometer controlled by a Bio-Rad SPC 3200 computer and were referenced using polystyrene (1601 cm^{-1}). Melting points were obtained using an Electrothermal Digital Melting Point Apparatus F0290 and are uncorrected. Thin layer chromatography was performed using either Whatman Silica Gel 60A MK6F plates (250 μm thickness) with a fluorescent indicator (254 nm) or Alltech Aluminumoxid 60 F254 Neutral (type E) TLC plates (thickness 0.2 mm). Radio-thin layer chromatography was performed using Alltech Aluminumoxid 60 F254 Neutral (type E) TLC plates (thickness 0.2 mm) which were analyzed by a Bioscan System 200 Imaging Scanner controlled by an IBM Personal Computer. Column chromatographic separations were accomplished using either Baxter C4582-86 60A (230-400 mesh) silica gel or Alltech aluminum oxide, 150 mesh, Brockmann 1 (activated, neutral). High pressure liquid chromatography was accomplished by two methods: Method A [column =

Waters Resolve™ Millipore high resolution column (8mm X 10cm) packed with silica (5μ particle size) P/N 86016; mobile phase = dichloromethane/methanol (95:5); flow rate = 1.0 ml/min] and Method B [column = Alltech Adsorbosphere C18 (5μ particle size) column (4.6mm X 250mm) P/N 288105; mobile phase = methanol/aqueous phosphate buffer solution (85:15), the phosphate buffer consisted of 150 ml 0.1 M KH_2PO_4 and 122.7 ml 0.1 M NaOH solution; flow rate = 1.0 ml/min]. The equipment utilized for the HPLC analyses included 2 Waters Millipore Model 510 pumps, a Waters Millipore WISP Automated Injection System with a 100 μL injection loop and a Waters System Interface Model (SIM). Detection was accomplished using a Waters Millipore 486 Tunable Absorbance Detector and a Harshaw NaI (2in X 2in) HD-420 Integral Line Detector with an ORTEC Model 276 photomultiplier base with a preamplifier. The HPLC system was controlled by a Compaq Desk Pro 286 computer. Elemental analysis were performed by Galbraith Laboratories, Knoxville, Tennessee and Atlantic Microlabs, Norcross, Georgia and all experimental values are within +/-0.40% of the theoretical values. The *in vitro* binding studies were carried out by NovaScreen, Inc., Baltimore, Maryland and were paid for by The National Institutes of Health. Mass spectra were obtained on a VG Analytical ZAB-EQ instrument (8 KV). All reagents were analytical grade and were used without further purification. The standards used for the log P experiment were purchased from Alltech Associates, Inc. The commercially available chemicals and solvents used and their manufacturers are listed in Table 3-7.

Table 3-7: Commerially Available Chemicals and Their Manufacturers (Part B).

Reagent	Manufacturer
Butyllithium (1.6 M in hexanes)	A
Dichloromethane	A
Diethyl Ether, anhydrous	B
Ethanol	A
Ethyl Acetate	A
Tetrahydrofuran, anhydrous	A
Iodine	A
Sodium Hydrogen Sulfite	B
Chloroform-d_6 (1% TMS)	A
4-Chloro-1-(2-thienly)-1-butanone	A
1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-one	A
Dimethylformamide (anhydrous)	A
Dihydrogen Phosphate	B
Potassium Carbonate	B
Iodomethane	A
Iodoethane	A
1-Iodopropane	B
1-Iodobutane	A
Benzyl Bromide	A
BOC-ON	A

Table 3-7 (continued)

Reagent	Manufacturer
Water	A
Sodium Iodide	B
Chloramine-T	A
Methanol	B
Sodium Bicarbonate	A
Sodium Hydroxide	A
Silica Gel	B
Silica Gel TLC Plates	W
Alumina Oxide (neutral)	B
Alumina TLC plates	L
Sodium Carbonate	A
Sodium Hydride	A
Hydrochloric Acid (concentrated)	B
Sodium Sulfate	A
Sodium ^{123}I	N

*Manufacturers

A = Aldrich Chemical Company

B = Baxter Chemical Company

N = New England Nuclear, Inc.

M = Mallinckrodt Chemical Company

W = Whatman Chemical Company

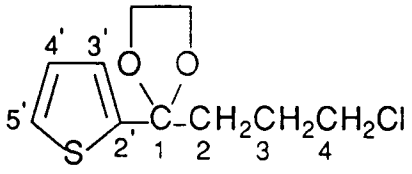
L= Alltech Associates, Inc.

2. Synthetic Procedures

a. 4-Chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane (2)

A 200 ml two-necked, round-bottomed flask was equipped with a Dean-Stark trap, a water-cooled condenser, a gas outlet (oil bubbler), a rubber septum, and a magnetic stirring bar. Anhydrous benzene (200 ml) was added to the flask, followed by 4-chloro-1-(2-thieny)-1-butanone (1.68 g, 8.90 mmol) , anhydrous ethylene glycol (3.0 ml, 53.4 mmol) and p-toluenesulfonic acid (100 mg, 0.55 mmol). The mixture was refluxed for 20 hours under a blanket of argon and all water that formed was collected in the Dean-Stark trap (approx. 0.3 ml). The resulting dark brown reaction mixture was cooled to room temperature and neutralized with an aqueous, saturated sodium bicarbonate solution (55 ml). The product was then extracted into dichloromethane (4 x 70 ml) and the organic layers were combined, dried over anhydrous sodium sulfate, and the solvent evaporated under reduced pressure to give 2.1 grams of a brown oil. Thin layer chromatography of the crude reaction mixture (mobile phase = dichloromethane/hexane (1:1); stationary phase = silica) showed two components: one corresponding to the product at R_f= 0.68, and the other corresponding to the starting ketone at R_f= 0.41. The product was purified by column chromatography, under the same conditions as described for the thin layer chromatography, to give 1.36 g (5.86 mmol, 65.8%) of the desired ketal as a clear, colorless oil which solidified when cooled to 0°C; ^{1}H-NMR ($CDCl_3$): δ 1.68-2.26 (m, 4H, H_2 and H_3), 3.54 (t, 2H, J = 6.35, H_4), 3.85-4.10 (m, 4H, -OC$\underline{H_2}$C$\underline{H_2}$O-), 6.81-7.0 (m, 2H, $H_{3'}$ and $H_{5'}$), 7.23 (dd, $J_{4'5'}$ = 4.5 Hz, $J_{3'4'}$ = 1.47 Hz, 1H, $H_{4'}$); ^{13}C-NMR ($CDCl_3$): δ27.08 (C_3), 37.86 (C_2), 44.66 (C_4), 64.80 (-O$\underline{C}H_2\underline{C}H_2$O-), 108.74 ($C_1$), 124.61 ($C_{5'}$), 125.23 ($C_{4'}$), 126.94 ($C_{3'}$),

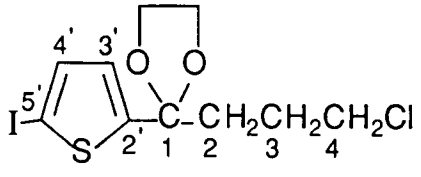
146.36 (C_2'); elemental analysis: (Calculated for $C_{10}H_{13}O_2SCl$) C, 51.61%; H, 5.63%; S, 13.78%; Cl, 15.23% (found) C, 51.50%; H, 5.65%; S, 13.68%; Cl, 15.26%.



b. <u>4-Chloro-1,1-(ethylenedioxy)-1-[2(5-iodothienyl)]butane (4)</u>

4-Chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane (1.20 g, 5.16 mmol) and anhydrous diethyl ether (50 ml) were added to a 250 ml oven-dried, round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and a gas outlet (oil bubbler). This mixture was cooled to 0°C and *n*-butyllithum (4.84 ml, 7.74 mmol, 1.6 M in hexanes) was added via syringe. This mixture became cloudy 10 minutes after the addition of the *n*-butyllithum. After 30 minutes, trimethyltin chloride (1.50 g, 7.74 mmol) was added in one portion and the reaction mixture quickly became clear and then cloudy again as copious amounts of a white precipitate formed. After stirring for 1.0 hour, 10 drops of water were added to destroy excess *n*-butyllithium. Next, iodine (1.57 g, 6.18 mmol) in diethyl ether (30 ml, anhydrous) was added to the reaction mixture and stirring continued for another 30 minutes. The resulting clear red mixture was washed with an 10% aqueous sodium hydrogen sulfite (75 ml) solution to

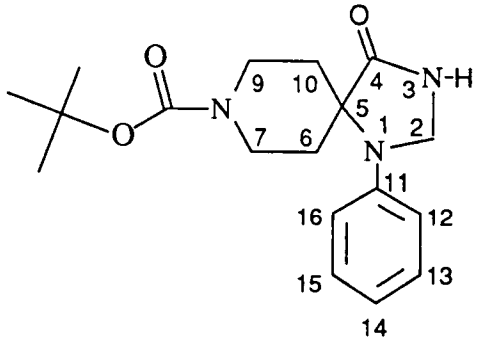
destroy the excess iodine. The product was extracted into ethyl acetate (3 X 50 ml) and the organic layers were combined, dried over anhydrous sodium sulfate, filtered by gravity filtration, and the solvent removed to give an orange oil. Thin layer chromatography (dichloromethane/hexane (1:1); stationary phase = silica) revealed a major component (R_f= 0.79), corresponding to the iodinated product, and a minor component (R_f= 0.63) corresponding to the starting material. The product was purified by column chromatography under the same conditions described for the TLC to afford 1.45 g (4.13 mmol, 80%) of the desired compound as a clear, colorless oil; ^{1}H-NMR ($CDCl_3$); δ1.80-2.25 (m, 4H, H_2 and H_3), 3.55 (t, 2H, J = 6.37, H_4), 3.90-4.11 (m, 4H, -OC$\underline{H_2}$C$\underline{H_2}$O-), 6.69 (d, 1H, $J_{3'4'}$ = 3.74 Hz, $H_{3'}$), 7.14 (d, 1H, $J_{3'4'}$ = 3.74 Hz, $H_{4'}$); ^{13}C-NMR ($CDCl_3$): δ27.04 (C_3), 37.77 (C_2), 44.92 (C_4), 65.10 (-O$\underline{C}H_2\underline{C}H_2$O-), 73.35 ($C_{5'}$), 108.30 ($C_1$), 126.94 ($C_{3'}$), 136.91 ($C_{4'}$), 152.43 ($C_{2'}$); elemental analysis: (calculated for $C_{10}H_{12}O_2SICl$) C, 33.49%; H, 3.37% (found) C, 33.57%; H, 3.47%.



c. 1-Phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (**6a**)

1-Phenyl-1,3,8-triazospiro[4.5]decan-4-one (3.00 g, 12.90 mmol), dichloromethane (210 ml), di-*tert*-butyl dicarbonate (3.70 g, 14.2 mmol) and freshly distilled triethylamine (2.17 ml, 0.155 mmol) were added to a 500 ml

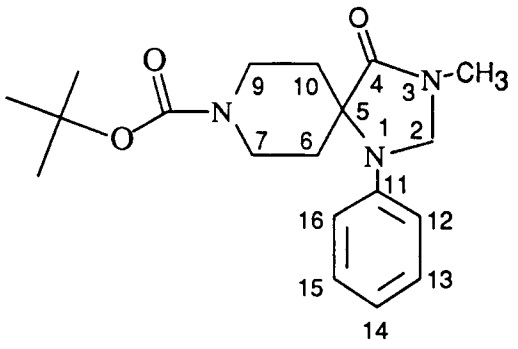
round-bottomed flask equipped with a magnetic stirring bar. The resulting clear yellow mixture was stirred at room temperature for 24 hours and then the volatile components were removed under reduced pressure, leaving behind a yellow solid. The product was purified by recrystallization [30 ml dichloromethane/ethyl acetate (1:1)] to give 3.93 g (11.8 mmol, 91.5%) of the desired product as a white solid and the chemical and spectroscopic properties correlated with those reported in literature; mp = 208-210°C (lit. mp = 209-212°C); ^{1}H-NMR ($CDCl_3$): δ 1.51 (s, 9H, -($C\underline{H}_3)_3$), 1.81 (d, 2H, J = 13.70 Hz, H_6(eq) and H_7(eq), 2.45-2.95 (m, 2H, H_6(ax) and H_{10}(ax)), 3.12-4.26 (m, 4H, H_7 and H_9), 4.77 (s, 2H, H_2), 6.70-7.40 (m, 5H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 28.56 (-C-($\underline{C}H_3)_3$), 28.83 (C_6 and C_{10}), 40.75 (C_7 and C_9), 59.94 (C_2), 79.75 (-$\underline{C}$-$(CH_3)_3$), 114.96 (C_{12} and C_{16}), 119.14 (C_{14}), 129.43 (C_{13} and C_{15}), 142.97 (C_{11}), 155.27 ($O_2\underline{C}$-N), 178.08 (C_4).



d. 3-Methyl-1-phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (**6b**).

