To the Graduate Council:

I am submitting herewith a thesis written by Anthony Michael Condo, Jr. entitled "The Synthesis of Compounds for the Treatment of Current Health Concerns: Potent Anti-HIV Agents and Cholesterol-Lowering Functional Food Products." I have examined the final paper copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

Linda J. Magid

Linda J. Magid, Major Professor

We have read this thesis
and recommend its acceptance:

Elizabeth E. Howell

Elizabeth E. Howell

Ronald M. Magid

Accepted for the Council:

Vice Provost and
Dean of Graduate Studies

The Synthesis of Compounds for the Treatment of Current Health Concerns: Potent Anti-HIV Agents and Cholesterol-Lowering Functional Food Products

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Anthony Michael Condo, Jr.
December 2001

DEDICATION

This thesis is dedicated to the following people, for my successes are due in large part to their guidance, support, and encouragement. Much love and thanks to all of you.

My parents:

Anthony and Debra Condo

My sister:

Courtney Condo

and my maternal grandparents:

Frederick and Audrey Sheppard

ACKNOWLEDGMENTS

There are so many people who have made my graduate career at the University of Tennessee a rewarding personal and professional experience. I am particularly grateful to my research advisor, Dr. David C. Baker, for providing me with the resources which merit the publication of this thesis. Many thanks to him for research funding, lab resources, and many helpful insights and conversations about the projects I was very fortunate enough to be a part of.

Many thanks to my M. S. Thesis Committee: Dr. Linda J. Magid, Dr. Elizabeth E. Howell, and Dr. Ronald M. Magid, for their encouragement, support, and for standing by me in the end. I appreciate the cooperation from Academic Press, LTD (London, U. K.) In giving me permission to reprint **Figure 1** of this manuscript (see Appendix II).

Some compounds discussed in this thesis were prepared by my colleagues in the Baker Research group. They are as follows: **NSC 708199, NSC 703774, and NSC 704673** by Dr. Jianmin Mao; **NSC 694444** by Dr. Anand Mayasundari; and **NSC 701751** by James Miller.

I am indebted to the Department of Chemistry for the financial assistance that they have provided me with throughout my career in the form of a graduate teaching assistantship as well as a Science Alliance supplement for maintaining a portion of the UTK NMR Facility.

To my many friends and colleagues at the University of Tennessee, thanks

for all of your support and your friendship throughout my career. And to my friends and family outside of the University community, thank you for listening and for being there when you were most needed.

ABSTRACT

In an ongoing effort to develop pharmaceutically active compounds for use in treating patients with HIV, we have investigated a series of 3-aryl-2-methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxides. Some of these compounds, commonly known as "sultams", have shown potent activity in *in vitro* screens as non-nucleoside reverse transcriptase inhibitors (NNRTIs) of HIV-1 (EC_{50}'s in the sub-micromolar range). The compounds that have been identified as the most potent in the biological screens have been substituted analogs at the 3-position of the C ring. In light of these results, we have extended our studies to include an investigation of the effects of substitution at the 3-position on the biological activity. The synthesis of a number of analogs bearing a range of functional groups at this position has been accomplished.

The ability of phytosterols and phytostanols to lower serum low-density lipoprotein–cholesterol (LDL-C) levels in humans is well documented. Commercially, "functional foods" such as Benecol® and Take Control®, contain fatty acid esters of both phytosterols and phytostanols, respectively. The purpose of the esterification is to improve the solubility of the otherwise insoluble free sterol or stanol. These esters are hydrolyzed in the intestine to the proposed physiologically active sterol or stanol. The aim of this project was to synthesize multi-gram quantities of these fatty acid esters to be used in an appropriate hamster-feeding study by our USDA collaborators. We were able to synthesize these esters under

mild laboratory conditions which we thought to be more amenable to large-scale production and reproducibility than that which was previously reported in the literature.

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LIST OF SYMBOLS AND ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AZT	2'-azido-2'-deoxythymidine
DCC	*N,N*-Dicyclohexylcarbodiimide
DCU	*N,N*-Dicyclohexylurea
4-DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
EC_{50}	50% Effective Concentration
ee	Enantiomeric Excess
EIMS	Electron Impact Mass Spectrometry
EtOAc	Ethyl Acetate
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IC_{50}	50% Inhibitory Concentration
INT	Integrase
LDL-C	Low Density Lipoprotein—Cholesterol
NCI	National Cancer Institute
NIH	National Institutes of Health

NMR	Nuclear Magnetic Resonance
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
PI	Protease Inhibitor
PLC	Preparative Liquid Chromatography
ppm	Parts Per Million (δ)
PR	Protease
RNA	Ribonucleic Acid
R_f	Retention Factor (for TLC)
RT	Reverse Transcriptase
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
t_R	Retention Time (for HPLC)
TsDPEN	1,2-*p*-Toluenesulfonyl-1,2-Diphenylethylenediamine
2-PrOH	2-Propanol

INTRODUCTION

INTRODUCTION

The content of this thesis centers around the use of synthetic organic chemistry to prepare compounds with the potential for treating diseases affecting our society and other societies around the world. Two of the more prevalent (and preventable) diseases include HIV and coronary artery disease, and this is where the work discussed within has focused.

HIV has become a worldwide epidemic and has seen a tremendous increase in the number of reported cases[1] as well as in the magnitude of research devoted to treating the disease. In addition, vaccinations have and continue to be investigated as a preventative measure against this dreaded affliction.[2] Research done in the Baker laboratory and reported within deals with treating the disease from a replication standpoint, inhibiting the ability of the virus to perform a crucial step during the replication process. In particular, the focus has been on the reverse transcriptase enzyme and potential inhibitors of the non-nucleoside class. The synthesis and biological evaluation of compounds that are potential drug candidates is reported.

Elevated serum cholesterol levels and an associated higher incidence of blockages and buildup can lead to coronary artery disease. Methods involving the use of cholesterol–lowering drugs have been used, but dietary management and functional foods having the ability to lower serum cholesterol levels have also been investigated for elevated cholesterol treatment.[3] In particular, the fatty-acid esters

of phytostanols and phytosterols have been studied and have been found to lower serum cholesterol levels by significant amounts.[4,5] The scope of this project involved the synthesis and purification of a large quantity of these esters. The synthesis reported within utilizes published synthetic methods applied to the production of these esters. We feel that our methodology represents an advantage of the previously published methods in a few key aspects and would be amenable to large-scale reactions to produce these and other related ester products.

PART ONE

THE SYNTHESIS AND BIOLOGICAL EVALUATION OF ANALOGS OF A NEW NON-NUCLEOSIDE REVERSE INHIBITOR OF HIV-1

1. INTRODUCTION

In 1981, research groups led by Friedman-Klein, Siegel, and Masur first diagnosed a new disease: *acquired immune deficiency syndrome* (AIDS) among young homosexual men.[6] Associated with AIDS is Kaposi's Sarcoma, an unusual form of cancer seen in the patients presenting symptoms of AIDS infection.[6,7] Both of these diseases are characterized by a deficiency in the number of helper T cells present to help fight and ward off various infectious diseases. By January 1985, the viral genome of AIDS was determined by groups at the Pasteur Institute[8], the NCI/NIH[9], Genentech, Inc.[10], and Chiron.[11] The virus causing AIDS was determined to be a retrovirus and was named appropriately as the *human immunodeficiency virus*.

A. The Human Immunodeficiency Virus (HIV)

HIV belongs to a subfamily of viruses known as lentiviruses,[12] characterized by the sometimes long period of time between initial viral infection and the signs or symptoms of illness. These are part of a larger group known as Retroviridae.[12] Viruses classified under the Retroviridae family name possess the following features:

- A single stranded genome composed of RNA material
- Common viral strucure containing three polyprotein genes—group specific antigen (*gag*), polymerase (*pol*), and envelope (*env*)

- Integration of the viral genome into the genetic material of the host as an essential step in viral reproduction
- The ability to mutate in response to environmental stimuli (i. e. drug treatment)

B. Replication of the HIV Virus

The HIV virus replicates using the host's cellular machinery to produce healthy virions. A diagram depicting the events leading to viral infection is shown in Figure 1. A summary of the HIV life-cycle is as follows:

- Attachment to the host cell by a healthy virion
- Entry into the host cell's interior
- Transcription of viral RNA into DNA (reverse transcription)
- Viral integration into the host cell's genome (integration)
- Synthesis and expression of new viral RNA
- Assembly of the virus and budding of new virions
- Release of the virions from the host cell (proteation)

A few of the key proteins involved in viral replication, namely reverse transcriptase, integrase, and protease, have been studied extensively in an effort to produce viable treatment options for those infected with HIV.

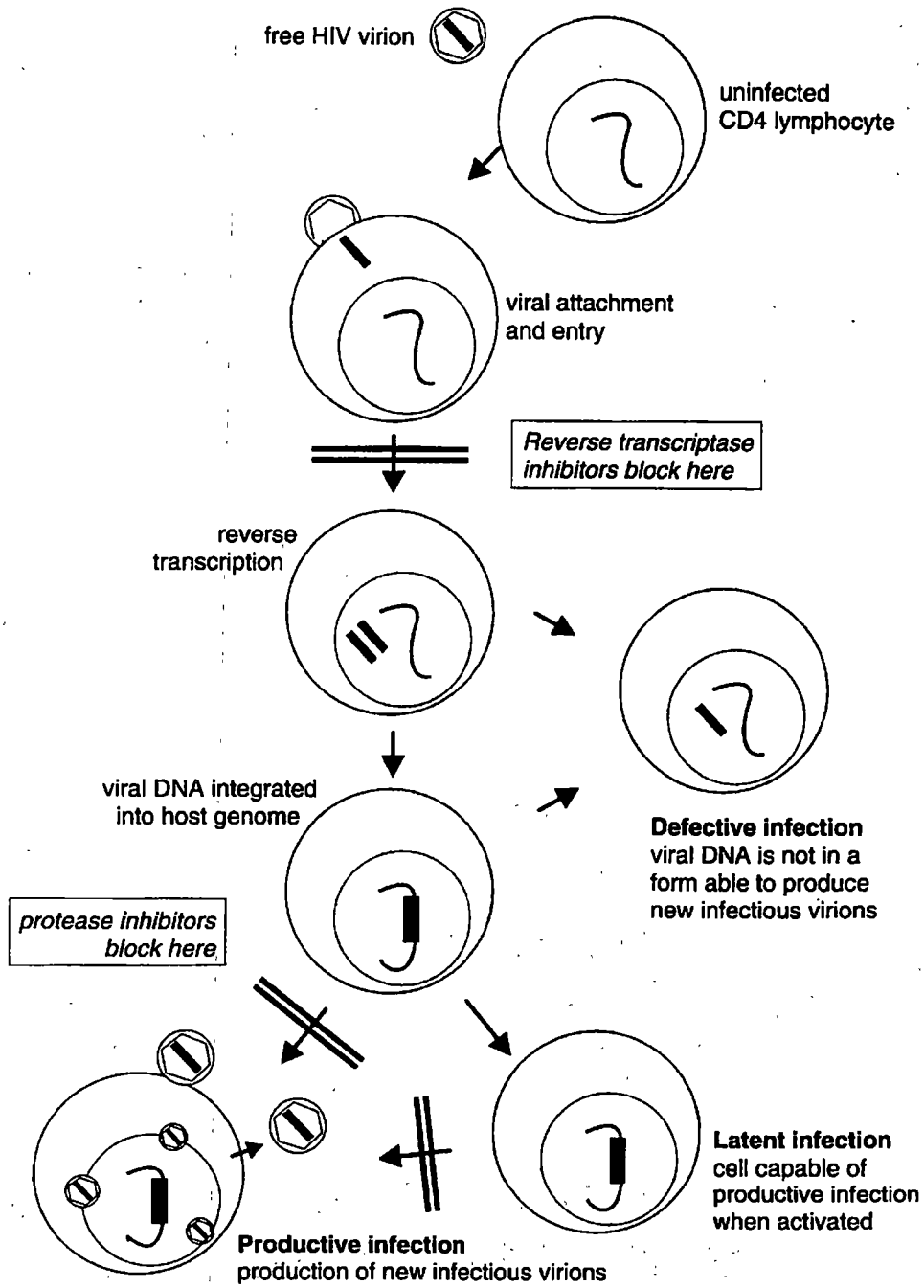


Figure 1. The Replication of HIV-1 in CD4 Lymphocytes

Source: Mann, D. A. In *The Molecular Biology of HIV/AIDS*; Lever, A. M. L., Ed.; John Wiley & Sons LTD: Chichester, 1996; p. 61

C. Targeting the HIV Virus: The Antiviral Approach

Treating patients with HIV has proven to be a difficult task undertaken by researchers and physicians. This is largely due to the resistance of HIV to antiviral treatment as a result of key mutations in the wild-type virus to produce infectious virions undeterred by current drugs used to treat the disease.[13-21]

The three major targets for treating HIV infection fall into the following classes:

- Reverse Transriptase Inhibitors
- Integrase Inhibitors
- Protease Inhibitors

There are other targets that have been identified as possible targets for treatment of HIV, however the above classes remain the most studied to date and will be the focus of this introduction.

HIV Reverse Transcriptase (RT)

The reverse transcriptase (RT) enzyme of HIV-1 is a heterodimer consisting of p51 and p66 subunits (see **Figure 2**). RT catalyzes the conversion of viral RNA into double stranded DNA.[22,23] This differs from the normal flow of genetic information which proceeds in the DNA to RNA direction. This is a crucial step in the replication process which has attracted a great deal of attention among researchers. Efforts to develop inhibitors of this enzyme are ongoing and have revealed some valuable insight into the mechanics of viral replication as well as

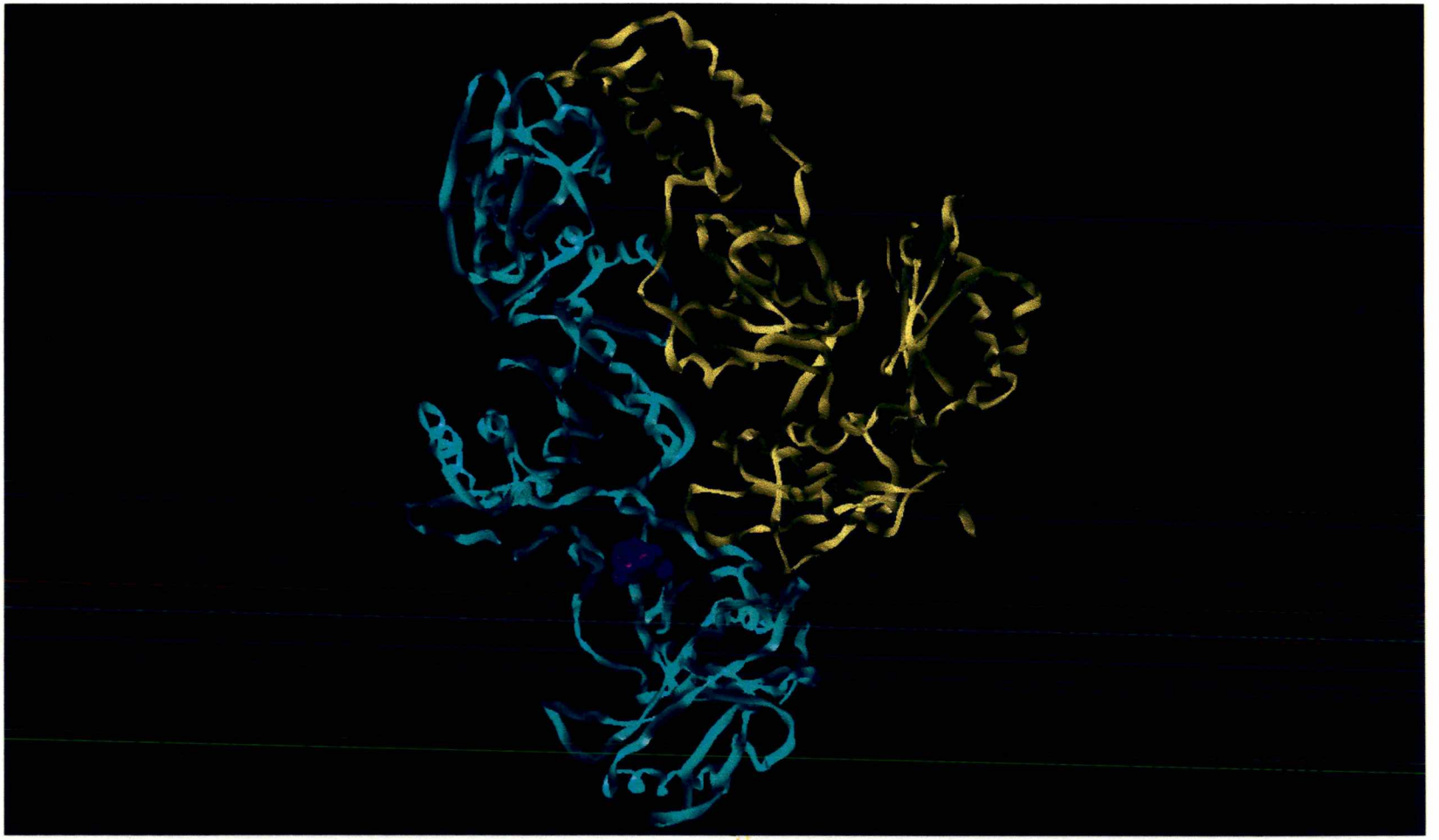


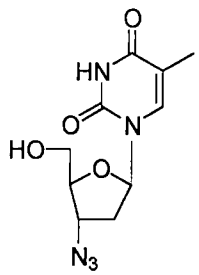
Figure 2. HIV-1 Reverse Transcriptase Complexed with Nevirapine (p66 in cyan, p51 in yellow, Nevirapine in purple) *Source*: Protein Data Bank (PDB ID 3HVT)

strategies to manage the patients' viral load.

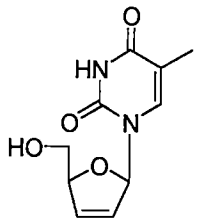
Inhibitors of the RT enzyme are classified according to their molecular structure. Also, their mode of action is indicated according to which class they fall into. The two main categories of RT inhibitors developed to date are *nucleoside reverse transcriptase inhibitors* (NRTIs) and *non-nucleoside reverse transcriptase inhibitors* (NNRTIs).

The nucleoside inhibitors of HIV-1 act as chain terminators to the growing viral DNA chain.[7,22,24-29] Since they are analogs of the natural nucleosides, except that they lack 3'-OH groups, they are immune to the attachment of an incoming 2'-deoxynucleotide triphosphate (dNTP) and therefore terminate the chain once they are incorporated. These inhibitors are competitive inhibitors relative to incoming dNTPs and bind to the catalytic site in the enzyme. Some of the current nucleoside inhibitors are shown in **Figure 3**.

The non-nucleoside inhibitors of HIV bind non-competitively to an allosteric site proximal to but separate from that in which the nucleoside analogs bind.[30-35] This binding has an effect on the ability of the catalytic binding site to function, and this is how these inhibitors are thought to operate. The NNRTIs are an asset to treatment regiments as they exhibit high efficacy even at sub-micromolar and nanomolar concentrations and minimal toxicity to healthy cells.[36-39] Some of the current non-nucleoside inhibitors are shown in **Figure 4**.



Zidovudine, AZT (RETROVIR®)



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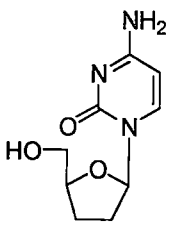
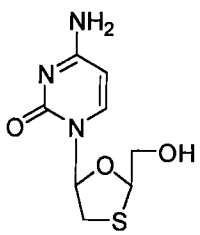
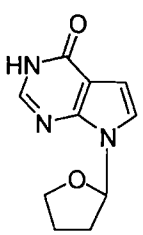


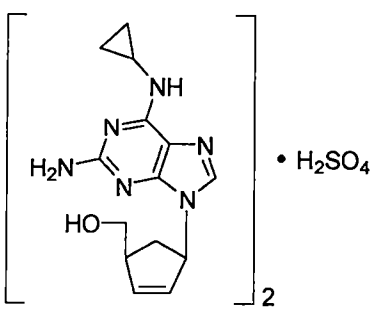
Zalcitabine, ddC (HIVID®)



Lamivudine, 3TC (EPIVIR®, ZEFFEX®)

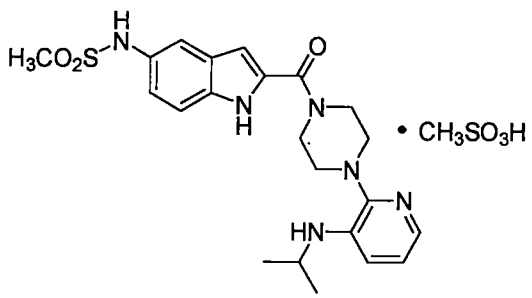


Didanosine, ddI (VIDEX®)

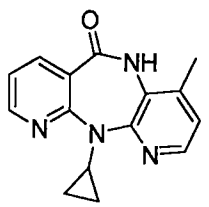


Abacavir, ABC (ZIAGEN®)

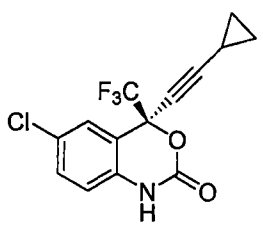
Figure 3. Nucleoside Inhibitors of HIV-1



Delavirdine mesylate (RESCRIPTOR®)



Nevirapine (VIRAMUNE®)



Efavirenz (SUSTIVA®)

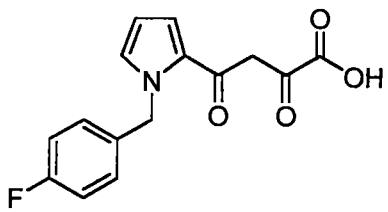
Figure 4. Non-Nucleoside Inhibitors of HIV-1

HIV Integrase (INT)

The integrase enzyme of HIV-1 mediates the connection of the host cell's chromosome to the generated proviral DNA.[40,41] The viral integrase enzyme and the integration process is an attractive target to study given that there is no known analogous mammalian counterpart or process.[40,42] Several limitations, however, have prevented the development of efficient inhibitors and antiviral assays of the integrase enzyme.[40] Only one class of compouds, the diketo acid inhibitors (see **Figure 5**), are found to inhibit the integrase enzyme in a manner which translates to cellular (*in vivo*) inhibition and therefore has the potential to be developed into a viable drug candidate.[41,42]

HIV Protease (PR)

The viral protease enzyme of HIV is essential for the cleavage of precursor polyproteins that contain proteins and enzymes that make up an infectious virion.[43-46] This cleavage is necessary to produce mature viral particles, and hence, necessary to lead to HIV infection. Several small molecule inhibitors have been approved for human use and are shown in **Figure 6**.



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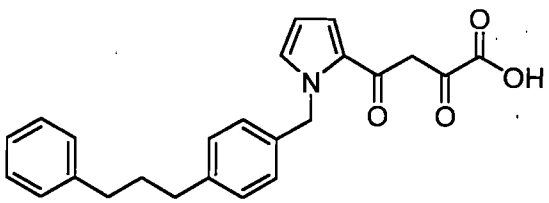

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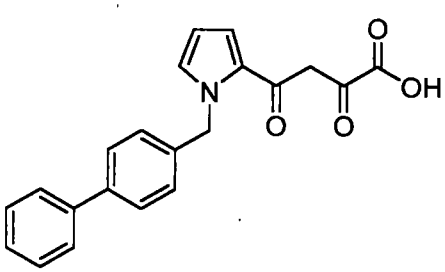

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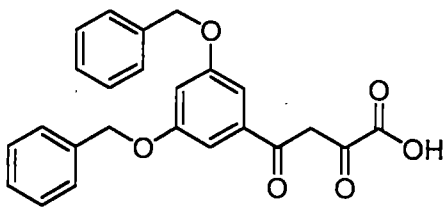
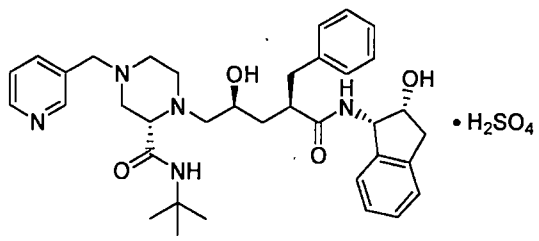
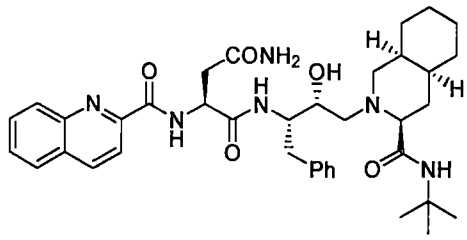


L-708,906

Figure 5. Some of the Diketo Acid Integrase Inhibitors of HIV-1



Indinavir Sulfate (CRIXIVAN®)



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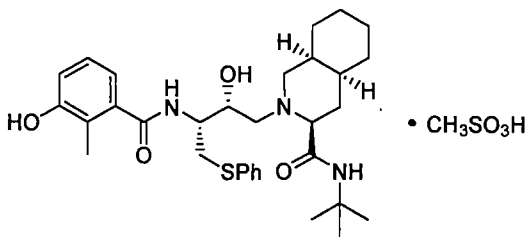
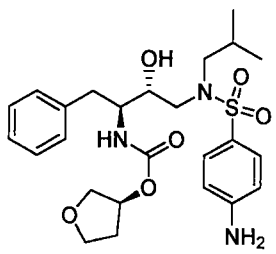
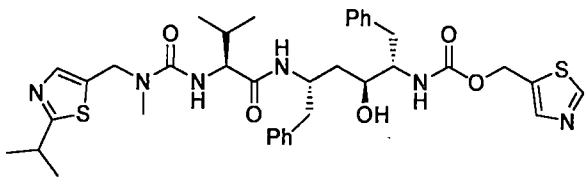


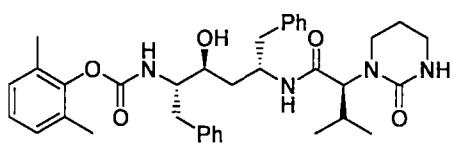
Nelfinavir Mesylate (VIRACEPT®)



Amprenavir (AGENERASE®, PROZEI®)



Ritonavir (NORVIR®)



Lopinavir (co-administered w/ Ritonavir (4:1), KALETRA®)

Figure 6. Protease Inhibitors of HIV-1

D. Multiple Drug and Combination Therapy

In the early stages of development, treatment of HIV infection involved the use of monotherapy with one of the nucleoside reverse transcriptase inhibitors such as zidovudine (AZT).[25,27-29,47] Mono and dual therapies are more or less obsolete now because of the temporary reduction in plasma HIV-1 RNA levels, and the emergence of drug-resistant strains.[47] More recently, combination therapies consisting of three or more antiviral drugs are being utilized in order to keep the viral load beyond detectable levels.[7,47-49] This multiple combination therapy is termed "Highly Active Antiretroviral Therapy" (HAART) and includes the use of any three drugs from the NRTI, NNRTI, and PI classes. The treatment currently recommended according to the HAART guidelines is a combination of two NRTIs in addition to one or two PIs or an NNRTI.[47] Studies suggest that combination therapy offers greater success for the overall reduction of infection and the progression to AIDS.[2,42-44]

2. STATEMENT OF THE PROBLEM

A. Objective

The main objective of this project was to develop a series of 3-substituted sultams. Progressing from a 3-vinyl derivative, we were able to make a series of compounds containing various substituents via derivatization of the vinyl function. Included in our study was the possibility of developing a sultam capable of covalent inhibition (covalent attachment to the enzyme or protein target).

