University of Nevada, Reno

"Syntheses And Photo-Reactivity Of Chromene Natural Products And Photo-degradation of Steroidal Hormones"

A dissertation submitted in partial fulfillment of the

requirements for the degree of Doctor of Philosophy in Chemistry

By

Trang Thi Nguyen

Dr. Christopher S. Jeffrey/Dissertation Advisor

December 2015

THE GRADUATE SCHOOL

We recommend that the dissertation
prepared under our supervision by

TRANG THI NGUYEN

Entitled

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be accepted in partial fulfillment of the
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DOCTOR OF PHILOSOPHY

Christopher S. Jeffrey, Ph. D, Advisor

Vincent J. Catalano, Ph. D , Committee Member

Benjamin T. King, Ph. D, Committee Member

Lee A. Dyer, Ph. D, Committee Member

Marjorie D. Matocq, Ph. D, Graduate School Representative

David W. Zeh, Ph. D., Dean, Graduate School

December, 2015

Abstract

Over the past twenty years natural products from *Piper,* a tropical genus of plant, have been extensively studied due to the number of biologically active natural products with unique structures. The genus *Piper* provides an excellent model system for exploring the relationship between natural product diversity and its impact on biotic interactions. Our group has isolated three prenylated benzoic acid derivatives, **36-38**, from *Piper kelleyi* Tepe, a newly described species of *Piper* that grow in the Eastern Andes of Ecuador and Peru. These unique natural products have recently been found to significantly vary in their relative concentration and this variation was found to be a main driver of diversification of associated herbivores specifically feeding on *P. kelleyi*. Phytochemical variation was found to be associated with elevation, Chapters 3 of this dissertation describes our exploration of the unique photochemistry.

Our biosynthetic hypothesis suggests that these three metabolites share a common pathway, where one is the precursor to the other. Recent studies unveiled that these metabolites varied significantly between individuals and that plants growing at higher elevation were chemically distinct, producing a higher concentration of all three metabolites. Given that the UV-B concentration is established to increase 15%/1,000m of elevation at the equator and the extreme phototoxicity of these metabolites to generalist herbivores, we proposed that a photochromic process involving the chromene metabolite could be driving both the phytochemical variation and the associated toxicity of the metabolites. Studies of this photochromism in the context of the natural products variation and toxicity were conducted and are the subject in chapter 2 & 3.

The photochemistry of environmental contaminants, especially agricultural pharmaceuticals, has been largely understudied. While it is widely accepted that photochemical degradation of these contaminants is a primary mode of environmental clearing, reversible photochemical transformations that provide selective avenues could result in long-lived environmental contaminants and can also provide reactive pathways that produce contaminants with enhanced biological activity. Trenbolone acetate (TBA) is an anabolic steroid injected to beef cattle for muscle growth, and trace amounts of TBA leach from their manure into our aquatic systems. Risk from this contamination is eliminated as TBA rapidly photodegrades in the exposure of sunlight. Unfortunately, at night TBA regenerates causing potential risk to aquatic organisms. We have recently extended this study to understand the aquatic photochemistry of dienogest (DIE), a potent progestin analog that has structural similarities to TBA. My study demonstrates that DIE rapidly photodegrades under exposed light and reverts to dienogest when left in the dark. Additionally, we have found that continued irradiation of dienogest produces a major photoproduct that contains an aromatic A ring, like estrogen.

Dedication

For my family & friends. Without their love, support and guidance, I would not be the person I am today.

For William Brennan. It's been one crazy ride, but thank you for jumping on and providing me with support and unconditional love throughout this process.

Acknowledgements

First and foremost, I would like to thank my advisor, Dr. Christopher Jeffrey. There are no words that I can possibly combine together to express how thankful I am to have him as a mentor. He is the most motivating and supportive mentor any student could ever ask for. He mentors with such enthusiasm and encouragement it makes learning chemistry and working in the lab enjoyable, even when research goes terrible wrong. I did not officially thank him for allowing me to join his research group in Fall 2010, even though his group was "full" already, so thank you! I have no idea where I would be now and I only hope that he knows how much he is appreciated.

I would also like to thank the members of my doctoral committee: Dr. Vincent Catalano, Dr. Benjamin King, Dr. Lee Dyer and Dr. Marjorie Matocq for giving me their time in reading my manuscript and fitting me into their schedules. Thanks for your support, providing me with guidance and advice throughout my academic career. Thanks to Dr. Sheridan for his help and guidance throughout my studies. I would like to thank Dr. Stephan Spain for his patience and continued help with all the instrumentation. Thanks to Dr. Sarah Cummings for her teaching mentoring and assistance throughout my time as a teacher's assistant to organic chemistry, I'm not sure if I would have survived teaching that course without your help. Thanks to Jennifer Heck for her patience in my crazy graduate life and her constant help throughout this process. And thanks to Dr. John Spence (CSUS) for inspiring me to pursue organic chemistry research in the first place.

To my laboratory classmates: Korry Barnes, Arjun Acharya, Devendar Anumandla, we've been through a lot during our graduate years and I wouldn't change anything about it. Even with the tough and stressful times, I knew that eventually

everything would be okay because we had each other. You guys are apart of my life and had a tremendous impact on my career with your constant motivation and support. I will cherish these memories forever. In addition, to my laboratory coworkers: Michael Leonard, Ryan Littlefield, Kaitlin Ochsenrider, Karissa Montes, Celso Ricardo de Oliveira Junior, Casey Philbin. Thank you for bringing the joy and breath of fresh air back into the lab. And last, to some wonderful undergraduates I've had the pleasure in mentoring: Xibei Liu, Sarah Gebrazghi and Ashlyn Conlin, thanks for your hard work and dedication to research.

I would also like to thank my family, Mom & Dad, Thuy, Kenneth, Johnson, Tommy, Lance, Angie, Farm, Adalynn, Arilynn, Baby Ducky and Mochi. Thanks for being one big eccentric family; Its never a dull moment during family reunions. Thanks for your support, words of wisdom and encouragement throughout my endeavors. I want to thank my best friend, Delaina Geczi, for all her friendship and loyalty throughout the years. In addition I want to thank my Reno family for their friendship and support. To my in-laws, Bill and Joan Brennan, thank you for making me feel apart of the family by welcoming me to your home with open arms and thank you for raising an amazing son.

Finally, I wish to thank my husband, Liam, for his love and support throughout this process. It has undoubtedly improved everything about me and I feel that we push each other to be the best or better at any of our endeavors. I appreciate the passion and encouragement you have for me and my career. Thank you for settling me down when things get rough. Basically, there are no words that can possibly describe how much you mean to me. I love you and thank you.

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Chapter 1: Introduction

1.1 Natural Products from the *Genus Piper*

The plant *Genus Piper* belongs to the Piperaceae family and is considered one of the largest genera containing over 1000 species.[1] The greatest diversity of species within this genera are commonly found in the Neotropics and mostly grow in wet, warm, lowland rain forests.[1-2] *Piper* is an excellent model system to explore the diversification of phytochemicals due to the abundance of biologically active natural products found within the plants.[1-2] Although, *Piper* species vary in morphological diversity, there abundance in both species and phytochemical diversity is phenomenal.[1-2] Phytochemicals found within plants can be characterized as primary and secondary metabolites. Primary metabolites are considered metabolites that are directly involved in development and reproduction.[3] Secondary metabolites or natural products are considered as more specific compounds that play extremely important ecological roles, such as plant defense, significantly contributing to the organisms overall fitness.[3-4] Plants continuously produce a variety of these metabolites and utilize them for their defensive properties against herbivorous predators and microbial pathogens.[5] Organic chemists have widely studied the secondary metabolites found within *Piper* as it contains a highly diverse set of natural products that have unique structures and a suite of biological activities. The classes of naturally occurring compounds that have been isolated from *Piper* species commonly include alkaloids **A**, flavonoids **B**, chalcones and quinones **C**, terpenoids **D**.[2] (Figure 1.1.1). In addition, these secondary metabolites have been found to mediate the relationship between plants, herbivores and their predators.[5-6] Understanding these interactions provides important information for understanding the co-evolutionary

relationship between phytochemical diversity and associated communities of insect herbivores.[6-8] One specific example is the strong co-evolutionary relationship between *Piper* host plants and the *Eois* moth, a highly specialized genus of Lepidoptera that exclusively feeds on *Piper* host plants.[6-8]

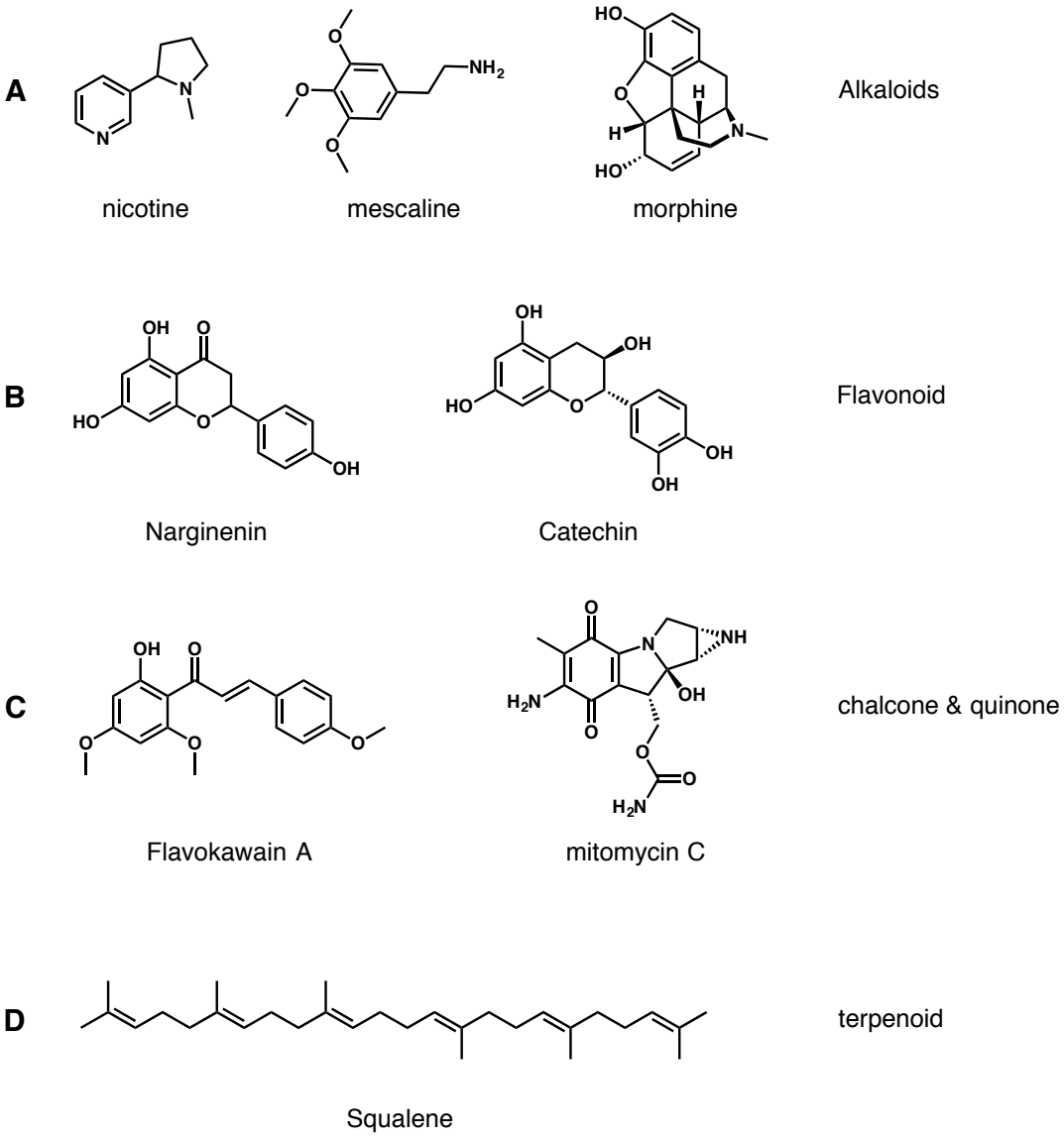


Figure 1.1.1 Classes of naturally occurring compounds.

Piper kelleyi, a newly described species within this genus, is documented to play host to a tremendous diversity of *Eois* caterpillars in addition to associated parasitoid wasps and flys.[9] In order to further understand the chemical underpinnings of these plant-insect interactions, Jeffrey and coworkers successfully isolated novel benzopyran natural products from *P. kelleyi*,[10] which is discussed in chapter 2.

1.2 The Ubiquity of Chromenes as Secondary Metabolites

Benzopyran, a well-known pharmacophore, appears as an important structural class of natural occurring compounds and have generated great interest due to their diverse biological activity.[11-14] This heterocyclic ring system consists of a benzene ring fused to a pyran ring. More specific subtypes of naturally occurring benzopyrans include various structural skeletons such as chromane **1**, 2*H*-chromene **2** and 4*H*-chromene **3**,[12-14] (Figure 1.2.1)

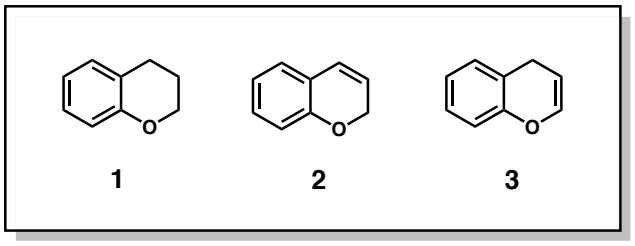


Figure 1.2.1 Chemical structures of chromane and chromene systems.

The first benzopyran prepared was 2,2-dialkyl-2*H*-chromene (alkyl = ethyl to *n*-heptyl) in 1939, which was used to test if the chromene core was responsible for the insecticidal activity found in deguelin.[15] In this study, Shriner and coworkers found that

these chromene analogs caused paralysis in goldfish in as little as three minutes. Subsequent studies found that an abundance of benzopyran natural products have been isolated from several natural sources and have been identified as antiviral[16], such as anti-HIV,[17] estrogenic,[18] antibacterial[19] antioxidant,[20] antifungal[21] and some containing antiherbivory activity.[10] Some natural products containing these moieties such as vitamin E **4**, having antioxidant activity,[20] tetrahydrocannabinol **5**, having CNS activity[14], chromene **6**, having antiherbivory activity[10] and deguelin **7**, an insecticide that recently show anti-cancer activity[21] (Figure 1.2.2).

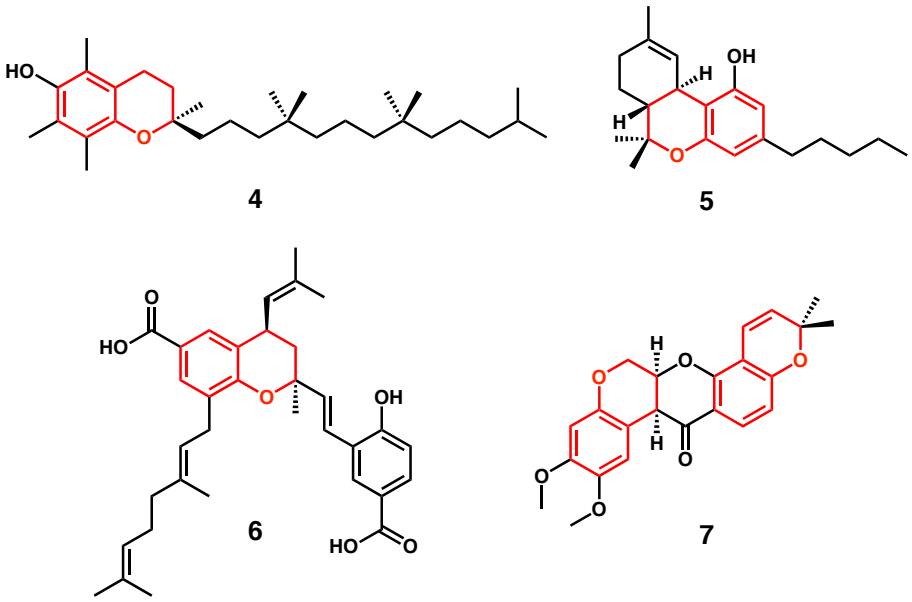


Figure 1.2.2 Examples of natural occurring benzopyran containing products

In addition, natural products containing a benzopyran core commonly incorporate isoprenyl side chain units (Figure 1.2.3). Chromenes **8** and **9** were isolated from the leaves of *Peperomia serpent* Loudon and are found to have antifungal activities against

Cladosporium cladosporioides and *C. sphaerospermum*.[22] Chromene **10** was isolated from the leaves of *Piper kelleyi* along with chromane **6** (Figure 1.2.2) and is found to exhibit antiherbivory activity evaluated against a lab colony of generalist caterpillar *Spodoptera exigua*.[10]

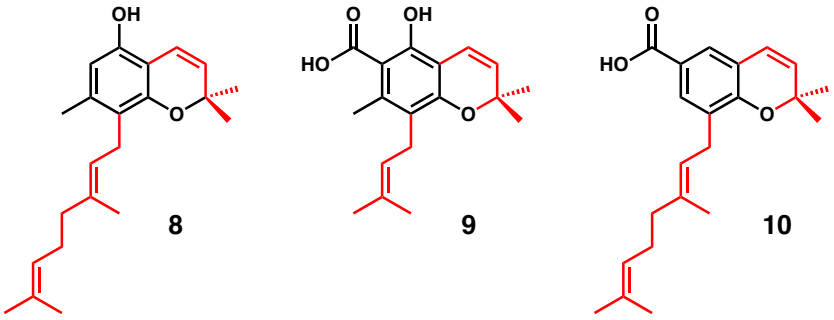
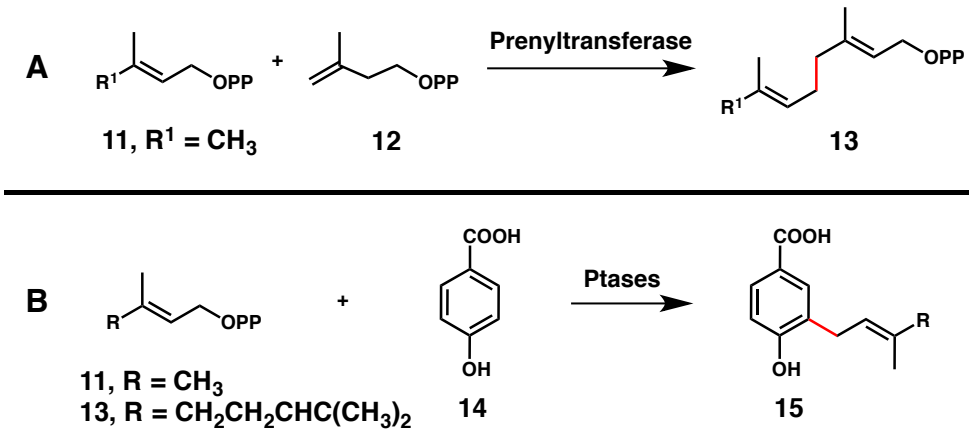


Figure 1.2.3 Examples of natural occurring benzopyran containing compounds with isoprenoid side chains

Prenylated chromene derivatives can be accessed by merging two natural building blocks, an isoprenoid and a phenol and upon oxidation will yield the chromene core. A prenyltransferase type enzyme catalyzes the reaction of an allylic pyrophosphate (DMAPP) **11** and isopentenyl pyrophosphate (IPP) **12** generating geranylpyrophosphate (GPP) **13** (Scheme 1.2.1, panel **A**), which originates from mevalonate (MVA) or non-mevalonate pathway (MEP).[23] This condensation is responsible for the fundamental chain elongation reaction of terpene biosynthesis, which leads to the formation of diverse set of natural products, such as sterols and carotenoids.[23] Similarly, aromatic prenyltransferase (PTases) catalyzes the transfer of isoprenyl moieties, such as DMAPP **11** or GPP **13** to aromatic acceptors, such as *para*-hydroxybenzoic acid **14** yielding a prenylated benzoic

acid **15** through an electrophilic aromatic substitution *ortho* to a phenol derived from shikimate or polyketide biosynthesis.[24] (Scheme 1.2.1, panel **B**)



Scheme 1.2.1 Prenyltransferase and aromatic prenyltransferase catalyzed reactions

The aromatic nucleophiles can be accessed through shikimate/prenylpropanoid pathway[25] to provide the *p*-hydroxybenzoic acid precursor **14** necessary for prenylation. Combinations of these metabolic pathways allow plants to produce an abundance of secondary metabolites that are essential for long-term survival.[3-4] In addition, secondary metabolites that contain these chromene cores were found to have photochromic properties, a reversible change of color initiated by light, which gives rise to ring-opening of the chromene core, providing a highly reactive intermediate speculated to be an *o*-quinomethide (*o*-QM).[26-27] It is our hypothesis that the photochemical generation of a quinomethide from chromenes could be responsible for chemical variation and diversity of secondary metabolites found in chromene containing plants. It is our expectation that strong selective pressures could be responsible for the ubiquity of chromenes.