1-Phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (732 mg, 2.21 mmol) was dissolved to anhydrous tetrahydrofuran (30 ml) in an

argon-flushed, 100 ml round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and a gas outlet. To this solution was added solid sodium hydride (98 mg, 2.4 mmol, 60% in mineral oil) and evolution of gas was immediately observed. The mixture was stirred at room temperature for 10 minutes and then methyl iodide (145 µl, 2.21 mmol) was added via an Eppendorf pipet. Stirring continued for another 2 hours, after which time the reaction was quenched with 5.0 ml water. The clear and colorless mixture was poured into a separatory funnel and the product was extracted into dichloromethane (3 x 20 ml). The organic layers were combined, dried over anhydrous sodium sulfate, filtered by gravity filtration and the solvent removed by vacuum to give 810 mg of a clear, colorless oil. Thin layer chromatography (mobile phase = dichloromethane/ethyl acetate (1:1); stationary phase = silica) revealed a major component with an R_f= 0.77, corresponding to the methylated compound, and a minor component with an R_f= 0.48, corresponding to starting material. Purification was accomplished via column chromatography (same conditions as given for the TLC) to yield 680 mg (1.97 mmol, 89.1%) of the desired compound as a white solid; mp 164-165°C; ^{1}H-NMR ($CDCl_3$): δ 1.51 (s, 9H, (-C($C\underline{H}_3$)$_3$), 1.80 (d, 2H, J = 13.70 Hz, H_6(eq) and H_7(eq)), 2.35-2.80 (m, 2H, H_6(ax) and H_{10}(ax)), 3.01 (s, 3H, N-$C\underline{H}_3$), 3.26-4.10 (m, 4H, H_7 and H_9), 4.69 (s, 2H, H_2), 6.69-7.40 (m, 5H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 28.56 (-C($\underline{C}H_3$)$_3$), 29.05 (C_6 and C_{10}), 40.65 (C_7 and C_9), 60.39 (C_2), 65.18 (N-$\underline{C}H_3$), 79.67 (-$\underline{C}$(CH$_3$)$_3$), 114.64 (C_{13} and C_{15}), 118.92 (C_{14}), 129.43 (C_{13} and C_{15}), 142.68 (C_{11}), 155.25 ($O_2\underline{C}$-N), 174.26 (C_4); elemental analysis: (calculated) C, 66.06%; H, 7.88%; N, 12.16% (found) C, 66.00%; H, 7.91%; N, 12.17%.



e. 3-Ethyl-1-phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5] decan-4-one (**6c**)

The procedure was followed as described on page 182 for the formation of 3-methyl-1-phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one. 1-Phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (732 mg, 2.20 mmol) was dissolved in tetrahydrofuran (20 ml, anhydrous) and treated with sodium hydride (200 mg, 5.0 mmol, 60% in mineral oil) followed by iodoethane (185 µl, 2.31 mmol). The reaction mixture was stirred at room temperature for 6.0 hours and 648 mg of the product (1.80 mmol, 81.8%) was obtained after purification as a clear, coloress oil; ^{1}H-NMR ($CDCl_3$): δ 1.24 (t, 3H, J=7.30 Hz, -$NCH_2C\underline{H}_3$), 1.80 (d, 2H, J=13.70 Hz, H_6(eq) and H_{10}(eq)), 1.55 (s, 9H, -C-($C\underline{H}_3)_3$), 2.30-2.76 (m, 2H, H_6(ax) and H_{10}(ax)), 3.30-4.10 (m, 4H, H_7 and H_9), 3.45 (q, 2H, J=7.30Hz, $NC\underline{H}_2CH_3$), 4.66, (s, 2H, H_2), 6.69-7.35, (m, 5H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 12.64 (-$NCH_2\underline{C}H_3$), 28.83 (-$C(\underline{C}H_3)_3$), 29.64 (C_6 and C_{10}), 35.60 ($N\underline{C}H_2CH_3$), 40.64 (C_7 and C_9), 60.57 (C_2), 79.48 (-$\underline{C}(CH_3)_3$), 114.64 (C_{12} and C_{16}), 118.81 (C_{14}), 129.27 (C_{13} and C_{15}), 142.71 (C_{11}), 155.11 ($O_2\underline{C}$-N), 173.73 (C_4); elemental analysis:

(calculated for $C_{19}H_{27}O_3N_3$) C, 66.83%; H, 8.13%; N, 11.69% (found) C, 67.09%; H, 8.26%; N, 11.42%.

f. 1-Phenyl-3-propyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro [4.5]decan-4-one (6d).

The procedure was followed as described on page 182 for the formation of 3-methyl-1-phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one. 1-Phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (551 mg, 1.66 mmol) was dissolved in tetrahydrofuran (20 ml, anhydrous) and was treated with sodium hydride (122 mg, 3.1 mmol, 60% in mineral oil) followed by 1-iodopropane (356 µl, 3.65 mmol). The reaction mixture was stirred at 40°C for 7.0 hours and 527 mg of the product (1.39 mmol, 83.7%) was obtained after purification as a clear, coloress oil; ^{1}H-NMR ($CDCl_3$): δ 0.96 (t, 3H, J=7.32Hz, $N(CH_2)_2C\underline{H}_3$), 1.51-1.82 (m, 13H, $NCH_2C\underline{H}_2CH_3$, H_6(eq), H_{10}(eq) and $C(C\underline{H}_3)_3$), 2.37-2.79 (m, 2H, H_6(ax) and H_{10}(ax)), 3.25-4.10 (m, 6H, H_7, H_9 and $NC\underline{H}_2CH_2$-), 4.69 (2, 2H, H_2), 6.69-7.25 (m, 5H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ11.23 ($N(CH_2)_2\underline{C}H_3$), 20.76 ($NCH_2\underline{C}H_2$-), 28.45 ($C(\underline{C}H_3)_3$), 28.99 ($C_6$ and C_{10}), 40.10 (C_7 and C_9), 42.43 ($N\underline{C}H_2CH_2$-), 63.39 (C_2), 79.59 ($\underline{C}(CH_3)_3$), 114.59 (C_{12} and C_{16}), 118.76 (C_{14}), 129.34 (C_{13} and C_{15}), 142.71 (C_{11}), 155.17 (-$O_2\underline{C}$-N), 174.18 (C_4); elemental analysis: (calculated for $C_{20}H_{29}O_3N_3$) H, 67.53%; H, 8.37%; N, 11.25% (found) C, 67.40%; H, 8.52%; N, 11.11%.

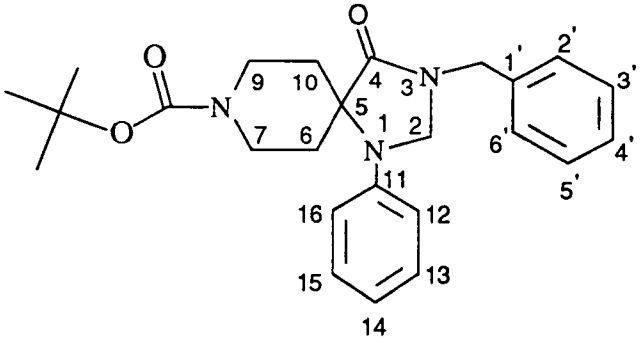
g. 3-Butyl-1-phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro [4.5]decan-4-one (6e)

The procedure was followed as described on page 182 for the formation of 3-methyl-1-phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one. 1-Phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (600 mg, 1.81 mmol) was dissolved in tetrahydrofuran (20 ml, anhydrous) and was treated with sodium hydride (248 mg, 6.20 mmol, 60% in mineral oil) followed by 1-iodobutane (612 µl, 5.43 mmol). The reaction mixture was stirred at 55°C for 14.0 hours and 684 mg of the product (1.76 mmol, 97%) was obtained, after purification, as a clear, coloress oil; ^{1}H-NMR ($CDCl_3$): δ 0.95 (t, 3H, J=6.85 Hz, $N(CH_2)_2C\underline{H}_3$), 1.20-1.75 (m, 15H, $-(C\underline{H}_2)_2CH_3$, H_6(eq) and H_{10}(eq) and $-C(C\underline{H}_3)_3$), 2.35-2.75 (m, 2H, H_6(ax) and H_{10}(ax)), 3.25-3.20 (m, 4H, H_7 and H_9), 4.68 (s, 2H, H_2), 6.68-7.32 (m, 5H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 13.75 ($N-(CH_2)_3\underline{C}H_3$), 20.05 ($N(CH_2)_2\underline{C}H_2-$), 28.56 ($-C(\underline{C}H_3)_3$), 29.04 ($C_6$ and C_{10}), 29.45 ($N-CH_2\underline{C}H_2-$), 40.91 (C_7 and C_9), 42.38 ($N\underline{C}H_2CH_2-$), 63.47 (C_2), 79.65 ($-\underline{C}(CH_3)_3$), 114.62 (C_{12} and C_{16}), 118.86 (C_{14}), 129.41 (C_{13} and C_{15}), 142.76 (C_{11}), 155.98 (O_2CN-), 174.18 (C_4); elemental analysis: (calculated for $C_{21}H_{31}O_3N_3$) C, 68.21%; H, 8.58%; N, 10.84% (found) C, 68.50%; H, 8.51%; N, 10.88%.

h. 3-Benzyl-1-phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (6f)

The procedure was followed as described on page 182 for the formation of 3-methyl-1-phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one. 1-Phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (300 mg,

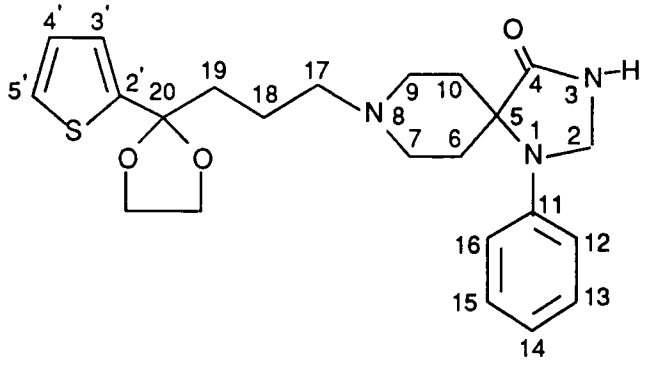
0.91 mmol) was dissolved in tetrahydrofuran (20 ml, anhydrous) and was treated with sodium hydride (73 mg, 1.8 mmol, 60% in mineral oil) followed by benzyl bromide (0.22 µl, 1.0 mmol). The reaction mixture was stirred at room temperature for 4.0 hours and 359 mg of the product (0.86 mmol, 94%) was obtained after purification as a white solid; mp = 102-103°C; ^{1}H-NMR ($CDCl_3$): δ 1.55 (C, 9H, C($C\underline{H}_3$)$_3$), 1.80 (d, 2H, J = 13.70 Hz, H_6(eq) and H_{10}(eq)), 2.35-2.76 (m, 2H, H_6(ax) and H_{10}(ax)), 3.26-4.20 (m, 4H, H_9 and H_7), 4.55 (s, 2H, $C\underline{H}_2$Ph), 4.68 (s, 2H, H_2), 6.70-7.42, (m, 10H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 28.56 (C($\underline{C}H_3$)$_3$), 29.10 (C_6 and C_{10}), 40.66 (C_7 and C_9), 44.89 ($\underline{C}H_2$Ph), 65.08 (C_2), 79.67 ($\underline{C}$(CH$_3$)$_3$), 114.64 (C_{12} and C_{16}), 118.92 (C14), 127.81 ($C_{2'}$ and $C_{6'}$), 128.01 ($C_{4'}$), 128.90 ($C_{3'}$ and $C_{5'}$), 129.43 (C_{13} and C_{15}), 135.60 ($C_{1'}$), 142.68 (C_{11}), 155.25 ($O_2\underline{C}$-N), 174.55 (C_4); elemental analysis: (calculated for $C_{22}H_{33}O_3N_3$) C, 71.23%; H, 7.41%; N, 9.97% (found) C, 71.14%; H, 7.46%; N, 9.92%.



i. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (7a)

A 100 ml round-bottomed, two-necked flask was equipped with a magnetic stirring bar, a water-cooled condenser, a thermometer held by a rubber septum and a gas outlet (oil bubbler). 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-one (2.23 g, 9.63 mmol) was added to the flask, followed by 4-chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane (2.24 g, 9.63 mmol), potassium carbonate (6.00g, 43.3 mmol), sodium iodide (290 mg, 1.90 mmol) and N,N-dimethyl formamide (35 ml, anhydrous). The mixture was stirred at 50°C under an argon atmosphere for 48 hours, resulting in a yellow solution containing a white precipitate. The reaction mixture was cooled to 0°C, diluted with 60 ml of water and stirred genty for 2 minutes. The white solid that remained undissolved was filtered from the water and dimethylformamide mixture by vaccuum filtration, dissolved in a minimal amount of dichloromethane (25 ml) and dried over anhydrous sodium sulfate. Thin layer chromatography [stationary phase = alumina, mobile phase = dichloromethane / ethanol (50:2)] showed one major component (R_f= 0.40) corresponding to the desired product, and a second spot (R_f= 0) which might have been due to unreacted amine or degradation products. The dichloromethane solution was then applied directly to a silica column and the product eluted with a mixture of dichloromethane and ethanol (50:2) to provide 3.50 g (8.2 mmol, 85%) of the product as a white solid; mp= 161-162°C; ^{1}H-NMR ($CDCl_3$): δ 1.55-1.80 (m, 4H, H_6(eq), H_{10}(eq) and H_{18}), 2.0-2.15 (m, 2H, H_{19}), 2.43 (t, 2H, J= 6.95Hz, H_{17}), 2.55-2.90 (m, 6H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.90-4.11 (m, 4H, -OC$\underline{H_2}$C$\underline{H_2}$O-), 4.69 (s, 2H, H_2), 6.78-7.35 (m, 8H, aromatic H); ^{13}C-NMR

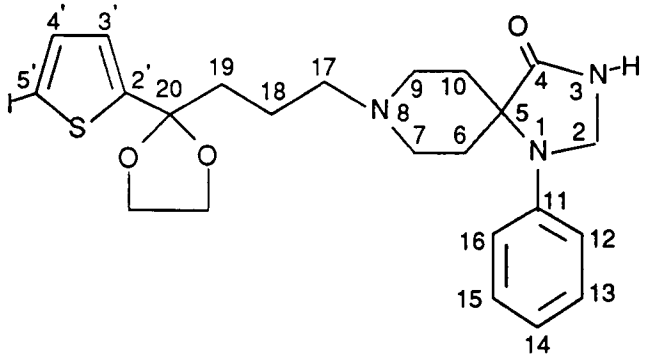
($CDCl_3$): δ21.44 (C_{18}), 29.00 (C_6 and C_{10}), 38.59 (C_{19}), 49.59 (C_{17}), 59.48 (C_7 and C_9), 57.93 (C_5), 60.11 (C_2), 64.95 (-O<u>C</u>H_2<u>C</u>H_2O-), 109.17 (C_{20}), 115.46 (C_{12} and C_{16}), 118.88 (C_{14}), 124.40 ($C_{5'}$), 124.94 ($C_{4'}$), 126.73 ($C_{3'}$), 129.20 (C_{13} and C_{15}), 143.17 (C_{11}), 146.64 ($C_{2'}$), 178.47 (C_4); MS (FAB) m/e (rel intensity) 428 (100, M+1); elemental analysis: (calculated for $C_{23}H_{29}O_3N_3S$) C, 64.61%; H, 6.84%; N, 9.83%; S, 7.50% (found) C, 64.54%; H, 6.87%; N, 9.84%; S, 7.41%.