B. Research Plan

This project was a continuation of one developed in our group designed to synthesize and evaluate a series of 2-methyl-1,3-phenyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxides, commonly known as "sultams". The parent compound (**NSC 108406**) was determined by the NCI to be a potent inhibitor of HIV-1 reverse transcriptase.[50] Studies done in our laboratory concluded that the active isomer has "*S*" stereochemistry at the asymmetric center.[46] The structure of the "active" parent compound (**NSC 694444**) is shown in **Figure 7**.

Initial studies focused on substitution of the A ring of the parent compound. These modifications, however, led to diminished activity relative to the parent. Modifications on the C ring, however, were found to lead to increased activity.

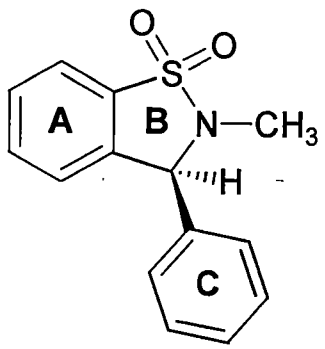


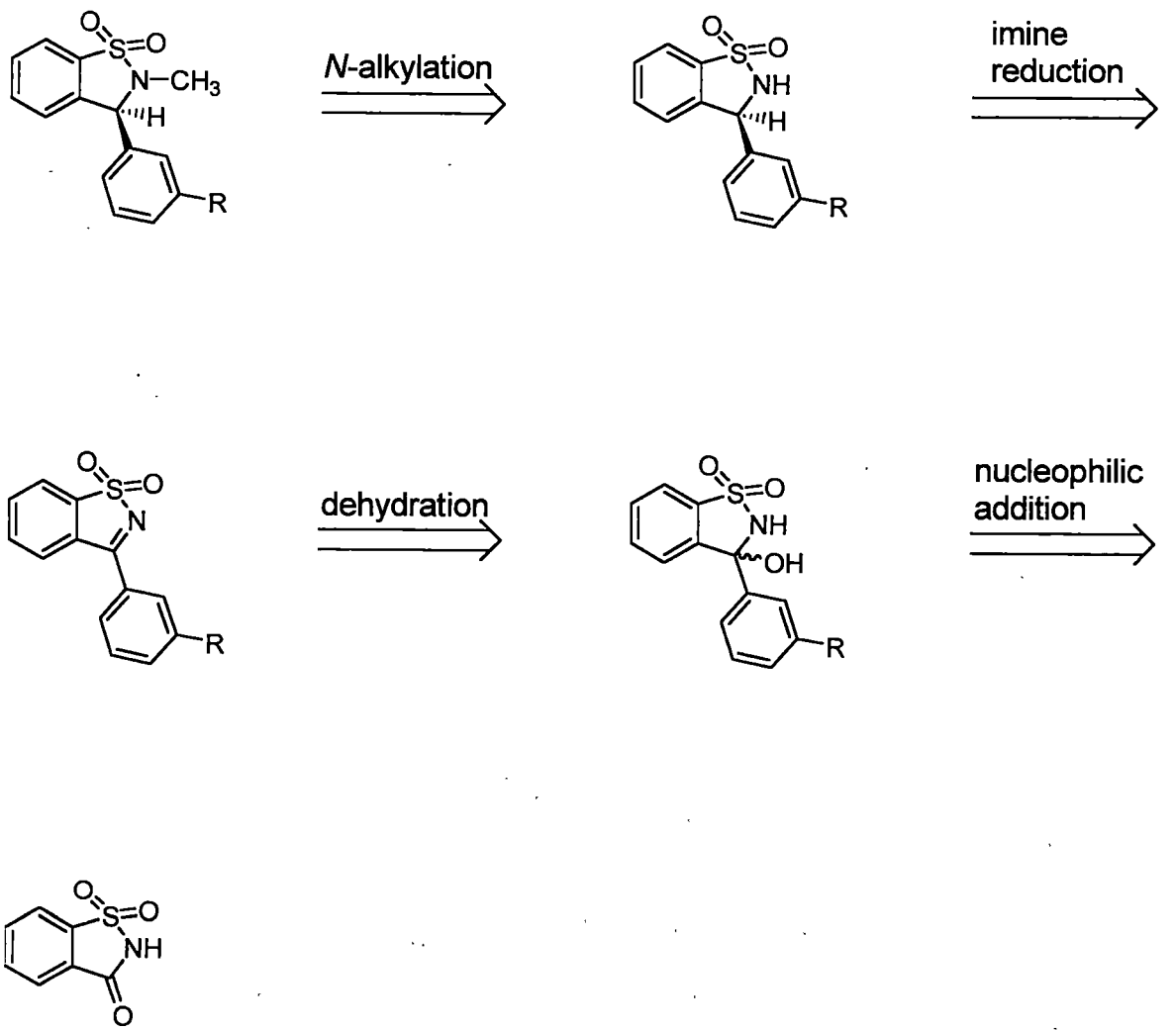
Figure 7. Structure of the NCI's Lead Compound **NSC 694444**

Note: This is the active "S" stereoisomer determined via separation of the two enantiomers by A. Mayasundari and subsequent testing. The racemate is referred to as **NSC 108406**.

Some of the most active compounds are listed in **Table 1**. Given that these compounds are structurally similar and all contain substituents at the 3-position of the C ring, we decided that further study into the effects of substituents at the 3-position with varying steric and electronic properties would be in order. In addition, the investigation into the potential for covalent inactivation was proposed.

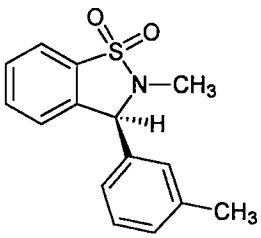
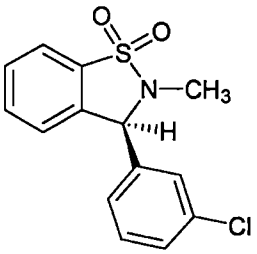
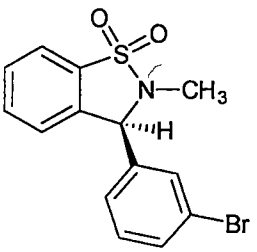
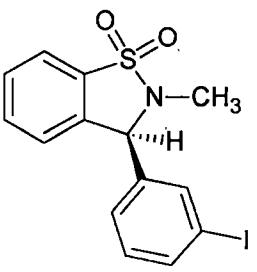
The retrosynthesis of these compounds, initially made in racemic form prior to the determination of the active isomer,[51] led us to ascertain that some key asymmetric reaction would be the crux of our scheme if we were to make these compounds in an optically active fashion. Taking the lead from some chemistry done by Abramovitch,[52] we decided that sulfonimide compounds would be ideal in these cases. The retrosynthetic analysis is shown in **Scheme 1**. The key step in the synthesis of these compounds will involve the asymmetric reduction of a sulfonimine intermediate to produce the sulfonamide of the proper stereochemistry. A catalyst developed by Noyori for the transfer hydrogenation of imines was well-

suited for the task.[53-56] Later studies[55] done by Mao and Baker led to the development of a new rhodium catalyst system that is superior to the ruthenium system we used in this synthesis. Previous chiral-phase HPLC analysis confirmed the presence of one isomer and crystallographic studies done by our group determined the absolute structure of the isomer to be correct.[51] Alkylation of the sulfonamide nitrogen would complete the synthesis.



Scheme 1. Retrosynthetic Analysis of 3-substituted Sultam Analogs

Table 1. Anti-HIV Activity of Some Highly Active 3-Substituted Sultam Analogs

Compound	EC_{50} (M)	IC_{50} (M)	TI_{50}	Activity
 **(NSC 708199)**	0.037×10^{-6}	$> 2.0 \times 10^{-6}$	$> 5.4 \times 10^{1}$	Active
 **(NSC 701751)**	0.086×10^{-6}	$> 2.0 \times 10^{-6}$	$> 3.1 \times 10^{1}$	Active
 **(NSC 703774)**	0.074×10^{-6}	1.0×10^{-4}	1.4×10^{2}	Active
 **(NSC 704673)**	0.076×10^{-6}	6.2×10^{-5}	8.2×10^{2}	Active

3. RESULTS AND DISCUSSION

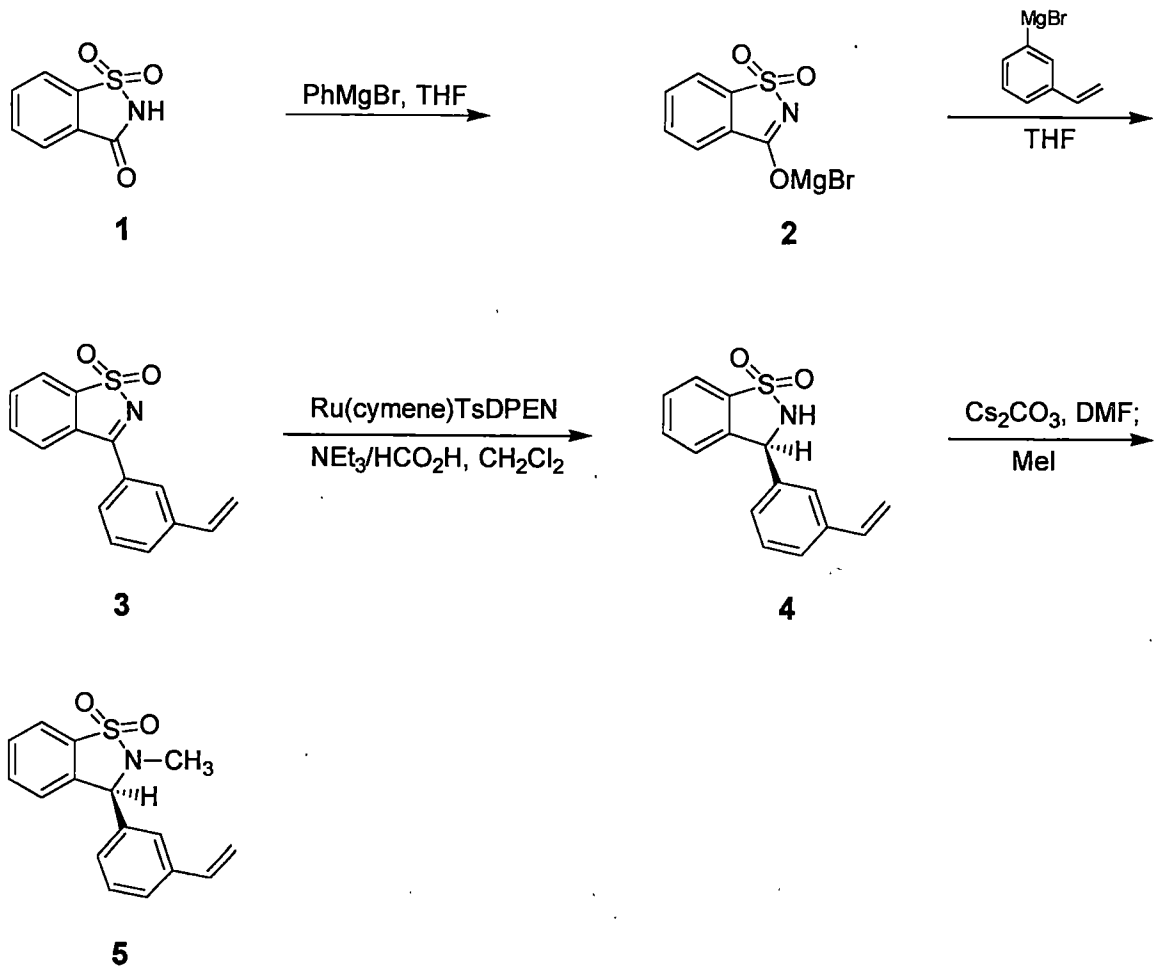
A. Synthesis of the 3-Vinyl Derivative

The gateway to the various 3-substituted sultams we wished to synthesize would best be a compound with a functional group capable of transformation into other groups which we wished to see incorporated into the 3-position of ring C on the parent compound. Initial attempts to utilize a protected 3-bromobenzyl alcohol as the precursor failed at any and all metallation steps, which included the attempt to make Grignard reagents and organolithiums using published methods. Our attention then turned to the production of a 3-vinyl analog **5** which we could then conceivably transform into other potential drug candidates using common laboratory methods. The starting point for our synthesis was saccharin, a cheap, readily available raw material, adding to the attractiveness and economic feasibility of our approach. Addition of one equivalent of phenylmagnesium bromide to remove the acidic proton followed by one equivalent of 3-vinylphenylmagnesium bromide leads to the hemiaminal which we found was dehydrated to the sulfonimide **3** under the conditions of the workup,[52] eliminating the necessity for a separate dehydration step. Our attention then focused on a stereoselective reduction of the imine functionality to produce the desired "*S*" stereoisomer. Reduction of the imine using Noyori's catalyst[53,54,56] in methylene chloride in the presence of a formic acid—triethylamine azeotrope[57] at room temperature yielded the desired product **4** in high enantiomeric excess as evidenced by chiral-phase HPLC analysis.

Recrystallization of the material increased the ee to > 99 %. A simple and clean alkylation of the sulfonamide nitrogen with cesium carbonate suspended in DMF and methyl iodide completed the synthesis of the target compound **5**.

B. Elaboration of 5 To Yield Analogs 6-8

After having the 3-vinyl derivative in hand, we decided that we could quickly and easily make analogs via catalytic hydrogenation and ozonolyis—reduction. This would lead to 3-ethyl (**6**), a 3-methanol (**7**), and a 3-formyl (**8**) derivatives. The scheme detailing these transformations is given in **Scheme 3**. High pressure hydrogenation of **5** over palladium on carbon in ethyl acetate cleanly afforded the ethyl derviative. Ozonolysis of **5** was performed in methylene chloride at -78 °C.[58] Reduction of the ozonide using borane—dimethylsulfide complex[59] afforded the 3-methanol analog **7**, while mild reduction using dimethylsulfide[58] led to the 3-formyl derivative **8**.



Scheme 2. Synthesis of Sultam Analog **5**

C. Covalent Inactivation and Analogs 9 and 10

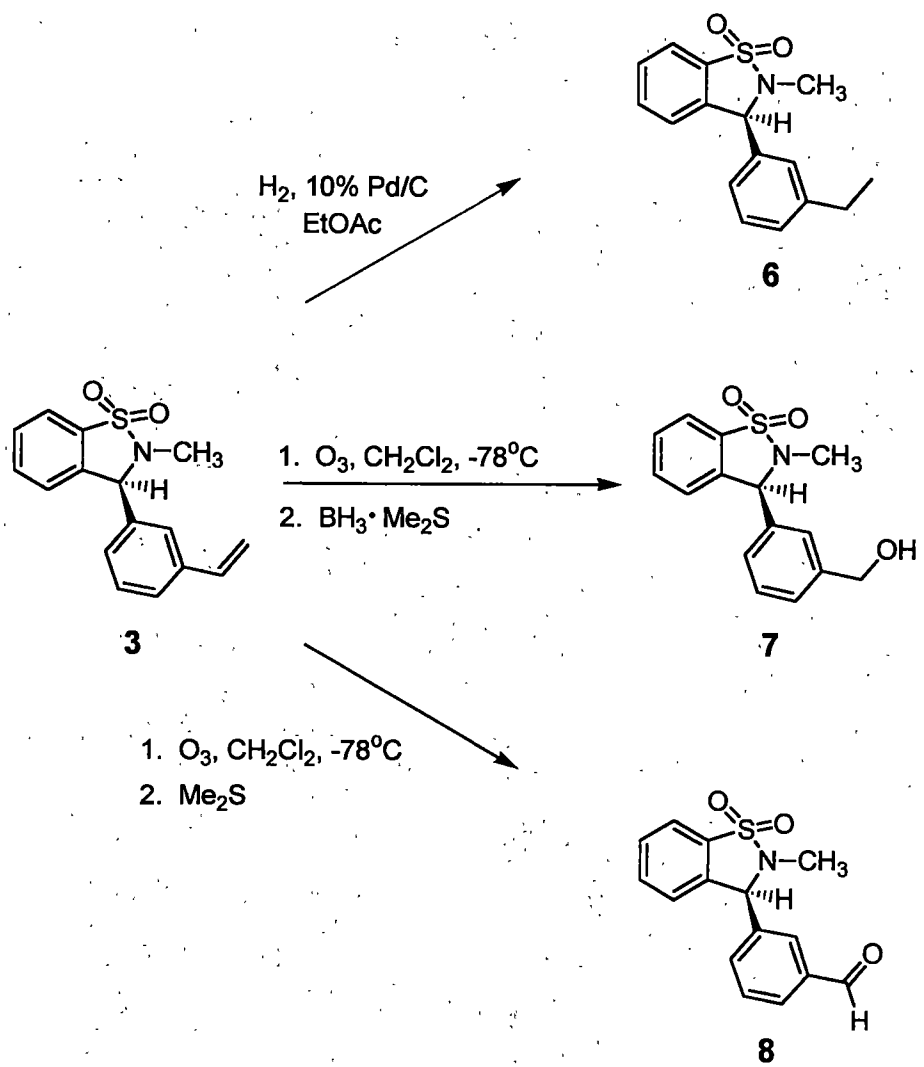
Since the NNRTI class of HIV-1 RT inhibitors bind reversibly with the enzyme, an attractive target to synthesize would incorporate functionality conducive to covalent attachment to the enzyme. If a nucleophilic amino acid side chain (e. g. lysine, serine, cysteine) were proximal to a function capable of nucleophilic displacement (e. g. an alkyl or benzyl halide), a substitution reaction might occur, leading to the irreversible binding of the inhibitor to the enzyme, and therefore complete inhibition of the catalytic process.[60,61] This is an exceedingly attractive method to try and inhibit HIV replication, and one that we felt was worth looking into.

Since we already had the 3-methanol compound in hand, transformation into an appropriate electrophile would be trivial. Treatment of **7** with methanesulfonyl chloride and triethylamine in methylene chloride[62,63] gave the corresponding mesylate **9**. This would be a viable candidate for this study and would be evaluated as well. Reaction of **9** with lithium bromide in DMF[62] gave the benzylic bromide. **Scheme 4** details the chemistry we used to accomplish these transformations.

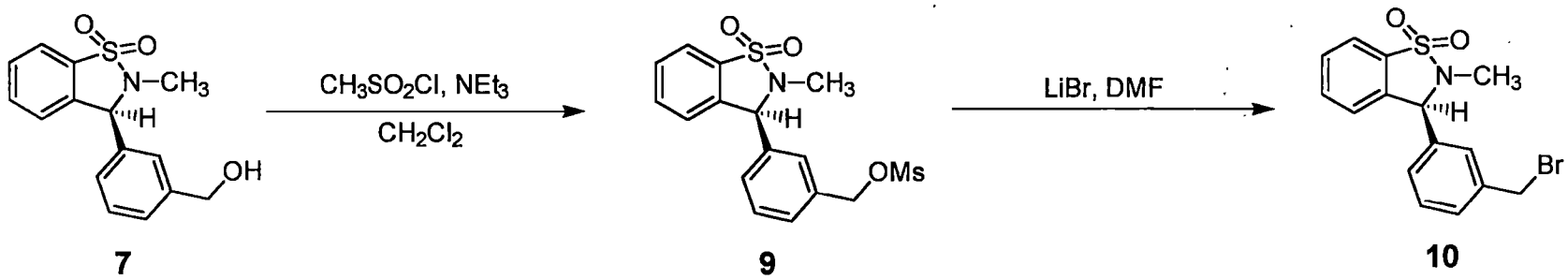
D. Anti-HIV Evaluation Results

The results of the anti-HIV evaluation of sultam analogs **6** - **10** are summarized in **Table 2**. These compounds were evaluated for anti-HIV-1 activity

using the XTT assay at the National Cancer Insitute of the National Institues of Health, Bethesda, Maryland.[64] Clearly the results are promising and represent a step in the right direction. Though the activities are less than those that were deemed the most potent in the initial studies, their sub-micromolar activities make them attractive as possible drug candidates. Our hopes of covalent attachment did not quite work out as planned, however the promise of developing an inhibitor capable of doing so likely warrants further study. In addition, the use of the "sultam" as a molecular scaffold to develop analogs which may overcome the resistance problem can be explored.



Scheme 3. Elaboration of **5** to Yield Analogs **6**, **7**, and **8**



7 9 10

Scheme 4. Synthesis of Potential Covalent Inactivators of HIV-1 RT

Table 2. Anti-HIV Activity of the Elaborated Series of 3-Substituted Sultam Analogs

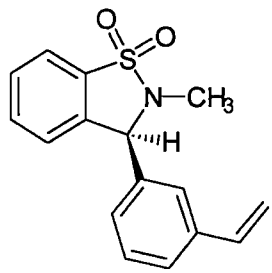
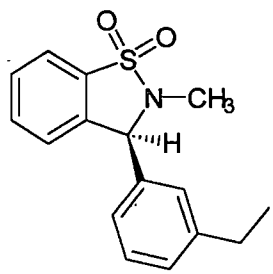
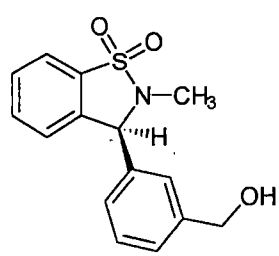
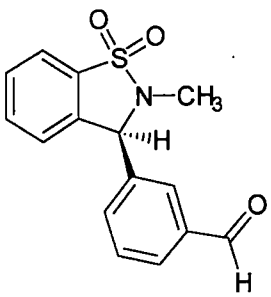
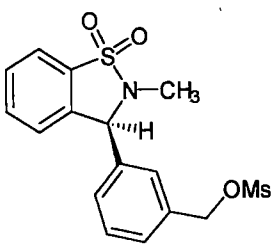
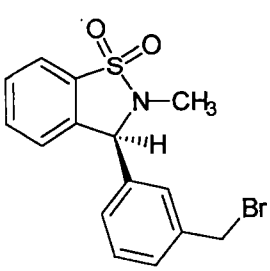
Compound	EC_{50} (M)	IC_{50} (M)	TI_{50}	Activity
 **(NSC 710028)** **5**	0.86×10^{-6}	4.3×10^{-6}	6.4×10^{1}	Active
 **(NSC 710027)** **6**	0.12×10^{-6}	3.5×10^{-5}	4.3×10^{1}	Active
 **(NSC 710828)** **7**	6.0×10^{-6}	$> 2.00 \times 10^{-4}$	$> 5.0 \times 10^{1}$	Active

Table 2, cont'd.