Additionally, it is our hypothesis that the photochromic behavior of chromenes could be responsible for their toxicity to insect herbivores, which will demonstrate enhanced toxicity upon exposure of the insect to UV light. Specifically, *in-vivo* photogeneration of a quinomethide intermediate from an ingested chromene could alkylatively modify biomolecules that can permanently disrupt or alter their function.

1.3 **Photochromism**

Photochromism is photochemical process that is characterized by a molecule that undergoes a reversible color change upon irradiation with light.[28] Photochromism has been extensively studied for over a century[26], however, its relevance to natural products variation and toxicity has not been evaluated. Metabolites containing a chromene core can absorb light and undergo a reversible transformation between two forms, **A** and **B**, and having distinct absorption spectra[26] (Figure 1.3.1). Usually, photochromic molecules have a colorless or pale yellow form **A**, which converts to a colored form **B** (e.g., red or blue) upon irradiation.

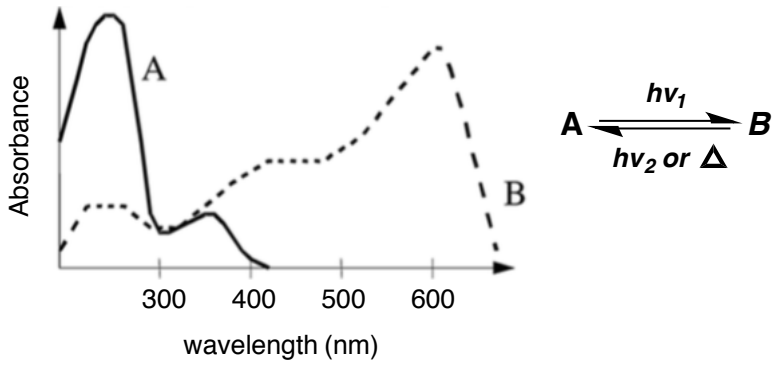
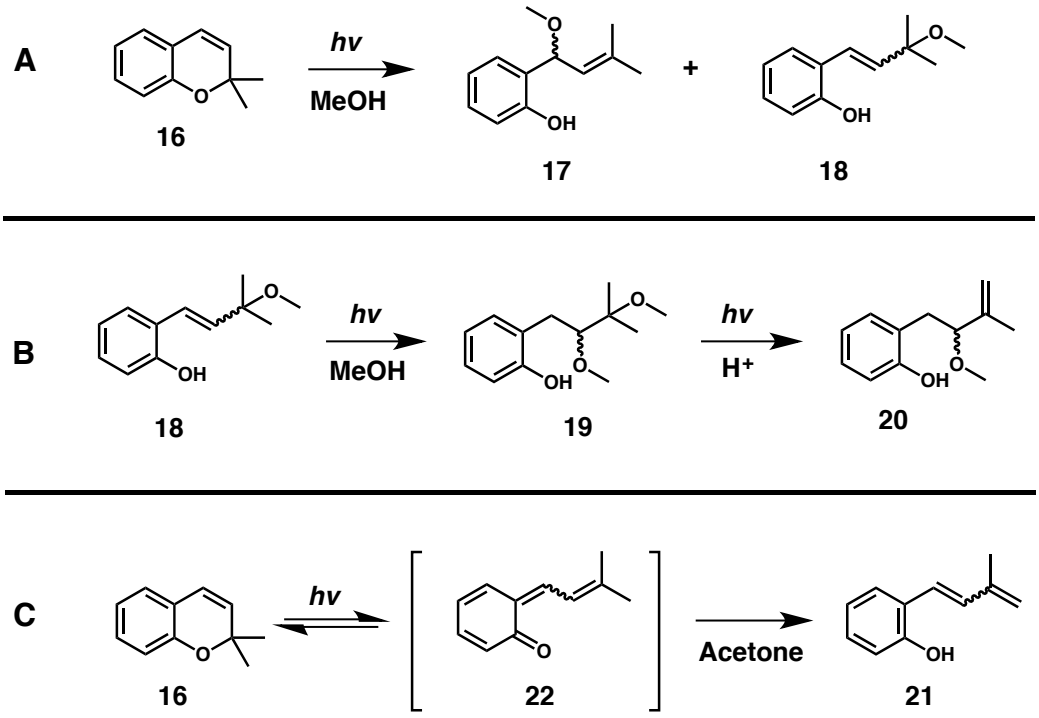


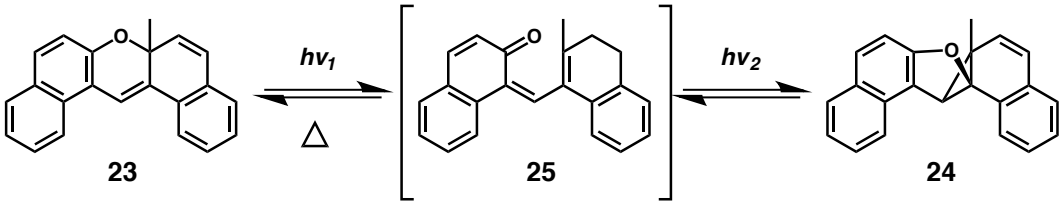
Figure 1.3.1 Different absorption spectra observed in a reversible transformation of a chemical species induced by absorption of electromagnetic radiation between two forms, A and B.

In 1972, Padwa and coworkers reported that the simple, unsubstituted 2*H*-chromene **16** can undergo photochemical ring opening to give a variety of photoproducts.[27a] In methanol, upon irradiation, chromene **16** can undergo an intermolecular nucleophilic addition giving a multitude of methanol adducts, such as 1,4- and 1,6- addition to a putative *o*-quinone methide generating the isomeric adducts **17** and **18**, respectively (Scheme 1.3.1, panel **A**). They also observed that further irradiation of this product led to an additional nucleophilic addition of methanol across the double bond in the 1,6-methanol adduct **18** providing a unique anti-Markovnikov addition product, 2,3-dimethoxy-2-methyl-4-(*o*-hydroxyphenyl)butane, **19**.[27] When an acid was added to the methanol adduct **19**, the compound undergoes an acid-catalyzed elimination to provide photoproduct **20** (Scheme 1.3.1, panel **B**). Padwa also investigated the effect of solvent on the type and ratio of photoproducts of the chromene reaction. Direct irradiation of chromene **16** in acetone gave 2-methyl-4-(*o*-hydroxyphenyl)-1,3-butadiene **21**, as the exclusive photoproduct.[27] From these observations, the mode of reactivity was best rationalized as a photochemical ring opening of **16** to give an *o*-quinomethide intermediate **22** (Scheme 1.3.1, panel **C**), which rapidly transforms to the variety of phenolic compounds.



Scheme 1.3.1 Photochemical transformation of chromene **16**.

In 1993, Uchida and coworker studied the photoisomerization of naphthopyran derivatives, which could find applications in materials science as rewritable optical memory media.[29] They found that the yellow colored naphthopyran derivative **23** (Scheme 1.3.2) isomerized to the colorless bicyclohexene isomer **24** via an unstable intermediate **25**. This photoreaction involves a step-wise two-photon process as one photon is absorbed to produce the reactive intermediate and the second photon absorbed converts **25** to the bicyclohexene isomer **24** (Figure 1.3.2). This phototransformation provides evidence of the versatility in reactivity of the *o*-quinomethide intermediate as it undergoes a unique intramolecular photorearrangement.



Scheme 1.3.2 Photochemical transformation of naphthopyran **23**.

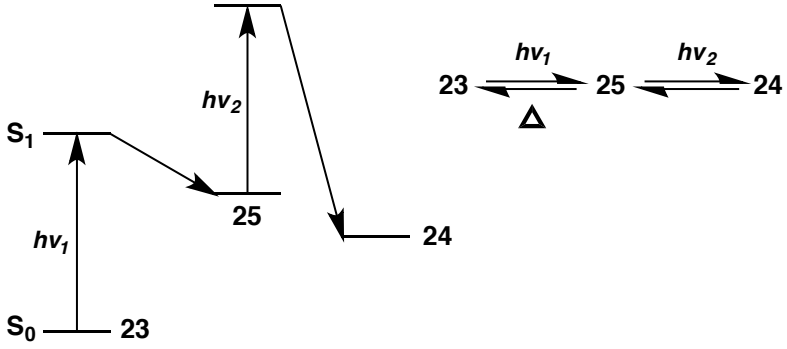


Figure 1.3.2 Two-photon absorption system of the photochemical transformation of naphthopyran **23**.

In 1976, Bowers and coworkers isolated the first naturally occurring 2*H*-chromenes called precocene I **26** and precocene II **27** (Figure 1.3.3) from *Ageratum houstonianum*.[30] These ageratochromenes are the simplest forms of natural occurring chromenes to show photochromic behavior.[31] In 2005, Gartner and coworkers used precocene I **26** as a photochromic agent to potentially label cytochrome P450 3A4.

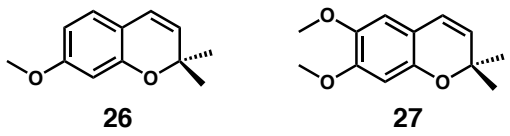


Figure 1.3.3 Naturally occurring 2*H*-chromenes

In their study, precocene I **26** was dissolved in ethanol and irradiated under UV light at 266 nm. The ring-opened product absorbed strongly at 380 nm and was found to be long lived with the observation of substantial amounts of the *o*-quinone methide after 150 ms. Irradiation in the presence of glutathione (GSH), a cysteine rich protein, yielded the 1,4- and 1,6- SG GSH adducts **28** and **29**, respectively (Figure 1.3.4), as well as other unidentified conjugates, establishing that chromenes can serve as photoactivatable conjugating agents and could covalently modify biomolecules of exposed organisms.

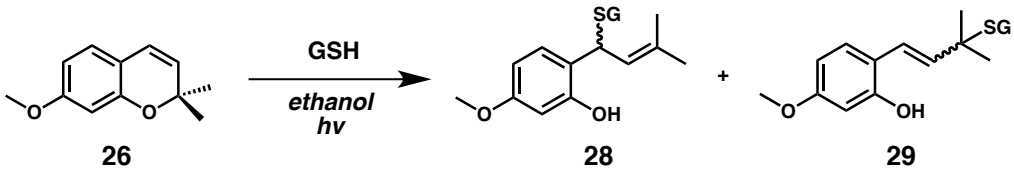


Figure 1.3.4 Photochemical ring-opening of precocene I **26**

1.4 Steroidal Photo-transformation

Steroidal hormones are naturally produced within the endocrine system of the human body. The endocrine system is composed of endocrine glands and hormone-producing tissues, hormones and hormone receptors.[32] Steroidal hormones, such as estradiol **30**, progesterone **31** and testosterone **32** (Figure 1.4.1), are natural compounds

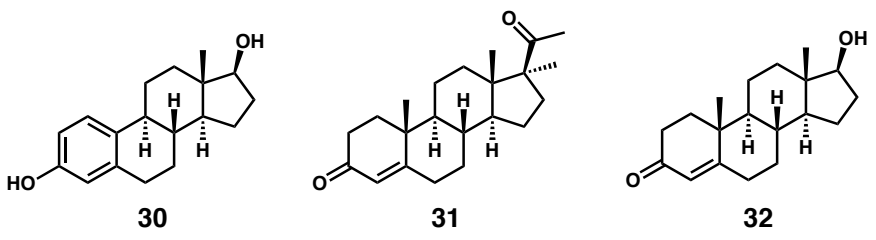


Figure 1.4.1 Naturally occurring steroids produced in the endocrine system

responsible for the regulation of metabolism, growth and development and reproduction. Semisynthetic compounds are synthetically derived to imitate these natural hormones to modify growth or other endocrine mediated physiological responses.[33] Mammals treated with these agricultural steroids discharge a magnitude of potential bioactive organic material, which could directly affect wildlife species and organisms in the aquatic system.[36]

In the past two decades, the demand for agricultural pharmaceuticals has rapidly increased as human population increases.[33b] In 2000, the US produced over 16,000 tons of antibiotics, of which 70% was used for livestock.[33, 34] These pharmaceuticals are used in the US animal agriculture to satisfy a profit motive and to cope with the approach used by operation of confined animal feeding operations (CAFOs).[35] Antibiotics are injected into animals to prevent infection due to CAFOs in addition to promoting muscle growth in animals at feed lots.[35] Monensin, for example, is an antibiotic that is given to beef cattle to prevent infection and to promote growth.[33b] Feed efficiency was substantially improved and that monensin-fed cows out-gained controls, 0.43 to 0.23 kg/day.[33b] In addition, CAFOs typically collect wastewater in lagoons and is applied to fields to function as fertilizer, which could contribute to environmental contaminants. Fortunately, the monensin antibiotic was found to be extensively metabolize by laboratory animals.[33] However, antibiotics or other pharmaceuticals not rapidly metabolized by exposed animals can eliminate these contaminants through their urine and feces.[33b] These metabolites can enter the environment through fertilizer application and runoff remains in soil that may lead to adverse effects in aquatic organisms.[36] These adverse affects that can interfere with the endocrine or hormone system of an organism are called endocrine

disruptors (EDs). Endocrine disruptors found in natural water systems have been demonstrated to cause physiological abnormalities, which negatively effect populations of fish and amphibians.[37] Many of these agricultural pharmaceuticals are synthetic androgenic steroids that incorporate polyunsaturated functional groups and are photoactive. The androgenic steroid trenbolone acetate, TBA, is a synthetic compound with a trienone unit used as an anabolic growth promoter given to millions of beef cattle annually.[39] Steroidal analog 17β –TBOH **33** as well as other known metabolites, such as its diastereoisomer, 17α-TBOH, and analog, trendione (TBO), **34** and **35** respectively (Figure 1.4.2), are potent endocrine disruptors with concentrations as low as 10 ng/L can cause changes in sex ratios and reduce fecundity in fish.[38-39] TBA was found to leach from cattle's urine and manure and eventually reach into streams and ponds; however, there were no concerns for biological risk as TBA rapidly photodegrades.[40]

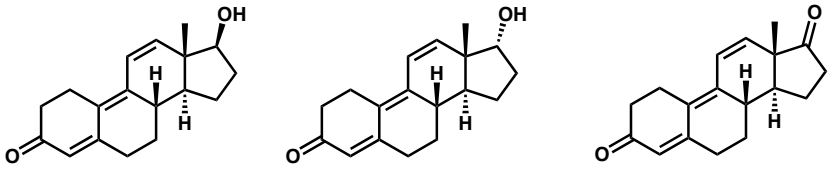
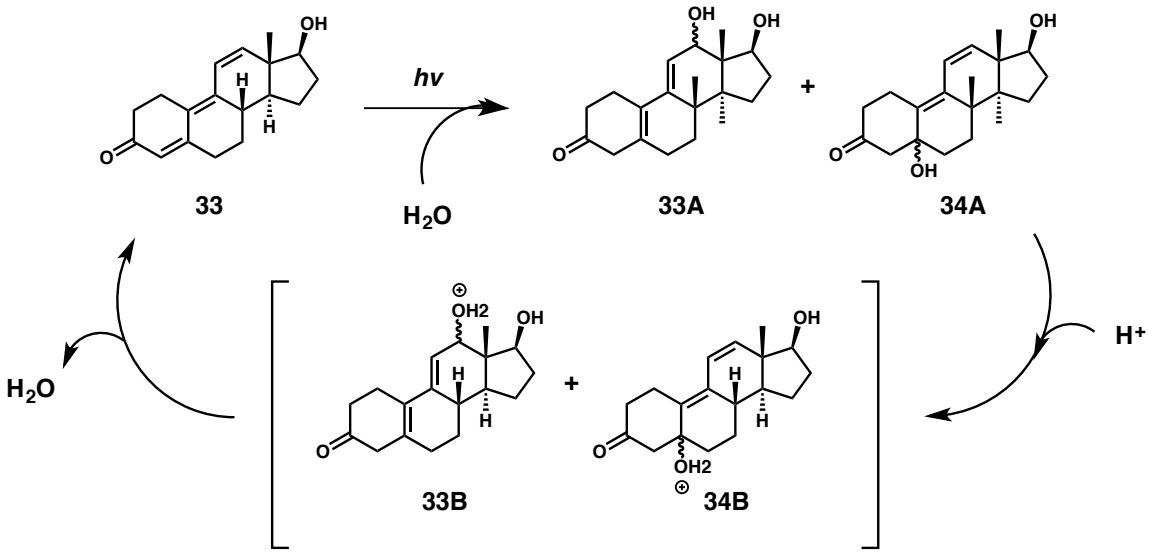


Figure 1.4.2 Semisynthetic compounds derived from naturally occurring steroids

In 2013, Qu and coworkers noticed that although TBA rapidly photodegrades, the risk was hidden as photohydrated products **33A** and **34A**, which in the absence of light under slight acidic conditions, lose water from protonated intermediates **33B** and **34B**, and can regenerate to the parent TBA compound **30** (Scheme 1.4.1) in the dark.[39] In

addition, estrogen-like endocrine disrupting chemicals (EEDC) are known to affect puberty in humans[40] and produce adverse effects on fish and wildlife in aquatic systems.[39, 42] Dienogest (DIE) is a potent, steroidal progestin used as an oral contraceptive in addition to a treatment for endometriosis, a chronic, estrogen-dependent disease that affects 10% of women of reproductive age.[43]



Scheme 1.4.1 Proposed acid-catalyzed reversible photohydration of TBA

DIE, structurally and synthetically similar to trenbolone acetate, has been found to photo-transform with evidence of reversion to its parent DIE structure in the dark.[39, 42] Therefore, it appears that semisynthetic steroids, containing similar moieties, may also exhibit similar behavior to TBA and require additional study of their aquatic photochemistry in order to fully understand their fate. The aquatic photochemistry of DIE will be discussed in chapter 4.

1.5 Research Interest

This dissertation is divided into four major sections, with additional chapters including an introduction, and the appendix. Chapter 2 describes the secondary metabolites from *P. kelleyi* and total syntheses. Chapter 3 introduces the use of photolysis in synthesis, which describes a unique and highly reactive *o*-quinone methide intermediate responsible for the effortless constructions of chromene and chromane cores. Chapter 4 discusses the utility of photolysis in the investigation of dienogest photo-transformation products as potential endocrine disruptors. And last, chapter 5 ends with concluding remarks and future directions to these projects.