j. <u>8-[4,4-Ethylenedioxy-4-(5-iodo-2-thienyl)butyl]1,3,8-triazaspiro[4.5]decan-4-one (**9a**)</u>

A 100 ml round-bottomed, two-necked flask was equipped with a magnetic stirring bar, a water-cooled condenser, a thermometer and a gas outlet (oil bubbler). 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-one (580 mg, 1.62 mmol) was added to the flask, followed by 4-chloro-1,1-(ethylenedioxy)-1-[2-(5-iodothienyl])butane (374 mg, 1.62 mmol), potassium carbonate (1.00 g, 7.28 mmol), sodium iodide (48.5 mg, 0.32 mmol) and N,N-dimethyl formamide (20

ml, anhydrous). The mixture was stirred at 50°C under an argon atmosphere for 48 hours, resulting in a light yellow solution containing a fair amount of white precipitate. The reaction mixture was cooled to 0°C, dilluted with 60 ml of water and stirred genty for 2 minutes. All undissolved solids were then filtered from the water and dimethylformamide by vacuum filtration, dissolved in a minimal amount of dichloromethane (20 ml) and dried over anhydrous sodium sulfate. Thin layer chromatography [stationary phase = alumina, mobile phase = dichloromethane / ethanol (50:2)] showed one major component (R_f= 0.48) corresponding to the desired product, and a second spot (R_f= 0) which might have been due to unreacted amide or degradation products. The dichloromethane solution was then applied directly to a silica column and the product eluted with a mixture of dichloromethane and ethanol (50:2) to provide 797 mg (1.44 mmol, 88.9%) of the product as a white solid; mp = 191-193 °C; ^{1}H-NMR ($CDCl_3$): δ 1.56-1.80 (m, 4H, H_6(eq), H_{10}(eq) and H_{18}), 1.97-2.14 (m, 2H, H_{19}), 2.42 (t, 2H, H= 6.94Hz, H_{17}), 2.55-2.90 (m, 6H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.90-4.10 (m, 4H, -OC$\underline{H}_2$C$\underline{H}_2$O-), 4.72 (s, 1H, H_2), 6.70 (d, 1H, J= 3.59Hz, $H_{3'}$), 6.83-6.92 (m, 3H, H_{12}, H_{14}, and H_{16}), 7.10 (d, 1H, J= 3.59Hz, $H_{4'}$); 7.20-7.32 (m, 3H, H_{13}, H_{15} and N-$\underline{H}$); ^{13}C-NMR ($CDCl_3$): δ 21.79 (C_{18}), 29.20 (C_6 and C_{10}), 38.78 (C_{19}), 49.96 (C_{17}), 59.62 (C_7 and C_9), 59.79 (C_2), 65.40 (-O$\underline{C}$H$_2$$\underline{C}H_2$O-), 73.01 ($C_{5'}$), 109.09 ($C_{20}$), 115.70 ($C_{12}$ and C_{16}), 119.14 (C_{14}), 126.40 ($C_{3'}$), 129.32 (C_{13} and C_{15}), 136.85 ($C_{4'}$), 143.22 (C_{11}), 152.60 ($C_{2'}$), 178.25 (C_4); MS (FAB) m/e (rel intensity) 554 (100, M+1); elemental analysis: (calculated for $C_{24}H_{28}O_3N_3SI$) C, 49.91%; H, 5.10%; N, 7.59%; S, 5.76%; I, 22.93% (found) C, 49.84%; H, 5.11%; N, 7.44%; S, 5.76%; I, 23.08%.



k. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**7b**)

Method A: 3-Methyl-1-phenyl-8-(*tert*-butoxycarbonyl)-1,3,8-triazaspiro-[4.5] decan-4-one (494 mg, 1.43 mmol) was placed into a 100 ml round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and a gas outlet. The flask was flushed with argon and 10 ml of anhydrous HCl in dioxane (4.0 M, 40 mmol) was added via syringe. The resulting solution solution was stirred for 1.5 hours and then all volatile components were removed under vacuum. The remaining yellow solid was mixed with 20 ml of an aqueous, saturated sodium carbonate solution and the product extracted into dichloromethane (3 x 20 ml). The organic layers were combined, dried over anhydrous sodium sulfate and the solvent removed under vacuum to give 320 mg (1.31 mmol, 91.2%) of a yellow crystalline solid. This solid was not characterized but was used directly in the next synthetic step. A 100 ml round-bottomed, two-necked flask was equipped with a magnetic stirring bar, a water-cooled condenser, a thermometer and a gas outlet (oil bubbler). To this flask was added 3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (297 mg, 1.21

mmol), 4-chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane (333 mg, 1.43 mmol), potassium carbonate (606 mg, 5.72 mmol), potassium iodide (47.5 mg, 0.290 mmol) and N,N-dimethyl formamide (25 ml, anhydrous). This mixture stirred at 60°C under an argon atmosphere for 24 hours, resulting in a yellow solution containing a white precipitate. The reaction mixture was cooled to room temperature, diluted with 30 ml of distilled water and then the product was extracted into dichloromethane (3 X 25 ml). The organic layers were combined, dried over anhydrous sodium sulfate, decanted, and the solvent removed under vacuum to give 520 mg of a yellow oil. Thin layer chromatography [stationary phase = alumina, mobile phase = dichloromethane / ethanol (50:2)] showed one major component (R_f= 0.69) corresponding to the desired product, and a second spot (R_f= 0) which might have been due to unreacted amine or degradation products. The dichloromethane solution was then applied directly to a silica column and the product eluted with a mixture of dichloromethane and ethanol (50:2) to provide 452 mg (1.02 mmol, 84.6%) of the product as a white solid.

Method B: 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one was dissolved in tetrahydrofuran (10 ml, anhydrous) in an argon-flushed, 100 ml round-bottomed flask fitted with a magnetic stirring bar, a rubber septum and a gas outlet. Sodium hydride (12 mg, 0.28 mmol) was then added and evolution of gas was immediately observed. The mixture was stirred at room temperature for 30 minutes and then iodomethane (15 µl, 0.25 mmol) was added via Eppendorf pipet. Stirring continued for another 3 hours and the reaction mixture remained clear and colorless throughout the reaction period. The reaction was quenched by the addition of 10 ml of water and the product

was extracted into dichloromethane (3 x 15 ml). The organic layers were combined, dried over anhydrous sodium sulfate and the solvent evaporated to give a light yellow solid. The product was purified by column chromatography [stationary phase = alumina; mobile phase = dichloromethane / ethanol (50 : 1)] to provide 79 mg (0.17 mmol, 77%) of the product as a white solid.

Product Characterization: mp = 127-128°C; ^{1}H-NMR ($CDCl_3$): δ 1.58-1.80 (m, 4H, H_6(eq), H_{10}(eq) and H_{18}), 2.10-2.15 (m, 2H, H_{19}), 2.42 (t, 2H, J= 6.94 Hz, H_{17}), 2.97 (s, 3H, N-CH_3), 2.55-2.90 (m, 6H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.90-4.10 (m, 4H, -OCH_2CH_2O-), 4.66 (s, 2H, H_2), 6.80-7.40 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 21.52 (C_{18}), 29.43 (C_6 and C_{10}), 38.72 (C_{19}), 49.62 (C_{17}), 58.28 (C_7 and C_9), 60.50 (C_2), 109.20 (C_{20}), 115.16 (C_{12} and C_{16}), 118.76 (C_{14}), 124.53 ($C_{5'}$), 125.04 ($C_{4'}$), 126.83 ($C_{3'}$), 129.32 (C_{13} and C_{15}), 142.94 (C_{11}), 146.79 ($C_{2'}$), 174.61 (C_4); elemental analysis: (calculated for $C_{24}H_{31}O_3N_3S$) C, 65.28%; H, 7.08%; N, 9.52%; S, 7.26% (found) C, 65.39%; H, 7.11%; N, 9.46%; S, 7.20%.

l. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-3-ethyl-1-phenyl-1, 3,8-triazaspiro[4.5]decan-4-one (**7c**)

Method A: The procedure was carried out as described on page 188 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5b**. **6c** (423 mg, 1.18 mmol) was reacted with anhydrous HCl (4.0 M in dioxane, 12.0 ml, 48.0 mmol) at room temperature for 1.0 hour to provide 291 mg of 3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (1.12 mmol, 95.4%) as a white solid. Next, a 200 mg portion of 3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.77 mmol) was reacted with 4-

chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane (180 mg, 0.77 mmol), potassium iodide (26 mg, 0.15 mmol) and potassium carbonate (515 mg, 4.86 mmol) in anhydrous dimethylformamide (10 ml) at 70 °C for 14 hours. After purification, 270 mg (0.770 mmol, 65.3%) of the product was obtained as a white solid.

Method B: The reaction was carried out as described on page 189 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (400 mg, 0.94 mmol) was dissolved in anhydrous tetrahydrofuran (10 ml,) and treated with sodium hydride (40 mg, 1.0 mmol) followed by iodoethane (76 µl, 1.1 mmol). The resulting mixture was stirred at room temperature for 18 hours and, after purification, 380 mg (0.83 mmol, 89%) of the product was obtained as a white solid.

Product Characterization: mp = 100.5-102°C; ^{1}H-NMR ($CDCl_3$): δ 1.22 (t, 3H, J= 7.26 Hz, -$NCH_2C\underline{H}_3$), 1.60-1.80 (m, 4H, H_6(eq), H_7(eq) and H_{18}), 2.10-2.19 (m, 2H, H_{17}), 2.44 (t, 2H, J= 6.92 Hz, H_{19}), 2.55-2.90 (m, 6H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.47 (q, 2H, J= 7.26 Hz, -$NC\underline{H}_2CH_3$), 3.90-4.10 (m, 4H, -$OC\underline{H}_2C\underline{H}_2O$-), 4.66 (s, 2H, H_2), 6.81-7.40 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 12.72 (-$NCH_2\underline{C}H_3$), 21.49 (C_{18}), 29.37 (C_6 and C_{10}), 35.60 (-$N\underline{C}H_2CH_3$), 38.72 (C_{19}), 49.66 (C_{17}), 58.25 (C_7 and C_9), 60.82 (C_5), 62.99 (C_2), 65.07 (-$O\underline{C}H_2\underline{C}H_2O$-), 109.17 ($C_{20}$), 115.32 ($C_{12}$ and C_{16}), 118.81 (C_{14}), 124.50 ($C_{5'}$), 125.04 ($C_{4'}$), 126.83 ($C_{3'}$), 129.30 (C_{13} and C_{15}), 143.20 (C_{11}), 146.70 ($C_{2'}$), 174.21 (C_4); IR (film, cm^{-1}): 2956, 2940, 2828, 2815 (C-H stretch), 1699 (amide C=O stretch), 1600, 1502 (aryl C-C stretch), 1194, 1298 (amine C-N stretch), 1035, 1105 (C-O-C stretch), 748, 735, 685 (aryl C-H bend); elemental analysis: (calculated for $C_{25}H_{33}N_3O_3S$) C, 65.91%; H, 7.30%; N,

9.22%; S, 7.04% (found) C, 65.81%; H, 7.32%; N, 9.21%; S, 6.97%.

m. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one (**7d**)

Method A: The procedure was carried out as described on page 188 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one except that the HCl salt of 1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one was obtained as the neutralization step was omitted. **6d** (525 mg, 1.41 mmol) was reacted with anhydrous HCl (4.0 M, in dioxane, 10 ml, 40.0 mmol) at room temperature for 1.0 hours to provide 431 mg of the HCl salt of 1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one (1.39 mmol, 98.9%) as a white powder. Next, a 230 mg portion of 1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one hydrochloride (0.74 mmol) was reacted with 4-chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane (172 mg, 0.74 mmol), potassium iodide (26 mg, 0.15 mmol) and potassium carbonate (530 mg, 3.70 mmol) in anhydrous dimethylformamide (10 ml) at 70 °C for 16 hours. After purification, 595 mg (1.23 mmol, 71.1%) of the product was obtained as a clear, colorless, viscous oil.

Method B: The reaction was carried out as described on page 189 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (150 mg, 0.35 mmol) was dissolved in tetrahydrofuran (8 ml, anhydrous) and treated with sodium hydride (42 mg, 1.05 mmol) followed by 1-iodopropane (168 µl, 1.76 mmol). The resulting mixture

was stirred at room temperature for 24 hours and, after purification, 139 mg (0.31 mmol, 84.4%) of the product was obtained as a clear, colorless oil. Product Characterization: ^{1}H-NMR ($CDCl_3$): δ 0.98 (t, 3H, J= 6.83 Hz, -N(CH_2)$_2$C$\underline{H}_3$), 1.60-1.85 (m, 6H, H_6(eq), H_{10}(eq), H_{18} and -NCH$_2$C$\underline{H}_2$-), 2.10-2.15 (m, 2H, H_{19}), 2.45 (t, 2H, J= 6.92 Hz, H_{17}), 2.55-2.90 (m, 6H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.41 (t, 2H, J= 6.84 Hz, -NC$\underline{H}_2$CH$_2$-), 3.90-4.10 (m, 4H, -OC$\underline{H}_2$C$\underline{H}_2$O-), 4.67 (s, 2H, H_2), 6.82-7.42 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 11.28 (-N(CH_2)$_2$C$\underline{H}_3$), 20.79 (-NCH$_2$C$\underline{H}_2$-), 21.46 (C_{18}), 29.48 (C_6 and C_{10}), 38.69 (C_{19}), 42.43 (-NC$\underline{H}_2$CH$_2$-), 49.63 (C_{17}), 58.25 (C_7 and C_9), 60.79 (C_5), 63.45 (C_2), 65.05 (-OC$\underline{H}_2$C$\underline{H}_2$O-), 109.17 (C_{20}), 115.21 (C_{12} and C_{16}), 118.73 (C_{14}), 124.48 ($C_{5'}$), 124.99 ($C_{4'}$), 126.81 ($C_{3'}$), 129.27 (C_{13} and C_{15}), 143.06 (C_{11}), 146.50 ($C_{2'}$), 174.59 (C_4); IR (film, cm^{-1}): 2960, 2927, 2880, 2829 (C-H stretch), 1700 (amide C=O stretch), 1599, 1503 (aryl C-C stretch), 1196, 1298 (amine C-N stretch), 1036, 1105 (C-O-C stretch), 748, 735, 698 (aryl C-H bend); elemental analysis: (calculated for $C_{26}H_{35}N_3O_3S$) C, 66.50%; H, 7.50%; N, 8.95%; S, 6.83% (found) C, 66.49%; H, 7.57%; N, 8.97%; S, 6.80%.

n. 3-Butyl-8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**7e**)

Method A: The procedure was carried out as described on page 188 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5b**, except that the HCl salt of 3-butyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one was obtained as the neutralization step was omitted. **6e** (684 mg, 1.77 mmol) was reacted with anhydrous HCl

(4.0 M. in dioxane, 12 ml, 48.0 mmol) at room temperature for 4.0 hours to provide 560 mg of the HCl salt of 3-butyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (1.73 mmol, 97.1%) as a white powder. Next, a 560 mg portion of 3-butyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one hydrochloride (1.73 mmol) was reacted with 4-chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane (402 mg, 1.73 mmol), potassium iodide (57 mg, 0.35 mmol) and potassium carbonate (916 mg, 8.65 mmol) in anhydrous dimethylformamide (20 ml) at 70 °C for 24 hours. After purification, 595 mg (1.23 mmol, 71.1%) of the product was obtained as a clear, colorless, viscous oil.