Compound	EC_{50} (M)	IC_{50} (M)	TI_{50}	Activity
 **(NSC 710829)** **8**	1.41×10^{-6}	3.2×10^{-5}	2.3×10^{1}	Active
 **(NSC 710832)** **9**	1.92×10^{-6}	7.2×10^{-5}	3.9×10^{1}	Active
 **(NSC 710831)** **10**	0.9×10^{-6}	3.1×10^{-6}	4.4	Moderate

4. EXPERIMENTAL

A. General Methods

All reactions were monitored by thin-layer chromatography (TLC). Adsorption chromatography was carried out using E. Merck silica gel products: (a) TLC on 0.2 mm aluminum-backed plates, (b) column chromatography using 230–400 mesh silica gel. Visualization of the TLC plates was by 254 nm light. Anhydrous solvents were obtained as follows: THF was refluxed over sodium–benzophenone ketyl and distilled, CH_2Cl_2 was distilled from calcium hydride, and DMF was distilled from calcium hydride under reduced pressure. All reactions where carried out under a nitrogen atmosphere unless otherwise indicated. Solvents were evaporated at aspirator vacuum at 25 °C unless otherwise indicated. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were furnished by Atlantic Microlab, Inc., Norcross, GA. ^1H and ^{13}C NMR spectra were recorded at 300.087 MHz and 75.464 MHz, respectively, on a Varian *MERCURY* spectrometer. ^1H NMR chemical shifts are reported as δ (ppm) downfield from an internal standard of tetramethylsilane (TMS); multiplicities are first-order values in Hz: s, singlet; br s, broad singlet; d, doublet; t, triplet; dd, doublet of doublets; ψt, pseudotriplet; m, multiplet. ^{13}C NMR chemical shifts are reported as δ (ppm) relative to $CDCl_3$ (77.00 ppm) as standard. Optical rotations were measured with a Perkin-Elmer Model 241 automatic polarimeter equipped with a sodium lamp for

solutions in a 1.0 dm cell at the indicated temperature. Enantiopurities were determined using a Beckman System Gold HPLC system equipped with a Chiralcel OD column (25 cm x 1 cm) using a flow rate of 3.0 mL/min of the indicated solvent system and a detection wavelength of 254 nm.

B. Synthesis of Compounds 3-10

3-(3-Vinylphenyl)benzo[*d*]isothiazole 1,1-dioxide (3). To a three-necked round-bottomed flask fitted with a condenser and an addition funnel was added magnesium metal shavings (0.210 g; 8.60 mmol). The apparatus was hooked up to a nitrogen inlet and purged for 20 min. 10 mL of THF was added and the mixture was stirred vigorously for 20 min. A solution of 3-bromostyrene (1.50 g; 8.19 mmol) in THF (25 mL) was prepared in the addition funnel and added dropwise to the reaction vessel over a period of 0.5 h. The mixture was brought to reflux temperature upon which time a cloudy yellow-brown mixture resulted which was accompanied by disappearance of the Mg metal. The Grignard reagent was refluxed for a total of 2 h before being used. To a 100 -mL round-bottomed flask was added saccharin (1.51 g; 8.27 mmol; 1.01 equiv) which was dissolved in 25 mL of THF and purged with nitrogen. The saccharin solution was cooled to 0 °C in an ice bath and a 3.0 M solution of phenylmagnesium bromide (2.76 mL; 8.27 mmol; 1.01 equiv) was added dropwise via syringe over a period of 10 min. The ice bath was removed and the 3-vinylphenylmagnesium bromide solution was added over a 10 min. period at which time the reaction mixture turned from pale yellow to

midnight blue. The mixture was stirred for a total of 18 h and hydrolyzed by pouring it into a mixture of ice-water (200 mL). The solution was adjusted to pH 1 using 5 % HCl and then extracted with Et_2O (4 x 50 mL). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (2 x 15 mL) to remove unreacted saccharin. The ethereal solution was dried over anhydrous $MgSO_4$ and concentrated to yield a yellow solid. Column chromatography (2:1 hexane–EtOAc) gave **1** as pale yellow crystals (998 mg, 45% over two steps): TLC: R_f (2:1 hexane–EtOAc) 0.29; mp 179–180 °C ; ^{1}H NMR ($CDCl_3$): δ 5.41 (d, 1H), 5.88 (d, 1H), 6.77 (dd, 1H), 7.57(yt, 1H), 7.71–8.04 (m, 7H); ^{13}C NMR ($CDCl_3$): δ 116.17, 123.03, 126.44, 127.09, 128.46, 129.27, 130.42, 130.61, 130.80, 133.33, 133.58, 135.29, 138.69, 140.92, 170.87. Anal. Calcd for $C_{15}H_{11}NO_2S$: C, 66.90; H, 4.12; N, 5.20; S, 11.91. Found: C, 66.80; H, 4.15; N, 5.17; S, 11.92.

3*S*-(3-Vinylphenyl)-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (4). In a 25-mL round-bottomed flask fitted with a reflux condenser was added dichloro(*p*-cymene)ruthenium(II) dimer (43.17 mg; 0.07 mmol) and (1*S*, 2*S*)-(*p*-tolylsulfonyl)-1,2-diphenylethylenediamine (TsDPEN) (51.68 mg; 0.141 mmol). TEA was added until the components dissolved, resulting in a clear orange-red solution. The mixture was refluxed at 80 °C for 0.5 h. 5 mL of dry CH_2Cl_2 was added to the flask to completely dissolve any of the precipitated catalyst. **3** (3.8 g; 14.1 mmol) was dissolved in 25 mL of CH_2Cl_2 in a 100-mL round-bottomed flask and kept under nitrogen. The prepared catalyst was slowly added via syringe. 2.0 mL of a 5:2 solution of Et_3N–HCO_2H was added to the solution. The reaction was monitored by

TLC and was complete after 9.5 h. The reaction was quenched with aqueous Na_2CO_3 until the aqueous layer tested basic to litmus paper (5 mL). The layers were separated and the organic phase was dried over $MgSO_4$ and concentrated to yield a brown solid. Column chromatography (2:1 hexane–acetone) afforded **4** as a slightly pink solid (3.0 g, 79%). Determination of the enantiomeric excess (ee) was accomplished using chiral HPLC (3:1 hexane–isopropanol) and revealed that the reaction produced an ee of 80% in favor of the desired *S* enantiomer. Recrystallization of the solid from EtOAc–hexane resulted in enantiomerically pure **4** (>99% ee by chiral HPLC) as white crystals (2.32 g, 61%): TLC: R_f (2:1 hexane–acetone); 0.20 mp 164–165 °C ; $[\alpha]_D^{20}$ +128° (*c* 0.46, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.95 (br s, 1H), 5.30 (d, 1H), 5.72 (d, 1H), 5.77 (d, 1H), 6.69 (dd, 1H), 7.14–7.17 (m, 1H), 7.23–7.59 (m, 6H), 7.83–7.86 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 61.32, 115.09, 121.08, 125.24, 125.29, 126.71, 126.78, 129.41, 129.44, 133.27, 134.60, 135.83, 138.50, 138.79, 139.47. Anal. Calcd for $C_{15}H_{13}NO_2S$: C, 66.40; H, 4.83; N, 5.16; S, 11.82. Found: C, 66.28; H, 4.92; N, 5.12; S, 11.70.

(3*S*)-2-Methyl-3-(3-vinylphenyl)-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (5). Compound **2** (2.30 g; 8.55 mmol) was dissolved in 50 mL of DMF in a 100 -mL round-bottomed flask. Cs_2CO_3 (4.17 g; 12.8 mmol; 1.5 equiv) was added and was allowed to stir at room temperature for 0.5 h. MeI (0.53 mL; 1.5 mmol; 1.5 equiv) was added slowly via syringe. The reaction was allowed to stir at room temperature for 1 h and poured into 200 mL of ice-water. The mixture was allowed to stand overnight upon which the product **3** crystallized from the solution. The

crude product was collected by vacuum filtration and subjected to column chromatography (3:1 hexane–EtOAc) and recrystallization from EtOAc–hexane to yield **3** as white crystals (2.01 g; 82%). Chiral HPLC analysis (9:1 hexane–isopropanol) confirmed that epimerization of the stereocenter did not occur under the reaction conditions (>99% ee). TLC: R_f (3:1 hexane–EtOAc) 0.30; mp 132–133 °C; $[\alpha]_D^{20}$ +179° (*c* 0.14, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.79 (s, 3H), 5.18 (s, 1H), 5.30 (d, 1H), 5.78 (d, 1H), 6.71 (dd, 1H), 7.04–7.07 (m, 1H), 7.21–7.55 (m, 6H), 7.85–7.88 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 27.58, 66.94, 114.96, 121.06, 124.89, 125.82, 126.75, 127.42, 129.27, 129.31, 132.91, 133.87, 135.94, 136.84, 138.12, 138.43. Anal. Calcd for $C_{16}H_{15}NO_2S$: C, 67.34; H, 5.30; N 4.91; S, 11.24. Found: C, 67.10; H, 5.24; N, 4.96; S, 11.09.

3*S*-(3-Ethylphenyl)-2-methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (6). Compound **5** (150 mg; 0.53 mmol) was dissolved in 10 mL of EtOAc in a 50 mL test tube and placed in a Parr bottle. 10% Pd/C (150 mg) was added and the contents were stirred in a high pressure hydrogenator at 50 psi for 2 h. The reaction mixture was filtered through Celite and concentrated to afford a white solid which was purified by recrystallization from hexane to afford **6** as white needles (143 mg; 94%). TLC: R_f (2:1 hexane–EtOAc) 0.44; mp 106–107 °C ; $[\alpha]_D^{20}$ +133° (*c* 0.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.23 (t, 3H), 2.65 (q, 2H), 2.78 (s, 3H), 5.16 (s, 1H), 7.03–7.04 (m, 1H), 7.14–7.35 (m, 4H), 7.50–7.55 (m, 2H), 7.84–7.88 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 15.63, 27.52, 28.80, 67.06, 120.98, 124.90, 125.41, 127.37, 128.57, 128.98, 129.15, 132.83, 133.84, 136.38, 138.38, 145.26. Anal. Calcd for

$C_{16}H_{17}NO_2S$: C, 66.87; H, 5.96; N, 4.87; S, 11.16. Found: C, 66.83; H, 6.05; N, 4.84; S, 11.14.

3*S*-(2-Methyl-1,1-dioxo-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazol-3-yl)benzaldehyde (8). In a 100 -mL round-bottomed flask, compound **5** (403 mg; 1.41 mmol) was dissolved in 50 mL of CH_2Cl_2 and cooled to -78 °C in a dry ice-acetone bath. Ozone was bubbled through the solution until the solution remained blue in color for 0.5 h. The reaction vessel was purged with nitrogen for 15 min and Me_2S (0.15 mL; 1.97 mmol; 1.4 equiv) was added while the solution was maintained at -78 °C. The cooling bath was removed allowing the mixture to warm to room temperature. The reaction was stirred under nitrogen for 1.5 h and a TLC was taken which revealed that starting material still remained. The reaction was stirred overnight to ensure completion. The solvent was evaporated to afford a white solid which was subjected to column chromatography (2:1 petroleum ether–EtOAc) to afford **8** as white crystals (92 mg; 23% over two steps). TLC: R_f (2:1 petroleum ether–EtOAc) 0.30 mp 209–210 °C; $[\alpha]_D^{20}$ +188° (*c* 0.16, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.80 (s, 3H), 5.29 (s, 1H), 7.01–7.04 (m, 1H), 7.54–7.62 (m, 4H), 7.88–7.94 (m, 3H), 10.05 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 28.08, 66.83, 121.60, 125.07, 129.23, 129.91, 130.35, 130.93, 133.41, 134.06, 134.23, 137.31, 137.65, 138.39, 191.68. Anal. Calcd for $C_{15}H_{13}NO_3S$: C, 62.70; H, 4.56; N, 4.87; S, 11.16. Found: C, 62.53; H, 4.43; N, 4.86; S, 11.05.

3*S*-(2-Methyl-1,1-dioxo-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazol-3-yl)methanol (7). In a 100 -mL round-bottomed flask, compound **5** (300 mg; 1.05

mmol) was dissolved in 50 mL of CH_2Cl_2 and cooled to -78 °C in a dry ice-acetone bath. Ozone was bubbled through the solution until the solution remained blue in color for 0.5 h. The reaction vessel was purged with nitrogen for 15 min. and a 2.0 M solution of borane—dimethylsulfide complex (2.1 mL; 4.2 mmol; 4.0 equiv) was added while the solution was maintained at -78 °C . The cooling bath was removed allowing the mixture to warm to room temperature. The reaction was stirred under nitrogen for 24 h when a TLC was taken which revealed the consumption of starting material. The reaction was quenched with 2 mL of 5% HCl and allowed to stir vigorously for 1 h. Solid $NaHCO_3$ was added until the aqueous layer tested basic to litmus. $MgSO_4$ was added until flocculent material could be seen in the bottom of the flask. The solution was filtered and the solvent evaporated to yield a white solid which was subjected to column chromatography (2:1 hexane–acetone) and recrystallization from EtOAc–hexane to yield pure **7** as white crystals (166 mg; 54% over two steps). TLC: R_f (2:1 hexane–acetone) 0.20; mp 193–194 °C; $[\alpha]_D^{20}$ +134° (*c* 0.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.80 (br s, 1H); 2.78 (s, 3H); 4.72 (s, 2H); 5.20 (s, 1H); 7.03–7.06 (m, 1H); 7.26–7.56 (m, 6H); 7.85–7.88 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 27.60, 64.83, 66.95, 121.07, 124.90, 126.24, 127.33, 127.58, 129.28, 132.91, 133.85, 136.87, 138.10, 141.88. Anal. Calcd for $C_{15}H_{15}NO_3S$: C, 62.27; H, 5.22; N, 4.84; S, 11.08. Found: C, 62.23; H, 5.13; N, 4.83; S, 11.04.

3*S*-(2-Methyl-1,1-dioxo-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazol-3-yl)benzyl methanesulfonate (9). Compound **7** (100 mg; 0.35 mmol) was dissolved in 5 mL of CH_2Cl_2 and cooled to 0 °C in an ice bath. TEA (0.074 mL; 0.53 mmol; 1.5

equiv) was added slowly while the solution was stirred continuously under nitrogen. Mesityl chloride (0.030 mL; 0.39 mmol; 1,1 equiv) was added via syringe over a period of 5 min. After 1 h, TLC showed the complete consumption of starting material. The reaction was quenched with 10 mL of water and extracted with 5% HCl (2 x 10 mL), saturated $NaHCO_3$ (2 x 15 mL), and saturated brine (2 x 10 mL). The organic layer was dried over $MgSO_4$ and concentrated to yield a pale yellow oil which was subjected to column chromatography (2:1 hexane–EtOAc) to yield a colorless oil. Crystallization of the product was effected by dissolution in EtOAc followed by saturation with hexane and allowing the solution to stand overnight. Compound **9** was isolated as white crystals (72 mg; 57%). TLC: R_f (1:1 hexane–EtOAc) 0.25; mp 122–123 °C; $[\alpha]_D^{20}$ +126° (*c* 0.17, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.79 (s, 3H); 3.00 (s, 3H); 5.22 (s, 1H); 5.24 (s, 2H); 7.01–7.04 (m, 1H); 7.26–7.56 (m, 6H); 7.86–7.89 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 27.70, 38.20, 66.70, 70.55, 121.21, 124.82, 127.99, 128.95, 129.35, 129.48, 129.76, 133.03, 133.88, 134.60, 137.58, 137.69. Anal. Calcd for $C_{16}H_{17}NO_5S_2$: C, 52.30; H, 4.66; N, 3.81; S, 17.45. C, 52.32; H, 4.83; N, 3.81; S, 17.29.

3*S*-(3-Bromomethylphenyl)-2-methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (10). Compound **9** (35 mg; 0.095 mmol) was dissolved in 1 mL of DMF in a 10 -mL round-bottomed flask. Lithium bromide (25 mg; 0.29 mmol; 3.0 equiv) was added over a period of 5 min. After 15 min. TLC revealed that the starting material had been completely consumed. The DMF was removed on a high vacuum rotary evaporator as an azeotrope with toluene. The crude product was taken up in

EtOAc (25 mL) and extracted with H20 (2 x 10 mL) and saturated brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated to yield a colorless oil. Crystallization of the product occurred overnight following dissolution of the oil in EtOAc and saturation with hexane to yield **10** as white crystals (27 mg; 81%). TLC: R_f (1:1 hexane–EtOAc) 0.44; mp 113–114 °C; $[\alpha]_D^{20}$+106° (*c* 0.095, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.79 (s, 3H); 4.48 (s, 2H); 5.19 (s, 1H); 7.02–7.05 (m, 1H); 7.25–7.56 (m, 6H); 7.86–7.89 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 27.67, 32.74, 66.74, 121.14, 124.89, 128.04, 128.38, 129.39, 129.63, 129.84, 132.99, 133.88, 137.28, 137.81, 138.91. Anal. Calcd for $C_{15}H_{14}BrNO_2S$: C, 51.15; H, 4.01; N, 3.98; S, 9.10. Found: C, 51.31; H, 3.91; N, 3.86; S, 9.15.

PART TWO

AN IMPROVED METHOD FOR THE SYNTHESIS OF *trans*-FERULOYL-β-SITOSTANOL AND RELATED PHYTOSTANOL ESTERS

1. INTRODUCTION

Two of the most popular cholesterol-lowering "functional" foods currently on the market are Benecol® and Take Control®, with the former containing synthetic fatty acid esters of phytostanols (stanol esters) derived from tall oil and the latter containing synthetic fatty acid esters of phytosterols (sterol esters) derived from soybean oil.[3] Phytosterols and phytostanols are known to lower low-density lipoprotein–cholesterol (LDL-C) levels in humans by up to 15%, and the FDA has recently permitted a rare health claim for their use in low-fat diets.[4] Phytosterols and phytostanols are thought to lower LDL-C by inhibiting the absorption of cholesterol from the intestine.[5] Free phytosterols and phytostanols are poorly soluble in most foods, and this has led to problems with formulations and inconsistencies in efficacy. To solve this problem, they are usually esterified with fatty acids to make them soluble in margarines and other high-fat food products.[65] In the intestine, fatty acid esters are hydrolyzed by digestive enzymes, producing the physiologically active free sterol or stanol.[65] Researchers at the USDA laboratories have recently discovered and patented a natural phytosterol-containing oil from corn fiber (corn fiber oil) that contains high levels of natural phytosterol and phytostanol fatty acid esters and a unique class of stanol esters, the stanol ferulate esters (also called ferulate phytostanol esters). [66,67] Although many clinical studies have been performed on fatty acid esters of stanols and sterols, no studies have examined the efficacy of stanol ferulate esters. Because these compounds contain

a ferulic acid moiety, which is a powerful antioxidant, it is proposed that in addition to reducing LDL-C levels, they might also reduce the oxidation of LDL-C, further lowering the risk of coronary artery disease. To test this hypothesis, quantities of pure stanol ferulate esters are required. A method that uses 4-*O*-acetylferuloyl chloride as the acylating agent has been published for the synthesis of these types of esters.[68,69] The present study was undertaken to develop an improved method for the synthesis of β-sitostanol ferulate (**15a**). Also, since "vegetable stanols" are a more readily available commercial product, containing ~70% β-sitostanol and 30% campestanol, the ferulate ester synthesis was also evaluated with this mixed substrate.

2. RESULTS AND DISCUSSION

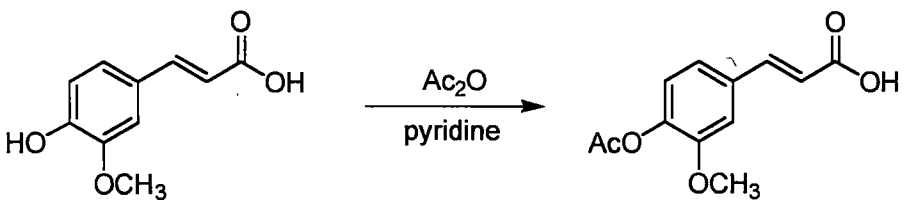
The process described herein proceeds directly from *trans*-4-*O*-acetylferulic acid (**12**), which is easily prepared by acetylation of ferulic acid (**11**) in acetic anhydride/pyridine, to give a product identical with that reported by Ebenezer (see **Figure 5**).[70] Thus, activation of **2** with *N*,*N*-dicyclohexylcarbodiimide (DCC)[71] and condensation with β-sitostanol in the presence of 4-dimethylaminopyridine (4-DMAP) proceeded smoothly to give the coupled product, *trans*-4-*O*-acetylferuloyl-β-sitostanol (**14a**, see **Figure 6**). The coupled product was purified by crystallization and filtration of the byproduct, *N*,*N*-dicyclohexylurea, and the crude product was subjected to preparative liquid chromatography (normal phase, silica gel), which served to separate the unreacted starting material and another byproduct, ethyl *trans*-4-*O*-acetylferulate. The origin of the latter was traced to ethanol, which was present in the β-sitostanol starting material (33 mol % ethanol; Research Plus, Inc.). Removal of the ethanol by thorough drying of the β-sitostanol raised the yield from 43% to 61% of pure **14a** and eliminated the ethyl ester byproduct.

Removal of the acetate protecting group required a selective hydrolytic procedure that favored acetate hydrolysis over hydrolysis of the *trans*-ferulate ester linkage. The published procedure[68,69] used sodium borohydride, a hydride reducing agent, which is not a common reagent to accomplish ester reductions, owing to the resistance of nonactivated esters to the action of borohydride. We found that a simple transesterification process that makes use of potassium carbonate in

refluxing chloroform–methanol was ideal for the task.[72] Thus, refluxing the acetate-protected **14a** with potassium carbonate in a 2:1 mixture of chloroform–methanol proceeded to give the product **15a** as an analytically pure material in 71% yield after recrystallization. The product was characterized by NMR spectroscopy, by mass spectrometry, and by elemental analysis. Normal-phase (silica gel) HPLC and TLC (silica gel) of the product showed a single substance.

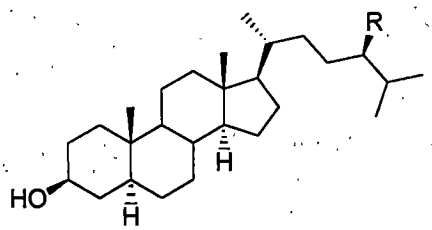
The acylation process was adapted to the processing of a mixture of stanols, termed "Vegetable Stanols" (AC Humko, Inc.), that was made up of a 7:3 mixture of β-sitostanol (**13a**) and campestanol (**13b**) (reversed-phase HPLC analysis showed 69.0% **13a** and 30.4% **13b**). Soy oil phytosterols are typically comprised of about 51% β-sitostanol, 16% sitosterol, and 22% campesterol,[73] and uponhydrogenation to reduce sterols to stanols, the composition of vegetable (soy) stanols is about 67% β-sitostanol and 22% campestanol. Processing the mixture as for pure β-sitostanol was carried out to give a ~7:3 mixture of the protected coupled products **14a** and **14b**, as well as the free-hydroxy *trans*-feruloyl esters **15a** and **15b**, in comparable yield. At no stage in the synthesis were the products separable by either crystallization or by normal-phase chromatography (silica gel); an analytical-scale separation was achieved by reversed-phase HPLC using a ternary solvent gradient. The products, either **14a** and **14b** or **15a** and **15b**, could be discerned most readily from one another by inspection of their ^{13}C NMR spectra, which gave separate signals for a number of the resonances. The process described herein offers a straightforward process to *trans*-feruloylated β-sitostanol,

as well as to a mixture of β-sitostanol and β-campestanol esters. The process should be amenable to modest-sized synthesis of related products. Compounds **15a** and **15b** are undergoing evaluation as cholesterol-lowering agents. Details of those studies will be reported elsewhere.