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Chapter 2: Chromenes in *Piper Kelleyi*

2.1 Introduction

Chromenes have been isolated from a variety of organisms and across all orders of plants. These metabolites are commonly found to occur in plants within the Piperaceae family, including species within the two most diverse genera, *Piper* and *Peperomia*. In 2004, Kato and coworkers isolated a novel chromene, gaudichaudianic acid **35** from the leaves of *Piper gaudichaudianum*[1] (Figure 2.1.1) that was found to have antifungal properties. Additional chromenes have been isolated from *Peperomia serpent* Loudon[2] as described in chapter 1 (section 1.2). In 2014, an isomeric chromene derivative was isolated in the leaves of *P. kelleyi*.[4]

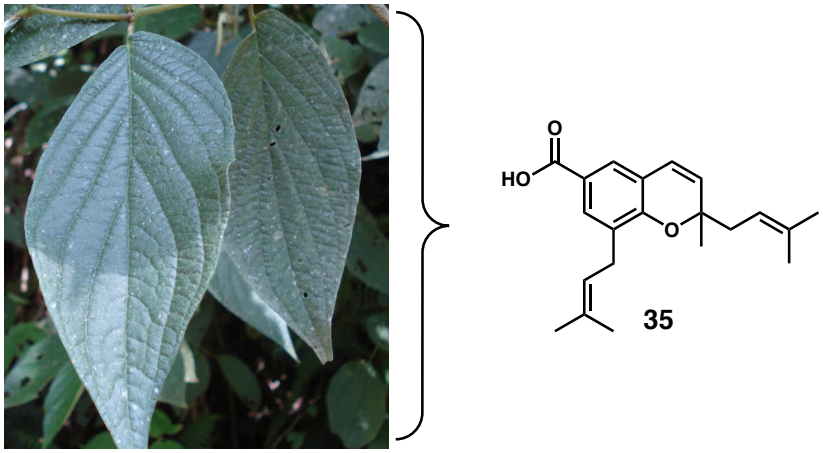


Figure 2.1.1 *Piper gaudichaudianum* in its natural habitat[5] and novel chromene metabolite **35**.

Piper kelleyi, is a newly described plant species found in the Eastern Andes of Ecuador and Peru at an altitudinal range between 1400-2400 meters.[3] It grows as shrub to the size of a small tree with broadly ovate adult green leaves (Figure 2.1.2, **A**) and is

characterized by its distinct pink undertone color on its young leaves giving its nickname, "*Piper* pink belly"[3,6] (Figure 2.1.2, **B**)

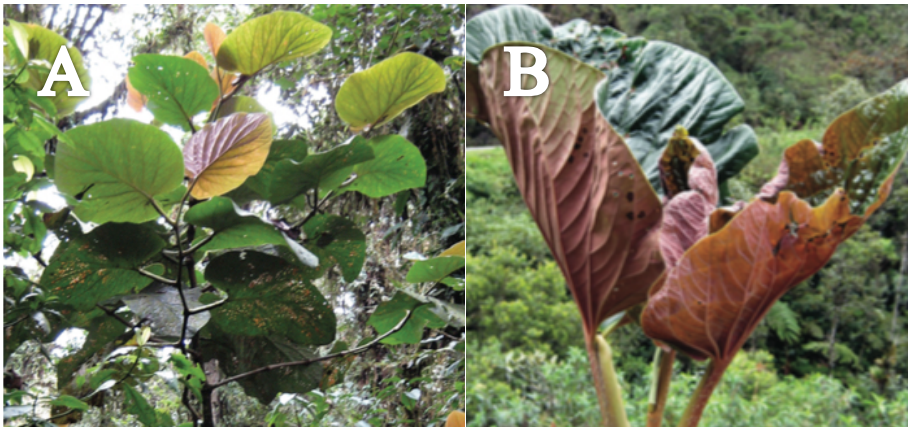


Figure 2.1.2 A *Piper kelleyi* in its habitat, **B** Close-up of leaves showing characteristic pink undertone color of young leaves

In 2014, the Jeffrey group isolated and characterized three prenylated benzoic acid (PBA) derivatives, a known PBA **36**, a chromene isomeric to gaudichaudianic acid **37**, and a chromane **38**[4] (Figure 2.1.3). From literature, the PBA **36** was found to be spectroscopically identical to 3-geranyl-4-hydroxy-5-(3" ,3" -dimethylallyl)benzoic acid

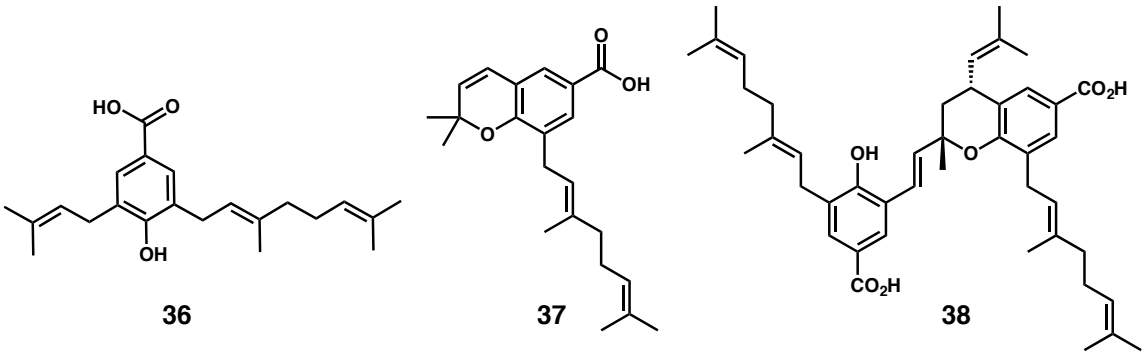


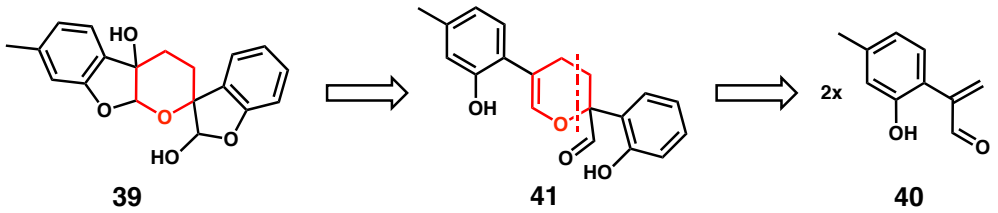
Figure 2.1.3 Secondary metabolites isolated from *P. Kelleyi*.

that has been previously isolated and characterized from species within Myrisine and Rapanea.[4,7-8] Novel chromene derivatives **37** and **38** were also isolated and structurally characterized via 1D and 2D NMR analysis.[4] Structure elucidation of **37** lead to the reassignment of cumanensic acid, which was isolated from *P. cumanense*,[9] and proposed to have the same chemical structure as **37**, but was found to be structurally identical to gaudichaudianic acid **35**. Recently, these secondary metabolites within *P. kelleyi* were found to dramatically vary in concentration between individuals growing across an elevational gradient of ~1,500m. This phytochemical variation was recently found to be associated with the genetic differentiation of the community of specialist herbivores, especially in high elevation with greater UV-B exposure.[9] Additionally, the crude plant extract and the individual compounds were found to have potent anti-herbivore properties and these negative effects were enhanced when the herbivores were reared in enhanced UV light. Given the known photochromic properties of chromenes, it is proposed that the chromene isolated from *P. kelley*i leaves is responsible for the phototoxicity of the crude extract through the photochromic ring opening to a highly reactive *o*-quinone methide intermediate. Additionally, it is proposed that PBA **36**, chromene **37**, and chromane **38** are biosynthetically linked and that the chromane is produced by a hetero-[4+2] cycloaddition reaction of the photogenerated quinone methide and its tautomer. We propose that this biosynthetic relationship and the role of light in the production of the chromane is strongly tied to the observed intraspecific variation of these metabolites, especially at high elevations with exposure to UV-B. In order to fully understand the relationship between UV light exposure of the plant and herbivores has on the observed phytochemical variation of the plant metabolites and the photoxicity of these metabolites,

we pursued a detailed study of the photochromic properties of naturally occurring chromenes, their reactivity, and a total synthesis of these metabolites.

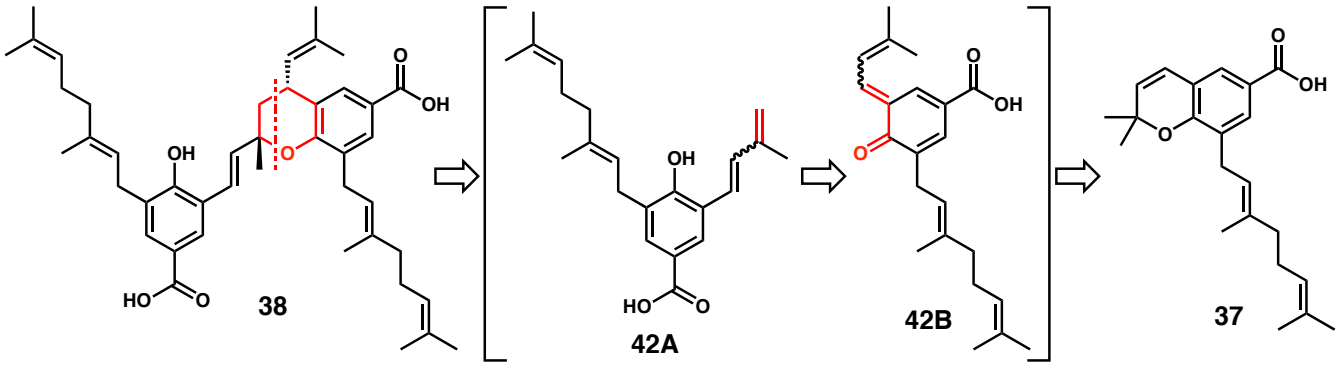
2.2 Retrosynthetic analyses of Natural Products from *Piper kelleyi*

The biosynthetic relationships between metabolites **36-38** were proposed upon careful retrosynthetic analysis of the chromene derivative **38** (Figure 2.1.3). The chromene derivative **38** was found to have the molecular formula $C_{44}H_{56}O_6$ by high-resolution mass spectrometry (HRMS), which is exactly double of what was found for the chromane derivative **37**.[4] This observation is indicative of a subsequent dimerization of **37** to afford **38**. Naturally occurring dimers are commonly isolated from natural sources and have demonstrated broad bioactivity.[10] Given the photochromic properties of chromenes, we propose that ring opening of the pyran lead to dimerization via [4+2]-hetero Diels-Alder (HDA) reaction. Though hetero Diels-Alder reaction of quinone methides has been established and are relevant to the biosynthesis of natural products, reactions of photochemically generated *o*-quinone methides have not been comprehensively explored as biomimetic entries to this reactive intermediate. Hetero-[4+2] Diels-Alder reactions have been established as common modes of dimerization within a variety of natural products classes. The biosynthesis of the dimeric thymol derivative **39** with a spiro[benzofuran-3-(2*H*)-2'-pyrano[2,3-*b*]benzofuran] is proposed to occur through a dimerization of the enal derivative **40** (Scheme 2.2.1).[10-11]

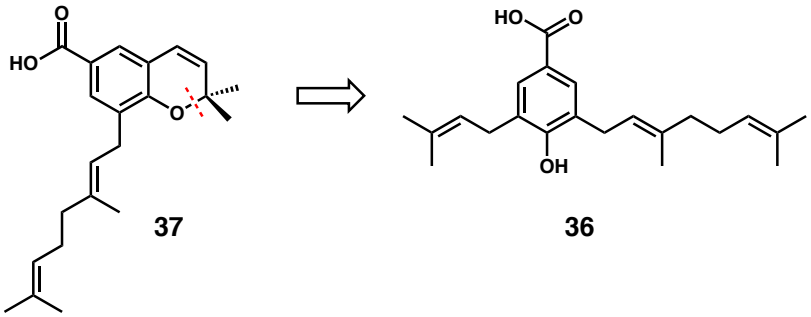


Scheme 2.2.1 Construction of key intermediate **41** in the biosynthesis of **39**.

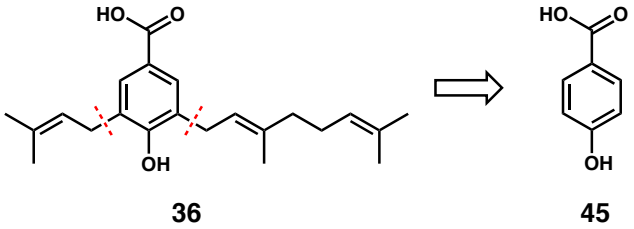
In this key step, the tetrahydropyran ring in **39** is formed via a [4+2]-HDA cycloaddition of the corresponding acrolein precursor **40**, which then spontaneously cyclizes to the acetal **39.**[11] We propose that the chromane **38**, is formed by dimerization of intermediate **42A** and **43B** via [4+2]-HDA cycloaddition (Scheme 2.2.2). Intermediates **42A** and **43B** are proposed to originate from an *o*-quinone methide that is formed from photo-induced ring opening of the chromene **37**. The chromene **37** is proposed to be derived from the PBA **36** via benzylic oxidation (Scheme 2.2.3). The PBA **36** is proposed to be accessed via aromatic prenyltransferase to install the isoprenoid side chains onto *p*-hydroxybenzoic acid **45** (Scheme 2.2.4). In order to explore the potential biosynthetic relationship between these metabolites, we first targeted the synthesis of the PBA **36**.



Scheme 2.2.2 Retrosynthetic analysis of chromene derivative **38.**



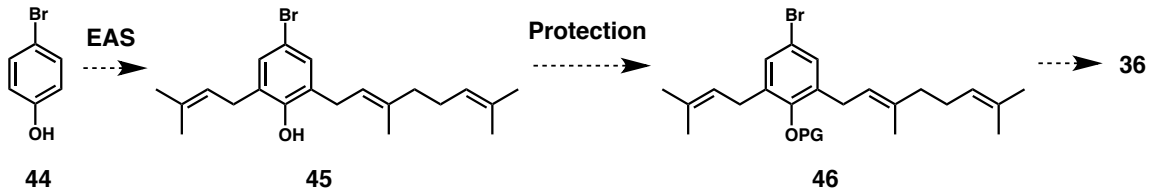
Scheme 2.2.3 Retrosynthetic analysis of chromene derivative **37.**



Scheme 2.2.4 Retrosynthetic analysis of chromene derivative **36.**

Our first approach was to prepare the chromene **37** by a biomimetic oxidation of a double prenylated phenol derivative. PBA **36** can be prepared via double C-alkylation of *p*-bromophenol **44** yielding the dialkylated precursor **45**, followed by protection of the phenolic OH **46** (e.g. PG = TMS or TBS) and functional group interconversion (FGI) of the aryl bromide to the desired carboxylate functionality found in **36** (Scheme 2.2.5). The carboxylation step can either be accomplished using palladium-catalyzed carbonylation or via metal-halogen exchange and quenching with an appropriate carbonyl carboxylate donor (e.g. CO_2, Ethyl chloroformate, or *N*,*N*-dimethylformamide). If *N*,*N*-dimethylformamide (DMF) is used to install the carbonyl functionality, the reaction

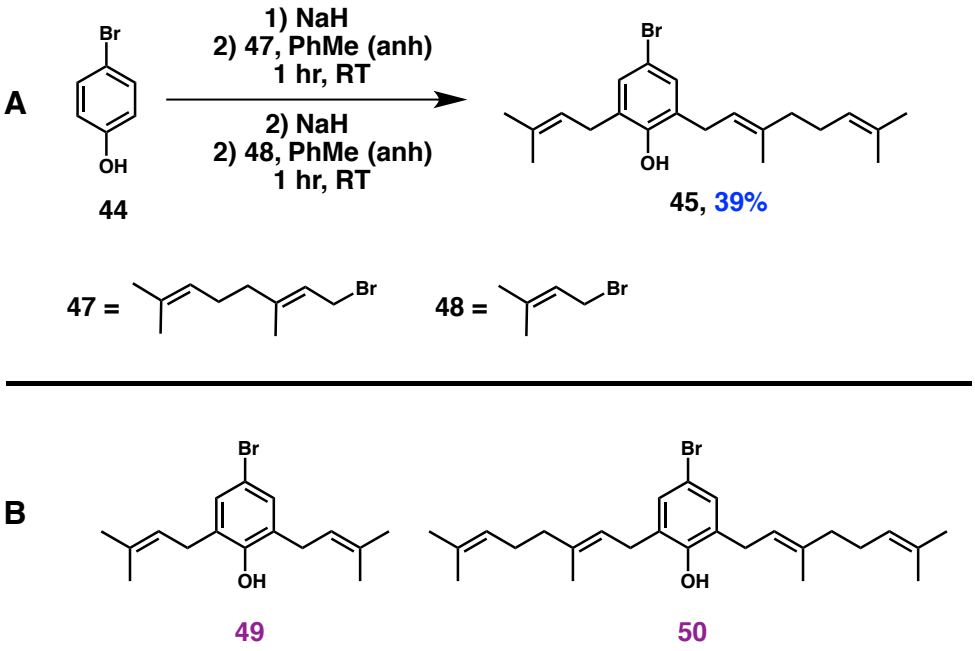
would require an additional oxidation from the aldehyde functionality to the desired carboxylic acid.



Scheme 2.2.5 Proposed biomimetic approach to achieve metabolite **36**

2.3 Total syntheses of Natural Products from *Piper kelleyi*

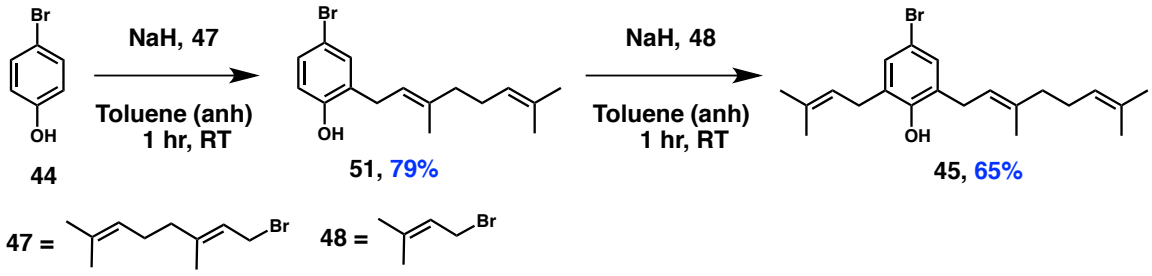
Our investigation began with synthesizing PBA **36** from a simple *p*-bromophenol building block **44** (Scheme 2.3.1, Panel **A**). Uto and coworker reported a one-pot synthesis of a related compound, which installs both the prenyl and geranyl side chain units. However, this method suffered from poor selectivity in our hands.[12] As expected for the reaction, the di-alkylation step lacked similar selectivity issues (Scheme 2.3.1, Panel **B**). Double alkylation of the *p*-bromophenol was achieved by dissolving **44** in anhydrous toluene with sodium hydride, followed by the addition of the appropriate alkyl halide, geranyl bromide **47**[13a] or prenyl bromide **48**[13b], which was repeated twice in one pot, to provide the desired mono-prenyl mono-geranylated *p*-bromophenol precursor **45**.



Scheme 2.3.1 Synthesis of di-alkylated *p*-bromophenol **45** precursor via double alkylation with sodium hydride and prenyl **47** or geranyl bromide **48** in a one-pot synthesis.