Method B: The reaction was carried out as described on page 189 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (189 mg, 0.44 mmol) was dissolved in anhydrous tetrahydrofuran (8 ml) and treated with sodium hydride (53 mg, 1.33 mmol) followed by 1-iodobutane (162 µl, 1.33 mmol). The resulting mixture was stirred at room temperature for 48 hours and, after purification, 136 mg (0.28 mmol, 62.8%) of the product was obtained as a clear, colorless oil.

Product Characterization: ^{1}H-NMR ($CDCl_3$): δ 0.96 (t, 3H, J= 6.96 Hz, $-CH_2C\underline{H}_3$), 1.20-1.85 (m, 8H, $-CH_2(C\underline{H}_2)_2CH_3$, H_6(eq), H_{10}(eq) and H_{18}), 2.10-2.16 (m, 2H, H_{19}), 2.52 (t, 2H, J= 6.94 Hz, H_{17}), 2.60-2.90 (m, 8H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.40 (t, 2H, J=6.35 Hz, $-NC\underline{H}_2CH_2-$), 3.90-4.10 (m, 4H, $-OC\underline{H}_2C\underline{H}_2O-$), 4.66 (s, 2H, H_2), 6.80-7.40 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 13.75 ($-CH_2C\underline{H}_3$), 20.05 ($-C\underline{H}_2CH_3$), 21.14 (C_{18}), 29.34 (C_6 and C_{10}), 29.44 ($-NCH_2\underline{C}H_2-$), 38.67 (C_{19}), 40.59 ($-N\underline{C}H_2CH_2-$), 49.61 (C_{20}), 59.22, (C_7 and C_9), 60.74 (C_5), 63.42 (C_2), 64.99 ($-O\underline{C}H_2\underline{C}H_2O-$), 109.18

(C_{20}), 115.13 (C_{12} and C_{16}), 118.68 (C_{14}), 124.45 ($C_{5'}$), 124.96 ($C_{4'}$), 126.77 ($C_{3'}$), 143.00 (C_{11}), 146.74 ($C_{2'}$), 174.42 (C_4); IR (film, cm^{-1}): 2957, 2940, 2934,2828, 2815 (C-H stretch), 1702 (amide C=O stretch), 1600, 1503 (aryl C-C stretch), 1298, 1192 (amine C-N stretch), 1105-1037 (C-O-C stretch), 747, 698 (aryl C-H bend); elemental analysis: (calculated for $C_{27}H_{37}O_3N_3S$) C, 67.05%; H, 7.71%; N, 8.69%; S, 6.63% (found) C, 67.12%; H, 7.75%; N, 8.66%; S, 6.59%.

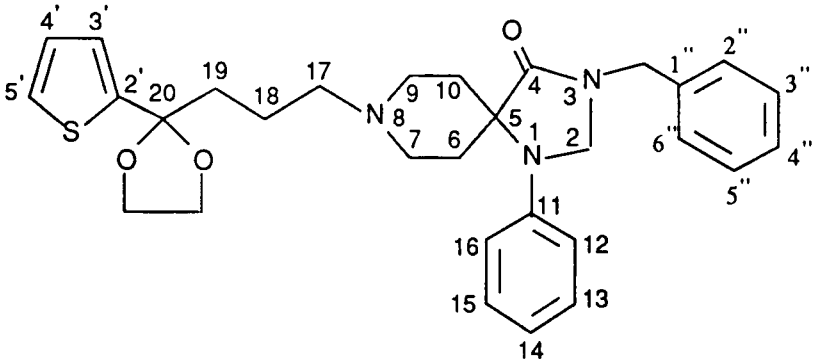
o. 3-Benzyl-8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**7f**)

Method A: The procedure was carried out as described on page 188 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5b**. **6f** (300 mg, 0.71 mmol) was reacted with anhydrous HCl (4.0 M in dioxane, 6.0 ml, 24.0 mmol) at room temperature for 2.0 hour to provide 217 mg 3-benzyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.68 mmol, 95%) as a white solid. Next, 217 mg of 3-benzyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.68 mmol) was reacted with 4-chloro-1,1-(ethylenedioxy)-1-(2-thienyl)butane (165 mg, 0.71 mmol), potassium iodide (24 mg, 0.14 mmol) and potassium carbonate (491 mg, 3.55 mmol) in anhydrous dimethylformamide (7 ml) at 65 °C for 16 hours. After purification, 251 mg (0.49 mmol, 71.3%) of the product was obtained as a clear, colorless, viscous oil.

Method B: The reaction was carried out as described on page 189 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-

1,3,8-triazaspiro[4.5]decan-4-one (400 mg, 0.94 mmol) was dissolved in anhydrous tetrahydrofuran (20 ml) and treated with sodium hydride (75 mg, 1.87 mmol) followed by benzyl bromide (92 µl, 0.98 mmol). The resulting mixture was stirred at room temperature for 4 hours and, after purification, 370 mg (0.77 mmol, 82%) of the product was obtained as a clear, colorless oil.

Product Characterization: ^{1}H-NMR ($CDCl_3$): δ 1.60-2.05 (m, 4H, H_6(eq), H_{10}(eq) and H_{18}), 2.10-2.17 (m, 2H, H_{19}), 2.53 (t, 2H, J= 6.95 Hz, H_{17}), 2.65-3.06 (m, 6H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.90-4.10 (m, 4H, -OC$\underline{H}_2$C$\underline{H}_2$O-), 4.54 (s, 2H, N-C$\underline{H}_2$Ph), 4.60 (s, 2H, H_2), 6.70-7.40 (m, 13H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 21.42 (C_{18}), 29.08 (C_6 and C_{10}), 38.41 (C_{19}), 44.83 ($\underline{C}H_2$Ph), 49.59 (C_{17}), 59.46 (C_7 and C_9), 59.51 (C_5), 62.99 (C_2), 65.10 (-O$\underline{C}H_2\underline{C}H_2$O-), 109.12 ($C_{20}$), 115.46 ($C_{12}$ and C_{16}), 118.86 (C_{14}), 124.40 ($C_{5'}$), 124.99 ($C_{4'}$), 126.71 ($C_{3'}$), 127.84 ($C_{2''}$ and $C_{6''}$), 127.98 ($C_{4''}$), 128.89 ($C_{3''}$ and $C_{5''}$), 129.21 (C_{13} and C_{15}), 135.61 ($C_{1''}$), 143.17 (C_{11}), 146.64 ($C_{2'}$), 174.58 (C_4); elemental analysis: (calculated for $C_{30}H_{35}O_3N_3S$) C, 69.60%; H, 6.82%; N, 8.12%; S, 6.19% (found) C, 69.35%; H, 6.88%; N, 7.99%; S, 6.09%.



p. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**9b**)

Method A: The procedure was carried out as described on page 188 for the preparation of 8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5b**. **6b** (310 mg, 0.90 mmol) was reacted with anhydrous HCl (4.0 M, in dioxane, 12.0 ml, 48.0 mmol) at room temperature for 0.5 hour to provide 218 mg of 1-phenyl-3-methyl-1,3,8-triazaspiro[4.5]decan-4-one (0.89 mmol, 99%) as a white solid. Next, 220 mg of 1-phenyl-3-methyl-1,3,8-triazaspiro[4.5]decan-4-one (0.89 mmol) was reacted with 4-chloro-1,1-(ethylenedioxy)-1-[2-(5-iodothienyl)]butane (336 mg, 0.891 mmol), potassium iodide (35 mg, 0.21 mmol) and sodium carbonate (333 mg, 3.14 mmol) in anhydrous dimethylformamide (6.0 ml) at 60 °C for 20 hours. After purification, 493 mg (0.751 mmol, 84.6%) of the product was obtained as a white solid.

Method B: The reaction was carried out as described on page 189 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (100 mg, 0.18 mmol) was dissolved in tetrahydrofuran (8 ml, anhydrous) and treated with sodium hydride (14 mg, 0.35 mmol) followed by iodomethane (20 µl, 0.22 mmol). The resulting mixture was stirred at room temperature for 1.0 hour and, after purification, 80 mg (0.14 mmol, 78%) of the product was obtained as a white solid.

Product Characteriztion: mp = 133.7-135 C; ^{1}H-NMR($CDCl_3$): δ 1.55-1.85 (m, 4H, H_6(eq), H_{10}(eq) and H_{18}), 1.97-2.14 (m, 2H, H_{19}), 2.41 (t, 2H, J= 6.94 Hz, H_{17}), 2.55-2.99 (m, 9H, H_6(ax), H_7, H_9, H_{10}(ax) and N-CH_3), 3.90-4.10 (m, 4H,

-OC$\underline{H_2}$C$\underline{H_2}$O-), 4.72 (s, 2H, H_2), 6.70 (d, 1H, J= 3.59 Hz, $H_{3'}$), 6.80-6.92 (m, 3H, H_{12}, H_{14} and H_{16}), 7.10 (d, 1H, J= 3.59 Hz, $H_{4'}$), 7.20- 7.32 (m, 2H, H_{13} and H_{15}); ^{13}C-NMR ($CDCl_3$): δ 21.35 (C_{18}), 27.53 (N-$\underline{C}H_3$), 29.37 (C_6 and C_{10}), 38.49 (C_{19}), 49.55 (C_{17}), 58.17 (C_7 and C_9), 60.44 (C_2), 65.07 (-O$\underline{C}H_2\underline{C}H_2$O-), 73.01 ($C_{5'}$), 108.74 ($C_{20}$), 115.16 ($C_{12}$ and C_{16}), 118.79 (C_{14}), 126.03 ($C_{3'}$), 129.32 (C_{13} and C_{15}), 136.80 ($C_{4'}$), 142.89 (C_{11}), 152.89 ($C_{2'}$), 174.53 (C_4); elemental analysis: (calculated for $C_{24}H_{30}O_3N_3SI$) C, 50.80%; H, 5.33%; N, 7.40%; S, 5.65%; I, 22.36% (found) C, 50.72%; H, 5.40%; N, 7.32%; S, 5.72%; I, 22.44%.

q. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**9c**)

Method A: The procedure was carried out as described on page 188 for the preparation of 8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5b**. **6c** (136 mg, 0.38 mmol) was reacted with anhydrous HCl (4.0 M, in dioxane, 8.0 ml, 32.0 mmol) at room temperature for 1.0 hour to provide 93 mg of 3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.37 mmol, 98%) as a white solid. Next, 93 mg of 3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.37 mmol) was reacted with 4-chloro-1,1-(ethylenedioxy)-1-[2-(5-iodothienyl)]butane (141 mg, 0.37 mmol), potassium iodide (50 mg, 0.30 mmol) and sodium carbonate (364 mg, 2.63 mmol) in anhydrous dimethylformamide (30.0 ml) at 60°C for 20 hours. After purification, 137 mg (0.240 mmol, 64.8%) of the product was obtained as a white solid.

Method B: The reaction was carried out as described on page 189 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro [4.5]decan-4-one (200 mg, 0.36 mmol) was dissolved in anhydrous tetrahydrofuran (6 ml) and treated with sodium hydride (21 mg, 0.53 mmol) followed by iodoethane (64 µl, 0.79 mmol). The resulting mixture was stirred at room temperature for 14 hours and, after purification, 170 mg (0.29 mmol, 80%) of the product was obtained as a white solid.