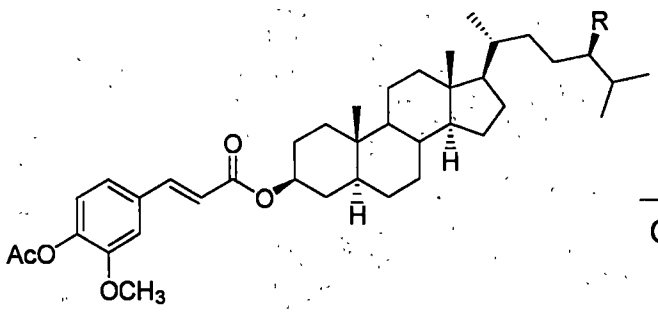
11 **12**

Scheme 5. Acetylation of *trans*-Ferulic Acid



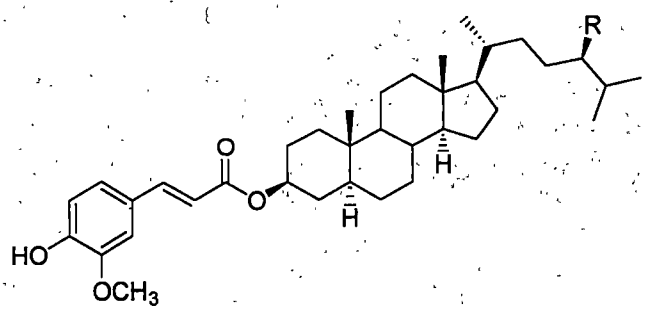
12, DCC, 4-DMAP
CH_2Cl_2

13a R = Et; stigmastanol
13b R = Me; campestanol



K_2CO_3
$CHCl_3$, MeOH

14a R = Et
14b R = Me



15a R = Et
15b R = Me

Scheme 6. Synthesis of *trans*-Feruloyl-β-Phytostanols

3. EXPERIMENTAL

A. General procedures

All reactions were conducted under a nitrogen atmosphere unless otherwise indicated. Anhydrous solvents were dried prior to distillation as follows: dichloromethane, chloroform, and pyridine were refluxed over calcium hydride; THF was refluxed over sodium–benzophenone ketyl; methanol was refluxed with magnesium metal shavings. *trans*-Ferulic acid and acetic anhydride were purchased from Aldrich Chemical Company and used without further purification. β-Sitostanol was purchased from Research Plus, Inc. and used without further purification. In order to maximize the yield of the coupled products **14a** and **14b**, the material was dried for 18 h to remove residual ethanol (78 °C, <1 Torr). "Vegetable Stanols" were obtained from AC Humko, Inc. (Memphis, TN) and used without further purification. Thin-layer chromatography (TLC) was carried out on E. Merck Silica Gel 60 aluminum–backed plates. Visualization was accomplished by a combination of UV exposure (254 nm) and treatment with phosphomolybdic acid dip. Solvents were evaporated at aspirator vacuum at 25 °C unless otherwise indicated. Preparative liquid chromatography (PLC) was carried out on a Waters Prep LC/System 500A with a flow rate of 250 mL/min of the indicated solvent system and with refractive index detection. Melting points were taken on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured at the sodium D line at 20 °C using a Perkin–Elmer 241

polarimeter (1-dm cell). ^{1}H and ^{13}C NMR spectra were recorded at 300.087 and 75.464 MHz, respectively, on a Varian *MERCURY* series spectrometer as solutions in $CDCl_3$ with an internal standard of TMS. ^{1}H chemical shifts are reported as δ (ppm) downfield from the TMS standard. ^{13}C chemical shifts are reported as δ (ppm) relative to $CDCl_3$ (77.00 ppm) as standard. Infrared spectra were obtained on a Bomem MB-Series spectrometer as KBr pellets. Elemental analyses were furnished by Atlantic Microlab, Inc. of Norcross, GA and are within ±0.4% of the theoretical values. Electron-impact mass spectra (EIMS) were obtained on a VG-ZAB-EQ mass spectrometer (Manchester, England) using an ionization potential of 70 eV.

B. HPLC Conditions

Ester products (β-sitostanol ferulate and campestanol ferulate, with retention times of 35.4 and 33.9 min, respectively) were separated and quantified via a reversed-phase HPLC system consisting of a Hewlett–Packard (Avondale, PA) Model 1100 Pump and autosampler, with the effluent entering first a Hewlett–Packard Model 1100 UV detector at 320 nm, and then an Alltech–Varex Model MKIII evaporative light-scattering detector (Deerfield, IL). The column (100 x 3 mm) was a Chromsep glass cartridge packed with LiChrosorb7RP18 (7μm) by Varian–Chrompack (Bridgewater, NJ). The 60-min solvent gradient program (0.5 mL/min) was for a ternary solvent system of solvents A/B/C: at 0 min 10/10/80; at 10 min 10/10/80; at 40 min 10/0/90; at 50 min 10/0/90; at 52 min 10/10/90; and at

60 min 10/10/90, A/B/C (v/v/v), where A = 2-propanol, B = water, and C = methanol. Normal-phase HPLC was used to separate various stanol esters from the free-OH stanols. Normal-phase HPLC was carried out using the same HPLC system and flow rate as above with a LiChrosorb Diol column (100 x 3 mm) and a 60-min solvent program consisting of solvents A/B: at 0 min 100/0; at 8 min 100/0; at 10 min 75/25; at 40 min 75/25; at 41 min 100/0; and at 60 min 100:0, A/B (v/v), where A = 1000:1 hexane–acetic acid and B = 100:1 hexane–2-PrOH.

C. Synthesis of Compounds 12-15

***trans*-4-*O*-Acetylferulic Acid (12).** *trans*-Ferulic acid (**11**, 25.0 g, 128.7 mmol) was dissolved in distilled pyridine (100 mL) in a 250-mL round-bottomed flask. Acetic anhydride (65 mL) was added, and the mixture was stirred overnight in the dark. The solution was diluted with toluene (50 mL) and evaporated to yield a yellow syrup. Subsequent addition and evaporation of toluene (3 x 50 mL) yielded an off-white solid that was dried for 18 h (78 °C,<1 torr) prior to use. ^{1}H and ^{13}C NMR data were consistent with published literature values.[70]

3-*O*-(*trans*-4-*O*-Acetylferuloyl)-β-sitostanol (14a). *trans*-4-*O*-Acetylferulic acid (**12**, 23.2 g; 98.2 mmol) and β-sitostanol (**13a**, 49.0 g, 108 mmol, 1.1 equiv) were dissolved in dry CH_2Cl_2 (750 mL). DCC (1.0 M in CH_2Cl_2, 108.0 mL, 108 mmol, 1.1 equiv) and DMAP (1.20 g, 9.82 mmol, 0.10 equiv) were added, and the mixture was stirred under nitrogen for 18 h. The DCU that formed was filtered off, and the solution was extracted with H_2O (2 x 150 mL), 10% HOAc (2 x 150 mL), and again

with H_2O (2 x 150 mL). The organic extracts were combined and dried over anhydrous $MgSO_4$ and evaporated to yield a white solid. The solid was dissolved in a minimal amount of THF and chilled at 0 °C overnight to precipitate any residual DCU. The solution was filtered, the solvent was evaporated, and the residual solid was chromatographed using PLC with 2:4:1 chloroform–hexane–ethyl acetate as the eluent to yield **14a** as a white solid (29.0 g, 43%). Two additional products were recovered and identified as ethyl 4-*O*-acetylferulate (5.8 g, 22%) and unreacted β-sitostanol (11.9 g, 29%). ^1H and ^{13}C NMR as well as EIMS were used to characterize the minor products. Characterization data for the major product **14a** are as follows: TLC: R_f (5:1 petroleum ether–ethyl acetate) 0.24; mp 162 °C (lit.[68] 156 °C); $[\alpha]_D^{20}$ +20.6° (*c* 0.7, $CHCl_3$); IR (KBr, cm^{-1}): 2938, 2867, 1760, 1711, 1639, 1600, 1512, 1155, 1073; ^1H NMR ($CDCl_3$): δ 0.6–2.0 (β-sitostanol moiety, ~50H), 2.33 (s, 3H), 3.86 (s, 3H), 4.83 (m, 1H), 6.37 (d, 1H, *J* = 16.2 Hz), 7.10 (m, 3H), 7.62 (d, 1H, *J* = 15.9 Hz); ^{13}C NMR ($CDCl_3$): δ 12.06, 12.15, 12.34, 18.80, 19.09, 19.91, 20.76, 21.27, 23.08, 24.28, 26.04, 27.63, 28.33, 28.67, 29.12, 32.04, 33.92, 34.15, 35.51, 36.21, 36.80, 39.98, 42.60, 44.68, 45.79, 54.19, 55.86, 56.12, 56.39, 73.96, 110.95, 118.88, 121.08, 123.08, 133.36, 141.06, 143.41, 151.11, 166.15, 168.64; EIMS (70 eV) *m/z*: 634.5 [M^+]; 592.4 [$M^+ - CH_3CO$]; 398.4 [$M^+ - C_{12}H_{12}O_5$]. Anal. Calcd for $C_{41}H_{62}O_5$: C, 77.56; H, 9.84. Found: C, 77.58; H, 9.88. ^1H and ^{13}C NMR data are consistent with published literature values.[68]

In a similar experiment, using a sample (633 mg, 1.52 mmol) of **13a** that had been dried for 18 h (78 °C, <1 torr), 489 mg (61%) of coupled product **14a** was

obtained, and no ethyl 4-*O*-acetylferulate was detected.

3-*O*-(*trans*-4-Feruloyl)-β-sitostanol (15a). Compound **14a** (28.5 g, 44.9 mmol) was dissolved in a 2:1 chloroform–methanol solvent mixture (450 mL). Potassium carbonate (1.24 g, 8.98 mmol, 0.20 equiv) was added, and the mixture was refluxed for 6 h. TLC analysis revealed the consumption of the starting material and the appearance of a new spot with a decreased R_f value. The reaction was quenched by the addition of satd aq NH_4Cl (100 mL). The layers were separated, and the organic layer was washed with H_2O (2 x 100 mL) and dried over $MgSO_4$. PLC using 2:4:1 chloroform–hexane–ethyl acetate as the eluent yielded a white solid (23.5 g, 88%) that was recrystallized (chloroform–methanol) to give the final product **15a** as fine white crystals (19.0 g, 71%): TLC: R_f (5:1 petroleum ether–EtOAc) 0.14; HPLC (normal phase): t_R 26.2 min; mp 156–157 °C (lit. [68] 156 °C); $[\alpha]_D^{20}$ +22.3° (*c* 1.33, $CHCl_3$); IR (KBr, cm^{-1}): 3528, 3408, 2938, 2867, 1707, 1634, 1594, 1514, 1173, 1074; ^1H NMR ($CDCl_3$): δ 0.6–2.0 (β-sitostanol moiety, ~50H), 3.91 (s, 3H), 4.83 (m, 1H), 5.96 (s, 1H), 6.2 (d, J = 15.9 Hz, 1H), 6.91 (d, J = 7.8 Hz, 1H), 7.05 (m, 2H), 7.60 (d, J = 16.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 12.06, 12.14, 12.33, 18.79, 19.09, 19.91, 21.26, 23.08, 24.27, 26.04, 27.67, 28.33, 28.67, 29.12, 32.03, 33.91, 34.19, 35.50, 36.20, 36.81, 39.97, 42.58, 44.66, 45.78, 54.19, 55.86, 56.12, 56.38, 73.68, 109.07, 114.57, 115.99, 122.88, 126.93, 144.25, 146.54, 147.63, 166.63; EIMS (70 eV) *m/z*: 592.4 [M^+], 398.4 [$M^+ - C_{10}H_{10}O_4$]. Anal. Calcd for $C_{39}H_{60}O_4$: C, 79.01; H, 10.20. Found: C, 78.73; H, 10.21.

3-*O*-(*trans*-4-*O*-Acetylferuloyl)phytostanols (14a and 14b). By the same procedure used for the preparation of **14a**, *trans*-4-*O*-acetylferulic acid (**12**, 8.86 g, 37.5 mmol) and "Vegetable Stanols" (69.0% **13a** and 30.4% **13b**, 17.2 g, 41.3 mmol, 1.1 equiv) were dissolved in dry CH_2Cl_2 (75 mL) and reacted with DCC (8.52 g, 41.3 mmol, 1.1 equiv) and DMAP (504 mg, 4.13 mmol, 0.10 equiv). Workup as for **14a** gave a residual solid that upon PLC (5:1 petroleum ether–EtOAc) gave **14a** and **14b** as a white solid (9.25 g, 39%) that was an inseparable mixture by chromatographic techniques. The characterization data for the mixture are as follows: TLC: R_f (5:1 petroleum ether–EtOAc) 0.24; mp 160–161 °C; $[\alpha]_D^{20}$ +22.2° (*c* 0.18, $CHCl_3$); IR (KBr, cm^{-1}): 2946, 2862, 1764, 1710, 1639, 1596, 1514, 1155, 1078; ^1H NMR ($CDCl_3$): δ 0.6–2.0 (β-sitostanol/campestanol moieties, ~50H), 2.32 (s, 3H), 3.85 (s, 3H), 4.83 (m, 1H), 6.36 (d, *J* = 16.2 Hz, 1H), 7.10 (m, 3H), 7.61 (d, *J* = 15.9 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 12.05, 12.14, 12.33, 15.44*, 15.51*, 17.65*, 18.32*, 18.72*, 18.80*, 18.91*, 19.10, 19.89, 20.28*, 20.59*, 20.70, 21.27, 23.10, 24.27, 26.09, 27.63, 28.32, 28.67, 29.16, 30.32*, 30.61*, 31.47*, 32.03, 32.44*, 33.71*, 33.93, 34.16, 35.51, 35.93*, 36.20, 36.81, 38.83*, 39.07*, 39.99, 42.60, 44.68, 45.81, 54.21, 55.84, 56.14, 56.19*, 56.40, 79.92, 110.99, 118.89, 121.04, 123.05, 133.35, 141.10, 143.37, 151.13, 166.10, 168.55; EIMS (70 eV) *m/z*: **14a**: 634.4 [M^+], 592.4 [$M^+ - CH_3CO$], 398.4 [$M^+ - C_{12}H_{12}O_5$], **14b**: 620.4 [M^+], 578.4 [$M^+ - CH_3CO$], 384.4 [$M^+ - C_{12}H_{12}O_5$]. Anal. Calcd for 70% $C_{41}H_{62}O_5$ (**14a**) and 30% $C_{40}H_{60}O_5$ (**14b**): C, 77.51; H, 9.81. Found: C, 77.51; H, 9.94. Note: Resonances in the ^{13}C spectrum attributable to the minor component **14b** are denoted by an

asterisk (*).

3-*O*-(*trans*-4-Feruloyl)phytostanols (15a and 15b). Compounds **14a** and **14b** (9.20 g, 14.6 mmol) were deacylated [1:1 chloroform–methanol (100 mL), potassium carbonate (202 mg, 1.46 mmol)] by the procedure used for **14a** to give the final products **15a** and **15b** as fine white crystals (7.30 g, 84%) after PLC (5:1 petroleum ether–EtOAc) and recrystallization (chloroform–methanol). The characterization data for the mixture of isomers is as follows: TLC: R_f (5:1 petroleum ether–EtOAc) 0.14; HPLC (reversed-phase): t_R 33.9 min (**15b**) and t_R 35.4 min (**15a**); mp 157 °C; $[\alpha]_D^{20}$ +19.8° (*c* 0.23, $CHCl_3$); IR (KBr), 3531, 3408, 2936, 2867, 1705, 1633, 1594, 1513, 1172, 1074; ^{1}H NMR ($CDCl_3$): δ 0.6–2.0 (β-sitostanol moiety/β-campestanol moieties, ~50H), 3.88 (s, 3H), 4.82 (m, 1H), 5.86 (s, 1H), 6.27 (d, *J* = 15.9 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 1H), 7.05 (m, 2H), 7.59 (d, J = 15.9 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 12.08, 12.18, 12.37, 15.47*, 15.54*, 17.67*, 18.34*, 18.74*, 18.82, 18.93*, 19.11, 19.92, 20.31*, 20.62*, 21.29, 23.12, 24.30, 26.11, 27.70, 28.35, 28.71, 29.18, 30.34*, 30.64*, 31.51*, 32.07, 32.48*, 33.74*, 33.96, 34.24, 35.55, 35.96*, 36.23, 36.85, 38.87*, 39.10*, 40.02, 42.63, 44.72, 45.85, 54.25, 55.92, 56.10*, 56.17, 56.22*, 56.43, 73.70, 109.10, 114.57, 116.10, 122.91, 127.01, 144.23, 146.56, 147.63, 166.63; EIMS (70 eV) *m/z*: **15a**: 592.4 [M^+], 398.4 [$M^+ - C_{10}H_{10}O_4$], **15b**: 578.4 [M^+], 384.4 [$M^+ - C_{10}H_{10}O_4$]. Anal. Calcd for 70% $C_{39}H_{60}O_4$ (**14a**) and 30% $C_{38}H_{58}O_4$ (**14b**): C, 78.96; H, 10.17. Found: C, 78.98; H, 10.24. Note: Resonances in the ^{13}C spectrum attributable to the minor component **15b** are denoted by an asterisk (*).

CONCLUSIONS

CONCLUSIONS

The syntheses of the compounds described in this thesis have been accomplished in order to try and develop potential drug and functional food candidates that can be used to treat HIV and coronary artery disease. Although the testing data on the sultams shows excellent potential as inhibitors of HIV-1 reverse transcriptase, further studies into their efficacy and the development of other analogs should be a priority to discover more potent compounds. In addition, the resistance issue is one which would need to be addressed. The treatment of HIV-1 often fails because of mutant strains of the virus developing over long periods of antiviral treatment.

The synthesis of *trans*-feruloyl-β-sitostanol and related phytostanol esters that has been developed represents an alternative to published literature methods in that it is a more mild esterification and possesses a mild deprotection step. We were able to produce large quantities of the required compounds for our USDA collaborators in a relatively short period of time using these methods and a large-volume purification process using preparative liquid chrmoatography. Hamster feeding studies to be conducted by the USDA are ongoing and will be published elsewhere.

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LIST OF REFERENCES

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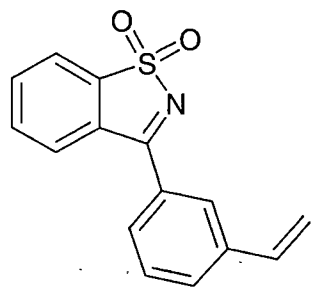
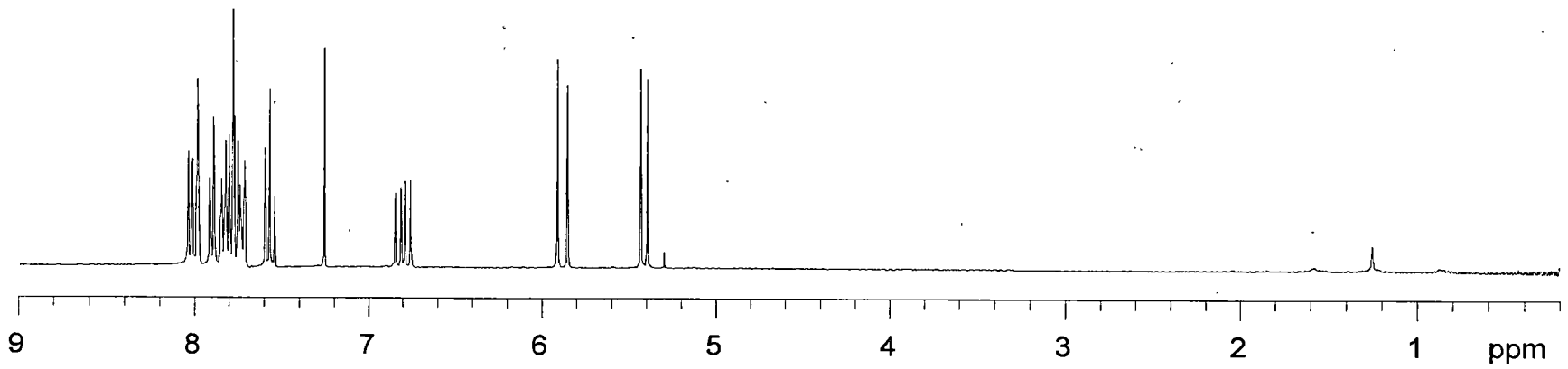
APPENDICES