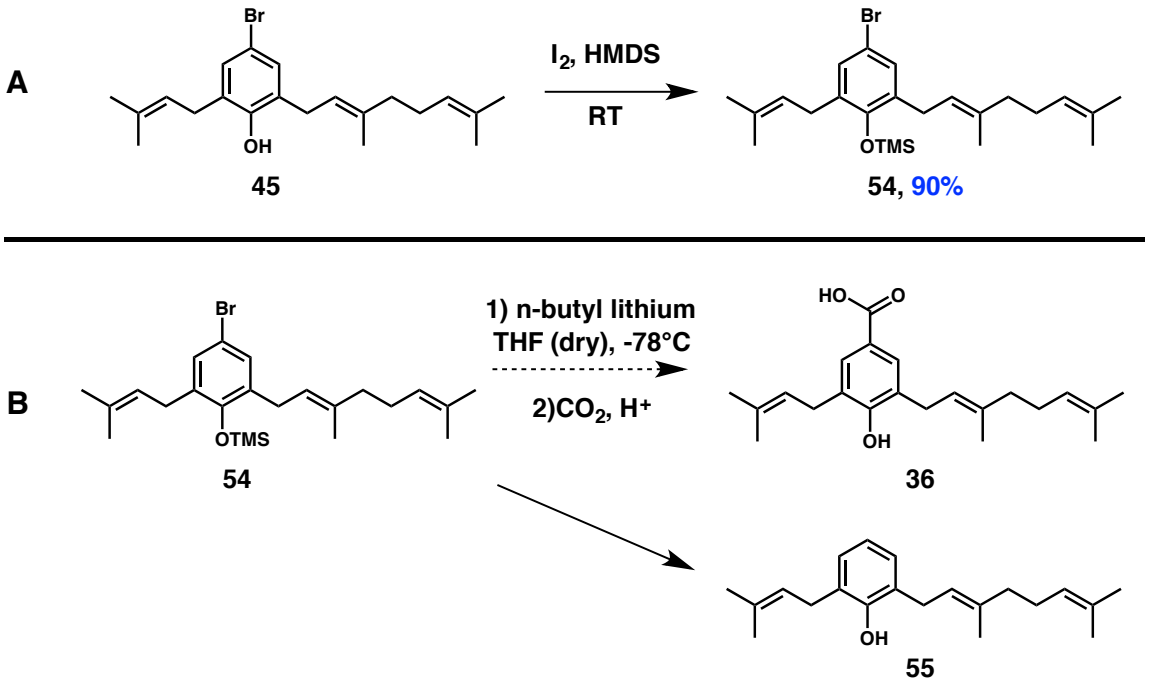
Although, the alkylating agents were added step-wise, both di-prenylated and di-geranylated *p*-bromophenol **49** and **50** respectively, were observed in addition to the desired product. Observation of the diprenylated product **49** lead us to the conclusion that there was either unreactive starting material prior to step 2 due to the consumption of geranyl bromide **47** via digeranylation **50** or insufficient amounts of sodium hydride were added in step 1 leading to leftover unreactive starting material. To test the latter, an excess of sodium hydride was added to step 1, but unfortunately similar results as described above were observed. To resolve this issue we expanded the one-pot reaction and performed the alkylation using a two-pot process. First, mono-geranylation of the *p*-bromophenol **44** providing the desired product **51** at moderate to high yields (70-79%)

followed by mono-prenylation of **51** to provide the desired dialkylated precursor **45** (Scheme 2.3.2).



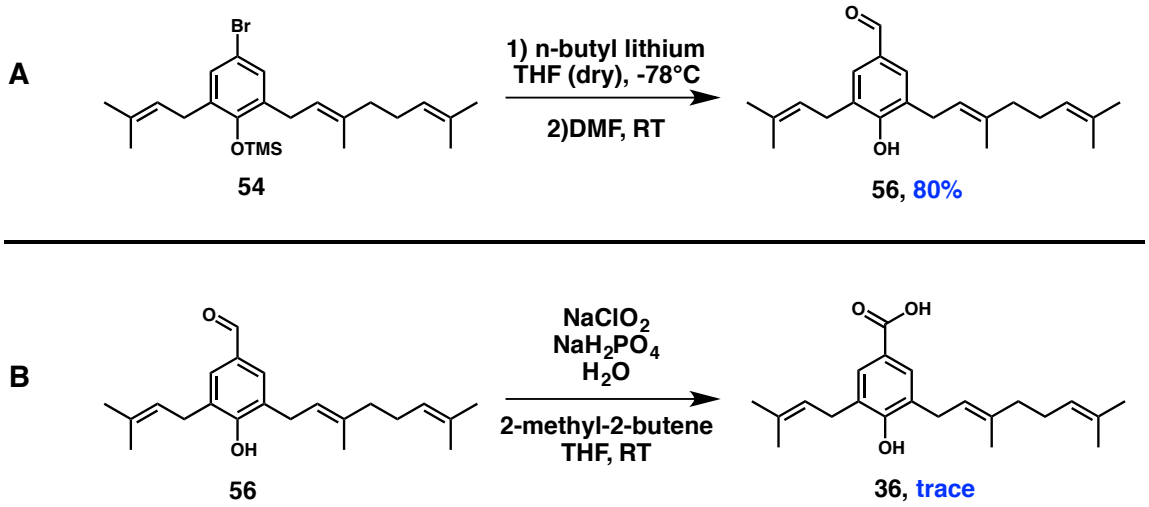
Scheme 2.3.2 Synthesis of di-alkylated *p*-bromophenol **45** precursor via double alkylation with sodium hydride and geranyl **47** or prenyl bromide **48** in a two-step fashion.

A functional group interconversion of the aryl bromide to the benzoic acid was required in order to complete this synthesis. Several synthetic routes were considered in the installment of the acid functionality from the aryl bromide. The most common method for FGI of the aryl halide involves a metal-halogen exchange utilizing *n*-butyllithium and quenching the reaction with dry ice. First, protection of the phenolic alcohol in **45** was achieved under solvent-free reaction conditions (SFRC) utilizing 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and elemental iodine to provide the TMS protected phenol ether **54** (Scheme 2.3.3, Panel **A**).[15] All attempts to install the acid functionality via metal-halogen exchange of the aryl bromide **54** with dry ice lead to dehalogenation and subsequent silyl deprotection of **54** to produce **55** as determined by ^{1}H-NMR spectroscopy (Scheme 2.3.3, Panel **B**).



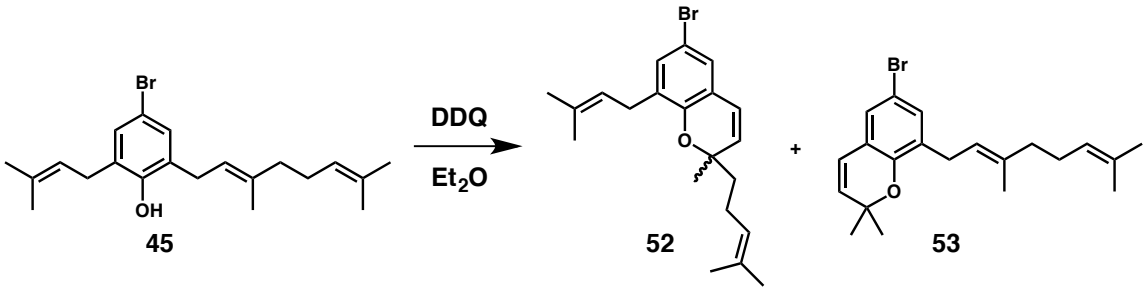
Scheme 2.3.3 TMS-protection of the phenolic alcohol of **45**, followed by installation of the carbonyl functionality.

An alternative route for installation of the carbonyl group was achieved in a two-step process where the bromoarene **54** was transformed to the aldehyde **56** by metal-halogen exchange followed by the addition of DMF (Scheme 2.3.4, Pane; **A**). Upon completion, the new-formed aldehyde was oxidized via Pinnick oxidation using $NaClO_2$[16] (Scheme 2.4.2, Panel **B**). Unfortunately, this method provided only dismal yields of the desired carboxylic acid, presumably due to the hypochlorous acid (HClO) is generated during the reaction, which would react with the tri-substituted alkenes of the geranyl and prenyl side chains, even in the presence of 2-methylbutene as a HClO scavenger. Further efforts will be directed toward developing a more scalable synthesis using a Pd-catalyzed carbonylation of the aryl bromide as an alternative method to the two-step oxidative approach.



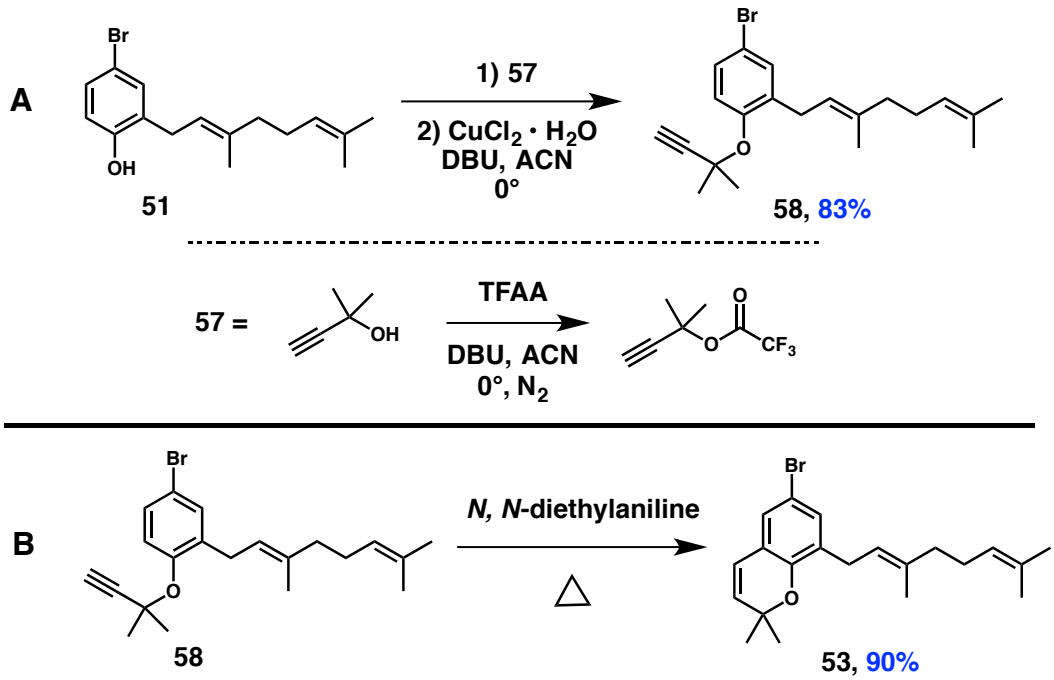
Scheme 2.3.4 Functional group interconversion of **54** via metal-halogen exchange with DMF, followed by oxidation via Pinnick oxidation.

Compound **45** was subjected to an oxidative cyclization reaction utilizing 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) as the oxidant (Scheme 2.3.5), as described by Cardillo and coworkers.[14] Unfortunately, the di-alkylated *p*-bromophenol **45** in the presence of DDQ lacked regioselectivity and oxidative cyclization occurred on either the geranyl side providing chromene **52** or the prenyl side providing chromene **53**. Although both regioisomers were formed, this oxidative cyclization reaction provides access to the desired chromene core in metabolite **37**, as well as a synthetic precursor that could be used to prepare gaudichaudianic acid **35**, an isomeric chromene isolated from *P. gaudichaudianum*.[1] Unfortunately, isolation and separation of the isomeric chromenes proved to be difficult, so our efforts were turned toward developing an alternative strategy to prepared the desired chromene **37**.



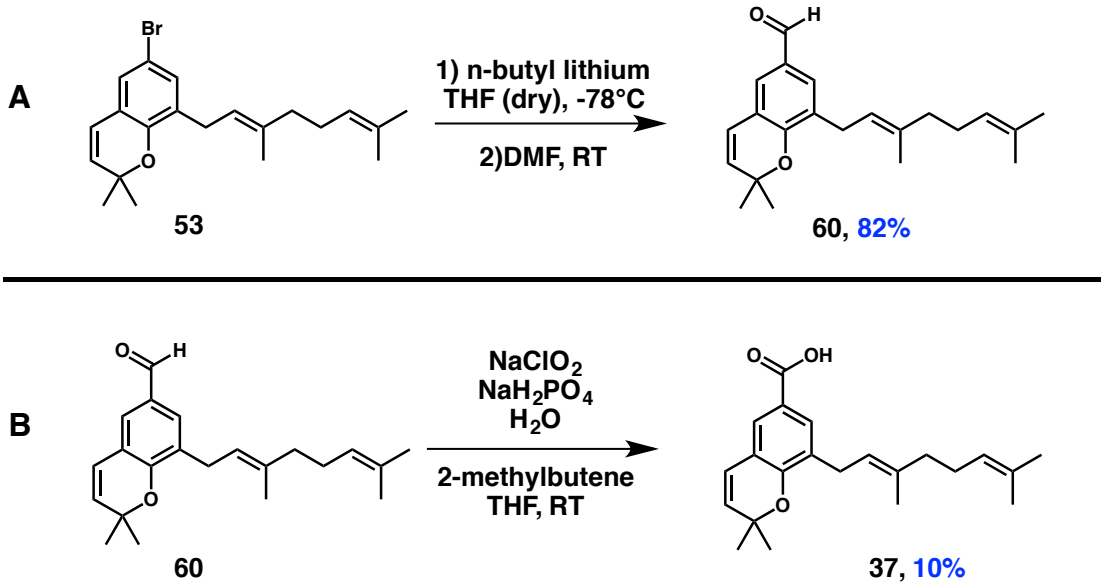
Scheme 2.3.5 Oxidative cyclization of **45** reaction utilizing DDQ.

In 1963, Iwai and coworkers observed thermal rearrangement in aryl propargyl ethers to provide chromenes.[17] As an alternative to the oxidative approach, we propose to adopt Iwai's approach, generating the chromene from the propargyl ether. *O*-alkylation of the phenol **51** using 2-methylbut-3-yn-2-yl 2,2,2-trifluoroacetate **57** would provide the propargyl ether[18] **58** (Scheme 2.3.6, Panel **A**), which is expected to produce the bromo-derived chromene **53** upon thermal rearrangement (Scheme 2.3.6, Panel **B**).[19a] To our delight, we found that this approach provided efficient access to the desired bromo-chromene **53** in good yield, which then could serve as a precursor to the desired chromene metabolite.



Scheme 2.3.6 Installation of a propargyl group via *O*-alkylation of **51**, followed by a subsequent thermal rearrangement.

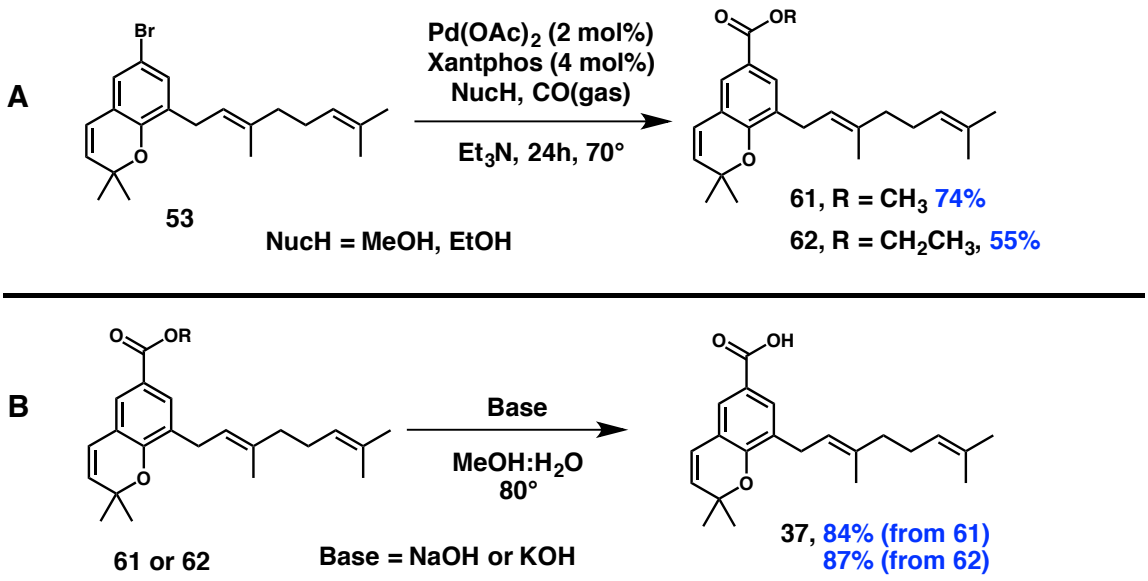
After successful cyclization of the propargyl intermediate **58** to the bromo-derived chromene **53**, the next step was to functionalize the halide to the desired acid chromene **37**. Two methods were employed similar to those attempted for the synthesis of metabolite **36** (Scheme 2.3.4). First, we attempted to directly install the carboxylic acid functionality through a metal-halogen exchange with *n*-butyllithium and quenching with CO_2 (dry ice). Unfortunately, this approach produced a dehalogenated chromene product as determined by ^{1}H NMR spectroscopy, presumably through protonation of the aryl lithium intermediate. In an alternative two-step approach, the bromide was successfully converted to the carboxylic acid via metal-halogen exchange and quenching with DMF to afford the corresponding aldehyde chromene **60** (Scheme 2.3.7 Panel **A**). Subsequent Pinnick oxidation provided the chromene **37** at 10% yield (Scheme 2.3.7 Panel **B**).



Scheme 2.3.7 Functional group interconversion via metal-halogen exchange with DMF, followed by oxidation via Pinnick oxidation.

The low yields of this process led us to consider an alternative method for installing the carboxylic acid directly from the aryl bromide by a palladium-catalyzed carbonylation reaction.[20] Buchwald and coworkers developed a general method for the palladium-catalyzed carbonylation of aryl bromides utilizing Xantphos as the ligand, which provides access to Weinreb amides, 1° and 2° benzamides and methyl esters at atmospheric pressure. Xantphos is a bidentate phosphine ligand, which has been used extensively for Pd-catalyzed C—N bond forming processes, contains a wide bite angle and flexibility range are believed to improve catalytic activity and stability in palladium and other transition-metal-catalyzed processes.[20] Although, the role of the Xantphos ligand remains unclear, an excess of the Xantphos ligand increases the stability of the catalyst system. Synthesis of methyl ester via Pd-catalyzed alkoxycarbonylating is achieved with an aryl halide in the presence of carbon monoxide gas (CO), a palladium catalyst, Xantphos as

the ligand (1:2, M:L ratio) in an excess of methanol to synthesize a variety of methyl ester derivatives. Utilizing the same approach as described by Buchwald and coworkers, bromo-derived chromene **53** was directly functionalized to the methyl or ethyl esters via palladium-catalyzed carbonylation using either methanol (MeOH) or ethanol (EtOH), respectively (Scheme 2.3.8, Panel **A**). These ester chromenes were hydrolyzed under basic conditions to successfully provide the chromene **37** (Scheme 2.3.8, Panel **B**). This represents a completed total synthesis of the chromene and provides access to material to study its conversion to the chromane dimer **38**, which will be discussed in Chapter 3.



Scheme 2.3.8 Palladium-catalyzed carbonylation in MeOH or EtOH of **53**, followed by hydrolysis to provide the desired acid functionality

2.4 Discussion

The three benzoic acid (PBA) derivatives can all be accessed through aryl bromide precursors **45**, **54**, **56** and **53**, which were prepared using modified literature

procedures.[12-13, 15-16] These PBA precursors are all prepared in good yields from inexpensive, commercially available starting materials. Attempts were made to directly oxidize the prenyl side chain to the chromene using DDQ.[14] This oxidation reaction provided our desired chromene precursor **52** and a regioisomeric product **53** due to the equal rates of oxidation at either the prenyl or geranyl side chains, respectively. This observation suggests that PBA compounds are biosynthetically linked to chromene through a selective enzymatic oxidation process and that gauchaudianic acid and the isomeric chromene from P. Kelleyi could be derived from a common PBA precursor.