Product Characterization: mp = 127-128°C; ^{1}H-NMR ($CDCl_3$): δ 1.23 (t, 3H, J= 7.33 Hz, -$NCH_2C\underline{H}_3$), 1.55-1.80 (m, 4H, H_6(eq), H_{10}(eq) and H_{18}), 1.96-2.15 (m, 2H, H_{19}), 2.43 (t, 2H, J= 6.95 Hz, H_{17}), 2.55-3.00 (m, 6H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.47 (q, 2H, J= 7.33 Hz, -$NC\underline{H}_2CH_3$), 3.90-4.10 (m, 4H, -$OC\underline{H}_2C\underline{H}_2O$-), 4.72 (2, 2H, H_2), 6.70 (d, 1H, J= 3.60, $H_{3'}$), 6.80-6.92 (m, 3H,. H_{12}, H_{14} and H_{16}), 7.10 (d, 1H, J= 3.60, $H_{4'}$), 7.20-7.35 (m, 2H, H_{13} and H_{15}); ^{13}C-NMR ($CDCl_3$): δ 12.72 (-$NCH_2\underline{C}H_3$), 21.44 (C_{18}), 29.40 (C_6 and C_{10}), 35.61 (-$N\underline{C}H_2CH_3$), 38.50 (C_{19}), 49.60 (C_{17}), 58.22 (C_7 and C_9), 60.85 (C_5), 62.99 (C_2), 65.10 (-$O\underline{C}H_2\underline{C}H_2O$-), 73.01 ($C_{5'}$), 108.79 ($C_{20}$), 115.40 ($C_{12}$ and C_{16}), 118.87 (C_{14}), 126.10 ($C_{3'}$), 129.30 (C_{13} and C_{15}), 136.83 ($C_{4'}$), 143.11 (C_{11}), 152.94 ($C_{2'}$), 174.21 (C_4); elemental analysis: (calculated for $C_{25}H_{32}O_3N_3SI$) C, 51.64%; H, 5.55%; N, 7.23%; S, 5.51%; I, 21.82%; (found) C, 51.65%; H, 5.61%; N, 7.16%; S, 5.57%; I, 21.78%.

r. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one (**9d**)

Method A: The procedure was carried out as described on page 188 for

the preparation of 8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5b**. **6d** (290 mg, 0.780 mmol) was reacted with anhydrous HCl (4.0 M in dioxane, 10.0 ml, 40.0 mmol) at room temperature for 1.5 hour to provide 210 mg of 1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one (0.77 mmol, 98.9%) as a white solid. Next, 210 mg 1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one (0.77 mmol) was reacted with 4-chloro-1,1-(ethylenedioxy)-1-[2-(5-iodothienyl)]butane (288 mg, 0.770 mmol), potassium iodide (26 mg, 0.15 mmol) and sodium carbonate (285 mg, 2.69 mmol) in anhydrous dimethylformamide (6.0 ml) at 60°C for 21 hours. After purification, 279 mg (0.470 mmol, 61.0%) of the product was obtained as a clear, colorless oil.

Method B: The reaction was carried out as described on page 189 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (150 mg, 0.270 mmol) was dissolved in anhydrous tetrahydrofuran (8 ml) and treated with sodium hydride (33 mg, 0.83 mmol) followed by 1-iodopropane (136 µl, 1.42 mmol). The resulting mixture was stirred at room temperature for 66 hours and, after purification, 135 mg (0.226 mmol, 83.7%) of the product was obtained as a clear, colorless oil.

Product Characterization: ^{1}H-NMR ($CDCl_3$): δ 0.95 (t, 3H, J= 6.84 Hz, -N(CH_2)$_2$C$\underline{H}_3$), 1.55-1.90 (m, 6H, H_6(eq), H_{10}(eq), H_{18} and -NCH_2C$\underline{H}_2$-), 1.96-2.15 (m, 2H, H_{19}), 2.40 (t, 2H, J= 6.95 Hz, H_{17}), 2.55-3.00 (m, 6H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.39 (t, 2H, J= 6.80 Hz, -NC$\underline{H}_2$CH_2-), 3.90-4.10 (m, 4H, -OC$\underline{H}_2$C$\underline{H}_2$O-), 4.66 (s, 2H, H_2), 6.70 (d, 1H, J= 3.60 Hz, $H_{3'}$), 6.80-6.95 (m, 3H, H_{12}, H_{14} and H_{16}), 7.10 (d, 1H, J= 3.60 Hz, $H_{4'}$), 7.20-7.34 (m, 2H, H_{13} and

H_{15}); ^{13}C-NMR ($CDCl_3$): δ 11.34 (-N(CH_2)$_2$$\underline{C}H_3$), 21.41 ($C_{18}$), 28.81 (-N$CH_2\underline{C}H_2$-), 29.54 ($C_6$ and C_{10}), 38.50 (C_{19}), 42.48 (-N$\underline{C}H_2CH_2$-), 49.70 (C_{17}), 58.25 (C_7 and C_9), 60.82 (C_5), 63.50 (C_2), 65.10 (-OCH_2CH_2O-), 73.01 ($C_{5'}$), 108.97 (C_{20}), 115.27 (C_{12} and C_{16}), 118.81 (C_{14}), 126.10 ($C_{3'}$), 129.32 (C_{13} and C_{15}), 136.83 ($C_{4'}$), 143.08 (C_{11}), 153.94 ($C_{2'}$), 174.59 (C_4); IR (film, cm^{-1}): 2960, 2927, 2882, 2829 (C-H stretch), 1703 (amide C=O stretch), 1599-1510 (aryl C-C stretch), 1297, 1196 (amide C-N stretch), 1105, 1034 (C-O-C stretch), 801, 749, 688 (aryl C-H bend); elemental analysis: (calculated for $C_{26}H_{34}O_3N_3SI$) C, 52.43%; H, 5.75%; N, 7.06%; S, 5.38%; I, 21.31% (found) C, 52.23%; H, 5.86%; N, 6.91%; S, 5.45%; I, 21.08%.

s. 3-Butyl-8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**9e**)

Method A: The procedure was carried out as described on page 188 for the preparation of 8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. **6e** (339 mg, 0.880 mmol) was reacted with anhydrous HCl (4.0 M in dioxane, 20.0 ml, 80 mmol) at room temperature for 4.0 hour to provide 244 mg of 3-butyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.855 mmol, 97.1%) as a white solid. Next, 150 mg of 3-butyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (0.52 mmol) was reacted with 4-chloro-1,1-(ethylenedioxy)-1-[2-(5-iodothienyl)]butane (196 mg, 0.52 mmol), potassium iodide (87 mg, 0.52 mmol) and potassium carbonate (577 mg, 4.17 mmol) in anhydrous dimethylformamide (10.0 ml) at 60 °C for 16 hours. After purification, 230 mg (0.33 mmol, 64 %) of the product was obtained

as a clear, colorless oil.

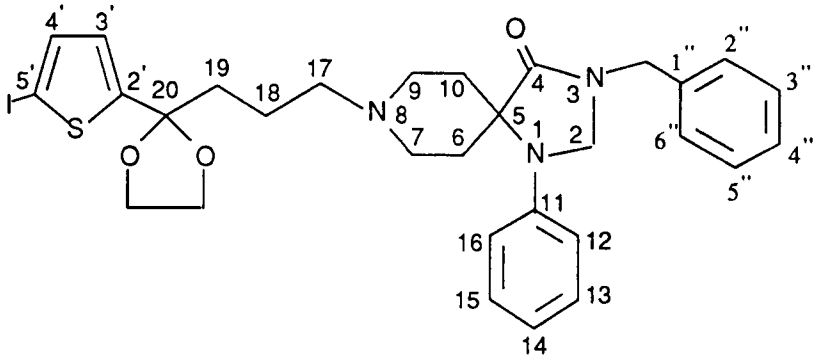
Method B: The reaction was carried out as described on page 189 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro [4.5]decan-4-one (200 mg, 0.360 mmol) was dissolved in anhydrous tetrahydrofuran (8 ml) and treated with sodium hydride (29 mg, 0.72 mmol) followed by 1-iodobutane (91 µl, 0.79 mmol). The resulting mixture was stirred at room temperature for 48 hours and, after purification, 200 mg (0.33 mmol, 92%) of the product was obtained as a clear, colorless oil.

Product Characterization: ^{1}H-NMR ($CDCl_3$): δ 0.97 (t, 3H, J= 6.96 Hz, -N(CH_2)$_3$C$\underline{H}_3$), 1.20-1.76 (m, 8H, H_6(eq), H_{10}(eq), H_{18} and -NCH_2C$\underline{H}_2$C$\underline{H}_2$-), 2.11-2.17 (m, 2H, H_{19}), 2.47-3.10 (m, 8H, H_6(ax), H_7, H_9, H_{10}(ax) and H_{17}), 3.42 (t, 2H, J= 6.35 Hz, -NC$\underline{H}_2$CH_2-), 3.90-4.10 (m, 4H, -OC$\underline{H}_2$C$\underline{H}_2$O-), 4.62 Hz (s, 2H, H_2), 6.70 (d, 1H, J= 3.60 Hz, $H_{3'}$), 6.81-6.95 (m, 3H, H_{12}, H_{14} and H_{16}), 7.10 (d, 1H, J= 3.60 Hz, $H_{4'}$), 7.20-7.32 (m, 2H, H_{13} and H_{15}); ^{13}C-NMR ($CDCl_3$): δ 13.80 (-N(CH_2)$_3$C$\underline{H}_3$), 20.10 (-NCH_2CH_2C$\underline{H}_2$-), 21.41 (C_{18}), 29.45 (NCH_2C$\underline{H}_2$-), 29.51 (C_6 and C_{10}), 38.48 (C_{19}), 40.62 (-N$\underline{C}H_2$CH_2-), 58.22 (C_7 and C_9), 60.78 (C_5), 63.48 (C_2), 65.10 (-O$\underline{C}H_2\underline{C}H_2$O-), 73.00 ($C_{5'}$), 108.79 ($C_{20}$), 115.27 ($C_{12}$ and C_{16}), 118.78 (C_{14}), 126.10 ($C_{3'}$), 129.23 (C_{13} and C_{15}), 136.83 ($C_{4'}$), 143.08 (C_{11}), 152.94 ($C_{2'}$), 174.48 (C_4); IR (film, cm^{-1}): 2956, 2928, 2880, 2872, 2830 (C-H stretch), 1702 (amide C=O stretch), 1599-1510 (aryl C-C stretch), 1299, 1195 (amine C-N stretch), 1105, 1034 (C-O-C stretch), 802, 747, 688 (aryl C-H bend); elemental analysis: (calculated for $C_{27}H_{36}O_3N_3SI$) C, 53.20%; H, 5.95%; N, 6.89%; S, 5.26%; I, 20.82% (found) C, 53.28%; H, 6.02%; N, 6.91%; S, 5.21%; I, 20.76%.

t. 3-Benzyl-8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (9f)

Method B: The reaction was carried out as described on page 188 for the preparation of 8-[4,4-ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)] butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (200 mg, 0.360 mmol) was dissolved in anhydrous tetrahydrofuran (5 ml) and treated with sodium hydride (18 mg, 0.45 mmol) followed by benzyl bromide (46 µl, 0.38 mmol). The resulting mixture was stirred at room temperature for 18 hours and, after purification, 225 mg (0.348 mmol, 96.8%) of the product was obtained as a clear, colorless oil.

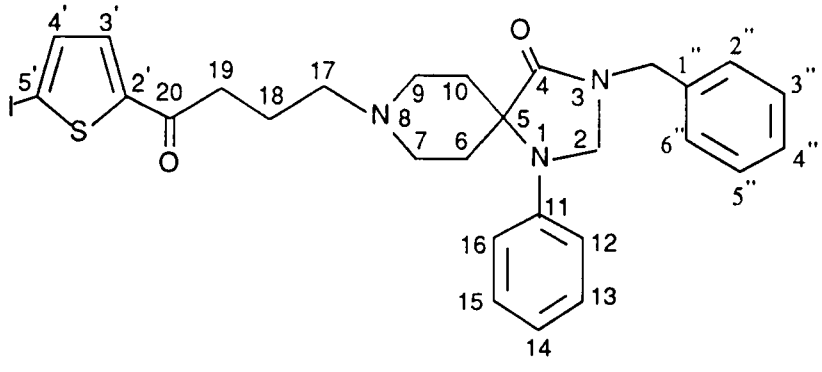
Product Characterization: mp 49.2-50.5 °C; ^{1}H-NMR ($CDCl_3$): δ 1.57-2.05 (m, 4H, H_6(eq), H_{10}(eq) and H_{18}), 2.10-2.15 (m, 2H, H_{19}), 2.53 (t, 2H, J= 6.94 Hz, H_{17}), 2.65-3.09 (m, 6H, H_6(ax), H_7, H_9 and H_{10}(ax)), 3.90-4.10 (m, 4H, -OC$\underline{H}_2$C$\underline{H}_2$O-), 4.53 (s, 2H, -C$\underline{H}_2$Ph), 4.59 (s, 2H, H_2), 6.65-7.40 (m, 12H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 21.44 (C_{18}), 29.51 (C_6 and C_{10}), 38.48 (C_{19}), 44.73 (-$\underline{C}H_2$Ph), 49.66 (C_{17}), 58.22 (C_7 and C_9), 60.79 (C_5), 62.96 (C_2), 65.10 (-O$\underline{C}H_2\underline{C}H_2$O-), 73.01 (5'), 108.79 (C_{20}), 115.35 (C_{12} and C_{16}), 118.87 (C_{14}), 126.10 ($C_{3'}$), 127.92 ($C_{2''}$ and $C_{6''}$), 128.00 ($C_{4''}$), 128.97 ($C_{3''}$ and $C_{5''}$), 129.24 (C_{13} and C_{15}), 135.74 ($C_{1''}$), 136.83 ($C_{4'}$), 142.95 (C_{11}), 152.94 ($C_{2'}$), 174.53 (C_4); IR (film , cm^{-1}): 2953, 2919, 2887, 2827, 2784 (C-H stretch), 1700 (amide C=O stretch), 1599, 1503 (aryl C-C stretch), 1295, 1195 (amine C-N stretch), 1030 (C-O-C stretch), 802, 747, 688 (aryl C-H bend); elemental analysis: (calculated for $C_{30}H_{34}N_3O_3SI$) C, 55.99%; H, 5.32%; N, 6.53%; S, 4.98%; I, 19.72%; (found) C, 56.04%; H, 5.38%; N, 6.42%; S, 5.08%; I, 19.56%.



u. 3-Benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**11f**)

3-Benzyl-8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (100 mg, 0.155 mmol) was placed in an argon-flushed, 100 ml round-bottomed flask equipped with a magnetic stirring bar, a water cooled reflux condenser and a gas outlet. A solution of hydrochloric acid and methanol was prepared by slowly adding 1.0 ml of the concentrated acid to 40 ml of methanol. A 10 ml portion of the acid solution was added to the ketal and the resulting mixture was heated to 40°C for 1 hour. No discoloration of the reaction mixture occurred, however, a white precipitate appeared which was likely the HCl salt of the product. The reaction mixture was then cooled to room temperature and neutralized slowly with an aqueous saturated sodium bicarbonate solution. The product extracted into dichloromethane (3 x 15 ml) and the organic extracts were combined, dried over anhydrous magnesium sulfate, filtered by gravity filtration, and the solvent evaporated to give 96 mg of a clear, colorless oil. The product was purified by column chromatography (dichloromethane / ethanol (50:1); stationary phase = alumina, R_f = 0.72) to give