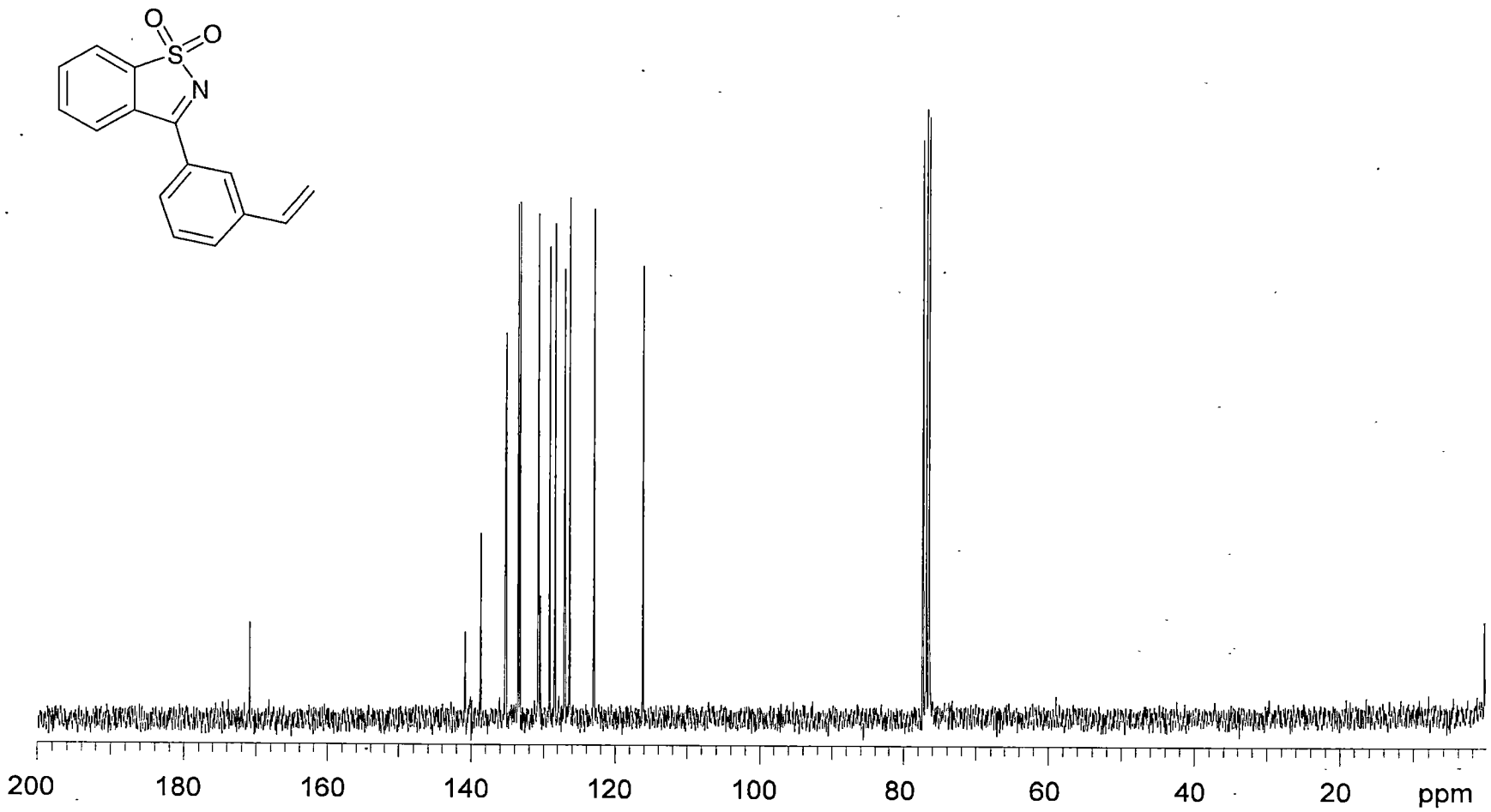
Appendix I

Compendia of ^{1}H and ^{13}C NMR spectra

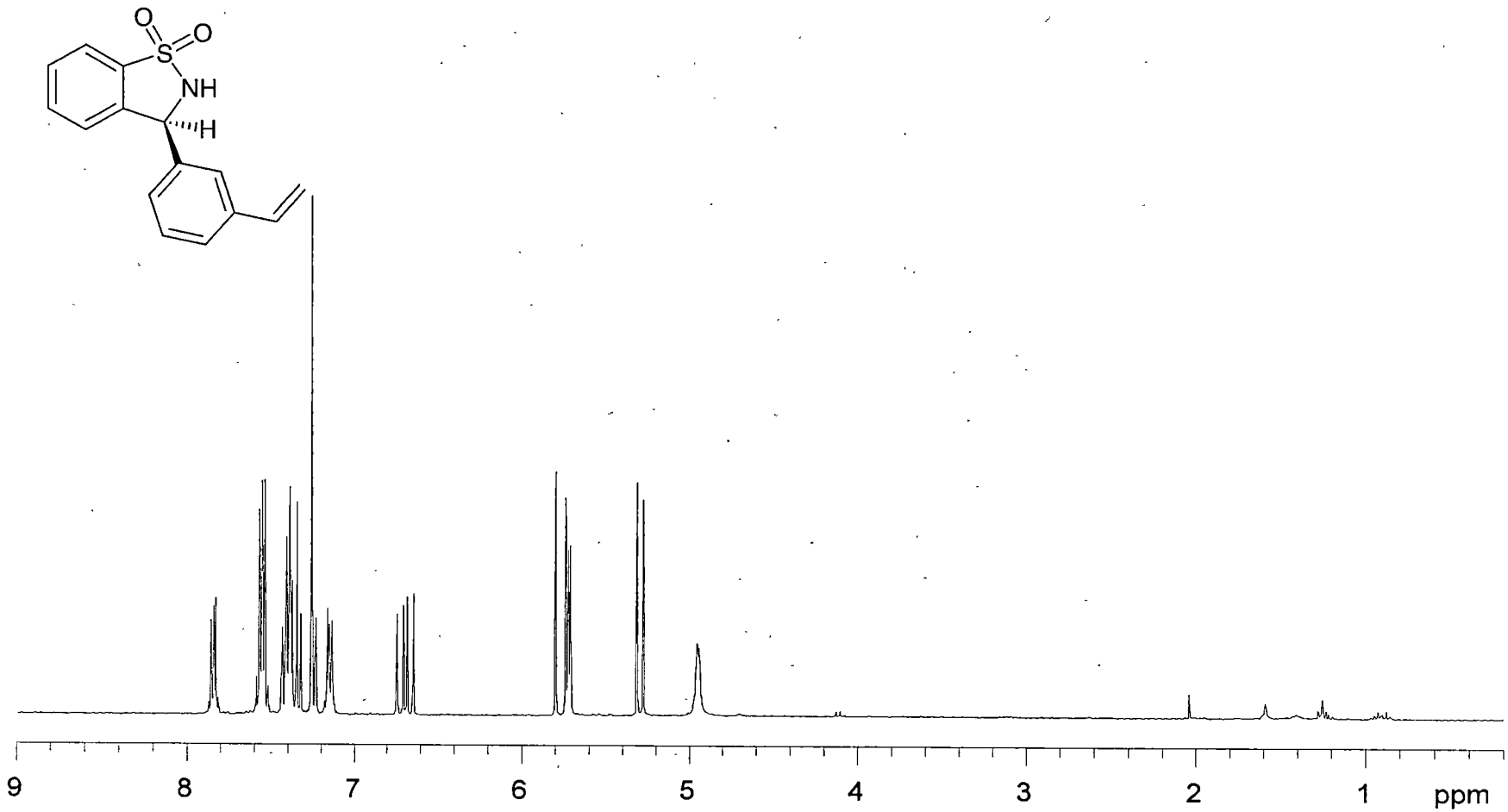




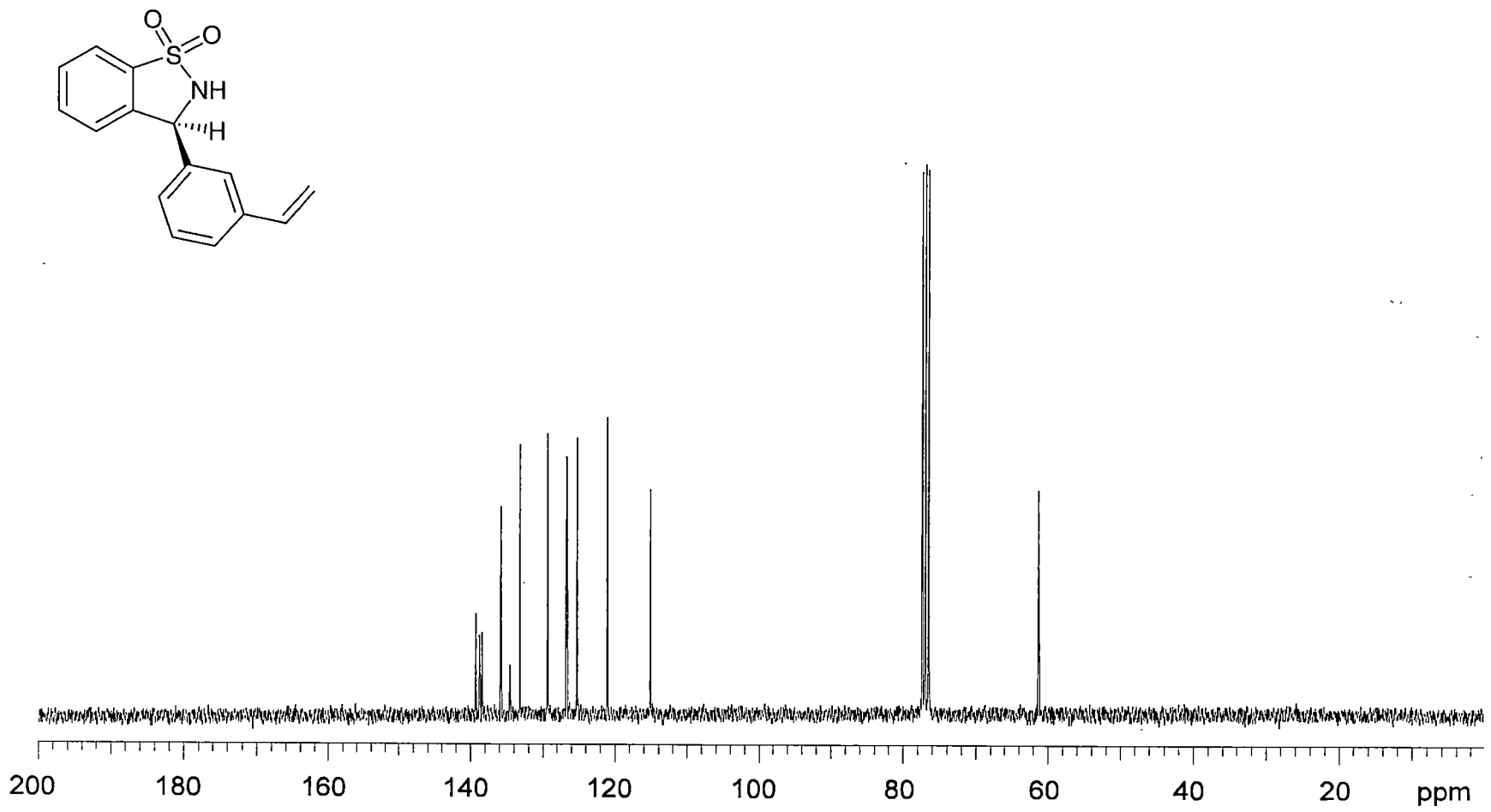
^{1}H NMR (300 MHz, $CDCl_3$) of 3-(3-Vinylphenyl)benzo[*d*]isothiazole 1,1-dioxide (**3**)



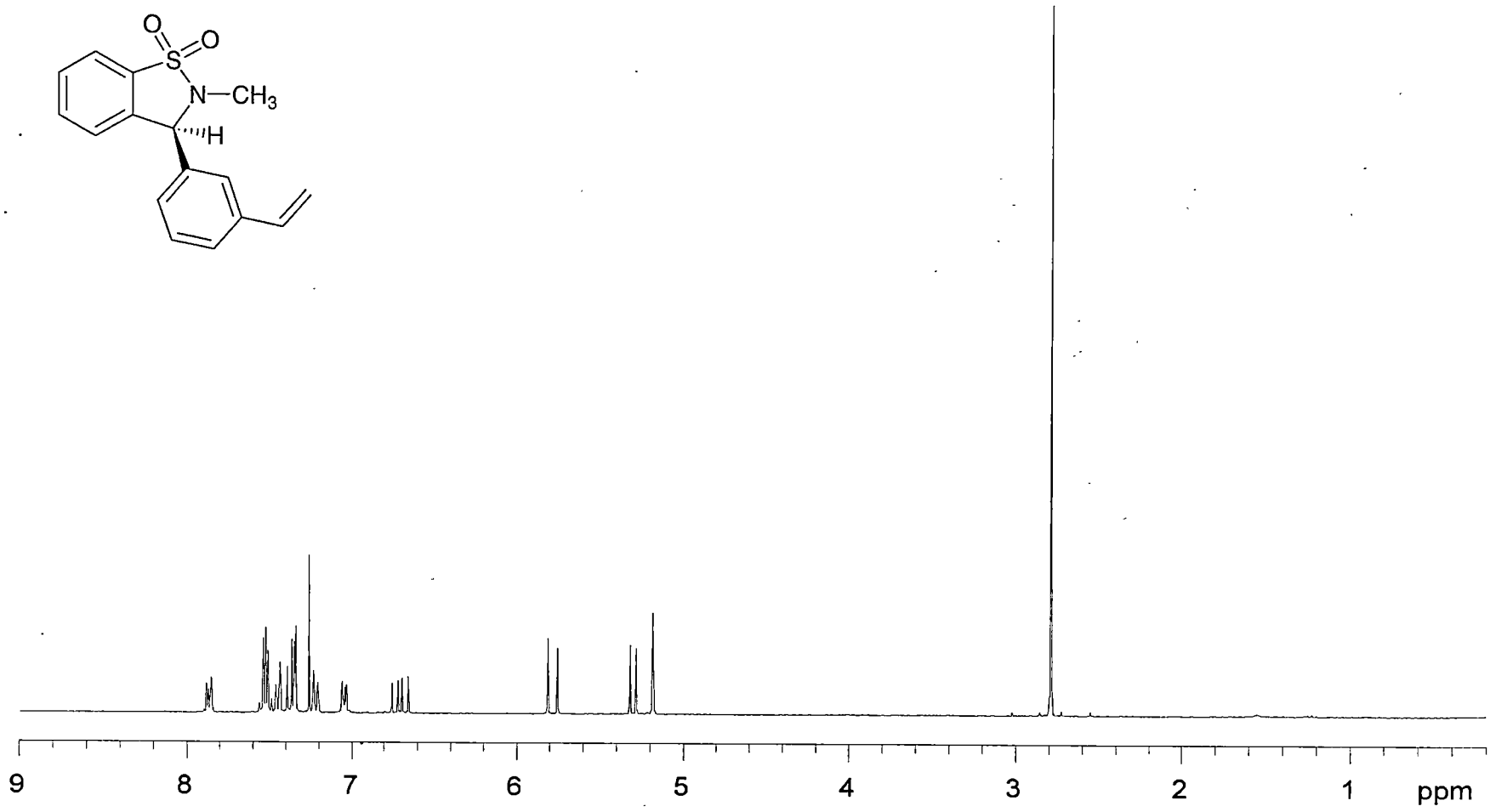
^{13}C NMR (75 MHz, $CDCl_3$) of 3-(3-Vinylphenyl)benzo[*d*]isothiazole 1,1-dioxide (**3**)



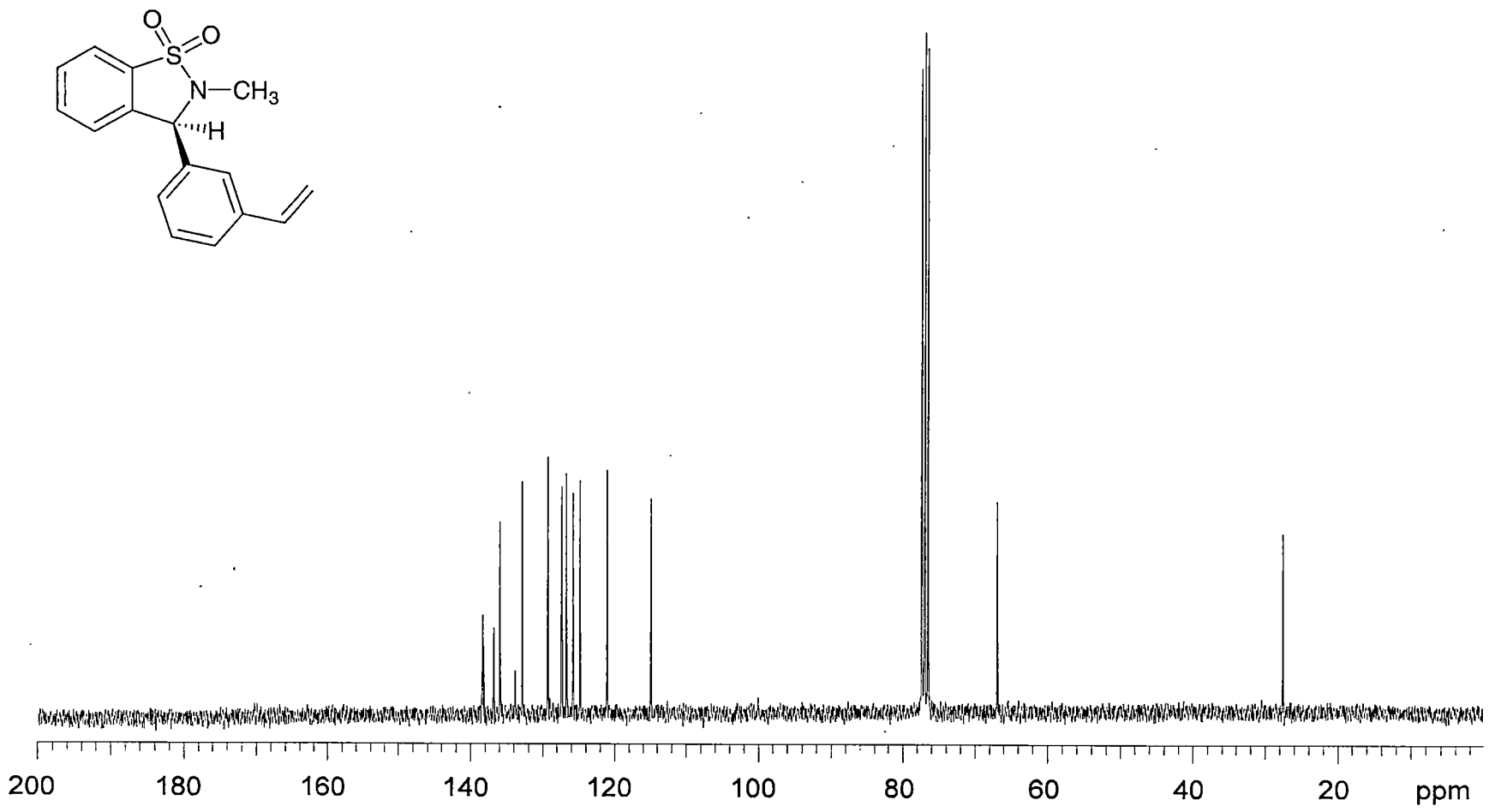
^{1}H NMR (300 MHz, $CDCl_3$) of 3*S*-(3-Vinylphenyl)-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (**4**)



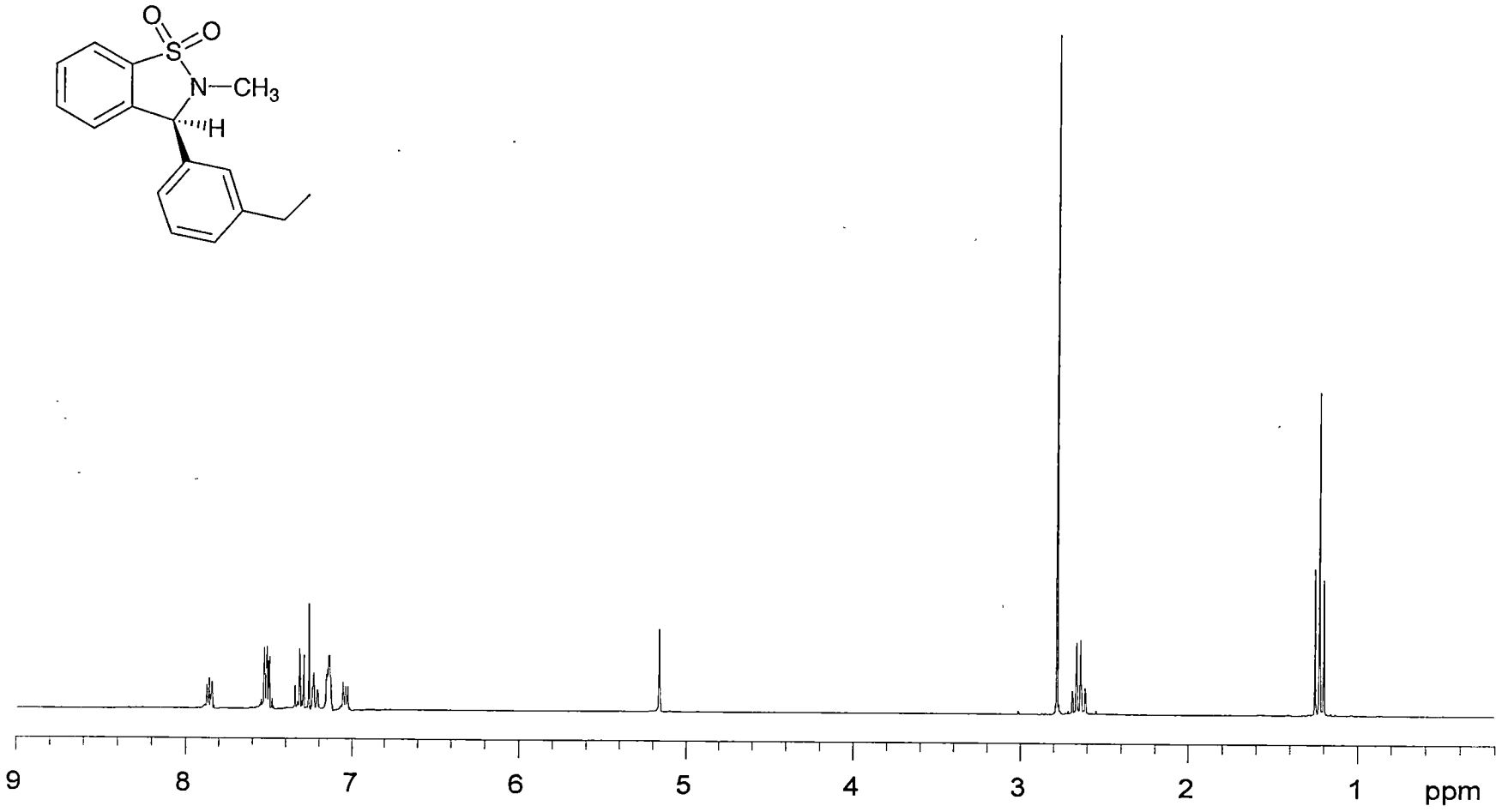
^{13}C NMR (75 MHz, $CDCl_3$) of 3*S*-(3-Vinylphenyl)-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (**4**)



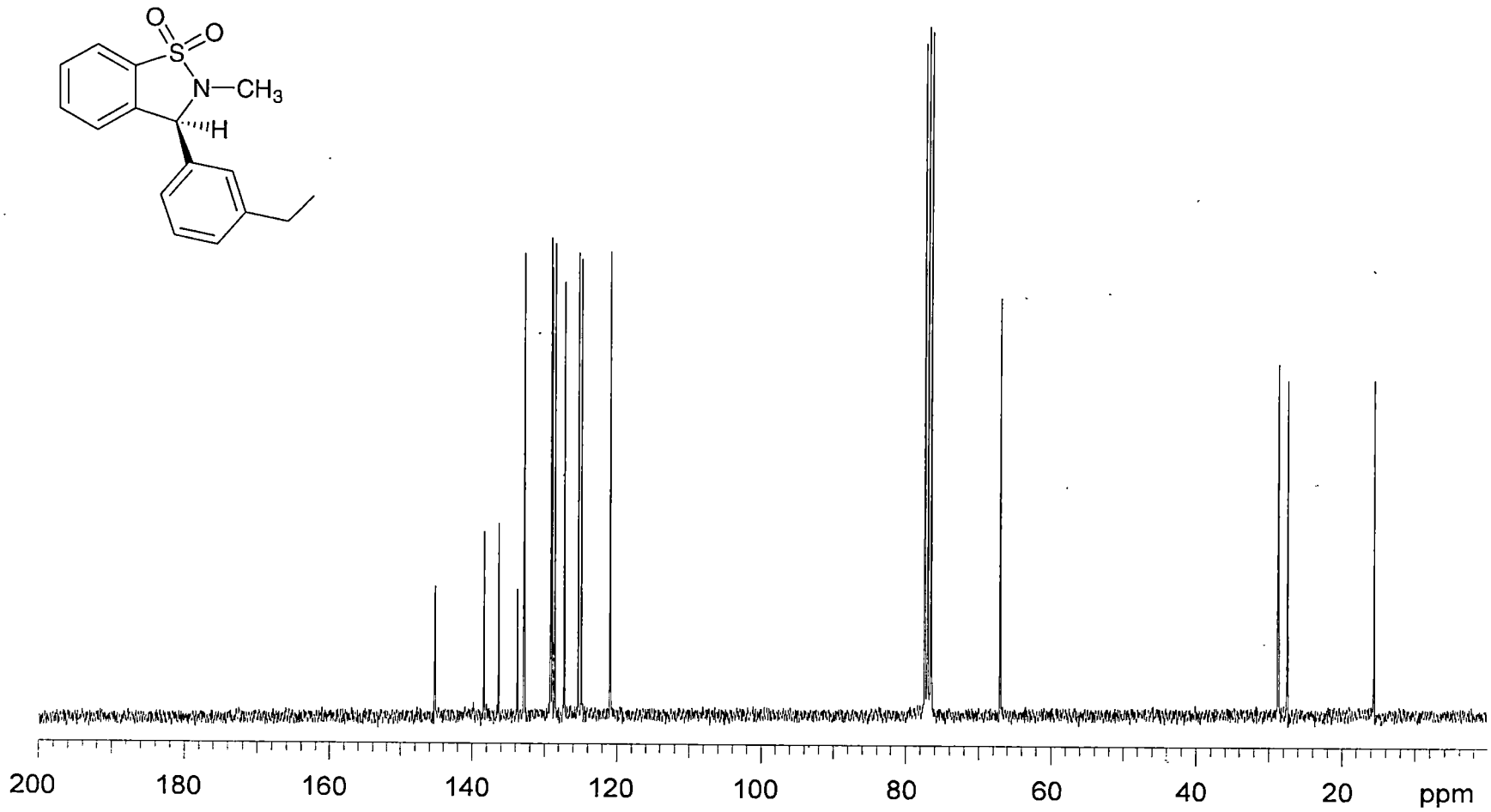
^{1}H NMR (300 MHz, $CDCl_3$) of (3*S*)-2-Methyl-3-(3-vinylphenyl)-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (**5**)



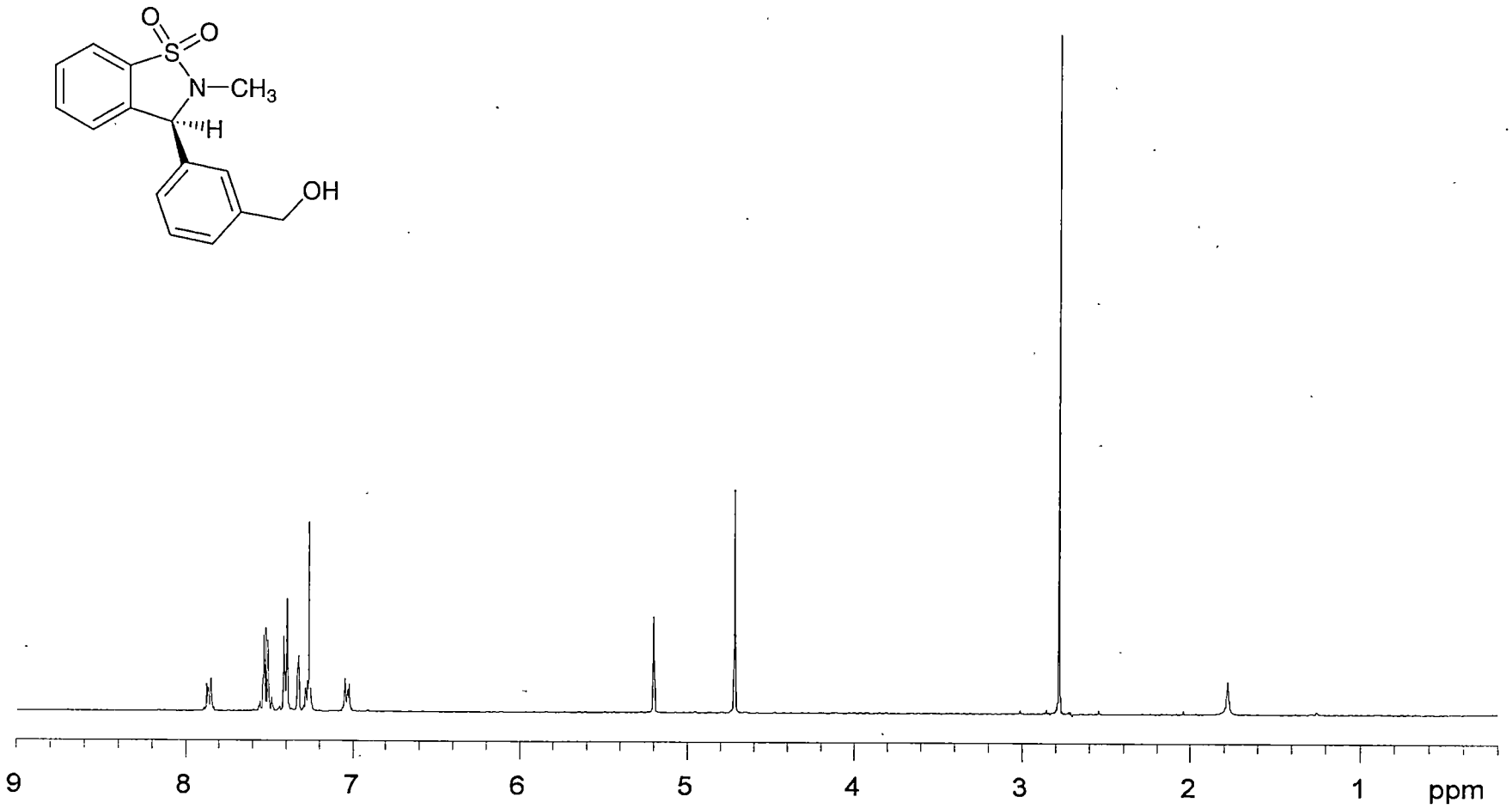
^{13}C NMR (75 MHz, $CDCl_3$) of (3*S*)-2-Methyl-3-(3-vinylphenyl)-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (**5**)



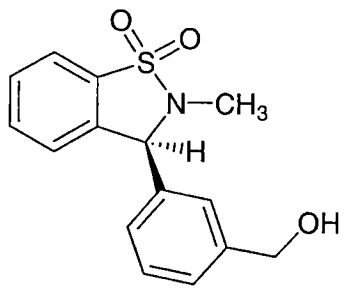
^{1}H NMR (300 MHz, $CDCl_3$) of 3*S*-(3-Ethylphenyl)-2-methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (**6**)