The PBA could be prepared by installation of an aldehyde functionality from lithium halogen exchange and quenching with DMF. However, oxidation of the aldehyde **56** via Pinnick oxidation, only provided dismal yields of the desired product. Attempts to improve the yield were unsuccessful, possible due to the generation of hypochlorous acid , which is generated under the Pinnick conditions and reacts rapidly with electron-rich double bonds. Therefore, methods for functionalizing the aryl bromide directly via palladium-catalyzed carbonylation in the PBA precursor **45** is currently being studied as an alternative route for installing the carbonyl functionality.[20]

Thermal rearrangement of the propargyl aryl ether **58** provides direct access to the desired chromene as shown in **53** as the sole product. Smith and coworkers observed similar observations when reporting the thermal rearrangement of propynyloxy benzoate compound to give a chromene.[13, 19] To functionalize the aryl halide in precursor **53** to an acid functionality, two methods were employed. The first method utilized *n*-butyllithium in a halogen-metal exchange and quenched the resulting aryl lithium intermediate with DMF to provide an in high yield. Unfortunately, oxidation to the acid functionality via

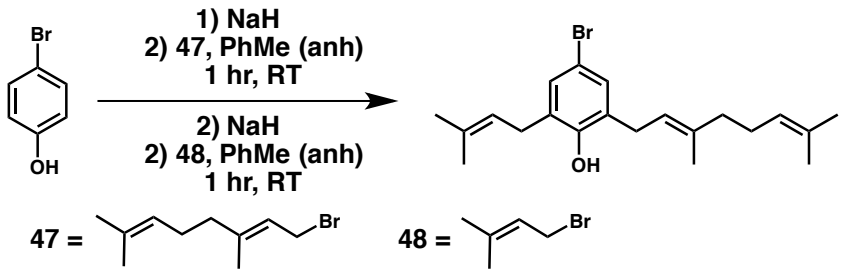
Pinnick oxidation only provided a 10 % isolated yield of desired product, which is likely due to the decomposition of the tri-substituted alkenes within the product or starting material. As a second approach, we chose functionalized the aryl bromide directly via palladium-catalyzed carbonylation in methanol or ethanol to provide the benzoate esters **61** and **62** respectively, at high yields. Saponification of the acid derivative **61** and **62**, provided the desired PBA chromene **37** in 5 synthetic steps with 37 % overall yield from **61** and 28 % overall yield from **62**. The second method for installing the acid derivative functionality provides an alternative route for synthesized metabolite **37** in moderate yield.

2.5 Experimental Procedures

Reactions were carried out under inert atmosphere of nitrogen (N_2) gas in clean, oven-dried glassware (Pyrex) with magnetic stirring, unless otherwise specified. All reagents and solvents were purchased from Sigma-Aldrich Chemical Company and used without further purifications.[21] TLC was recorded and performed using Silicycle glass 60 F254 plates[22] or on Sorbtech alumina N TLC plates, w/UV254, polyester backed (200 μm)[23] and visualized observed using UV light (254 nm) or developed by staining with $KMnO_4$ or CAM. Each reaction was purified using flash chromatography with Silicycle Siliaflash® P60 (230-400 mesh)[24] or basic alumina.[23] ^{1}H-NMR spectra were measured on Varian 400 (400 MHz)[25a] or Varian 500 (500 MHz) spectrometers[25a] and are reported in ppm (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; integration)[25b]; coupling constant(s) in Hz; using *d*-$CDCl_3$ (7.26 ppm, with 0.01% TMS at 0.00 ppm) or in d_4-MeOD (3.31 ppm) or d_3-ACN (1.94 ppm) as locking solvents. ^{13}C-

NMR spectra were measured on Varian 400 (101 MHz)[25a] or Varian 500 (126 MHz)[25a] spectrometers and are reported in ppm using *d*-$CDCl_3$ (77.16 ppm, with 0.01% TMS at 0.00 ppm) or in d_4-MeOD (49.00 ppm) or d_3-ACN (118.26 and 1.32 ppm) as locking solvents. Infrared (IR) spectra were recorded on a Nicolet 6700 FT-IR with a diamond ATR[25a] and the bands reported in cm^{-1} (br = broad, st = strong).[25b] High-resolution mass spectra were obtained using an Agilent 6230 TOF LC/MS with an atmospheric pressure photo-ionization (APPI, with C60 and anthracene internal standards) or electrospray (ESI, with purine and HP-0921 internal standards).[25a]

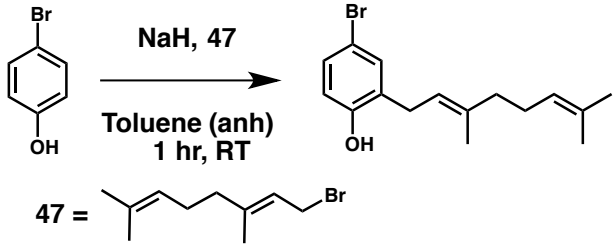
4-bromo 2-(3-methyl-2-buten-1-yl)-6-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]phenol (45, from *p*-bromophenol):



p-Bromophenol (2.00 g, 11.6 mmol) was dissolved in dry toluene (40 mL, 0.3M), and sodium hydride (NaH, 60% dispersion in mineral oil,[21] 0.50 g, 13 mmol) was added in portions. After five minutes of stirring, geranyl bromide (2.64 g, 12.7 mmol) was added, and the mixture was stirred at room temperature. After 1 hour, an additional portion of NaH was added slowly (60% dispersion in mineral oil,[21] 0.50 g, 13 mmol). After five minutes of stirring, prenyl bromide (1.88 g, 12.7 mmol) was added and the mixture was stirred at RT for 1 hr. The reaction was diluted with DI-water (50 mL) acidified with 2M acetic acid (pH 4-5) and the toluene layer was separated. The aqueous

layer was extracted with diethyl ether (2x50 mL). The combined diethyl ether layer was washed with saturated $NaHCO_3$, followed by saturated NaCl, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure.[25A] The crude mixture was purified with flash column chromatography (9:1 hexanes:ethyl acetate), and 1.72 g of a light yellow oil was isolated (39%). R_f was calculated = 0.67, using 9:1 hexanes:ethyl acetate; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.10 (s, 2H), 5.35 (s, 1H), 5.26-5.31 (m, 2H), 5.07-5.11 (m, 1H), 3.32 (t, *J* = 6.9 Hz, 4H), 2.17-2.06 (m, 4H), 1.79 (d, *J* = 1.5 Hz, 3H), 1.76 (d, *J* = 1.3 Hz, 6H), 1.71 (d, *J* = 1.4 Hz, 3H), 1.62 (d, *J* = 1.3 Hz, 3H);[25b] ^{13}C NMR (101 MHz, chloroform-*d*) δ 151.83, 138.84, 135.04, 134.81, 131.96, 130.19, 129.50, 129.17, 123.75, 121.24, 121.06, 112.32, 39.65, 29.41, 29.19, 26.32, 25.78, 25.70, 17.83, 17.70, 16.15;[25b] IR (neat): 3449.08 (br), 3024.80, 2974.14, 2910.82, 2850.66, 1666.49, 1448.02, 1368.87, 1191.56;[25b] HR-MS (ESI) calcd' $C_{16}H_{21}BrO$ $(M+H)^-$ 308.07758, observed 308.07624[25]

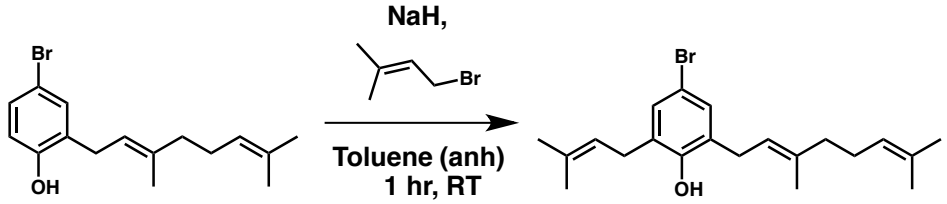
4-bromo 2-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]phenol (51):



p-Bromophenol (4.00 g, 23.1 mmol) was dissolved in dry toluene (80 mL, 0.3M), and NaH (60% dispersion in mineral oil,[21] 1.00 g, 25.0 mmol) was slowly added. After five minutes of stirring, geranyl bromide (13.8 g, 63.6 mmol) was added and the mixture was stirred at room temperature for 1.5 hours. The reaction was carefully diluted with DI-water (50 mL) and acidified with 2 M acetic acid (pH 4-5). The toluene layer was

separated. The aqueous layer was partitioned with diethyl ether (2x100 mL). The combined organic layer was washed with saturated $NaHCO_3$, followed by saturated NaCl, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure.[25A] The crude oil was purified via flash column chromatography (9:1 hexanes:ethyl acetate) and 5.64 g of product was isolated (79.1%) as a pale yellow oil. Yields varied (65-79%) due to competitive di-geranylation. R_f = 0.25 (9:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.25 – 7.16 (m, 2H), 6.68 (dd, *J* = 8.0, 0.7 Hz, 1H), 5.27 (tq, *J* = 7.2, 1.3 Hz, 1H), 5.10 – 5.03 (m, 2H), 3.32 (d, *J* = 7.2, 1H), 2.16 – 2.06 (m, 4H), 1.75 (d, *J* = 1.2 Hz, 1H), 1.69 (d, *J* = 1.1, 1H), 1.60 (d, *J* = 0.55, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): *δ* 153.55, 139.39, 132.45, 132.12, 130.18, 129.14, 123.66, 120.69, 117.54, 112.69, 39.64, 29.56, 26.33, 25.70, 17.72, 16.22; FT-IR (neat): 3439 (br), 2967, 2913, 1667, 1582, 1480, 1435, 1261, 1205, 1163, 1104; HR-MS (ESI) calcd' $C_{16}H_{21}BrO$ $(M+H)^-$ 308.07758, observed 308.07624

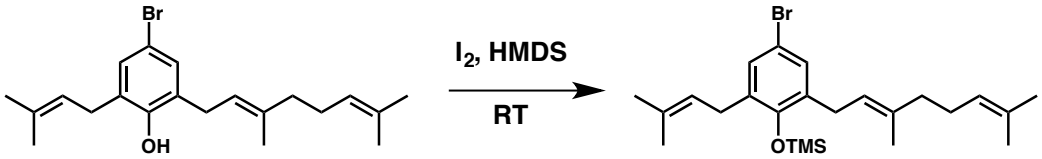
4-bromo-2-(3-methyl-2-buten-1-yl)-6-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]phenol (45, from 51):



Compound **51** (1.90 g, 2.91 mmol) was dissolved in dry toluene (30 mL, 0.1 M), and NaH (60% dispersion in mineral oil, 0.128 g, 3.20 mmol) was added slowly. After five minutes of stirring, prenyl bromide (0.46 g, 0.35 mL, 12.7 mmol) was added and the mixture was stirred at room temperature for an hour. The reaction was diluted with DI-

water (50 mL) acidified with 2M acetic acid (pH 4-5), and the toluene layer was separated. The aqueous layer was partitioned with diethyl ether (2x25 mL). The combined organic layer was then washed with saturated $NaHCO_3$, followed by saturated NaCl, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure.[25a] The crude oil was purified via flash column chromatography (9:1 hexanes:ethyl acetate), and 1.45 g of product was isolated (63%) as a light yellow oil. R_f = 0.67 (9:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.10 (s, 2H), 5.35 (s, 1H), 5.26-5.31 (m, 2H), 5.07-5.11 (m, 1H), 3.32 (t, *J* = 6.9 Hz, 4H), 2.17-2.06 (m, 4H), 1.79 (d, *J* = 1.5 Hz, 3H), 1.76 (d, *J* = 1.3 Hz, 6H), 1.71 (d, *J* = 1.4 Hz, 3H), 1.62 (d, *J* = 1.3 Hz, 3H); ^{13}C NMR (101 MHz, chloroform-*d*) δ 151.83, 138.84, 135.04, 134.81, 131.96, 130.19, 129.50, 129.17, 123.75, 121.24, 121.06, 112.32, 39.65, 29.41, 29.19, 26.32, 25.78, 25.70, 17.83, 17.70, 16.15; IR (neat): 3438.85 (br), 3040.10, 2966.53, 2912.61, 2845.20, 1480.05, 1409.25, 1261.47, 1205.42, 1163.10, 1103.92; HR-MS (APPI) calcd' for $C_{16}H_{21}BrO$ $(M+H)^+$ 308.07758, observed 308.07675.

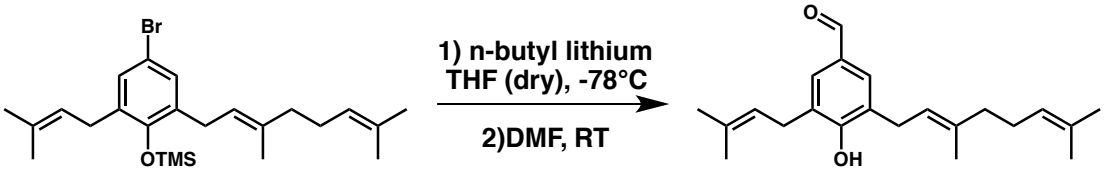
5-bromo-1-(3-methyl-2-buten-1-yl)-3-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]-2-[(trimethylsilyl)oxy]-benzene (54):



Iodine (5.6 mg, 0.022 mmol) was added to **51** (0.35 g, 0.78 mmol), followed by the addition of HMDS (0.081 g, 0.11 mL, 0.50 mmol). The mixture was stirred at room temperature until the complete consumption of the starting material (TLC check). The

crude reaction was dissolved in hexanes, and sodium thiosulfate was added until the disappearance of iodine. The solids were filtered through a plug of celite and concentrated under reduce pressure without further purification provided 0.388 g (90%) of a clear oil. R_f = 0.81 (95:5 hexanes:ethyl acetate, alumina plate); ^{1}H NMR (500 MHz, chloroform-*d*) 7.07 (dd, *J* = 1.5, 0.6 Hz, 2H), 5.26 (dddt, *J* = 7.2, 5.7, 2.8, 1.4 Hz, 2H), 5.13 (dddd, *J* = 6.9, 5.5, 2.9, 1.4 Hz, 1H), 3.27 - 3.23 (m, 4H), 2.17 - 2.06 (m, 4H), 1.78 (s, 3H), 1.71 (d, *J* = 1.3 Hz, 3H), 1.68 (d, *J* = 1.2 Hz, 3H), 1.63 (d, *J* = 1.0 Hz, 3H), 0.27 (d, *J* = 0.5 Hz, 9H); ^{13}C NMR (126 MHz, cdcl$_3$) δ 150.62, 150.61, 137.23, 134.34, 134.34, 134.32, 133.65, 133.64, 129.63, 124.11, 121.64, 114.24, 39.65, 28.89, 28.76, 26.53, 25.74, 17.85, 17.72, 16.17, 0.90; IR (neat): 3031.13, 2967.81, 2913.98, 2850.66, 1587.34, 1479.68, 1441.69, 1372.03, 1251.72; HR-MS (ESI) calcd' $C_{24}H_{37}BrOSi$ $(M+H)^+$ 448.18176, observed 448.17971.

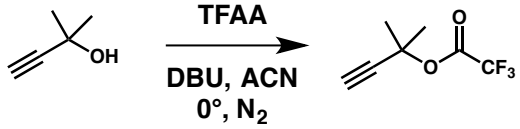
4-hydroxy-3-(3-methyl-2-buten-1-yl)-5-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]-benzaldehyde (56):



Compound **54** (0.50 g, 1.1 mmol) was dissolved in THF (3.5 mL, 0.3M) and cooled to -78°. A solution *n*-BuLi (0.75 mL of a 1.6 molar hexane solution, 1.2 mmol) was added drop-wise, and the reaction was stirred for 40 minutes. Dimethylformamide (0.42 mL, 5.5 mmol) was added and the reaction mixture was stirred for an additional 2 hours at room temperature. The reaction mixture was quenched with 1 M HCl (10 mL),

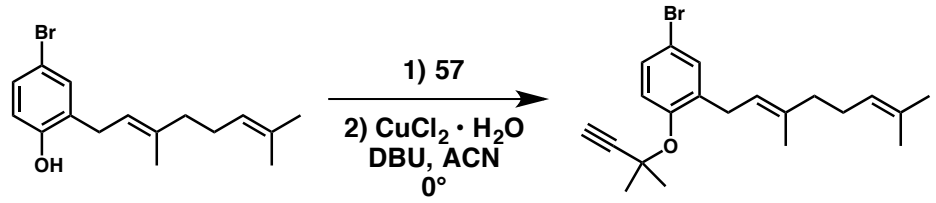
extracted with dichloromethane (3x5mL), washed with saturated $NaHCO_3$ and saturated NaCl and dried over anhydrous Na_2SO_4. The solvent was removed giving the product as a light yellow oil. R_f = 0.19 (95:5 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, chloroform-*d*) δ 9.83 (s, 1H), 7.55 (d, *J* = 3.2 Hz, 2H), 6.08 (s, 1H), 5.33 (ddt, *J* = 7.4, 3.0, 1.5 Hz, 2H), 5.09 (tdd, *J* = 5.4, 3.0, 1.4 Hz, 1H), 3.41 (dd, *J* = 10.9, 7.2 Hz, 4H), 2.16 – 2.10 (m, 4H), 1.80 (s, 3H), 1.79 – 1.78 (m, 6H), 1.70 (s, 3H), 1.62 (s, 3H); ^{13}C NMR (126 MHz, cdcl$_3$) δ 191.33, 158.65, 139.38, 135.22, 132.02, 130.15, 129.98, 129.50, 128.02, 127.54, 123.71, 121.03, 120.86, 39.68, 29.54, 29.22, 26.34, 25.82, 25.69, 17.89, 17.71, 16.23; HR-MS (ESI) calcd' $C_{22}H_{30}O_2$ $(M+H)^-$ 326.22447, observed 326.22458.

(2-methylbut-3-yn-2-yl)-2,2,2-trifluroacetate (57):



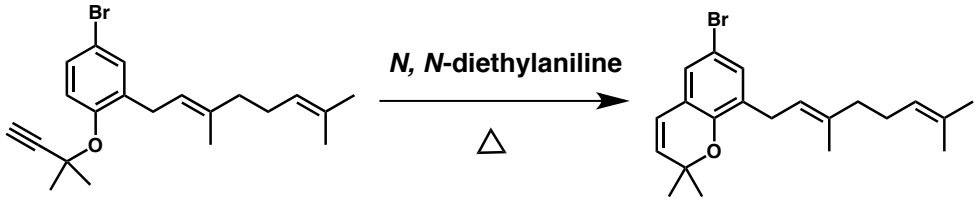
DBU (2.1 mL, 14 mmol) was added to a solution of 2-methyl-3-butyn-2-ol (1.04 mL, 11 mmol) in anhydrous CH_3CN (7 mL, 2M) and cooled in an ice-salt bath (0° C). Trifluoroacetic anhydride (1.5 mL, 10.6 mmol) was added drop-wise while keeping the temperature at 0°C for 30 minutes. The resulting solution was kept at 0°C and added to reaction **58**.