91 mg (0.15 mmol, 98%) of the product as a clear, colorless oil and the chemical purity determined by HPLC (Method A, ret. time = 3.64 minutes, 97.3%), (Method B, ret. time = 10.37 min., 98.9%); ^{1}H-NMR ($CDCl_3$): δ 1.70 (d, 2H, J = 13.70 Hz, H_6(eq) and H_{10}(eq)), 2.93-2.05 (m, 2H, H_2), 2.50-3.10 (m, 10H, H_6(ax), H_7, H_9, H_{10}(ax), H_{17} and H_{19}), 4.54 (s, 2H, -C$\underline{H}_2$Ph), 4.59 (s, 2H, H_2), 6.75-7.55 (m, 12H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ22.03 (C_{18}), 29.35 (C_6 and C_{10}), 37.01 (C_{19}), 44.79 (-$\underline{C}H_2$Ph), 49.61 (C_{17}), 57.49 (C_7 and C_9), 60.72 (C_5), 63.01 (C_2), 84.93 ($C_{5'}$), 115.43 (C_{12} and C_{16}), 119.03 (C_{14}), 127.94 ($C_{2''}$ and $C_{6''}$), 128.05 ($C_{4''}$), 129.03 ($C_{3''}$ and $C_{5''}$), 129.27 (C_{13} and C_{15}), 132.55 ($C_{3'}$), 135.69 ($C_{1''}$), 138.10 ($C_{4'}$), 142.92 (C_{11}), 150.34 ($C_{2'}$), 174.42 (C_4), 191.68 (C_{20}); MS (FAB) m/e (rel intensity) 600 (100, M+1); exact mass calcd for $C_{28}H_{31}O_2N_3SI$ (M+H) 600.1182, found 600.1193.



v. 8-[4-(2-Thienyl)-4-oxo-butyl]-3-methyl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one (**10b**)

The procedure was carried out as described on page 204 for the

preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, **11f**. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (220 mg, 0.500 mmol) was dissolved in 5 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated to 60 °C for 120 minutes. After purification, 186 mg (0.470 mmol, 94.0%) of the product was obtained as a white solid; mp = 145.0-146.8°C; ^{1}H-NMR ($CDCl_3$): δ 1.63 (d, 2H, J = 13.70 Hz, H_6(eq) and H_{10}(eq)), 1.92-2.07 (m, 2H, H_{18}), 2.48-3.10 (m, 13H, H_6(ax), H_7, H_9, H_{10}(ax), H_{17}, H_{19} and -NC$\underline{H}_3$), 4.65 (s, 2H, H_2), 6.70-7.75 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 22.44 (C_{18}), 27.58 (-N$\underline{C}H_3$), 29.43 (C_6 and C_{10}), 37.45 (C_2), 49.63 (C_{17}), 57.56 (C_7 and C_9), 60.52 (C_2), 115.29 (C_{12} and C_{16}), 118.17 (C_{14}), 128.13 ($C_{4'}$), 129.32 (C_{13} and C_{15}), 131.76 ($C_{5'}$), 133.33 ($C_{3'}$), 143.03 ($C_{2'}$), 144,20 (C11), 174.61 (C_4), 193.78 (C_{20}); elemental analysis: (calculated for $C_{23}H_{29}O_2N_3S$) C, 66.47%; H, 6.85%; N, 10.57%; S, 8.06%; (found) C, 66.36%; H, 6.79%; N, 10.50%; S, 8.02%.

w. 8-[4-(2-Thienyl)-4-oxo-butyl]-3-ethyl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one (**10c**)

The procedure was carried out as described on page 204 for the preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **11f**. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (110 mg, 0.24 mmol) was dissolved in 20 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated to 60°C for 120 minutes. After purification, 91 mg (0.22 mmol, 92%) of the product was obtained as a clear, colorless oil; ^{1}H-NMR

($CDCl_3$): δ 1.23 (t, 3H, J= 7.27 Hz, -$NCH_2C\underline{H}_3$), 1.64 (d, 2H, J = 13.70 Hz, H_6(eq) and H_{10}(eq)), 1.98-2.10 (m, 2H, H_{18}), 2.48-2.90 (m, 8H, H_6(ax), H_7, H_9, H_{10}(ax) and H_{17}), 2.98 (t, 2H, J= 7.23 Hz, H_{19}), 3.48 (q, 2H, J= 7.27 Hz, -$NC\underline{H}_2CH_3$), 4.67 (s, 2H, H_2), 6.70-7.75 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 12.72 (-$NCH_2\underline{C}H_3$), 22.33 (C_{18}), 29.35 (C_6 and C_{10}), 35.63 (-$NCH_2\underline{C}H_3$), 37.42 (C_{19}), 49.66 (C_{17}), 57.59 (C_7 and C_9), 60.80 (C_5), 63.01 (C_2), 115.46 (C_{12} and C_{16}), 118.92 (C_{14}), 128.08 ($C_{4'}$), 129.30 (C_{13} and C_{15}), 131.73 ($C_{5'}$), 133.33 ($C_{3'}$), 143.13 ($C_{2'}$), 144.21 (C_{11}), 174.15 (C_4), 193.22 (C_{20}); IR (film, cm^{-1}): 2950, 2930, 2827 (C-H stretch), 1697 (amide C=O stretch), 1662 (C=O stretch), 1599, 1503 (aryl C-C stretch), 1300, 1197 (amine C-N stretch), 747, 729, 698 (aryl C-H bend); elemental analysis: (calculated for $C_{23}H_{29}O_2N_3S$) C, 67.12%; H, 7.10%; N, 10.21%; S, 7.79% (found) C, 67.00%; H, 7.11%; N, 10.14%; S, 7.73%.

x. 8-[4-(2-Thienyl)-4-oxo-butyl]-1-phenyl-3-propyl-1,3,8-triaza-spiro[4.5]decan-4-one (**10d**)

The procedure was carried out as described on page 204 for the preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, **11f**. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one (149 mg, 0.32 mmol) was dissolved in 10 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated to 40°C for 30 minutes. After purification, 126 mg (0.30 mmol, 93%) of the product was obtained as a clear, colorless oil; ^{1}H-NMR ($CDCl_3$): δ 0.95 (t, 3H, J= 6.89 Hz, -$N(CH_2)_2C\underline{H}_3$), 1.55-2.05 (m, 6H, H_6(eq), H_{10}(eq), H_{18} and -$NCH_2C\underline{H}_2$-), 2.45-2.96 (m, 10H, H_6(ax), H_7, H_9, H_{10}(ax),

H_{17} and H_{19}), 3.40 (t, 2H, J= 6.89 Hz, -NC$\underline{H}_2$CH_2-), 4.64 (s, 2H, H_2), 6.70-7.80 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 11.31 (-N(CH_2)$_2$C$\underline{H}_3$), 20.81 (-NCH_2C$\underline{H}_2$-), 22.30 (C_{18}), 29.43 (C_6 and C_{10}), 37.42 (C_{19}), 42.48 (-N$\underline{C}H_2CH_2$-), 49.66 (C_{17}), 57.60 (C_7 and C_9), 60.77 (C_2), 115.35 (C_{12} and C_{16}), 118.87 (C_{14}), 128.08 ($C_{4'}$), 129.30 (C_{13} and C_{15}), 131.73 ($C_{5'}$), 133.33 ($C_{3'}$), 143.11 ($C_{2'}$), 144.62 (C_{11}), 174.53 (C_4), 193.22 (C_{20}); IR (film, cm^{-1}): 2961, 2930, 2871, 2828 (C-H stretch), 1699 (amide C=O stretch), 1301, 1198 (C-N amine stretch), 1599, 1573, 1503 (aryl C-C stretch), 747, 729, 696 (aryl C-H bend); MS (FAB) m/e (rel intensity) 426 (100, M+1); exact mass calcd for $C_{24}H_{32}O_2N_3S$ (M+H) 426.2209, found 426.2215; elemental analysis: (calculated for $C_{24}H_{31}O_2N_3S\cdot$ 1/3 H_2O) C,66.79%; H, 7.40%; N, 9.74%; S, 7.43% (found) C, 66.96%; H, 7.62%; N, 9.45%; S, 7.10%.

y. 3-Butyl-8-[4-(2-thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**10e**)

The procedure was carried out as described on page 204 for the preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **11f**. 3-Butyl-8-[4,4-ethylenedioxy-4-(2-thienyl) butyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (100 mg, 0.21 mmol) was dissolved in 10 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated to 50°C for 50 minutes. After purification, 88 mg (0.20 mmol, 97%) of the product was obtained as a clear, colorless oil; ^{1}H-NMR ($CDCl_3$): δ 0.96 (t, 3H, J= 6.37 Hz, -N(CH_2)$_3$C$\underline{H}_3$), 1.20-1.80 (m, 8H, H_6(eq), H_{10}(eq), H_{18} and -NCH_2C$\underline{H}_2$C$\underline{H}_2$-), 2.48-3.18 (m, 10H, H_6(ax), H_7, H_9, H_{10}(ax), H_{17} and H_{19}), 3.44 (t, 2H, J= 6.37 Hz, -NC$\underline{H}_2$CH_2-), 4.62 (s, 2H, H_2),

6.70-7.75 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 13.61 (-N(CH_2)$_3$$\underline{C}H_3$), 20.08 (-N($CH_2$)$_2$$\underline{C}H_2$-), 22.30 ($C_{18}$), 29.40 ($C_6$ and C_{10}), 29.45 (-N$CH_2$$\underline{C}H_2$-), 37.43 ($C_{19}$), 42.31 (-N$\underline{C}H_2CH_2$-), 49.72 ($C_{17}$), 57.62 ($C_7$ and C_9), 60.62 (C_2), 115.35 (C_{12} and C_{16}), 118.85 (C_{14}), 128.09 ($C_{4'}$), 129.31 (C_{13} and C_{15}), 131.73 ($C_{5'}$), 133.33 ($C_{3'}$), 143.11 ($C_{2'}$), 144.62 (C_{11}), 174.49 (C_4), 193.24 (C_{20}); IR (film, cm^{-1}): 2957, 2929, 2871, 2830 (C-H stretch), 1703 (amide C=O stretch), 1661 (C=O stretch), 1599, 1503 (aryl C-C stretch), 1301-1198 (amine C-N stretch), 747, 729, 688 (aryl C-H stretch); elemental analysis: (calculated for $C_{25}H_{33}O_2N_3S$) C, 68.30%; H, 7.57%; N, 9.56%; S, 7.29% (found) C, 68.04%; H, 7.54%; N, 9.41%; S, 7.15%.

z. 3-Benzyl-8-[4-(2-thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triaza spiro[4.5]decan-4-one (**10f**)

The procedure was carried out as described on page 204 for the preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, 11f. 3-Benzyl-8-[4,4-ethylenedioxy-4-(2-thienyl) butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (150 mg, 0.29 mmol) was dissolved in 10 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated to 50°C for 60 minutes. After purification, 130 mg (0.27 mmol, 94%) of the product was obtained as a clear, colorless oil; ^{1}H-NMR ($CDCl_3$): δ 1.62 (d, 2H, J = 13.70 Hz, H_6(eq) and H_{10}(eq)), 1.92-2.05 (m, 2H, H_{18}), 2.49-3.10 (m, 10H, H_6(ax), H_7, H_9, H_{10}(ax), H_{17} and H_{19}), 4.54 (s, 2H, -C$\underline{H}_2$Ph), 4.59 (s, 2H, H_2), 6.70-7.75 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 22.34 (C_{18}), 29.45 (C_6 and C_{10}), 37.45 (C_{17}), 44.78 (-$\underline{C}H_2$Ph), 49.69 (C_{19}), 57.65 (C_7 and C_9), 60.77 (C_2), 115.43 (C_{12} and C_{14}), 118.92 (C_{14}), 128.11

($C_{4'}$), 129.27 (C_{13} and C_{15}), 131.76 ($C_{5'}$), 133.36 ($C_{3'}$), 142.78 ($C_{2'}$), 144.60 (C_{11}), 174.53 (C_4), 193.22 (C_{20}); IR (film, cm-1): 2961, 2929, 2887, 2827 (C-H stretch), 1697 (amide C=O stretch), 1660 (C=O stretch), 1599, 1503 (aryl C-C stretch), 1301, 1198 (amine C-N stretch), 800, 747, 696 (aryl C-H bend); MS (FAB) m/e (rel intensity) 474 (100, M+1) exact mass calcd for $C_{28}H_{32}O_2N_3S$ (M+H) 474.2215, found 474.2231; elemental analysis: (calculated for $C_{28}H_{31}O_2N_3S$) C,71.01%; H, 6.60%; N, 8.87%; S, 6.77% (found) C, 70.80%; H, 60.65%; N, 8.80%; S, 6.84%.

aa. 8-[4-(2-Thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**10a**)

The procedure was carried out as described on page 204 for the preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **11f**. 8-[4,4-Ethylenedioxy-4-(2-thienyl)butyl]-1-phenyl-1,3,8-triazaspiro [4.5]decan-4-one (400 mg, 0.94 mmol) was dissolved in 30 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated to 40°C for 80 minutes. After purification, 330 mg (0.86 mmol, 94%) of the product was obtained as a white powder; mp = 182-183°C; ^{1}H-NMR ($CDCl_3$): δ 1.64 (d, 2H, J = 13.80 Hz, H_6(eq) and H_{10}(eq)), 1.90-2.08 (m, 2H, H_{18}), 2.48-2.90 (m, 8H, H_6(ax), H_7, H_9, H_{10}(ax) and H_{17}), 2.98 (t, 2H, J= 7.23 Hz, H_{19}), 4.69 (s, 2H, H_2), 6.70-7.75 (m, 8H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 22.32 (C_{18}), 29.02 (C_6 and C_{10}), 37.40 (C_{19}), 49.65 (C_{17}), 59.38 (C_7 and C_9), 59.49 (C_2), 115.37 (C_{12} and C_{16}), 118.91 (C_{14}), 128.10 ($C_{4'}$), 129.30 (C_{13} and C_{15}), 131.75 ($C_{5'}$), 133.35 ($C_{3'}$), 143.11 ($C_{2'}$), 144.61 (C_{11}), 178.52 (C_4), 193.22 (C_{20}); elemental analysis: (calculated for $C_{21}H_{25}O_2N_3S$)