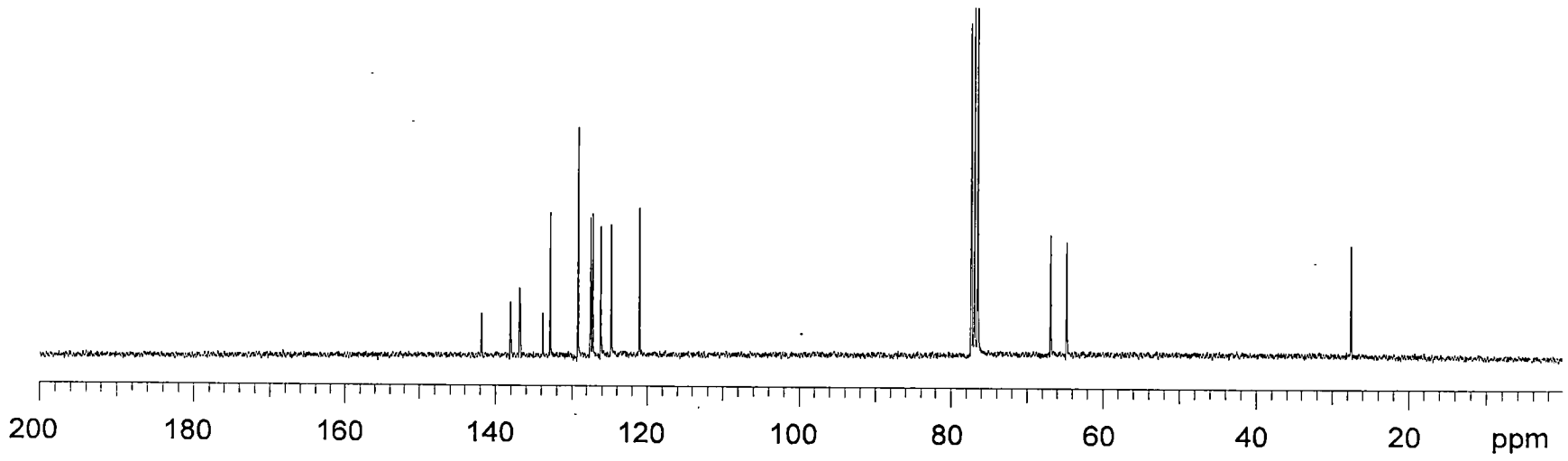


^{13}C NMR (75 MHz, $CDCl_3$) of 3*S*-(3-Ethylphenyl)-2-methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (**6**)

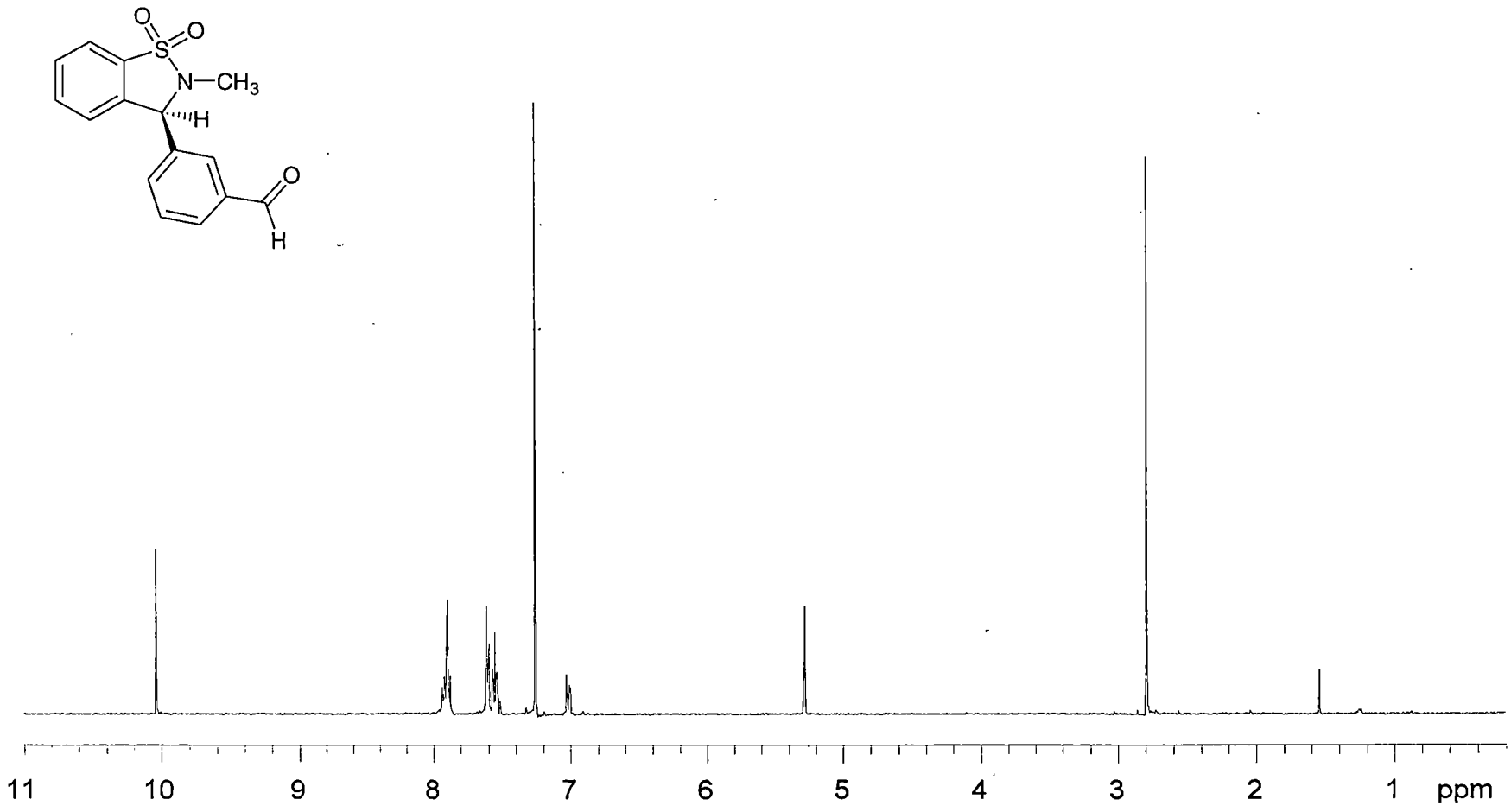


^{1}H NMR (300 MHz, $CDCl_3$) of 3*S*-(2-Methyl-1,1-dioxo-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazol-3-yl)methanol (**7**)

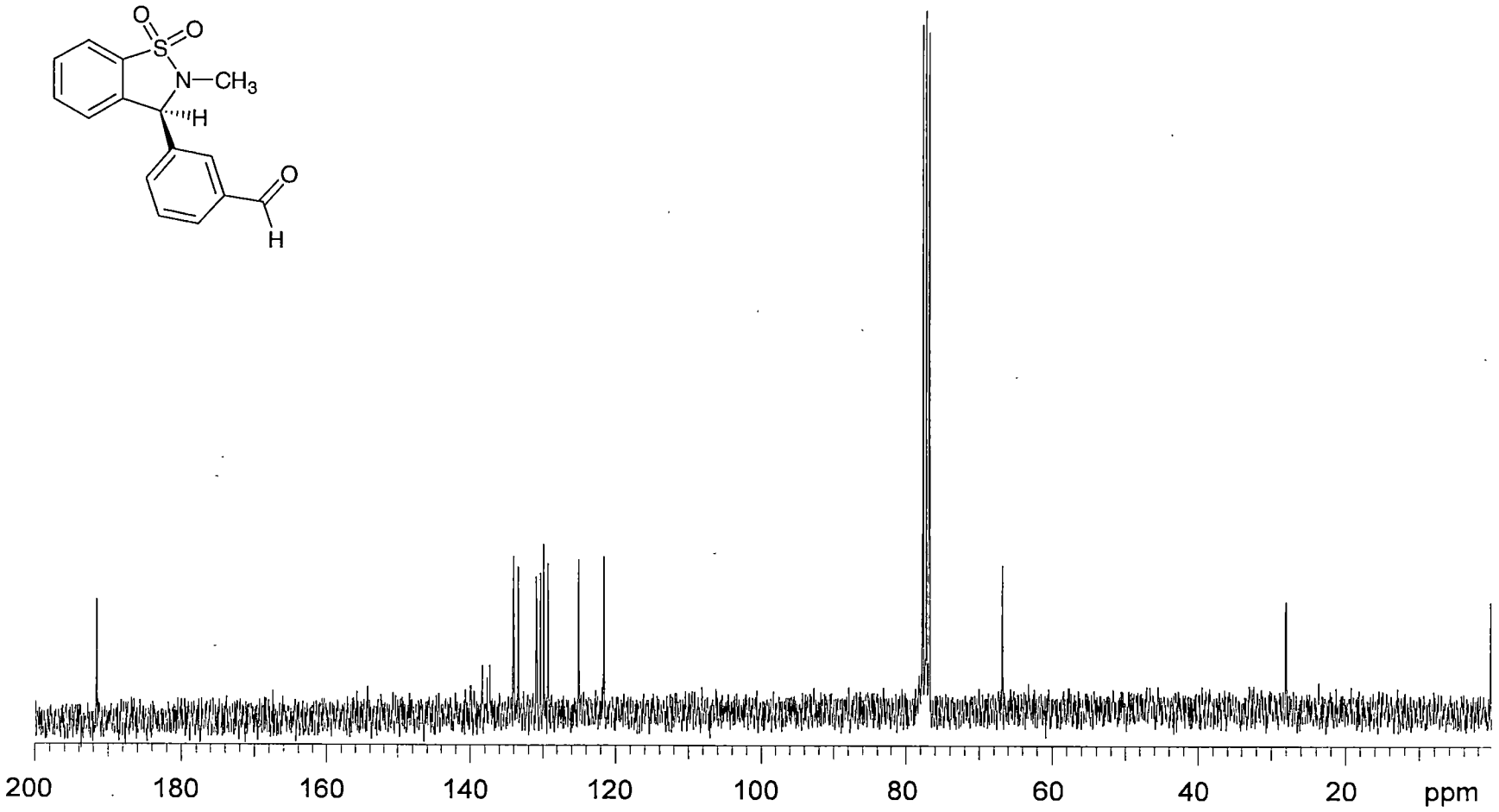




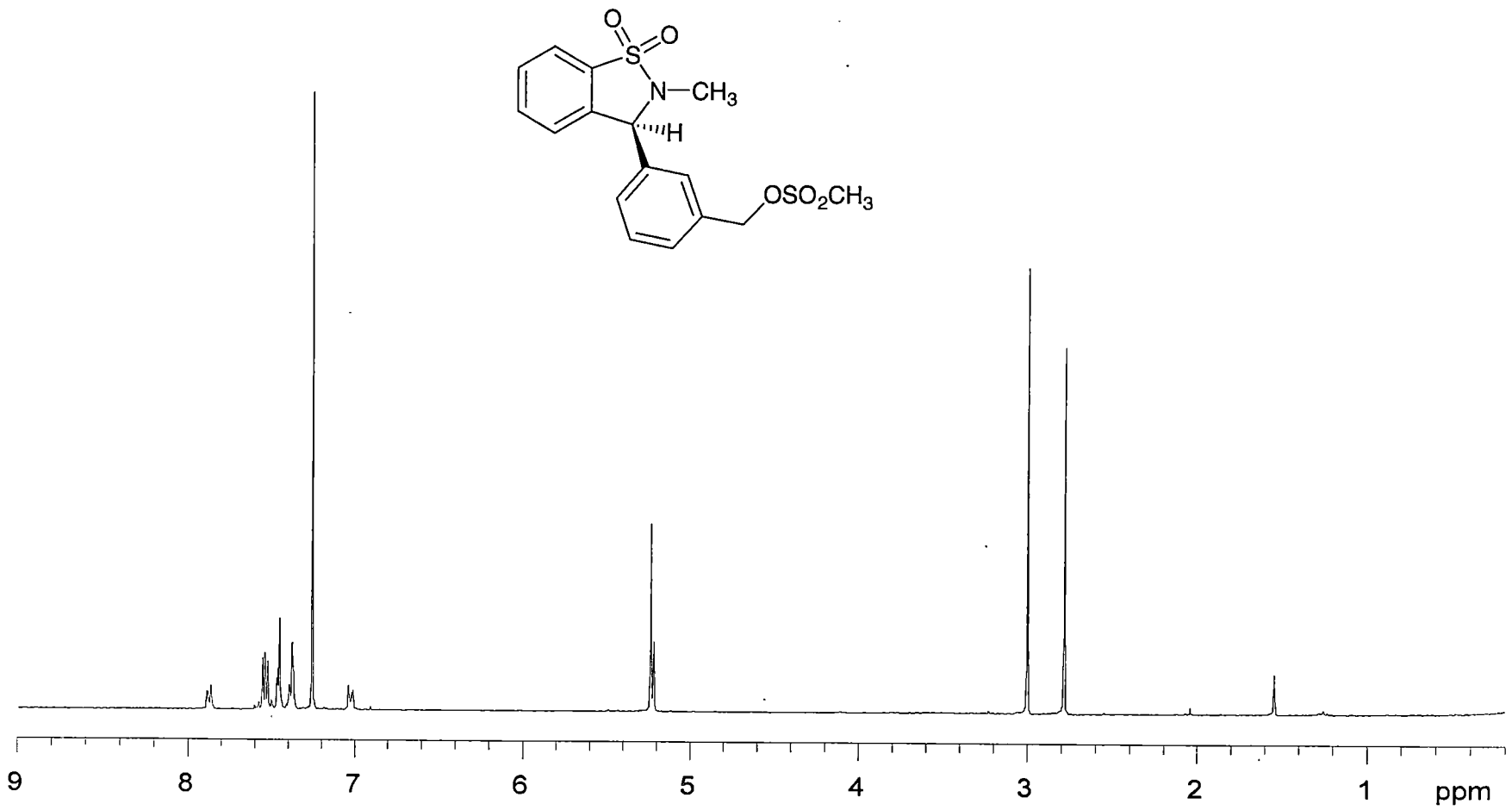
^{13}C NMR (75 MHz, $CDCl_3$) of 3*S*-(2-Methyl-1,1-dioxo-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazol-3-yl)methanol (**7**)



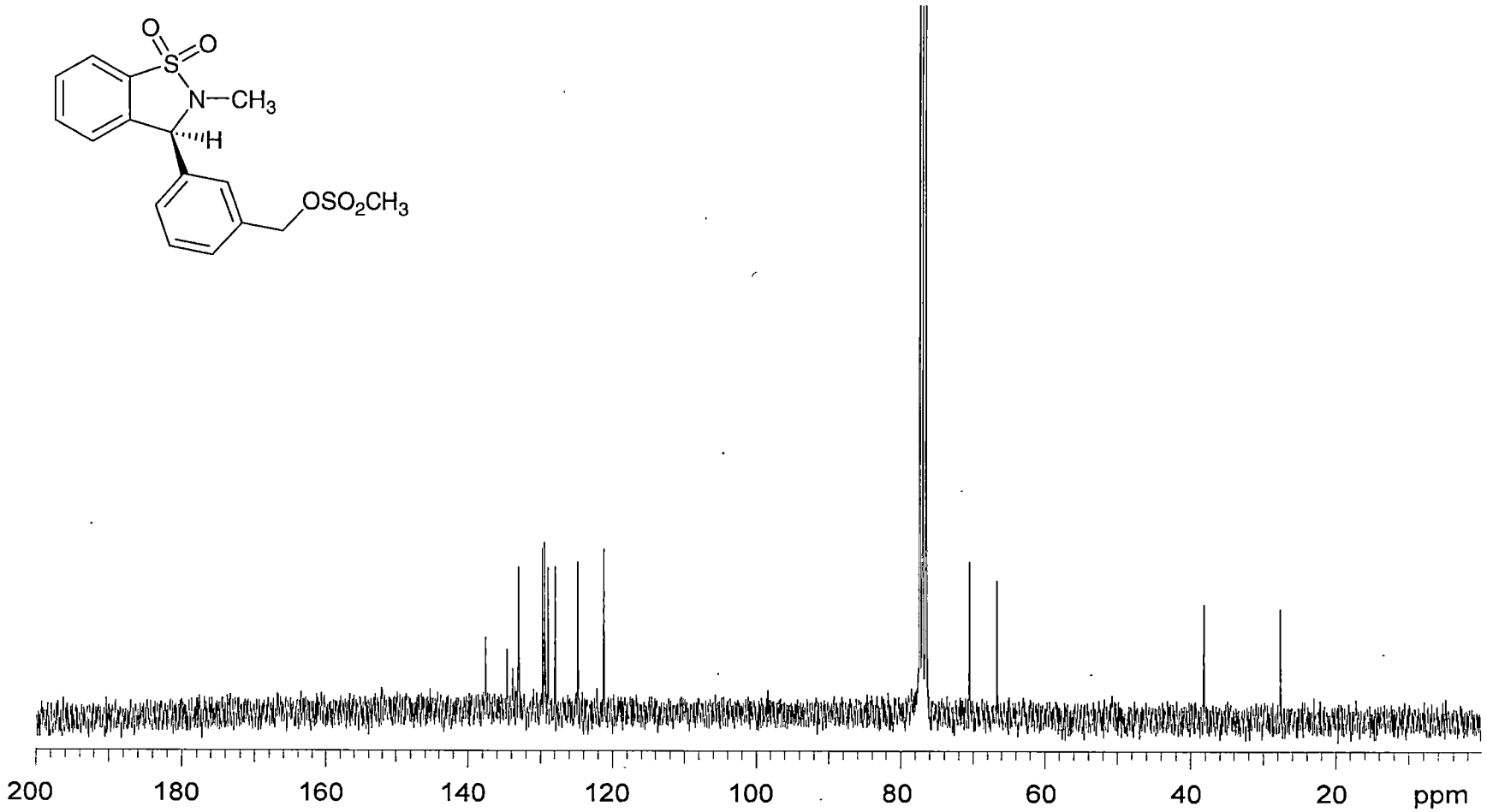
^{1}H NMR (300 MHz, $CDCl_3$) of 3*S*-(2-Methyl-1,1-dioxo-2,3-dihydro-1*H*-1λ^{6}-benzo[*d*]isothiazol-3-yl)benzaldehyde (**8**)



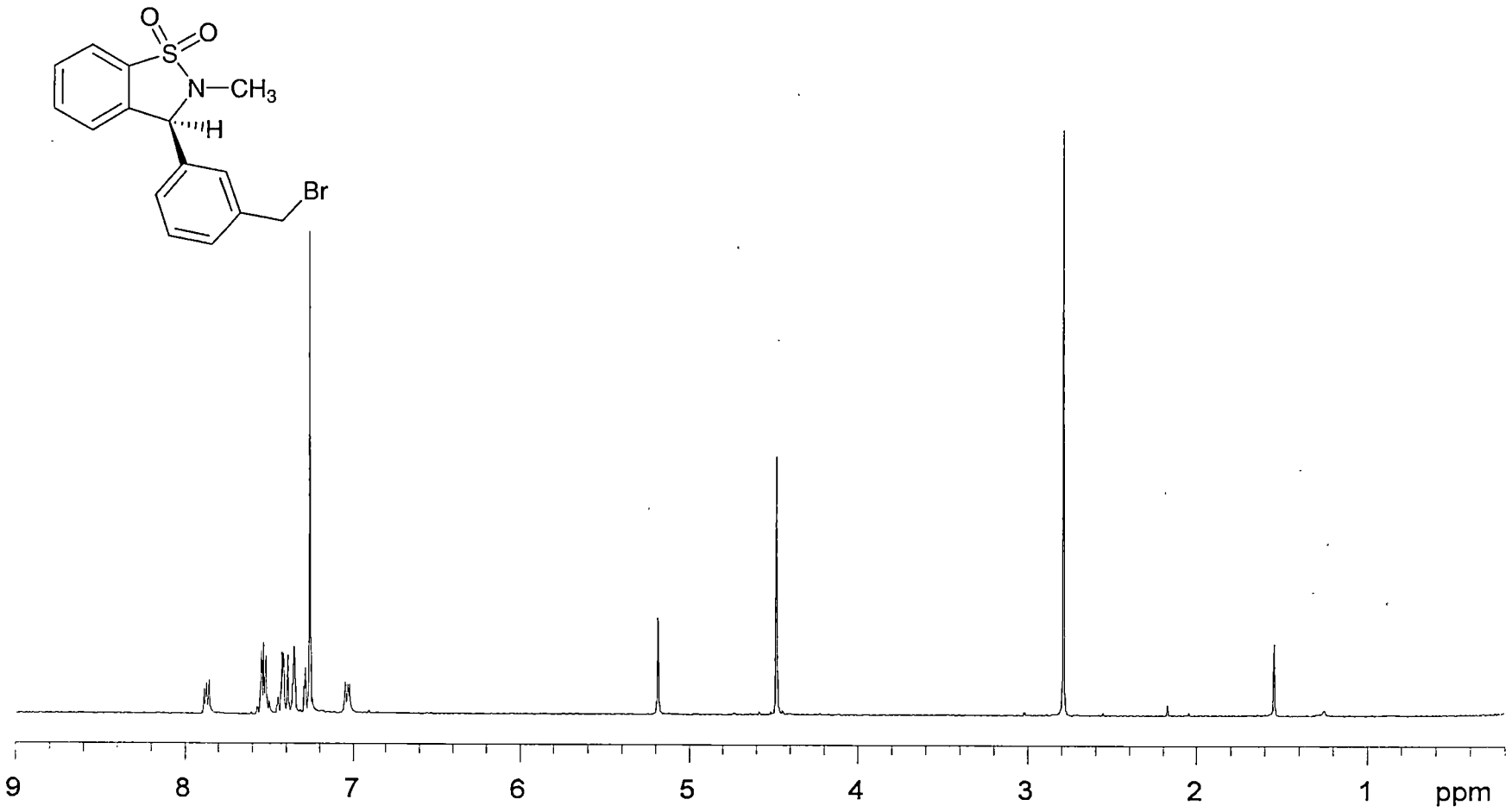
^{13}C NMR (75 MHz, $CDCl_3$) of 3*S*-(2-Methyl-1,1-dioxo-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazol-3-yl)benzaldehyde (**8**)



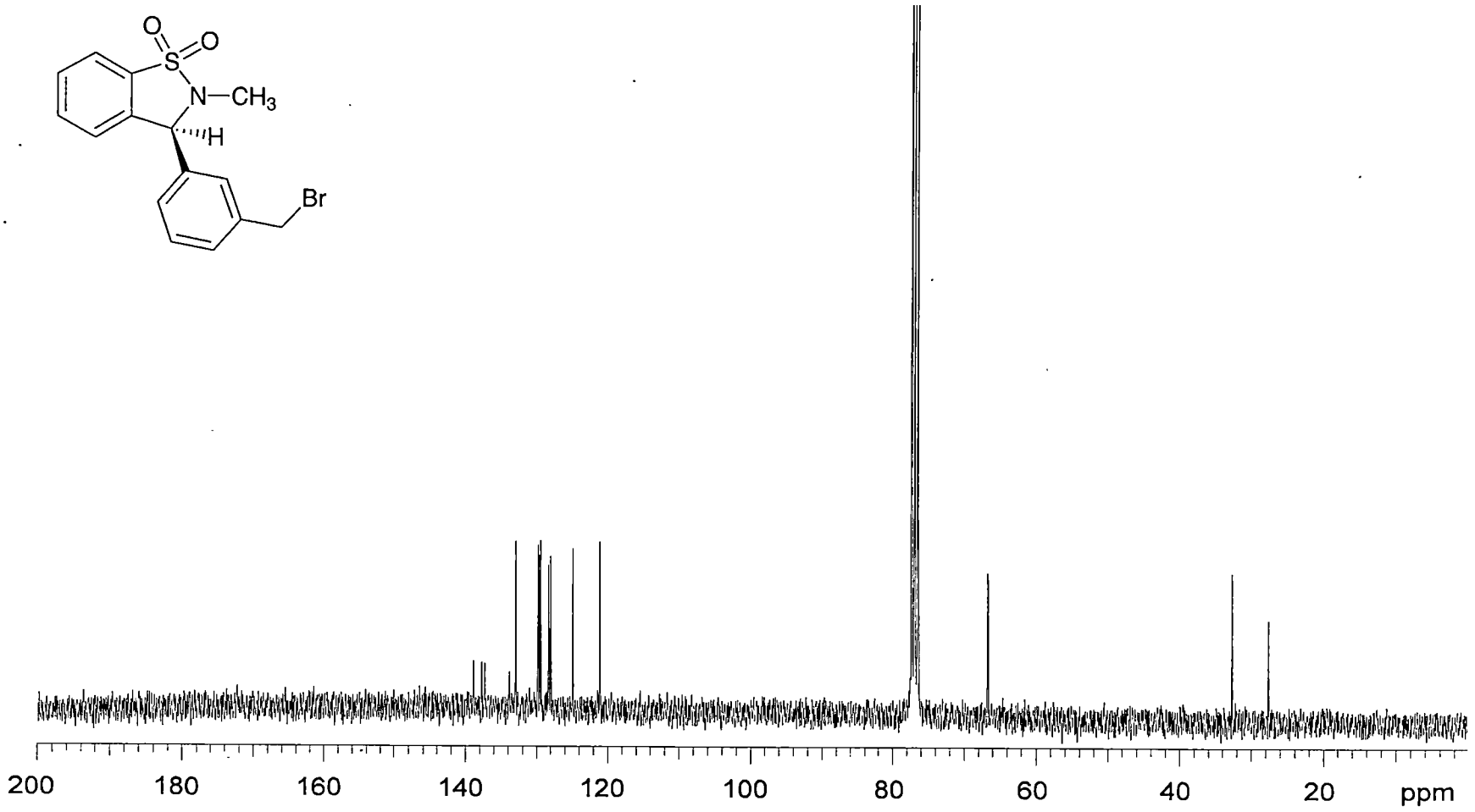
^{1}H NMR (300 MHz, $CDCl_3$) of 3*S*-(2-Methyl-1,1-dioxo-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazol-3-yl)benzyl methanesulfonate (**9**)