1-(1,1-dimethyl-2-propynyloxy)-2-[(2*E*)-3,7-dimethyl-2,6-octadienyl]-4-bromobenzene (58):



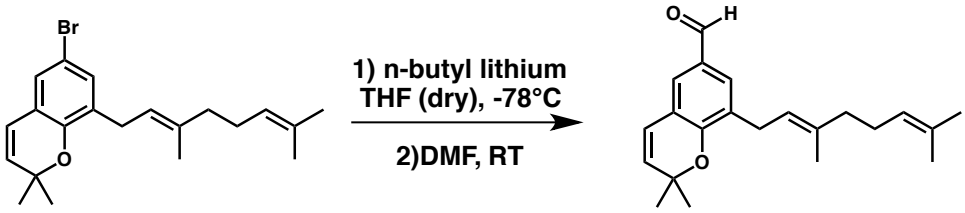
DBU (1.8 mL, 12 mmol) and $CuCl_2 \cdot 2H_2O$ (1.5 mg, 0.009 mmol) was added to a solution of **51** (2.9 g, 9.4 mmol) dissolved in anhydrous CH_3CN (30 mL, 0.3M) and cooled in an ice-salt bath under nitrogen. Solution **56** was then added drop-wise via cannulation addition. After stirring for 2 hours at 0°C, the mixture was concentrated at reduced pressure.[25a] The crude mixture dissolved toluene (100 mL). The organic layer was washed with 1 M HCl (3x30mL), aqueous $NaHCO_3$ (2x30mL) and saturated aqueous brine (2x30 mL) and dried with anhydrous Na_2SO_4. The solvent was removed at reduced pressure[25a] to give the desired product (87%) as a yellow oil. R_f = 0.55 (95:5 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (d, J = 8.6 Hz, 1H), 7.24 (d, J = 2.5 Hz, 1H), 7.22 (dd, J = 8.6, 2.5 Hz, 1H), 5.24 (tq, J = 7.2, 1.3 Hz, 1H), 5.11 (m, 1H), 3.29 (d, J = 7.2 Hz, 2H), 2.57 (s, 1H), 2.15 – 2.01 (m, 4H), 1.70 (d, J = 1.3 Hz, 3H), 1.69 (d, J = 1.3 Hz, J = 1.2 Hz, 3H), 1.66 (s, 6H), 1.60 (d, J = 0.87 Hz 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 152.76, 136.75, 136.11, 132.26, 131.54, 128.90, 124.14, 121.82, 120.38, 114.89, 86.04, 73.93, 72.34, 39.69, 29.63, 28.50, 26.57, 25.73, 17.72, 16.17; IR (neat): 3293.29, 2985, 2914, 2112, 1652, 1588, 1479, 1450, 1381, 1239, 1136, 1111; HR-MS (ESI) calcd' for $C_{21}H_{27}BrO$ $(M+H)^+$ 374.1245, observed 374.1243

6-bromo-2,2-dimethyl-8-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]-2*H*-1-benzopyran (53):



Compound **57** (3.01 g, 8.01 mmol) was dissolved in *N*, *N*-diethylaniline (10 mL) and was magnetically stirred to reflux for two hours. Upon completion, the reaction mixture was diluted with Et_2O (50 mL), washed (with caution; highly exothermic) with 6 M aqueous HCl (4x20 mL), followed by saturated $NaHCO_3$ (2x20 mL) and saturated NaCl (2x20 mL), and dried over anhydrous Na_2SO_4. The solvent was removed giving a crude amber oil that was flushed through a plug of silica yielding a light yellow oil (2.70 g, 89.7 %). R_f = 0.67 (95:5 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.08 (d, J = 2.4 Hz, 1H), 6.96 (d, J = 2.4 Hz, 1H), 6.25 (d, J = 9.8 Hz, 1H), 5.64 (d, J = 9.8 Hz, 1H), 5.27 (tq, J = 7.4, 6.1, 1.3 Hz, 1H), 5.13 (tq, J = 7.0, 5.5, 2.9, 1.4 Hz, 1H), 3.26 (d, J = 7.4 Hz, 2H), 2.21 – 1.96 (m, 4H), 1.72 (d, J = 0.8 Hz, 3H), 1.71 (d, J = 0.7, 3H), 1.62 (d, J = 0.7 Hz, 3H), 1.43 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ149.55, 136.39, 131.61, 131.57, 131.50, 131.47, 126.42, 124.21, 122.72, 122.45, 121.69, 112.31, 76.31, 39.74, 27.87, 27.78, 26.59, 25.73, 17.70, 16.17; IR (neat): 2967.14, 2924.25, 2853.98, 1640.61, 1571.39, 1479.70, 1444.92, 1382.40, 1360.84, 1255.59, 1203.03, 1164.35, 1123.22; HR-MS (APPI) calcd' for $C_{21}H_{27}BrO$ $(M+H)^+$ 374.12453, observed 374.12519

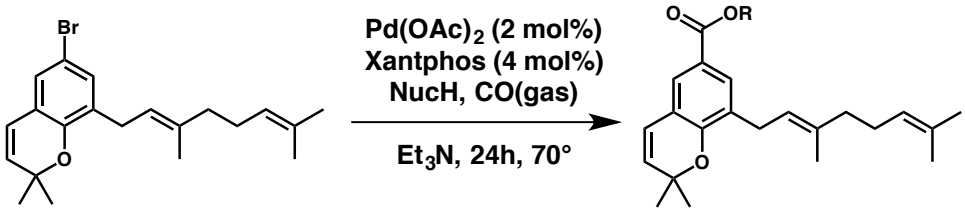
2,2-dimethyl-8-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]-2*H*-1-benzopyran-6-benzaldehyde (60):



Compound **53** (0.38 g, 1.0 mmol) was dissolved in THF (3.4 mL, 0.3M) and cooled to -78°. A solution *n*-BuLi (0.63 mL of a 1.6 molar hexane solution, 1.2 mmol) was added drop-wise and the reaction was stirred for 40 minutes. Anhydrous DMF (0.39 mL, 5.1 mmol) was added, and the reaction mixture was stirred for an additional 2 hours at room temperature. The reaction mixture was quenched with 1 M HCl (10 mL), extracted with dichloromethane (3x5mL), washed with saturated $NaHCO_3$ and saturated NaCl and dried over anhydrous Na_2SO_4. The solvent was removed giving the product as a light yellow oil (0.27 g, 82%). R_f = 0.38 (9:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, chloroform-*d*) δ 9.81 (s, 1H), 7.53 (d, *J* = 1.9 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 6.38 (d, *J* = 9.9 Hz, 1H), 5.69 (d, *J* = 9.9 Hz, 1H), 5.30 (tdd, *J* = 6.0, 2.6, 1.4 Hz, 1H), 5.11 (tdt, *J* = 5.7, 2.9, 1.4 Hz, 1H), 3.33 (d, *J* = 7.6 Hz, 3H), 2.14 – 2.03 (m, 4H), 1.74 (dd, *J* = 1.5, 0.8 Hz, 3H), 1.68 (d, *J* = 1.3 Hz, 3H), 1.60 (d, *J* = 1.3 Hz, 3H), 1.48 (s, 6H); ^{13}C NMR (126 MHz, chloroform-*d*) δ 191.06, 156.34, 136.61, 131.64, 131.47, 131.04, 129.90, 129.42, 125.95, 124.17, 121.78, 121.48, 120.74, 39.74, 32.04, 28.47, 27.97, 26.61, 25.68, 17.68, 16.22; IR (neat): 3045.02, 2970.02, 2916.02, 2859.02, 2808.02 & 2724.02 (Fermi doublet), 1689.05 (st), 1638.05, 1590.05, 1434.06, 1371.06, 1278.06,

1212.06, 1131.07 (st); HR-MS (APPI) calcd' for $C_{22}H_{28}O_2$ $(M+H)^+$ 324.20893, observed 324.20812.

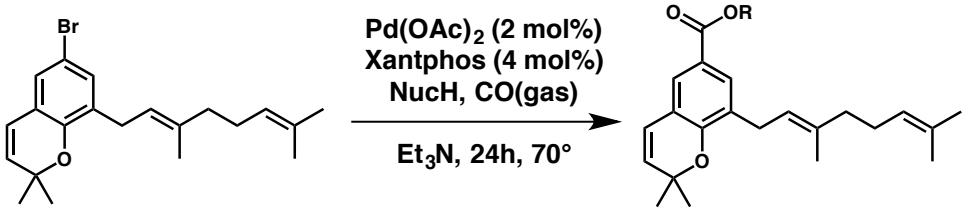
6-methoxycarbonyl-2,2-dimethyl-8-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]-2*H*-1-Benzopyran (61, R = CH_3):



Compound **53** (1.0 g, 2.7 mmol) was dissolved and triethylamine (5.3 mL, 0.5M) and methanol (1.1 mL, 27 mmol) was added. The solution was purged under nitrogen and then added to palladium(II)acetate (0.010 g, 0.050 mmol) and xantphos (0.060 g, 0.10 mmol) via syringe. The reaction mixture was purged with carbon monoxide (CO) for 1 minute and left under an atmosphere of CO for 24 hours at 70 °C. The solution was filtered through a plug of celite, concentrated at reduced pressure[25a] and the resulting crude mixture was purified via column chromatography (95:5 hexanes:ethyl acetate) providing a light yellow oil (0.580 g, 74% BRSM). R_f = 0.27 (95:5 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.70 (d, *J* = 2.2 Hz, 1H), 7.56 (d, *J* = 2.2 Hz, 1H), 6.35 (d, *J* = 9.8 Hz, 1H), 5.64 (d, *J* = 9.8 Hz, 1H), 5.30 (tq, *J* = 7.4, 1.3 Hz, 1H), 5.12 (ddt, *J* = 8.4, 5.6, 1.5 Hz, 1H), 3.87 (s, 3H), 3.30 (d, *J* = 7.3 Hz, 2H), 2.13 – 1.98 (m, 4H), 1.74 (d, *J* = 1.3 Hz, 3H), 1.67 (q, *J* = 1.2 Hz, 3H), 1.60 (d, *J* = 1.3 Hz, 3H), 1.45 (s, 6H); ^{13}C NMR (126 MHz, chloroform-*d*) δ 167.08, 154.81, 136.06, 131.36, 131.08, 130.66, 129.20, 125.97, 124.26, 122.10, 121.95, 120.33, 109.73, 77.18, 51.75, 39.77,

28.29, 28.28, 28.14, 26.68, 25.65, 17.66, 16.23; IR (neat): 3040.20, 2972.06, 2924.16, 2850.93, 1715.83 (st), 1602.80, 1433.31, 1310.90, 1195.77 (st), 1163.39; HR-MS (ESI) calcd' for $C_{23}H_{30}O_3$ $(M+H)^+$ 354.21950, observed 354.21890.

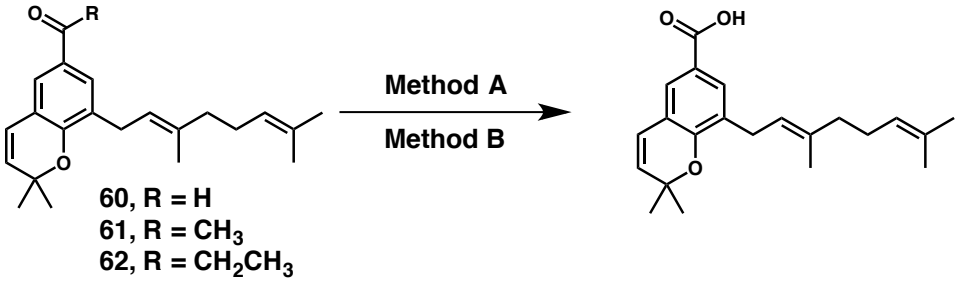
6-ethoxycarbonyl-2,2-dimethyl-8-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]-2*H*-1-Benzopyran (62, R = CH_2CH_3):



Compound **53** (1.0 g, 2.7 mmol) was dissolved and triethylamine (5.3 mL, 0.5M) and ethanol (1.6 mL, 27 mmol) was added. The solution was purged under nitrogen and then added to palladium(II)acetate (0.010 g, 0.050 mmol) and xantphos (0.060 g, 0.10 mmol) via syringe. The reaction mixture was purged with carbon monoxide (CO) for 1 minute and left under an atmosphere of CO for 24 hours at 70 °C. The solution was filtered through a plug of celite, concentrated at reduced pressure[25a] and the resulting crude mixture was purified via column chromatography (95:5 hexanes:ethyl acetate) providing a light yellow oil (0.580 g, 74% BRSM). R_f = 0.0.37 (95:5 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.70 (d, *J* = 2.0 Hz, 1H), 7.55 (d, *J* = 2.1 Hz, 1H), 6.39 – 6.30 (m, 1H), 5.64 (d, *J* = 9.8 Hz, 1H), 5.34 – 5.22 (m, 1H), 5.15 – 5.05 (m, 1H), 4.33 (1, *J* = 7.2 Hz, 2H), 3.30 (d, *J* = 7.3 Hz, 2H), 2.06 (m, 4H), 1.74 (s, 3H), 1.67 (s, 3H), 1.60 (s, 3H), 1.45 (s, 6H), 1.38 (t, *J* = 7.2, 3H); ^{13}C NMR (126 MHz, chloroform-*d*) δ 166.61, 154.73, 136.02, 131.36, 131.07, 130.64, 129.15, 125.90, 124.27,

122.26, 122.15, 122.01, 120.31, 77.13, 60.50, 39.78, 28.26, 28.17, 26.73, 25.65, 17.65, 16.24, 14.41. IR (neat): 3037.47, 2974.14, 2913.98, 2847.49, 1713.98 (st), 1600.00, 1375.20, 1302.37, 1194.72; HR-MS (ESI) calcd' $C_{24}H_{32}O_3$ $(M+H)^+$ 368.23514, observed 368.23600

2,2-dimethyl-8-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]-2*H*-1-benzopyran-6-benzoic acid (37 from 60, 61 and 62):



Method A, from 60: Compound **60** (0.33 g, 1.0 mmol) was dissolved in a mixture of THF/H_2O (0.1 M, 5:1 v:v) and 2-methyl-2-butene (0.27 mL, 2.5 mmol), Sodium dihydrogen phosphate (0.28 g, 2.3 mmol), and sodium chlorite (0.22 g, 2.4 mmol) were added. The reaction was stirred for 4 hours at room temperature. Upon completion the reaction was acidified with 1 M HCl (pH 4-5) and extracted with DCM (3x10 mL). The solution was concentrated at reduced pressure,[25a] dried under sodium sulfate anhydrous and the resulting solution was purified via column chromatography (1:1 hexanes:ethyl acetate) providing a light yellow oil (0.035 g, 10 %).

Method B, from 61: Compound **61** (0.095 g, 0.27 mmol) was dissolved in methanol/DI-water mixture (0.15 M, 1:1 v:v) and NaOH (0.107 g, 2.7 mmol) was added. The solution was stirred for 1 hour at 80 °C, upon completion the solution was acidified

with 1M HCl (pH 4-5) and extracted with ethyl acetate (3x10 mL). The organic layer was dried under sodium sulfate anhydrous and concentrated at reduced pressure[25a] providing a light yellow liquid (0.076 g, 84 %) without further purification.

Method B, from 62: Compound **62** (0.025 g, 0.068 mmol) was dissolved in methanol/DI-water mixture (0.15 M, 1:1 v:v) and NaOH (0.030 g, 0.68 mmol) was added. The solution was stirred for 1 hour at 80 °C, upon completion the solution was acidified with 1M HCl (pH 4-5) and extracted with ethyl acetate (3x5 mL). The organic layer was dried with anhydrous Na_2SO_4 and concentrated at reduced pressure[25a] providing a light yellow liquid (0.020 g, 87 %) without further purification. R_f was calculated to be 0.15 using 4:1 hexanes:ethyl acetate; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.64 (d, J = 2.2 Hz, 1H), 7.51 (d, J = 2.1 Hz, 1H), 6.36 (d, J = 9.9 Hz, 1H), 5.70 (d, J = 9.9 Hz, 1H), 5.25 (ddq, J = 7.4, 6.1, 1.3 Hz, 1H), 5.06 (tdt, J = 7.1, 2.9, 1.5 Hz, 1H), 3.28 – 3.23 (m, 2H), 2.04 – 2.11 (m, 2H), 2.04 – 1.97 (m, 2H), 1.74 – 1.69 (m, 3H), 1.62 – 1.59 (m, 3H), 1.57 – 1.52 (m, 3H), 1.41 (d, J = 1.4 Hz, 6H); ^{13}C NMR (101 MHz, cd_3od) δ 168.52, 154.59, 135.61, 130.82, 130.78, 130.61, 128.84, 125.79, 123.83, 122.13, 121.95, 121.57, 120.38, 77.02, 39.40, 27.53, 27.13, 26.22, 24.42, 16.31, 14.92; IR (neat): 3043.80, 2970.98, 2907.75, 2850.66, 2568.87 (br) 1679.16 (st), 1637.99, 1603.33, 1384.70, 134670, 1201.06, 1112.40; HR-MS (ESI) calcd' $C_{22}H_{28}O_3$ $(M+H)^-$ 340.20384, observed 340.20329

2.6 References

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22. Purchased from VWR International - Chemicals and Laboratory Scientific (https://en.wikipedia.org/wiki/VWR_International)

23. Purchased from Sorbent Technologies, Inc. (https://www.sorbtech.com)

24. Purchased from SiliaFlash – Silicycle (http://www.silicycle.com/products/siliaflash-irregular-silica-gels)

25. a) Instrumentation and use provided by the department of chemistry, UNR. b) Data and values are tabulated from the University instrumentation.

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Chapter 3: Utilizing Photolysis in a Biomimetic Approach in Synthesis

3.1 Introduction

Photochemistry is a specialized branch in chemistry that studies chemical processes that are caused by the absorption of light energy by organic molecules.[1-2] A photochemical reaction is initiated by infrared, visible, or ultraviolet light.[2] Most photochemical reactions can occur in three stages[3-4]:

1) Absorption of light to produce electronically excited states.
2) Primary photochemical reactions involving excited electronic states.
3) Secondary reactions of products of the primary photochemical reaction to provide stable products.