C, 65.77%; H, 7.40%; N, 9.74%; S, 7.43% (found) C, 65.51%; H, 6.60%; N, 10.92%; S, 8.28%.

bb. 8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**11a**)

The procedure was carried out as described on page 204 for the preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **11f**. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)]-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (180 mg, 0.330 mmol) was dissolved in 30 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated to reflux for 30 minutes and then stirred at room temperature for 40 hours. After purification, 143 mg (0.280 mmol, 86.3%) of the product was obtained as a white powder; (Method B, ret. time = 3.19 min); mp = 182-183°C; ^{1}H-NMR ($CDCl_3$): δ 1.65 (d, 2H, J = 13.80 Hz, H_6(eq) and H_{10}(eq)), 1.90-2.08 (m, 2H, H_{18}), 2.45-3.05 (m, 10H, H_6(ax), H_7, H_9, H_{10}(ax), H_{17}, and H_{19}), 4.68 (s, 2H, H_2), 6.65-7.40 (m, 8H, aromatic H and N-H); ^{13}C-NMR ($CDCl_3$): δ 22.23 (C_{18}), 29.39 (C_6 and C_{10}), 37.03 (C_{19}), 49.58 (C_{17}), 57.49 (C_7 and C_9), 60.42 (C_5), 60.18 (C_2), 84.87 ($C_{5'}$), 115.21 (C_{12} and C_{16}), 118.84 (C_{14}), 129.29 (C_{13} and C_{15}), 132.47 ($C_{3'}$), 138.05 ($C_{4'}$), 142.98 (C_{11}), 150.40 ($C_{2'}$), 178.56 (C_4), 192.89 (C_{20}); MS (FAB) m/e (rel intensity) 510 (100, M+1); exact mass calcd for $C_{21}H_{25}O_2N_3SI$ (M+H) 510.0712, found 510.0717.

cc. 8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**11b**)

The procedure was carried out as described on page 204 for the

preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, **11f**. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)] butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (219 mg, 0.390 mmol) was dissolved in 14 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated at 40°C for 30 minutes. After purification, 180 mg (0.35 mmol, 91%) of the product was obtained as a white powder; mp = 106-107°C; ^{1}H-NMR ($CDCl_3$): δ 1.62 (d, 2H, J = 13.70 Hz, H_6(eq) and H_{10}(eq)), 1.90-2.08 (m, 2H, H_{18}), 2.45-3.10 (m, 13H, H_6(ax), H_7, H_9, H_{10}(ax), H_{17}, H_{19} and -NC$\underline{H}_3$), 4.65 (s, 2H, H_2), 6.65-7.40 (m, 7H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 22.22 (C_{18}), 27.56 (-N$\underline{C}H_3$), 29.40 (C_6 and C_{10}), 37.03 (C_{19}), 49.58 (C_{17}), 57.49 (C_7 and C_9), 60.44 (C_5), 65.18 (C_2), 84.87 ($C_{5'}$), 115.21 (C_{12} and C_{16}), 118.84 (C_{14}), 129.30 (C_{13} and C_{15}), 132.47 ($C_{3'}$), 138.05 ($C_{4'}$), 142.98 (C_{11}), 150.40 ($C_{2'}$), 174.51 (C_4), 191.76 (C_{20}); elemental analysis: (calculated for $C_{22}H_{26}O_2N_3SI$) C, 50.48%; H, 5.01%; N, 8.03%; S, 6.12%; I, 24.24% (found) C, 50.74%; H, 5.25%; N, 7.82%; S, 6.26%; I, 24.04%.

dd. 8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**11c**)

The procedure was carried out as described on page 204 for the preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, **11f**. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)] butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (205 mg, 0.351 mmol) was dissolved in 15 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated at 40°C for 30 minutes. After

purification, 170 mg (0.32 mmol, 91%) of the product was obtained as a white solid; mp = 66.4-67.8°C; ^{1}H-NMR ($CDCl_3$): δ 1.23 (t, 3H, J= 7.32 Hz, -$NCH_2C\underline{H}_3$), 1.62 (d, 2H, J = 13.70 Hz, H_6(eq) and H_{10}(eq)), 1.85-2.02 (m, 4H, H_{18}), 2.45-3.00 (m, 10H, H_6(ax), H_7, H_9, H_{10}(ax), H_{17} and H_{19}), 3.47 (q, 2H, J= 7.32 Hz, -$NC\underline{H}_2CH_3$), 4.68 (s, 2H, H_2), 6.75-7.45 (m, 7H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 12.72 (-$NCH_2\underline{C}H_3$), 22.19 (C_{18}), 29.35 (C_6 and C_{10}), 35.63 (-$N\underline{C}H_2CH_3$), 37.07 (C_{19}), 49.66 (C_{17}), 57.52 (C_7 and C_9), 60.79 (C_5), 63.01 (C_2), 84.87 ($C_{5'}$), 115.46 (C_{12} and C_{16}), 118.97 (C_{14}), 129.32 (C_{13} and C_{15}), 132.52 ($C_{3'}$), 138.10 ($C_{4'}$), 143.14 (C_{11}), 150.39 ($C_{2'}$), 174.15 (C_4), 191.79 (C_{20}); MS (FAB) m/e (rel intensity) 538 (100, M+1); exact mass calcd for $C_{23}H_{29}O_2N_3SI$ (M+H) 538.1026, found 538.1033; elemental analysis: (calculated for $C_{23}H_{28}O_2N_3SI$) C, 51.40%; H, 5.25%; N, 7.82%; S, 5.97%; I, 23.61% (found) C, 51.26%; H, 5.33%; N, 7.77%; S, 5.89%; I, insufficient sample.

ee. 8-[4-(5-Iodo-2-thienyl)-4-oxo-butyl]-1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one (**11d**)

The procedure was followed as described on page 204 for the preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. 8-[4,4-Ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-3-propyl-1,3,8-triazaspiro[4.5]decan-4-one (100 mg, 0.170 mmol) was dissolved in 15 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated at 50°C for 2 hours. After purification, 82 mg (0.15 mmol, 89%) of the product was obtained as colorless oil; Chromatography: (Method A, ret. time = 3.19 min, 99.1%); ^{1}H-NMR ($CDCl_3$): δ 0.95 (t, 3H, J= 6.84

Hz, -N(CH_2)$_2$C$\underline{H}_3$), 1.55-2.05 (m, 6H, H_6(eq), H_{10}(eq) and H_{18} and -NCH_2C$\underline{H}_2$-), 2.45-3.05 (m, 10H, H_6(ax), H_7, H_9, H_{10}(ax), H_{17} and H_{19}), 3.40 (t, 2H, J= 6.84 Hz, -NC$\underline{H}_2$CH_2-), 4.66 (s, 2H, H_2), 6.65-7.40 (m, 7H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 11.34 (-N(CH_2)$_2$C$\underline{H}_3$), 20.81 (-NCH_2C$\underline{H}_2$-), 22.22 (C_{18}), 29.48 (C_6 and C_{10}), 37.09 (C_{19}), 42.48 (-N$\underline{C}H_2$CH_2-), 49.69 (C_{17}), 57.54 (C_7 and C_9), 60.79 (C_5), 63.53 (C_2), 84.85 ($C_{5'}$), 115.32 (C_{12} and C_{16}), 118.90 (C_{14}), 129.30 (C_{13} and C_{15}), 132.49 ($C_{3'}$), 138.07 ($C_{4'}$), 143.11 (C_{11}), 150.50 ($C_{2'}$), 174.56 (C_4), 191.79 (C_{20}); IR (film, cm^{-1}): 2961, 2928, 2880, 2827 (C-H stretch), 1697 (amide C=O stretch), 1660 (C=O stretch), 1599, 1503 (aryl C-C stretch), 1301, 1198 (amine C-N stretch), 799, 747, 696 (aryl C-H bend); ms (FAB) m/e (rel intensity) 552 (100, M+1); exact mass calcd for $C_{24}H_{30}O_2N_3SI$ (M+H) 552.1182, found 552.1213.

ff. <u>3-Butyl-8-[4-(5-iodo-2-thienyl)-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**11e**)</u>

The procedure was followed as described on page 204 for the preparation of 3-benzyl-8-[4-[2-(5-iodothienyl)]-4-oxo-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, **11f**. 3-Butyl-8-[4,4-ethylenedioxy-4-[2-(5-iodothienyl)]butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (240 mg, 0.480 mmol) was dissolved in 10 ml of a mixture of concentrated HCl and methanol (1:40) and the resulting solution was heated at 40°C for 60 minutes. After purification, 239 mg (0.431 mmol, 88.8%) of the product was obtained as a clear, colorless oil; ^{1}H-NMR ($CDCl_3$): δ 0.96 (t, 3H, J= 6.39 Hz, -N(CH_2)$_3$C$\underline{H}_3$), 1.20-1.80 (m, 8H, H_6(eq), H_{10}(eq), H_{18} and -NCH_2C$\underline{H}_2$C$\underline{H}_2$-), 2.47-3.18 (m, 10H, H_6(ax), H_7, H_9, H_{10}(ax), H_{17} and H_{19}), 3.44 (t, 2H, J= 6.39 Hz,

-NC$\underline{H}_2$CH_2-), 4.62 (s, 2H, H_2), 6.75-6.45 (m, 7H, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 13.64 (-N(CH_2)$_3\underline{C}H_3$), 20.10 (-N(CH_2)$_2\underline{C}H_2$-), 22.20 (C_{18}), 29.36 (C_6 and C_{10}), 29.45 (-NC$H_2\underline{C}H_2$-), 37.10 (C_{19}), 42.32 (-N$\underline{C}H_2CH_2$-), 49.65 (C_{17}), 57.53 (C_7 and C_9), 60.78 (C_5), 63.12 (C_2), 84.85 ($C_{5'}$), 115.46 (C_{12} and C_{16}), 118.96 (C_{14}), 129.34 (C_{13} and C_{15}), 132.54 ($C_{3'}$), 138.11 ($C_{4'}$), 143.14 (C_{11}), 150.40 ($C_{2'}$), 174.10 (C_4), 191.81 (C_{20}); elemental analysis: (calculated for $C_{25}H_{32}O_2N_3SI$) C, 53.10%; H, 5.70%; N, 7.43%; S, 5.67%; I, 22.44% (found) C, 53.23%; H, 5.92%; N, 7.27%; S, 5.73%; I, 22%.

gg. 8-[4-[2-(5-[123I]iodothienyl)]-4-oxo-butyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**13**)

Compound **7b** (120 mg, 0.272 mmol) was placed into a 100 ml, round-bottomed flask equipped with a magnetic stirring bar and a gas outlet. The flask was flushed with argon and then anhydrous diethyl ether (10 ml) was added to the flask via syringe. The resulting solution was stirred and cooled to 0°C using an ice water bath. A solution of *n*-butyllithium in hexanes (1.6 M, 0.20 ml, 0.33 mmol) was slowly added via syringe and the reaction mixture became cloudy within two minutes after the addition. The mixture was stirred for 10 minutes and then trimethyltin chloride (70 mg, 0.35 mmol) was added in one portion to the reaction mixture. The reaction mixture became clear and colorless upon addition of the trimethyltin chloride. Stirring continued for another 40 minutes and then the solution was allowed to warm to room temperature. Distilled water (0.5ml) was added to the reaction mixture and the product was extracted into dichloromethane (2x20ml). The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and the solvent evaporated to give a yellow

oil. The extracted material was analyzed by TLC [stationary phase = alumina; mobile phase = dichloromethane/ethanol (97:3)] to reveal the presence of a major product (Rf = 0.68) and a minor byproduct (Rf = 0.74). The major product (98 mg, 60%) was determined to be the desired trimethyltin intermediate **8** and was dissolved in ethanol (1.0 ml, 100%) in a 20 ml, round-bottomed flask. This solution was stirred at room temperature for 1 minute and 0.1 ml of an aqueous phosphate buffer solution was added (pH = 7.0). Next, a chloramine-T solution [0.02 M, in tetrahydrofuran/water (1:1)] was prepared and to this was added to a solution of 4.22 mCi of $Na^{123}I$ in 0.2 ml of 1.0 M aqueous sodium hydroxide. The resulting pink solution was added in one portion to the flask containing **8** and the resulting mixture immediately became clear and colorless. The pH of the reaction was determined to be between 9.0 and 10.0 using pH paper. The reaction mixture was stirred for 5 minutes at room temperature and then 1.0 ml of an aqueous sodium metabisulfite solution (0.1 M) was added, followed by 1.0 ml of an aqueous sodium chloride solution (5.0% w/w). The product was extracted into diethyl ether (2 x 1.0 ml) and the ether extracts were combined, dried over anhydrous sodium sulfate and analyzed by radio-TLC. One major radioiodinated product was detected and the R_f corresponded to that of **9b**. All other radioiodinated material remained at the origin and were most likely [^{123}I]iodide salts. The radioiodinated product was purified by HPLC [method A: stationary phase = silica; mobile phase = dichloromethane/methanol (95:5); retention time (**12**) = 9.10 minutes] and 2.04 mCi of **12** was obtained (60% radiochemical yield; corrected to time = 0 at the start of the reaction) . Compound **12** (2.04 mCi) was reacted with 1.0 ml of a solution of methanol and HCl (40:1) for 10 minutes at 50°C to provide the radioiodinated butyrothienone

13. The reaction mixture was cooled to room temperature and was neutralized with an aqueous potassium carbonate solution (1.0 ml, 0.1 M). The product was extracted into diethyl ether (3 x 1.0 ml) and the ether extracts were combined, dried, and checked by radio-TLC [mobile phase = alumina; stationary phase = dichloromethane/ethanol (97:3)]. The major radioiodinated component had an R_f value of 6.0, which corresponded to that of the ^{127}I analog **11b**. The radioiodinated product was purified by HPLC (Method A, retention time = 7.60 minutes) to provide 0.628 mCi of **13** in 20% overall radiochemical yield (corrected back to time = 0).