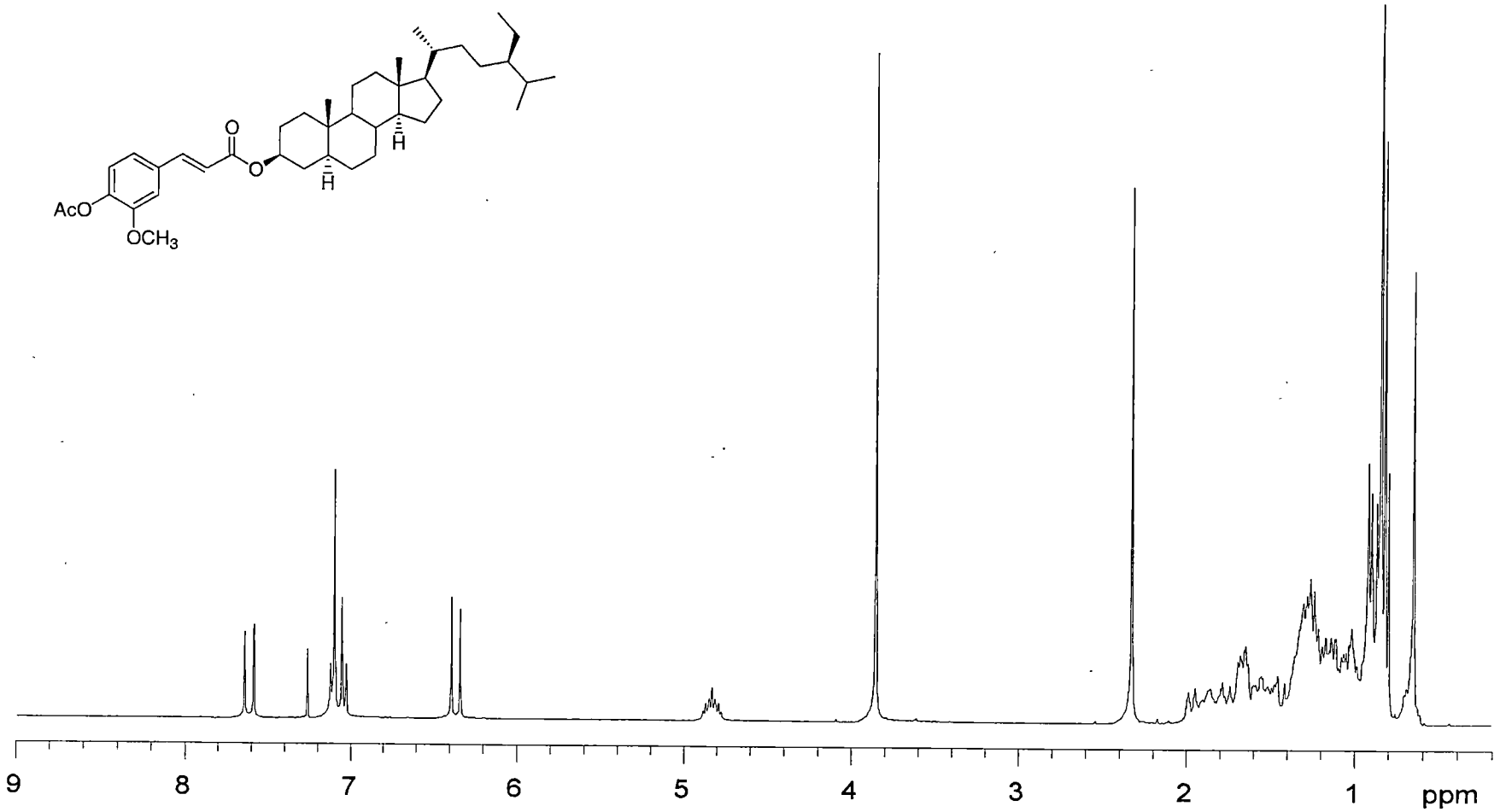
^{13}C NMR (75 MHz, $CDCl_3$) of 3*S*-(2-Methyl-1,1-dioxo-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazol-3-yl)benzyl methanesulfonate (**9**)



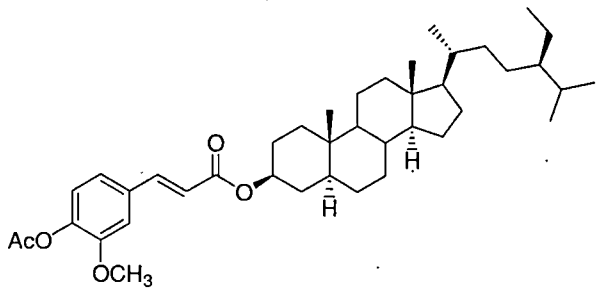
^{1}H NMR spectra of 3*S*-(3-Bromomethylphenyl)-2-methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (**10**)

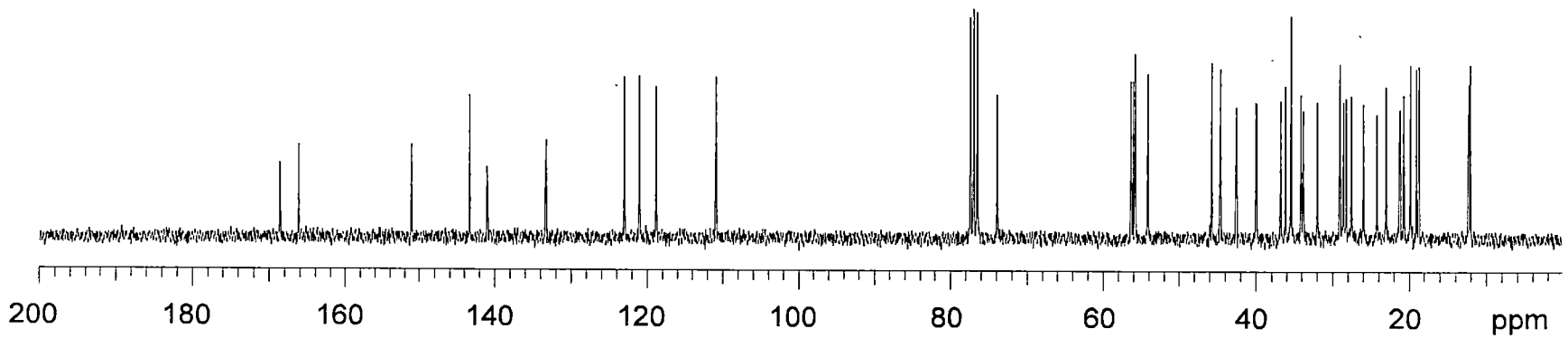


^{13}C NMR (75 MHz, $CDCl_3$) of 3*S*-(3-Bromomethylphenyl)-2-methyl-2,3-dihydrobenzo[*d*]isothiazole 1,1-dioxide (**10**)

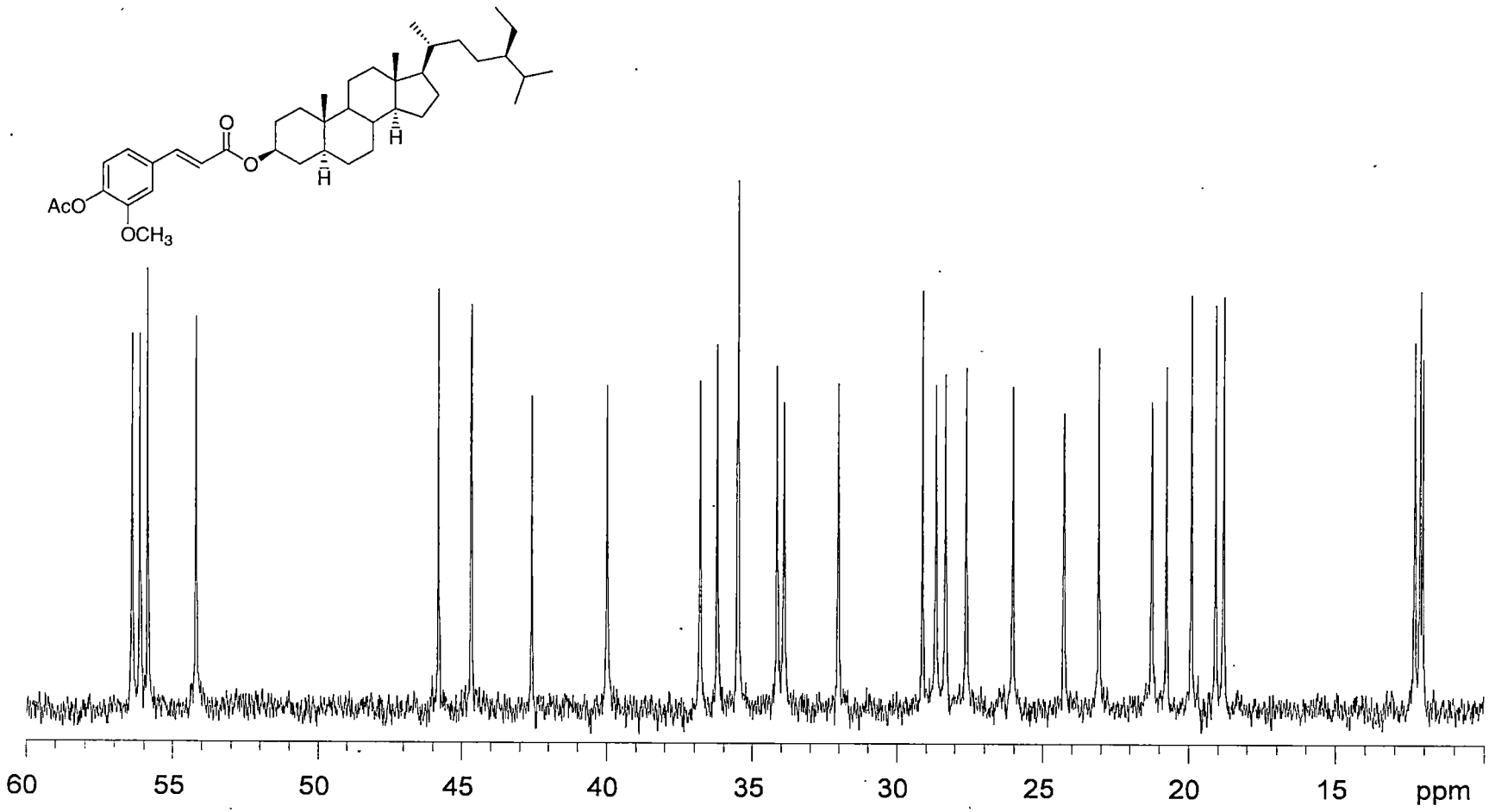


^{1}H NMR (300 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-*O*-Acetylferuloyl)-β-sitostanol (**14a**)

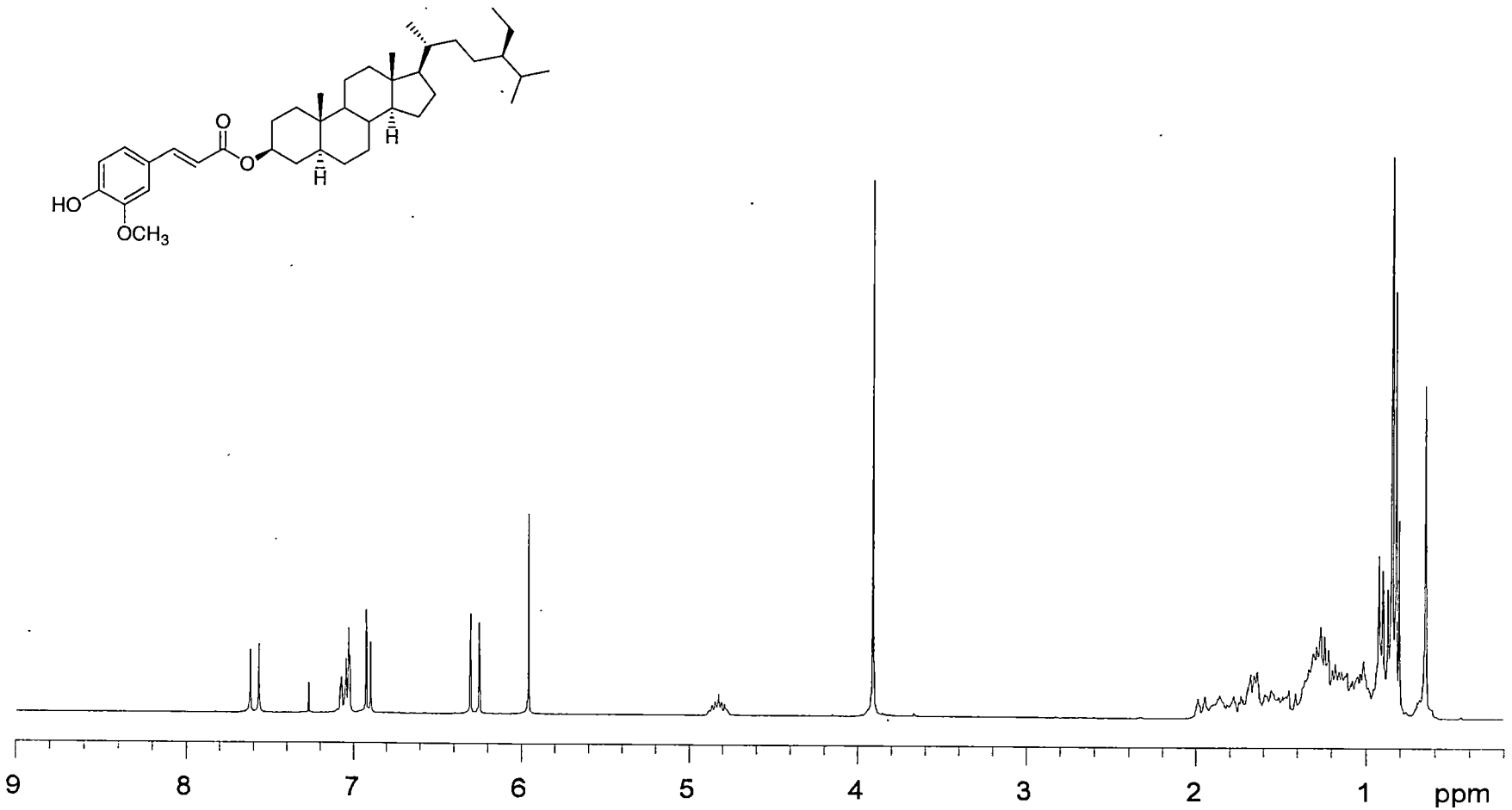




^{13}C NMR (75 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-*O*-Acetylferuloyl)-β-sitostanol (**14a**)



^{13}C NMR (75 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-*O*-Acetylferuloyl)-β-sitostanol (**14a**)



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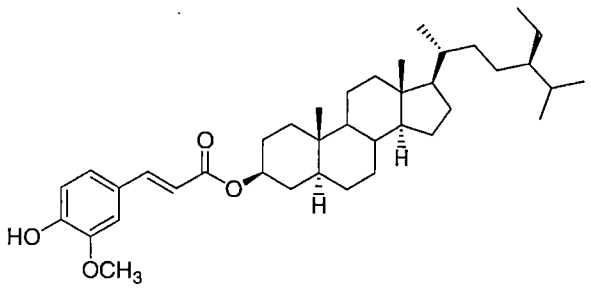
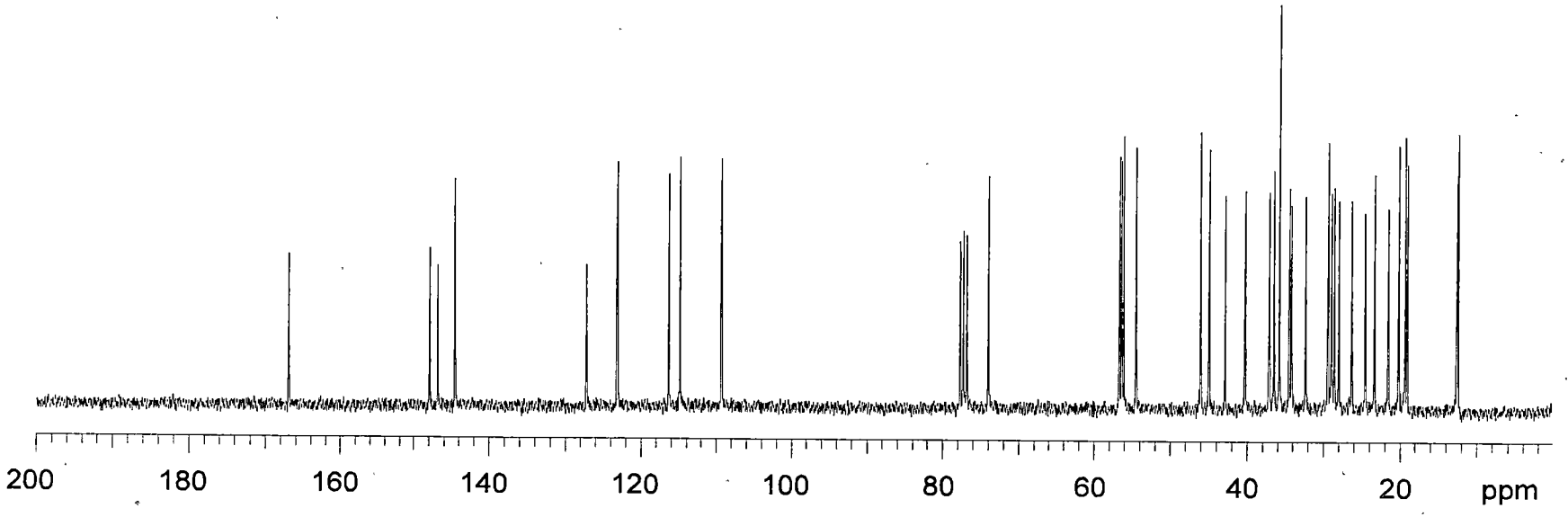
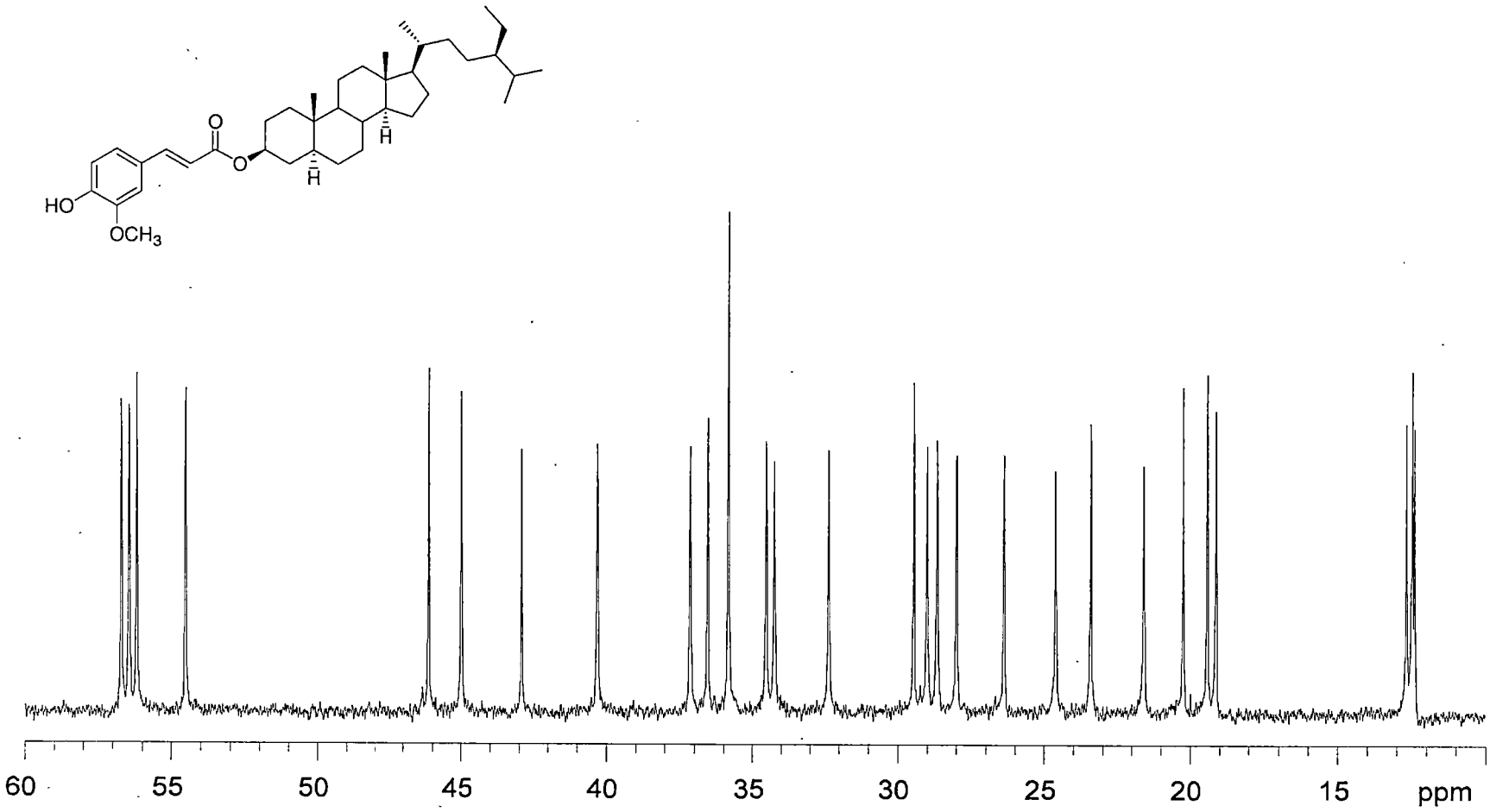
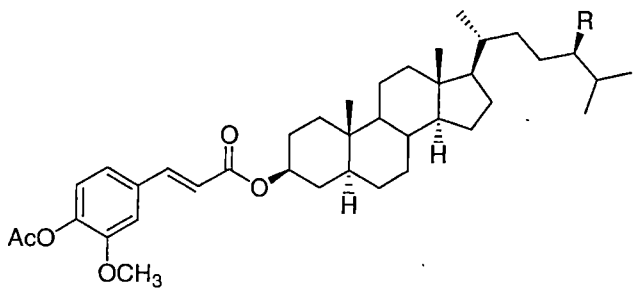


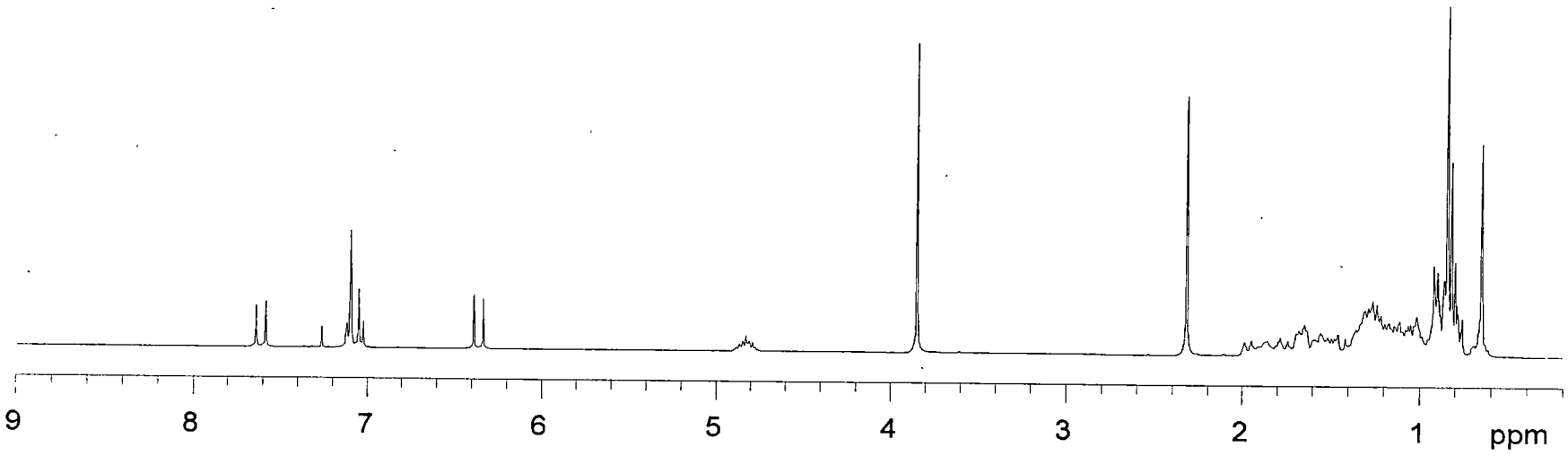


^{13}C NMR (75 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-Feruloyl)-β-sitostanol (**15a**)