Photochemical reactions are frequently used in organic synthesis to produce a variety of organic molecules.[2-4] In a photochemical process, molecule **A** must absorb energy of photon, which gives rise to an excited state **A***. The excited molecule can then undergo a number of primary photochemical processes (Figure 3.1.1)

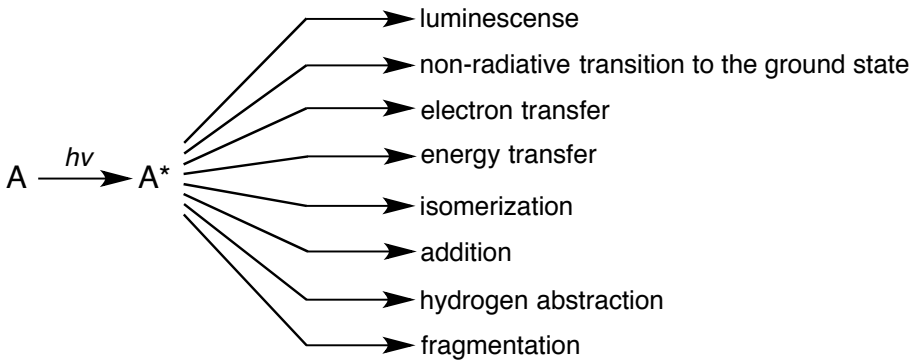
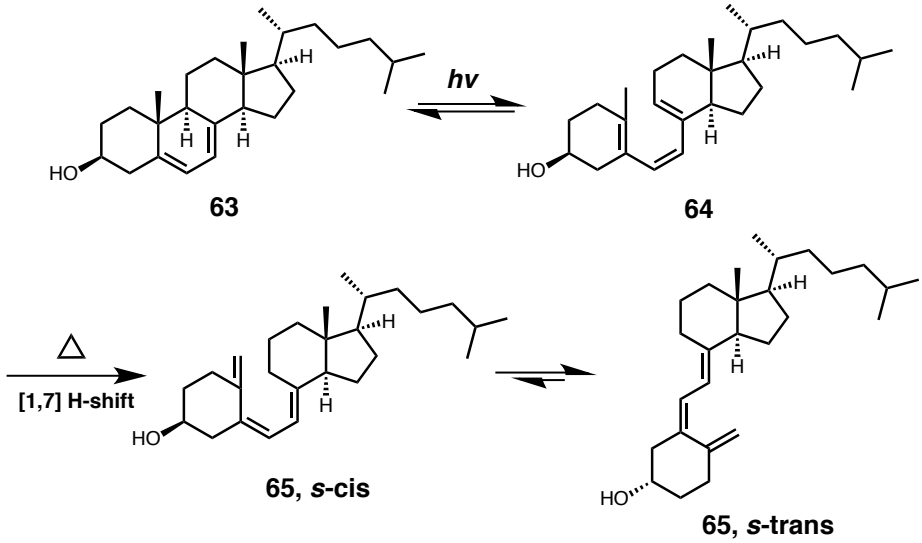


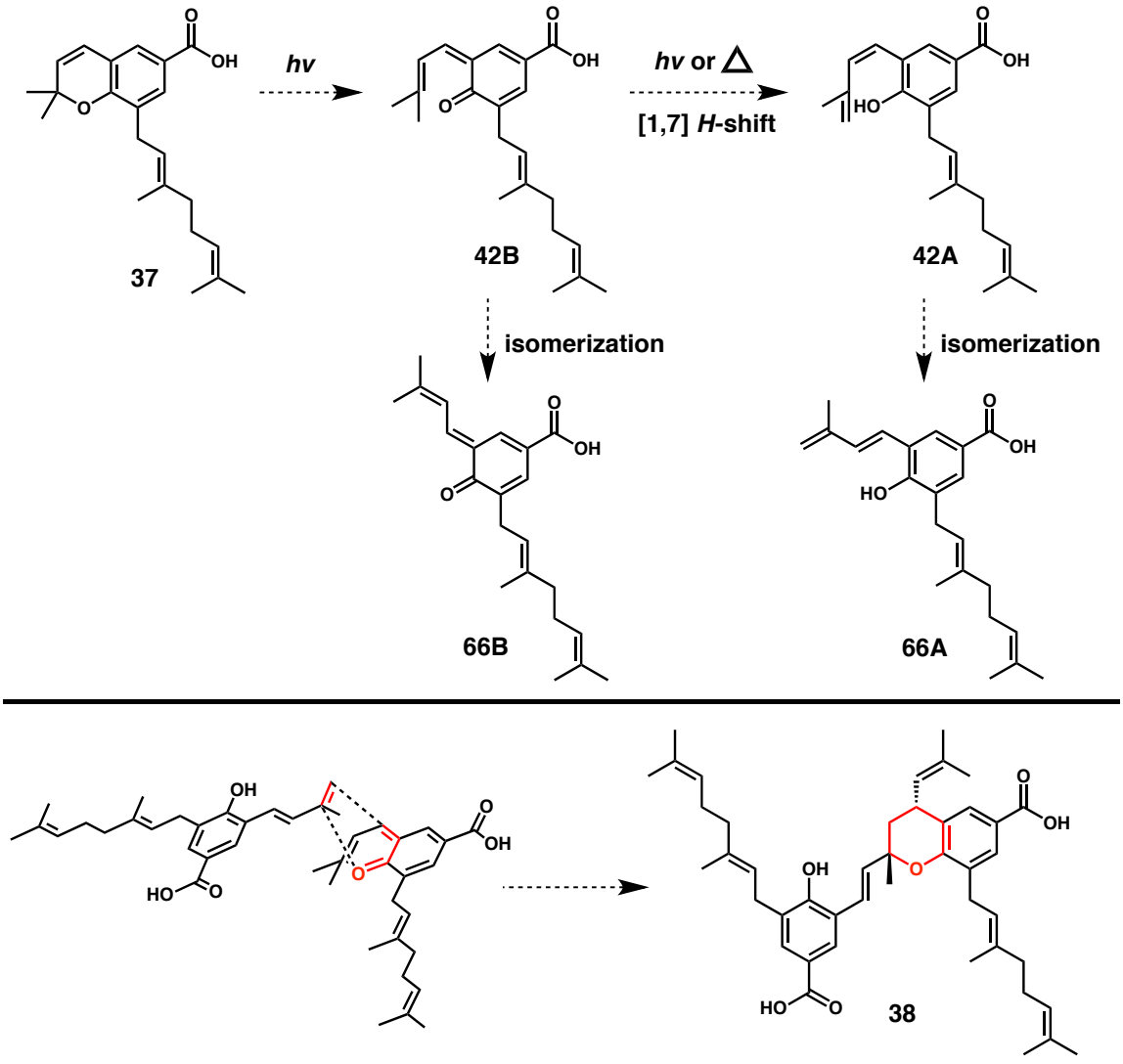
Figure 3.1.1 Excited state reaction pathways.

These primary photochemical processes are not only synthetically important and have important synthetic industrial applications. For example, the key step of the synthesis of vitamin D is initiated by a photochemical electrocyclic ring opening. Vitamin D is group of fat-soluble seco-steroids that is responsible for enhancing intestinal absorption and regulation of minerals found in the body.[6] Vitamin D_3 is one of five forms of vitamin D that our body naturally produces from 7-dehydrocholesterol found in our skin when exposed to UVB radiation (280-315 nm) from the sun.[5-6] The mass production of synthetic vitamin D_3 uses a similar photochemical process. This synthesis begins with irradiation of 7-dehydrocholesterol **63**, a vitamin D_3 precursor, which initiates ring opening of the B ring in the steroid core producing the previtamin D_3 **64** (Scheme 3.1.1). Upon gentle heating, **64** isomerizes to vitamin D_3 **65** as a result of a sigmatropic [1,7] hydrogen shift, which occurs thermally at body temperature.[4-6] Isomerization to vitamin D_3 can also occur photochemically, however this does not occur photochemically *in vivo*.[5]



Scheme 3.1.1 The photochemical conversion of 7-dehydrocholesterol to vitamin D_3.

A variety of photo-induced isomerization have been reported for organic molecules. We envisioned that both key intermediates **43A** and **43B** to the proposed hetero-Diels-Alder dimerization can be arrived at through photo-induced isomerization of a chromene. We propose the photolysis of the chromene will generate the o-quinomethide **43B**, which upon sigmatrophic [1,7]-hydrogen shift or tautomerization will produce **43A** (Scheme 3.1.2). These two intermediates can undergo a hetero [4+2] cycloaddition to provide the dimeric chromane **38.**

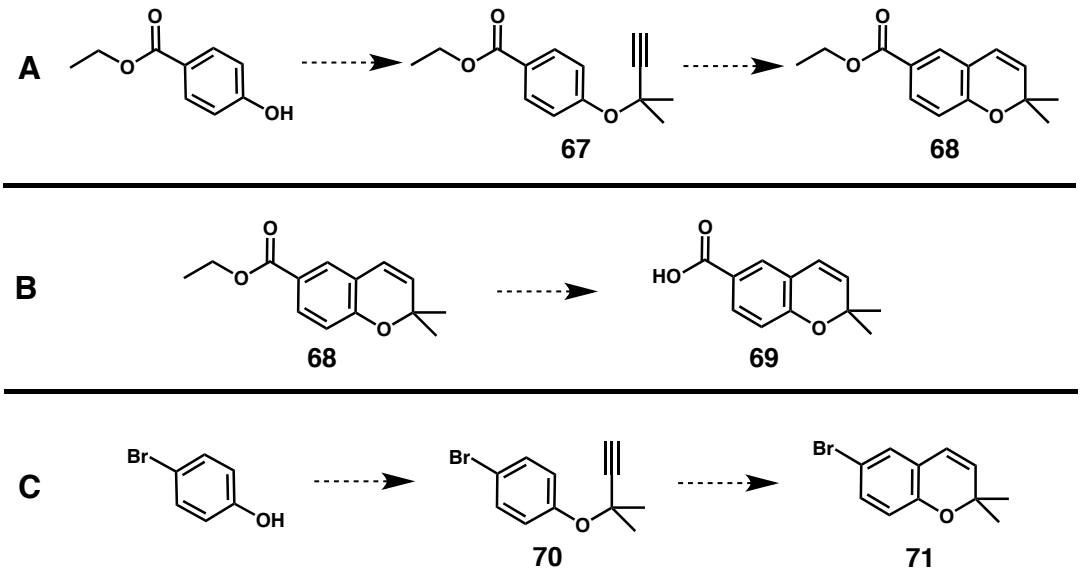


Scheme 3.1.2 The photochemical ring opening of PBA chromene **37**.

Our goal is to understand the photoisomerization of the chromene and its relevance to the biosynthesis of the chromane. This goal first requires a fundamental understanding of the photochromic behavior of naturally occurring chromenes, their isomerization, and their reactivity.

3.2 Chromene model systems

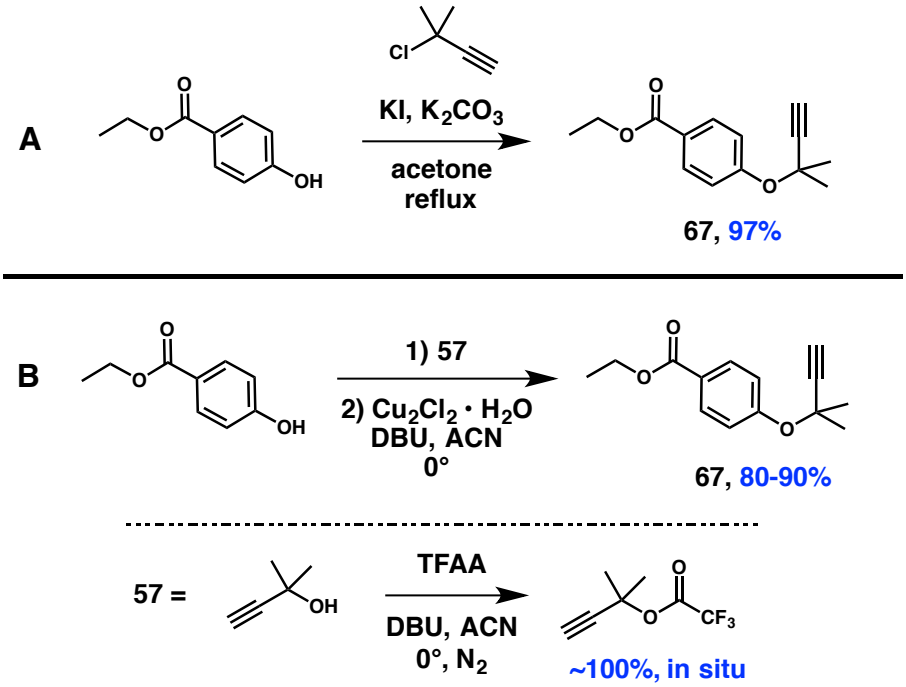
Our initial studies focused on a simplified naturally occurring chromene, which lacks the geranyl side chain present in the *P. Kelleyi*. Three simple chromenes were synthesized; the first chromene, 6-ethoxycarbonyl-2,2-dimethyl-2*H*-chromene **68**, was synthesized from an *O*-propargylated ethyl *p*-hydroxybenzoate **67** (Scheme 3.2.1, Panel **A**); the second chromene, 2,2-dimethyl-2*H*-chromene-6-carboxylic acid **69**, was synthesized with **68** (Scheme 3.2.1, Panel **B**); and the third chromene, 6-bromo-2,2-dimethyl-2*H*-chromene **71**, was synthesized with an *O*-propargylated *p*-bromophenol **70** (Scheme 3.2.1, Panel **C**). The corresponding *O*-propargylated arenes were synthesized utilizing the method described in chapter 2.4. These simple chromene systems without the geranyl side chain will allow us to efficiently study their photochromic properties and reactivity. In addition, these simplified studies to be used to prepare other natural products and their analogs and enable us to further understand the biological implications of the photochromic behavior of naturally occurring chromenes.



Scheme 3.2.1 Syntheses of simple chromene systems.

The first model chromene system was synthesized in a two-step process employing two different methods. Both methods include *O*-alkylation of the phenol, the first method[7] utilizes 3-chloro-3-methyl-1-butyne in the presence of potassium iodine and potassium carbonate in refluxing acetone to provide the ethyl *p*-(1,1-dimethyl-2-propynyloxy)-benzoate **67** with 97% yield at 100% conversion without further purification (Scheme 3.2.2, Panel **A**). The second method[8] uses 2-methylbut-3-yn-2-yl 2,2,2-trifluoroacetate to install the propargyl ether, as described in chapter 2.4, with 100% yield at 80-90% conversion (Scheme 3.2.2, Panel **B**). Although the first method provided slightly higher overall yield, the second method provides an alternative, inexpensive route for large-scale production of **67**. For method 2, (Scheme 3.2.2, Panel **B**), the yield seemed vary according to reaction scale. The second step involves a

subsequent thermal rearrangement[7] of the propargyl group to provide the desired 6-ethoxycarbonyl-2,2-dimethyl-2*H*-chromene **68** at 90% yield (Scheme 3.2.3).

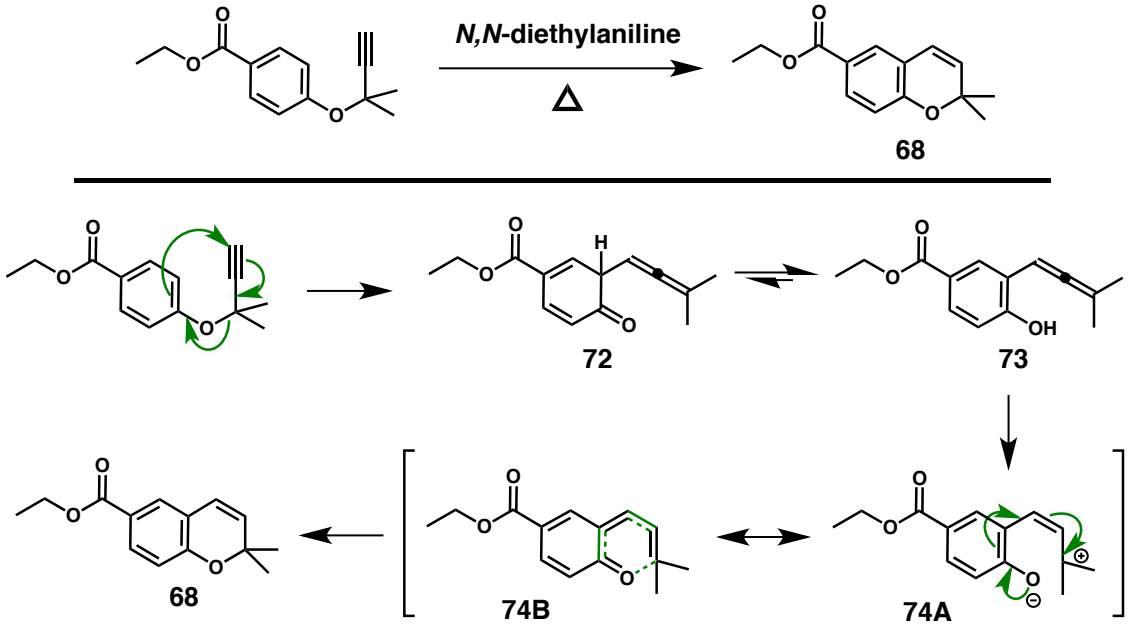


Scheme 3.2.2 Installation of a propargyl group via *O*-alkylation of ethyl *p*-hydroxybenzoate.

Scheme 3.2.3 Thermal rearrangement of the *O*-propargyl group to form the pyran ring of **67**.

Mechanistic analysis of this thermal rearrangement to the chromene suggests an *o*-quinomethide intermediate that undergoes an intramolecular [4+2]-HDA reaction to

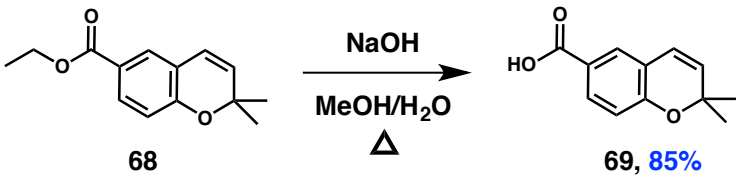
provide the pyran ring. Similar to the aromatic variation of the Claisen rearrangement, the first step is a [3,3]-sigmatropic rearrangement of the propargyl ether substituent to the unstable intermediate **72**, which rapidly tautomerizes to the allenyl phenol intermediate **73** re-establishing aromaticity of the benzene ring. The allenyl intermediate **73** then undergoes a [1,5]-hydride shift providing a neutral *o*-quinomethide intermediate **B**, which cyclizes to the chromene.



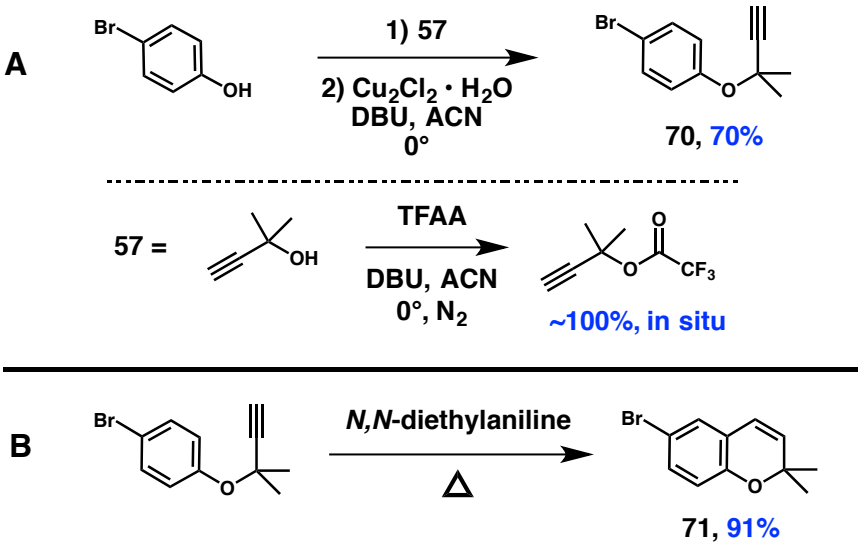
Scheme 3.2.4 Mechanistic analysis for the thermal rearrangement of the *O*-propargyl phenyl ether

The second chromene system was easily accessible upon base-catalyzed hydrolysis[9] of chromene **68**, which provides the acid derived chromene **69** in moderate to high yields (Scheme 3.2.5). Chromene **71** was synthesized utilizing method 2, (Scheme 3.2.2, Panel **B**[8]), by installation of the propargyl substituent on a phenol building block

yielding **70** (Scheme 3.2.6, Panel **A**) followed by a subsequent thermal rearrangement[7] to give the desired bromo-derived chromene **71** (Scheme 3.2.6, Panel **B**). Each of these chromenes were used to study photochromic properties and chromene containing compounds and determine if dimerization upon ring-opening was a viable process.



Scheme 3.2.5 Base-catalyzed hydrolysis of chromene **68** providing the acid-derived chromene.



Scheme 3.2.6 Synthesis of 6-bromo-2,2-dimethyl-2*H*-chromene from *p*-bromophenol.

3.3 Qualitative Kinetic Studies

Our first experimental evidence of photochromic ring opening of chromene **68** to a quinone methide was apparent after ultraviolet-visible (UV-vis) spectroscopic analysis. UV-vis spectrum of unreactive chromene **68** was obtained in spectroscopic grade

methanol and as expected we observed two absorption bands, a $\pi \rightarrow \pi*$ corresponding to the excitation of a pi-electron within the conjugated pi system and a $n \rightarrow \pi*$ corresponding to the excitation of a non-bonding electron from the oxygen within the system.