3. Evaluation of Lipophilicity of the Butyrothienones

The method utilized for the evaluation of the log P values of the butyrothienones **10a-f** and **11a-f** has been previously reported.[108] The chromatographic conditions (Method B) were designed to mimic blood pH and ionic strength in order to give accurate estimates of lipophilicity. A reversed phase C-18 column (Alltech) was used for the experiment and the mobile phase consisted of a mixture of aqueous phosphate buffer and HPLC grade methanol (15:85). The methanol/buffer solution was filtered through a 5 micron nylon filter prior to use. The phosphate buffer (PH= 7.5) was prepared by the addition of 150 ml of 0.1 M potassium dihydrogen phosphate solution to 122.7 ml of 0.1 M sodium hydroxide solution. The final pH of the methanol/buffer mobile phase was determined to be approximately 7.0 using pH paper.

Eight standards were used to create a plot of log retention time (relative to catechol) versus log P in order to derive the equation used to calculate the log P values of the butyrothienones. Solutions of the standards were prepared

by dissolving 100 mg of each standard in 10 ml of the mobile phase. Twenty microliters of the standard solutions was used for each analysis (2.0×10^{-7} grams injected). Standard solutions of the butyrothienones **10a-f** and **11a-f** were prepared by dissolving 10 mg of each compound into 1.0 ml of the mobile phase. Forty microliters of each butyrothienone solution was used for each retention time determination of compounds **10a-f** and **11a-f** (4.0×10^{-7} grams injected). The detector response was between 50% and 80% of the maximum response for each valid run. The retention times of the eight standards were obtained before and after the analysis of the butyrophenones in order to insure that the chromatographic conditions remained constant throughout the experiment. No significant change in the retention times of the standards was observed between the two runs. The eight standards and their corresponding log P values and retention times are listed in Table 3-7.

A plot of the log of the retention time of the standards (relative to catechol) versus the log P of the standard resulted in a parabolic curve with shown below (equation 3-9). This equation was obtained by least squares analysis and the correlation coefficient was 0.995. Substitution of the retention times obtained for **10a-f** and **11a-f** (relative to catechol) for **X** in equation 3-9 allowed for the calculation of the respective log P values (**Y**) for the butyrothienones. The retention times calculated log P values of **10a-f** and **11a-f** are summarized in Table 3-3 on page 159.

$$\mathbf{Y} = 1.8607 + 1.9022(\mathbf{X}) + 0.78606(\mathbf{X})^2 \qquad (3\text{-}9)$$

Table 3-7: Retention Times and Log P Values of Standards Used for the Log P Experiment

Standard	Rt (before) (Minutes)	Rt(after) (Minutes)	Log P
Catechol	3.03	3.02	0.95
Aniline	3.28	3.24	0.98
Benzene	4.59	4.61	2.13
Bromobenzene	5.60	5.58	2.99
Ethylbenzene	6.38	6.33	3.13
Trimethylbenzene	8.34	8.27	3.58
Hexachlorobenzene	35.41	35.60	6.35

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Appendix

Conformational Analysis of 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-one by Proton NMR Spectroscopy and Molecular Modeling.

The ^{1}H-NMR spectrum of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one is shown in Figure A-1. The pattern of splitting and the magnitude of the coupling constants ($J_{axial\text{-}axial}$ = 13.10 Hz) suggest that one chair conformation predominates. The integration of the four multiplets between 1.6 ppm and 3.5 ppm, corresponding to the piperidine ring protons, revealed that each multiplet represents 2 protons. Analysis of the coupling constants allowed for the assignment of each multiplet to either an axial or an equatorial proton (see Figure A-2). The tilt and coupling constants of each multiplet and were used to determine that the resonances at 3.43 ppm and 3.05 ppm originate from protons that are attached to the same carbon atom (see Figure A-2). Likewise, the resonances at 2.55 ppm and 1.73 ppm are from protons that are attached to the same carbon. The downfield position of the multiplets at 3.43 ppm and 3.05 ppm suggests that they originate from the four protons nearest to the amine (H_7 and H_9). The relatively large increase in the chemical shift of these protons when the amine is alkylated with a BOC group supports the assignments.

The five-membered ring appears to be flexible and the phenyl group can move to either side of the ring by way of nitrogen inversion. The presence of a singlet at 4.65 ppm, corresponding to the two methylene protons at position 2, is evidence of the relatively rapid movements. In addition, dynamic studies of conformations **A** and **B** using SYBYL suggests that the five membered ring moves into different conformations at room temperature.

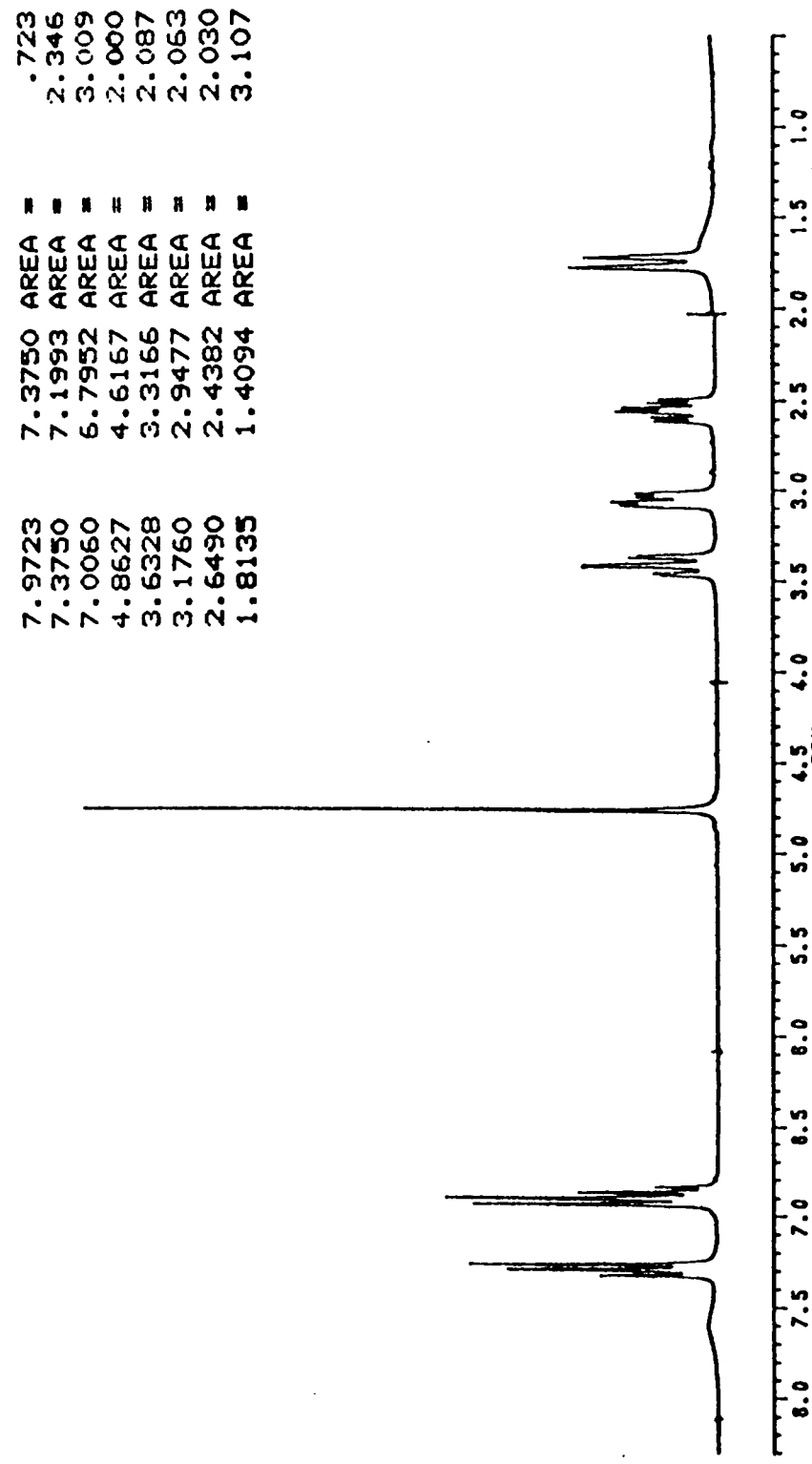


Figure A-1. 250 MHz Proton NMR Spectrum of 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5 a**, in $CDCl_3$ plus 0.3% TMS.

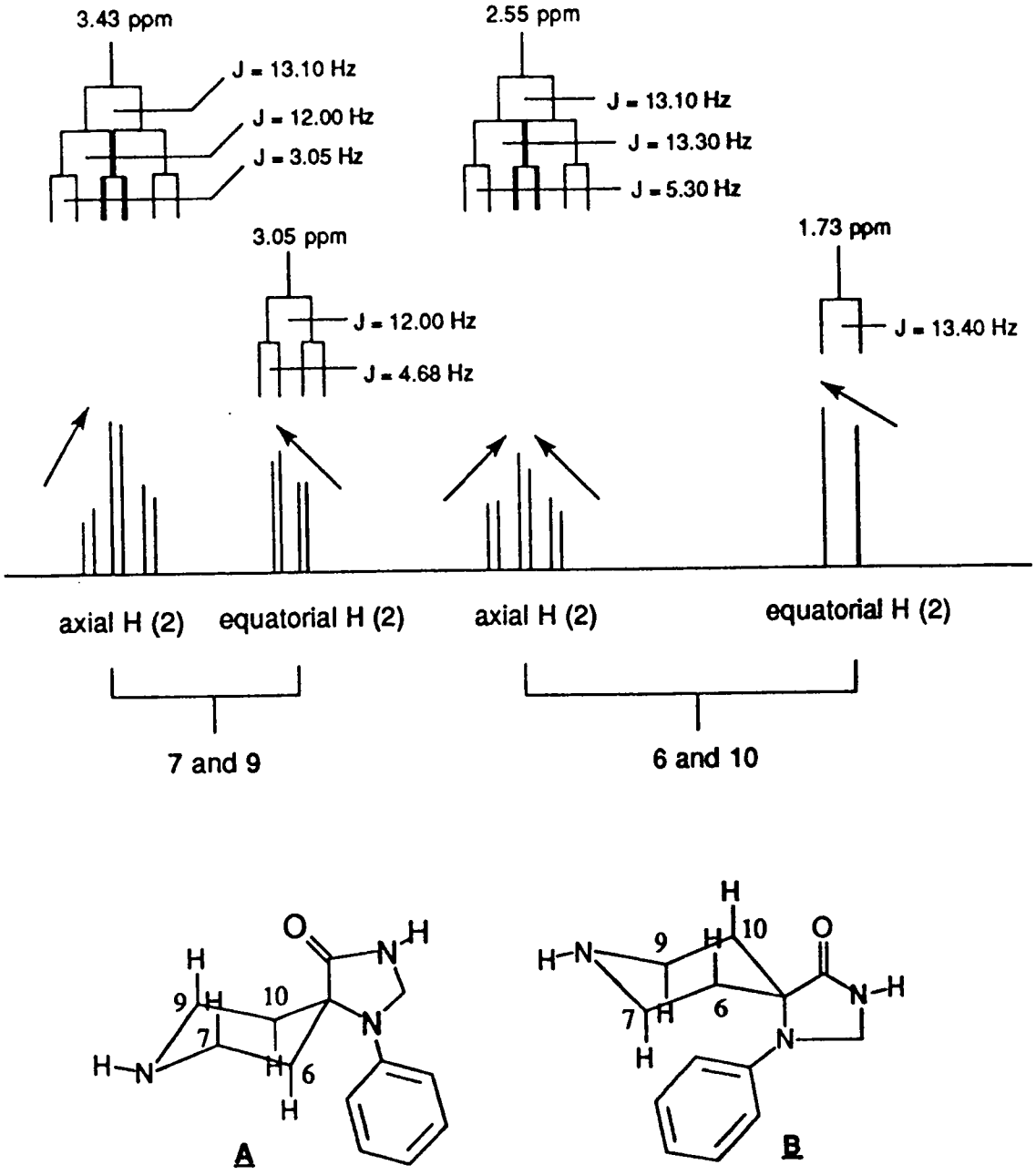


Figure A-2. ^{1}H-NMR Assignments for the Piperidene Ring Protons of 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **5a**.

It is impossible to conclude which chair conformation is present without performing further NMR studies. It is evident that all four axial protons are deshielded; however, both the carbonyl group and the phenyl group are capable of deshielding nuclei and arguments can be made for either chair conformation. The compound is not a quaternary salt because of the resonance of the N-H proton is too far upfield (broad singlet at 1.65 ppm superimposed on the doublet at 1.73 ppm). The resonances of a quaternary salt should appear between 6.0 ppm and 8.5 ppm and, therefore, the downfield shift of protons at positions 7 and 9 cannot be attributed to the presence of the quaternary salt. Analysis of the of the piperidine ring protons of conformations **A** and **B** by PCModel revealed no significant differences calculated dihedral angles of the respective vicinal axial and equatorial protons. This prevented distinguishing conformations **A** and **B** by the coupling constants obtained by ^{1}H NMR.

Energy minimization of conformations **A** and **B** using SYBYL reveal that the conformational energy of **A** [$E_{(min)}$ = 3.58 Kcal/mol] was 1.66 Kcal/mol lower than the conformational energy of **B** [$E_{(min)}$ = 5.24 Kcal/mol]. Dynamic studies of conformation **A** indicated that the chair inversion to form conformation **B** does not occur at room temperature. It is possible that the energy barrier for the interconversion of **A** and **B** is high enough that it does not readily occur at room temperature. If this hypothesis is true, then conformation **A** is most likely the predominate structure because it has the lowest conformational energy. Further computer analysis is needed to confirm an energy barrier for the interconversion of conformations **A** and **B**.

VITA

Rikki Noel Waterhouse was born in Shalimar, Florida on November 9, 1965. She attended elementary schools in Baltimore, Maryland, Glendale Tennessee, and Lenoir City, Tennessee. She attended high school in Greenback, Tennessee for three years and then graduated from Lenoir City High School in Lenoir City, Tennessee in the Spring of 1983. She entered The University of Tennessee in the Fall of 1983 and received the degree of Bachelor of Arts in Chemistry in August, 1988. She then continued her studies in chemistry at The University of Tennessee at the graduate level and, in May of 1993, received the degree of Doctor of Philosophy in Chemistry.

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