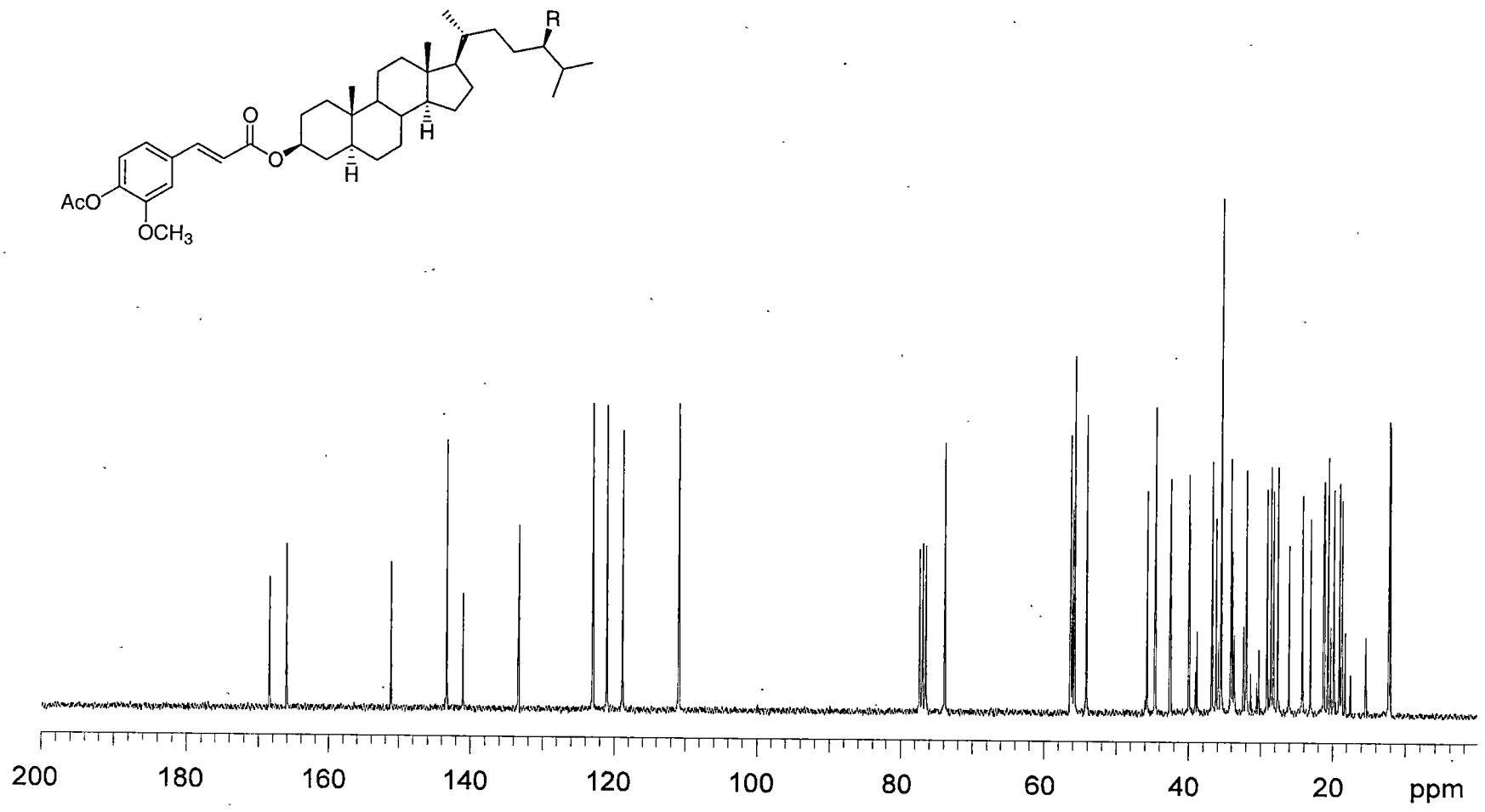


^{13}C NMR (75 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-Feruloyl)-β-sitostanol (**15a**)





^{1}H NMR (300 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-*O*-Acetylferuloyl)phytostanols (**14a** and **14b**)



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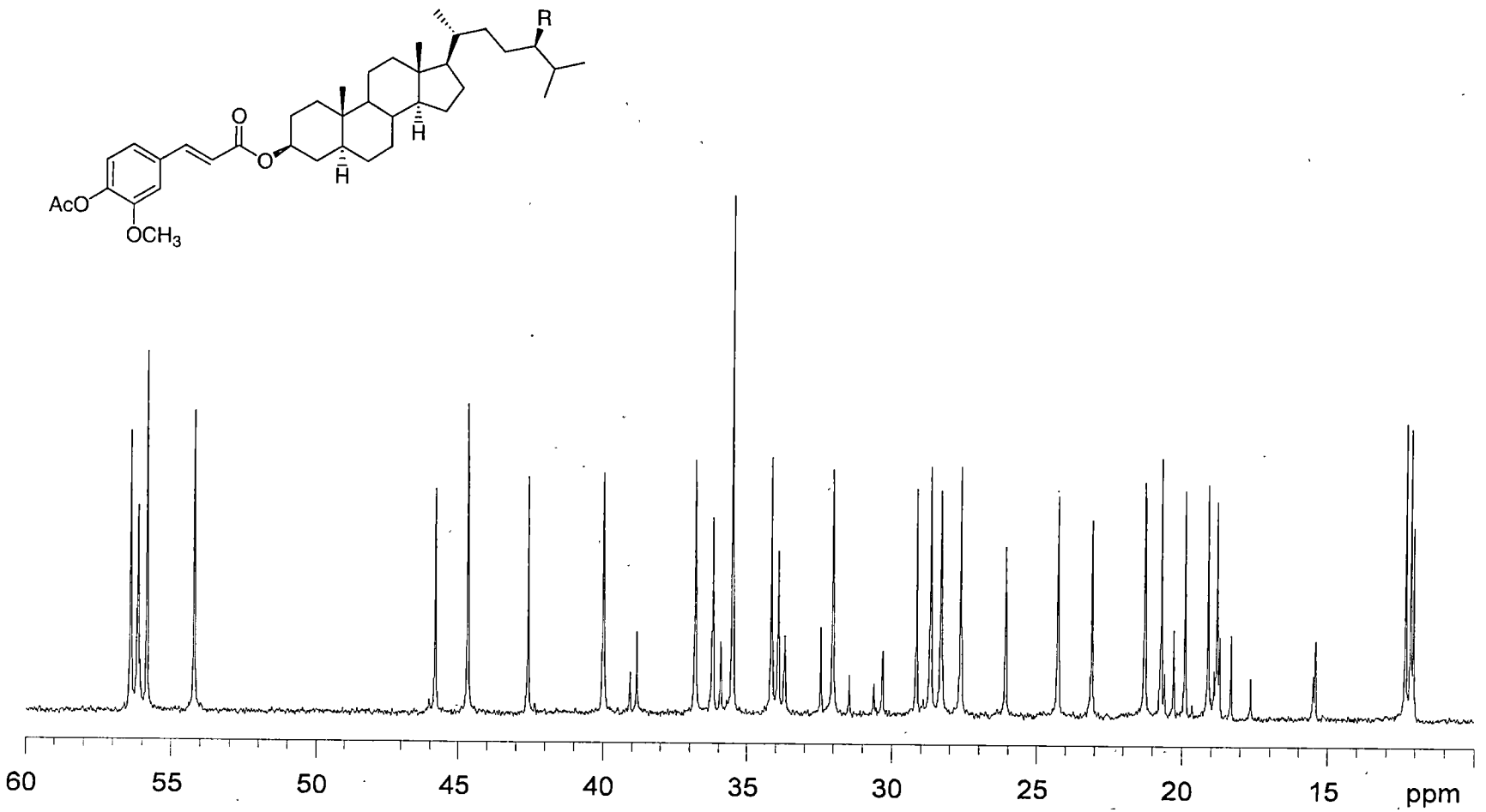
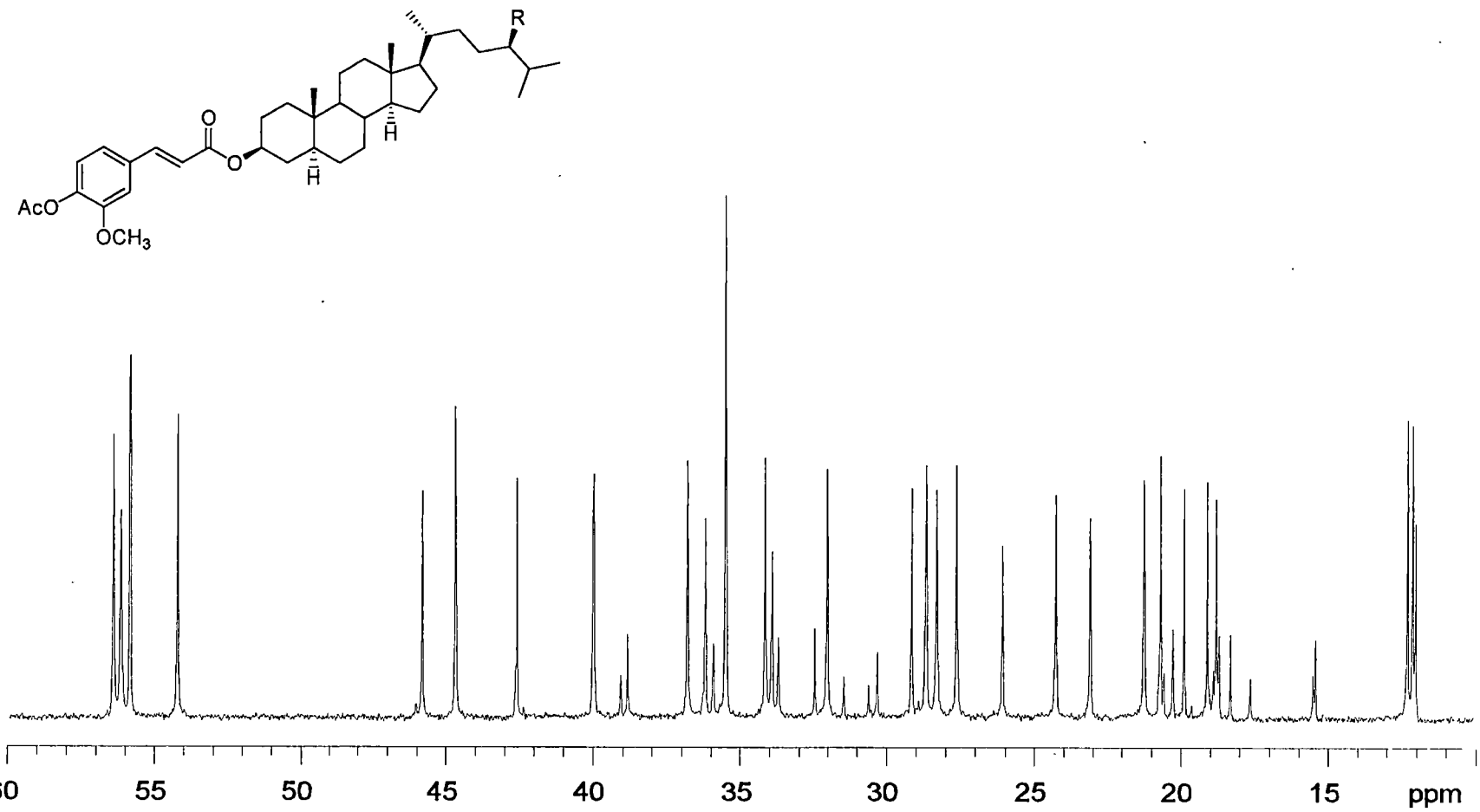
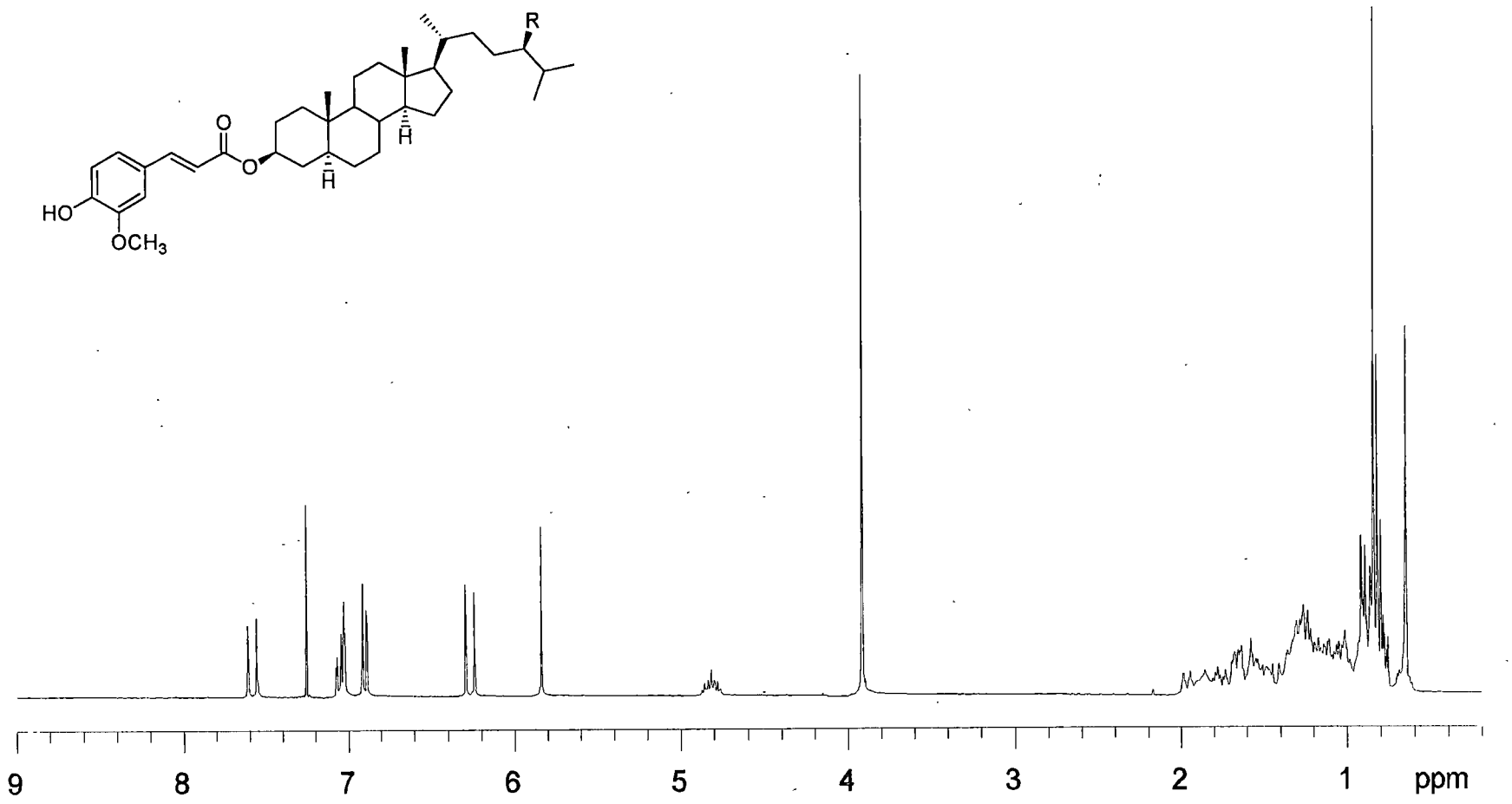


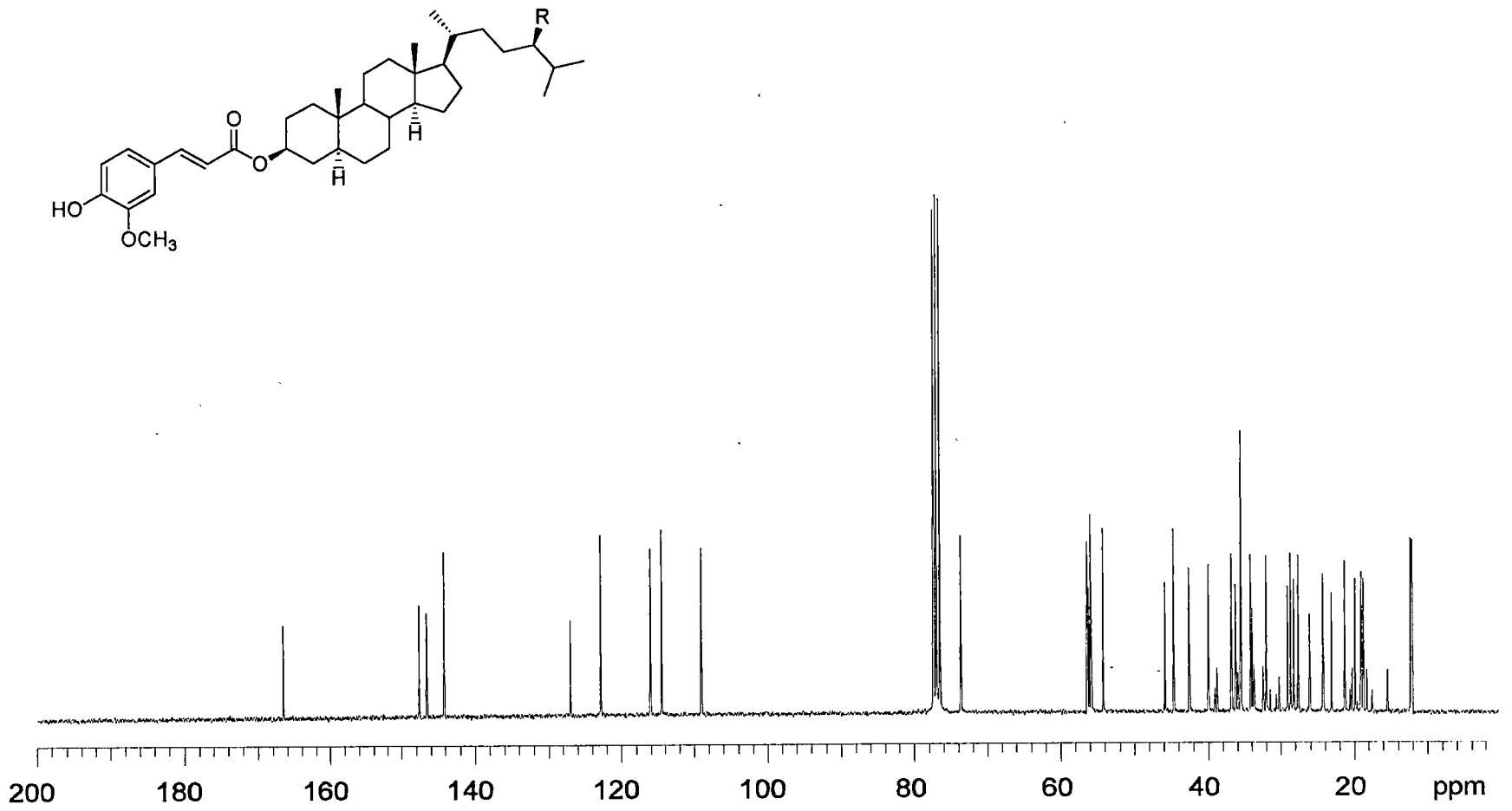
^{13}C NMR (75 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-*O*-Acetylferuloyl)phytostanols (**14a** and **14b**)



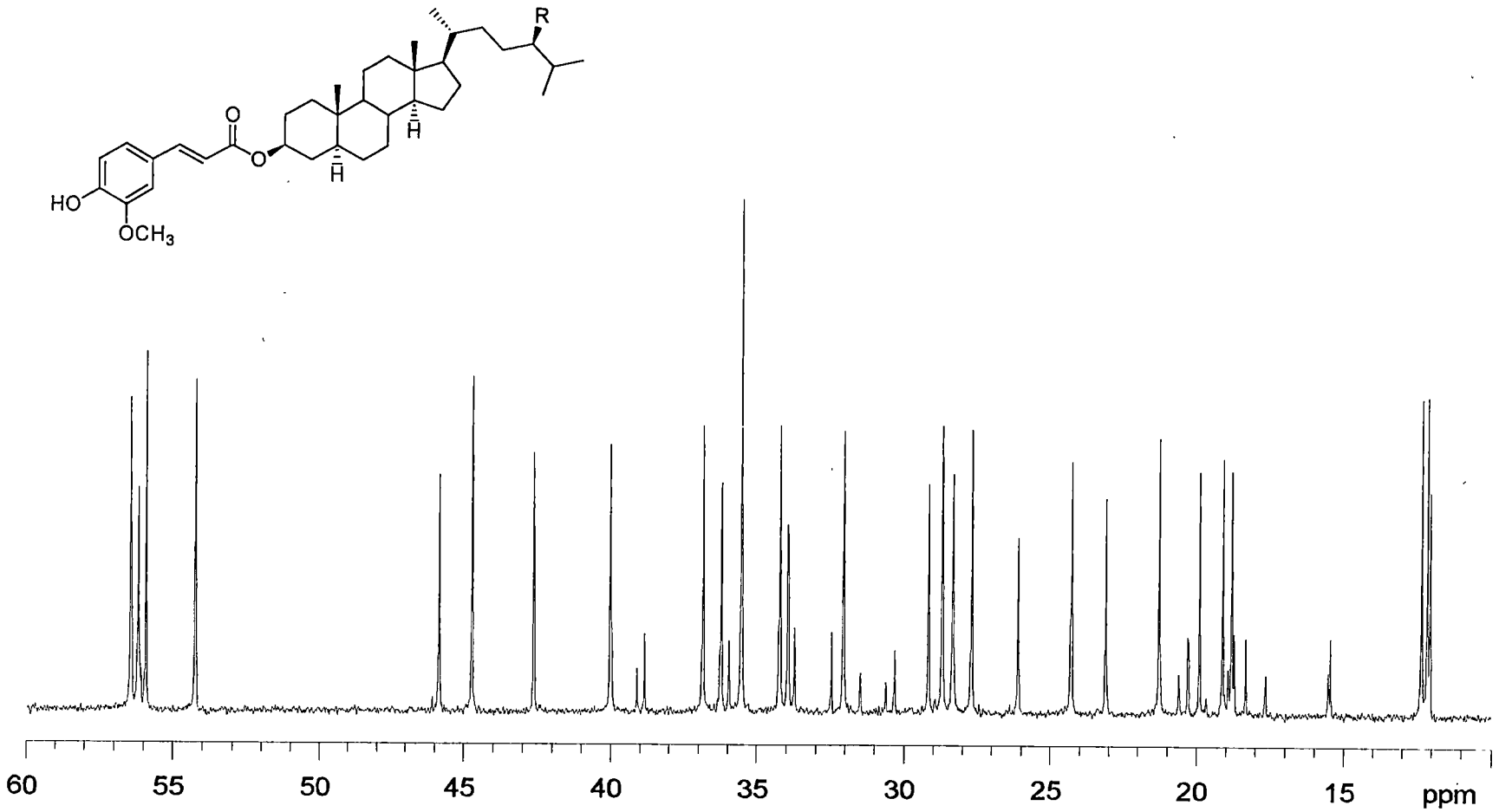
^{13}C NMR (75 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-*O*-Acetylferuloyl)phytostanols (**14a** and **14b**)



^{1}H NMR (300 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-Feruloyl)phytostanols (**15a** and **15b**)



^{13}C NMR (75 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-Feruloyl)phytostanols (**15a** and **15b**)



^{13}C NMR (75 MHz, $CDCl_3$) of 3-*O*-(*trans*-4-Feruloyl)phytostanols (**15a** and **15b**)

Appendix II

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Please consider my request to reprint from the following book published by your company:

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Editors: Angus G. Dalgleish, Robin A. Weiss
ISBN: 0-12-200741-7

I am in the process of writing a thesis for the M. S. degree with a major in chemistry at the University of Tennessee, Knoxville, and would like to include the following in my manuscript:

Page 61, Figure 4.1 "Replication of HIV in CD4 lymphocytes"

Thank you in advance for consideration of my request, and I hope to hear from you soon regarding the matter. My contact information, in addition to the above mailing address, is:

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FAX: (865) 974-3454
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Sincerely,

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VITA

Anthony Michael Condo, Jr. was born in New Brunswick, New Jersey on June 1, 1975. He attended public school in Brick Township, New Jersey, where he graduated from Brick Township High School in June, 1993. After entering Rutgers College of Rutgers University, New Brunswick/Piscataway, New Jersey, he was exposed to the realm of organic chemistry by Professors Robert A. Moss and Laurence W. Romsted, who served as co-lecturers for his undergraduate organic chemistry course sequence. His interest piqued by the field, he took an undergraduate research position with Dr. Leslie S. Jimenez, working in the field of synthetic organic chemistry. His major assignment had him assisting graduate students and postdoctoral associates with the large-scale synthesis of starting materials and intermediates in the synthesis of analogs of Mitomycin C and FR900482. Upon graduation in May, 1997, he enrolled in graduate school at the University of Tennessee, Knoxville. In March 1998 he joined the research group of Dr. David C. Baker, working in rational drug design and synthesis of bioactive compounds. He received the Master of Science degree in December 2001.