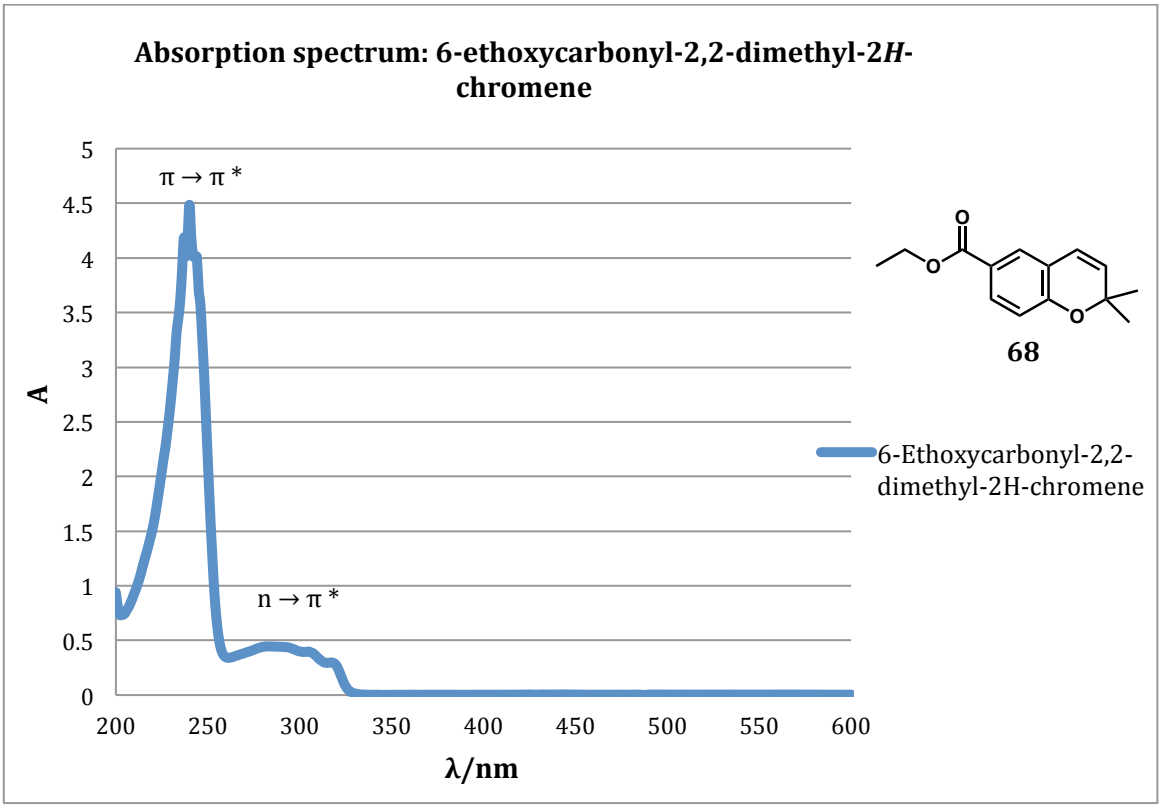


Figure 3.3.1 UV-visible spectrum of 6-ethoxycarbonyl-2,2-dimethyl-2*H*-chromene in methanol.

In collaboration with Dr. Robert Sheridan (University of Nevada, Reno, chemistry department) we were able to qualitatively observe ring opening of chromene **68** using a temperature-dependent UV-vis spectrometer. In this study, chromene **68** was dissolved in diethyl ether, and UV-vis spectra were taken at room temperature and at 193 K (ca. -78

°C). The solution was irradiated at 300 nm at low temperature for ten minutes, and UV-vis spectra were recorded before and after irradiation (Figure 3.2.2).

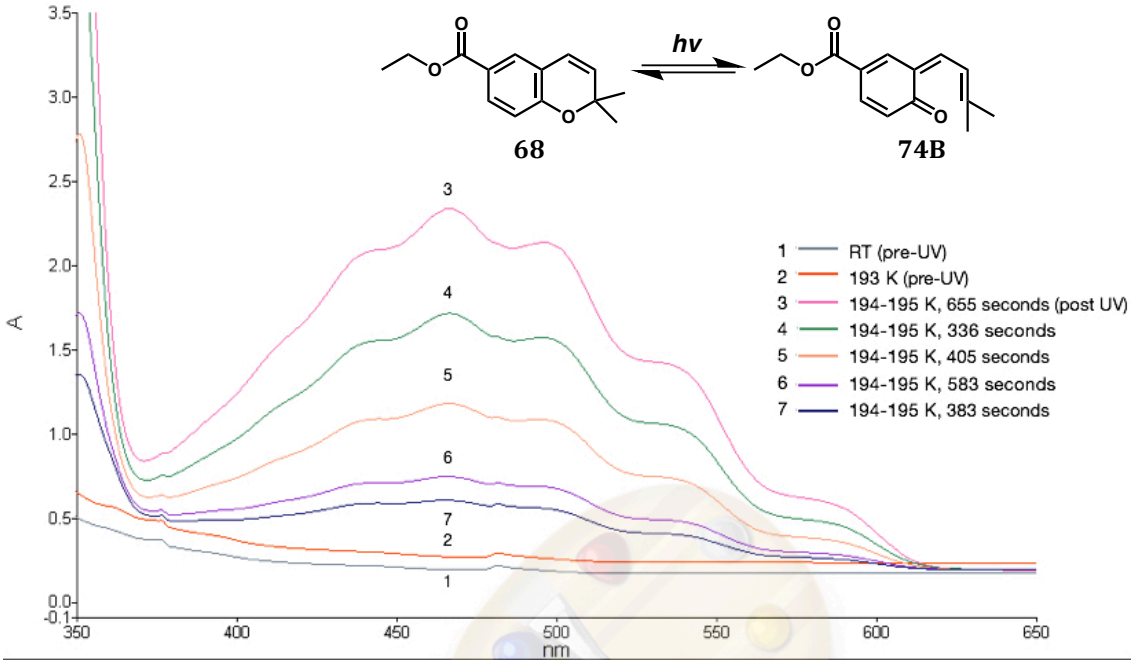


Figure 3.3.2 UV-visible spectra of chromene **68** upon irradiation in diethyl ether observed at 193-195 K.

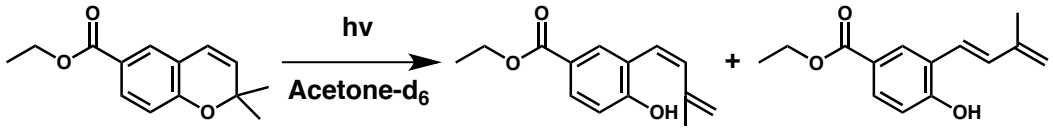
Analysis of the UV-vis spectra, revealed a new band at 450 nm, not apparent in the UV-Vis spectrum of **68**. This new absorption band was significantly longer wavelength (lower energy) and is consistent with the generation of an *o*-quinone methide intermediate. This absorption band was found to disappear with time, which further supports its assignment to the quinone methide, which undergoes a thermal reversion to the chromene over time. Both of these observations gave significant support to the hypothesis that these natural chromenes are photochromic, readily undergoing photo-

initiated ring opening to an *o*-quinone methide intermediate, which thermally reverts to the colorless chromene.

3.4 Photochemical transformation investigation

Photochemical transformations were performed primarily on chromene **68**, utilizing similar methods described by Padwa and coworker.[10] Our initial investigations explored the solvent effects on the photochromism and reactivity of the putative *o*-quinone methide intermediate. The chromene **68** was irradiated using 300 nm and 254 nm light in three different solvents: two aprotic solvents, acetone-d_6 and acetonitrile-d_3 (Table 3.3.1 and Table 3.3.2, respectively) and one protic solvent, methanol-d_4, (Table 3.3.3) and the results were monitored by ^{1}H-NMR spectroscopy.

Table 3.3.1 Qualitative photochemical analysis of chromene **68** in acetone-d_6.

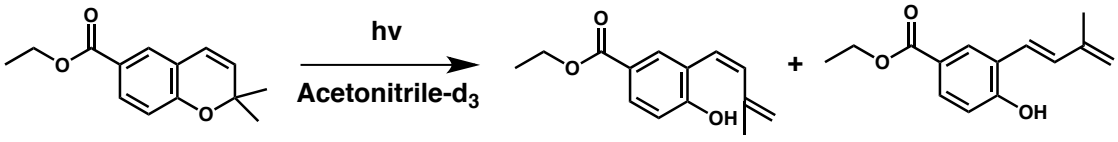


68 75 76

Entry	Time (mins)	[X] (M)	Solvent	Wavelength (nm)	% Ratios via ^{1}H-NMR 68 - 75:76
1	15	0.020	Acetone-d_6	300	85 - 11:4
	30	0.020	Acetone-d_6	300	68 - 22:10
	90	0.020	Acetone-d_6	300	47 - 34:19
2	15	0.20	Acetone-d_6	300	96 - 3:1
	30	0.20	Acetone-d_6	300	92 - 6:2
	90	0.20	Acetone-d_6	300	86 - 11:3

Each experiment was conducted in deuterated solvents so that qualitative NMR analysis could be used to understand the fate of the chromenes. The results observed for the acetone study (Table 3.3.1) correlates well with the literature.[10] Chromene **68** was dissolved in deuterated acetone at two different concentrations, 0.020 and 0.20 Molar (M), and irradiated at 300 nm over a course of 90 minutes. As expected, both *cis* and *trans* diene isomers **75** and **76**, respectively, were observed initially favoring the formation of the *cis*-isomer. In addition, a less concentrated solution (Entry 1) was consumed at a quicker rate than the more concentrated solution (Entry 2), presumably due to intermolecular quenching of the excited state of the chromene. Under these conditions, acetone absorbs rather strongly under UV light (330 nm) and can act as a photosensitizer, which either enhances photoreactivity or can also function to quench it.[11] To test this, the reaction was conducted similarly as Entry 1 (Table 3.3.1) utilizing acetonitrile as the solvent (Table 3.3.3, Entry 1).

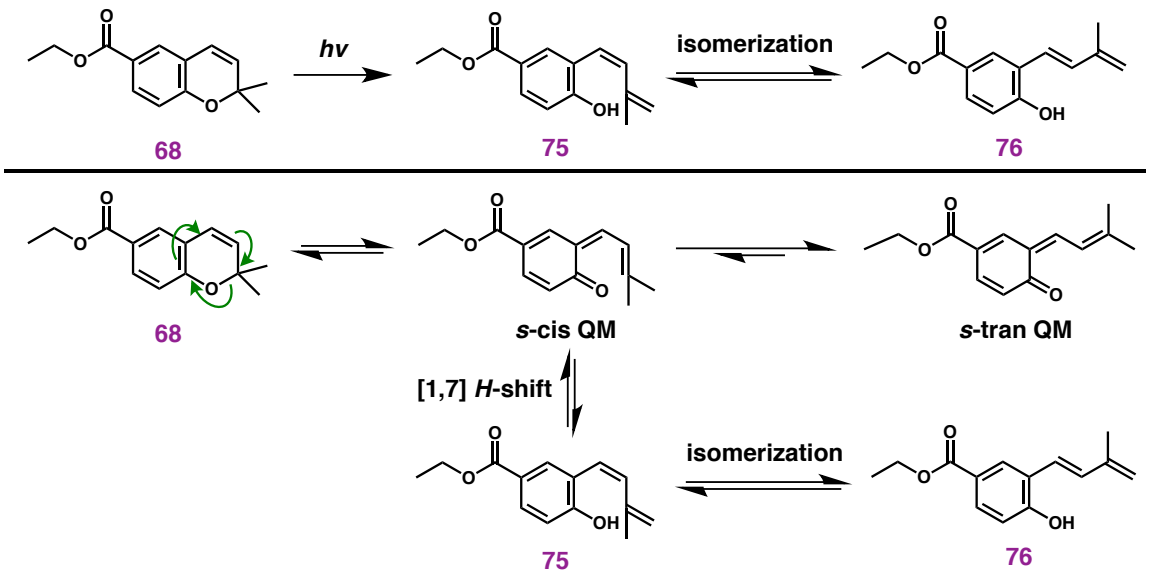
Table 3.3.2 Qualitative photochemical analysis of chromene **68** in acetonitrile-d_3.



68 75 76

Entry	Time (mins)	[X] (M)	Solvent	Wavelength (nm)	% Ratios via ^{1}H-NMR 68 - 75:76
1	15	0.020	Acetonitrile-d_3	300	86 - 11:3
	30	0.020	Acetonitrile-d_3	300	81 - 15:4
	90	0.020	Acetonitrile-d_3	300	77 - 17:6
2	15	0.020	Acetonitrile-d_3	254	69 - 12:19
	30	0.020	Acetonitrile-d_3	254	59 - 11:30
	90	0.020	Acetonitrile-d_3	254	48 - 19:33

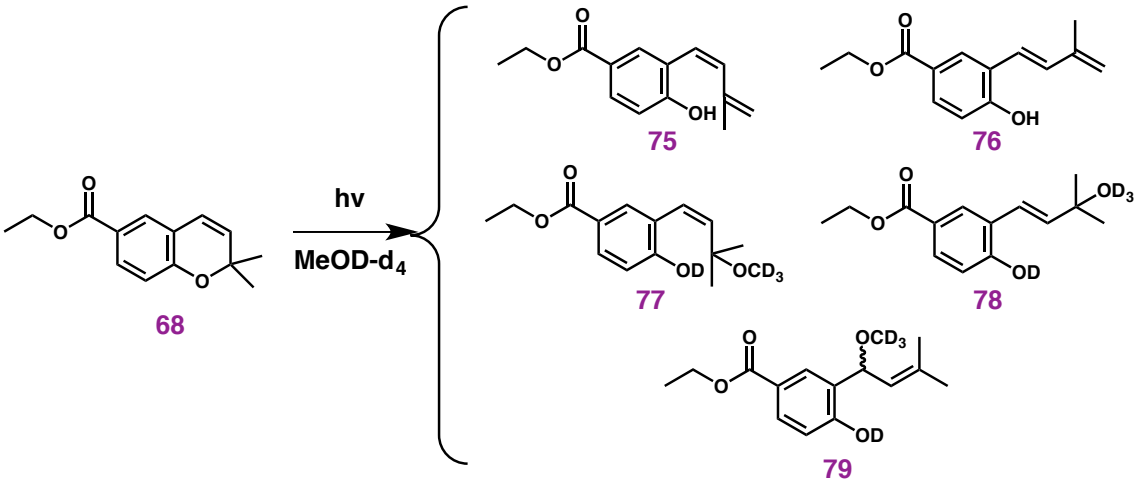
In both cases, phototransformation of chromene **68** to the dienes was observed; however, the conversion to the dienes occurred at a faster rate in acetone. The last experiment in this study was to explore the product ratio differences at upon irradiation with different wavelengths of UV light. In acetonitrile, Entry 2 (Table 3.3.2), irraddiation of the chromene at 254 nm efficeintly converted the *cis* and *trans* isomers were observed with the *trans* isomer **76** favored. Under these conditions, the formation of either *cis* or *trans* isomer **75** and **76** were best rationalized as photochemical ring opening of chromene **68** to give an *o*-quinomethide intermediate[10], *s*-cis or *s*-trans QM (Scheme 3.3.1). Once the *s*-cis QM intermediate is generated, it can undergo a [1,7] *H*-shift to provide the cis diene isomer. Alternatively, photochemical isomerization of cis diene isomer **75** can occur to produce trans diene isomer **75**. Unfortunately, no dimer formation was observed under these conditions.



Scheme 3.3.1 Photochemical ring opening of chromene **68** mechanism to cis and trans diene

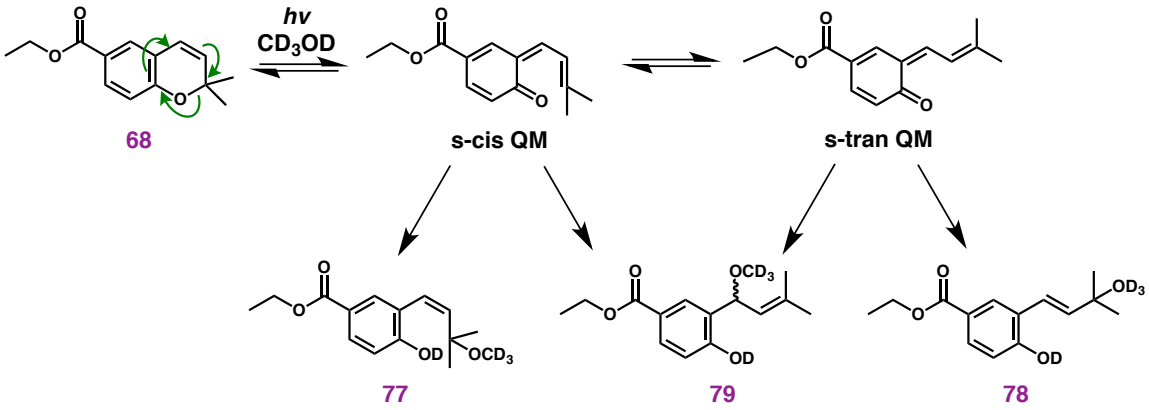
In the next study, photochemical transformation of chromene **68** was studied in methanol (Table 3.3.3). Chromene **68** was dissolved in deuterated methanol at 0.02 molar concentration, irradiated at 300 nm and analyzed by ^{1}H-NMR. After 90 minutes of irradiation, chromene **68** was completely consumed producing five different photoproducts: cis-diene **74**, trans-diene **75**, cis-1,6-methanol addition **76**, trans-1,6-methanol addition **77** and 1,4-methanol addition **78**.

Table 3.3.3 Qualitative photochemical analysis of chromene **68** in methanol-d_4.



Entry	Time (mins)	[X] (M)	Solvent	Wavelength (nm)	% Ratios via ^{1}H-NMR 68 - 75:76 - 77:78 - 79
1	15	0.022	Methanol-d_4	300	63 - 7:1 - 2:18 - 9
	30	0.022	Methanol-d_4	300	26 - 13:3 - 11:31 - 16
	90	0.022	Methanol-d_4	300	0 - 4:2 - 41:28 - 25
2	15	0.20	Methanol-d_4	300	74 - 7:0 - 0:12 - 7
	30	0.20	Methanol-d_4	300	74 - 5:0 - 0:16 - 5
	90	0.20	Methanol-d_4	300	44 - 10:5 - 0:30 - 11
3	15	0.20	Methanol-d_4	254	43 - 0:0 - 15:32 - 10
	30	0.20	Methanol-d_4	254	21 - 0:0 - 23:41 - 15
	90	0.20	Methanol-d_4	254	0 - 0:0 - 36:48 - 13

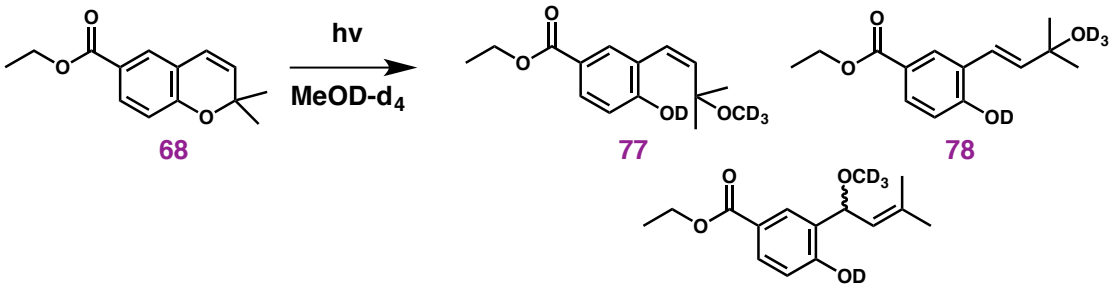
Increasing the concentration of the chromene 10-fold (Table 3.3.3, Entry 2) provided similar results, however, at slower conversion rates. Full conversion of chromene **68** was observed by irradiating at 254 nm (Table 3.3.3, Entry 3) and only the methanol addition photoproducts were produced, **77-79**. In the methanol solvent, the primary reactivity corresponds to the 1,4 and 1,6 methanol conjugate addition to either *s*-cis or *s*-trans *o*-quinomethide intermediates. From this observation, the reaction favors the trans 1,6 methanol addition **78** (Scheme 3.3.2).



Scheme 3.3.2 Photochemical ring opening of chromene **68** mechanism to 1,4 and 1,6 methanol addition photoproducts.

Our next study, we targeted the development of large scale production of either the *cis* or *trans* 1,6 methanol addition **78** or **78** respectively. Isolation of these methanol adducts allowed us to study modes of dimerization under thermal and acid catalyzed conditions. Additional attempts to directly produce the dimeric chromane we explored the differences in reactivity that the chromene had upon irradiation with the sensistizer xanthone (Table 3.3.4).

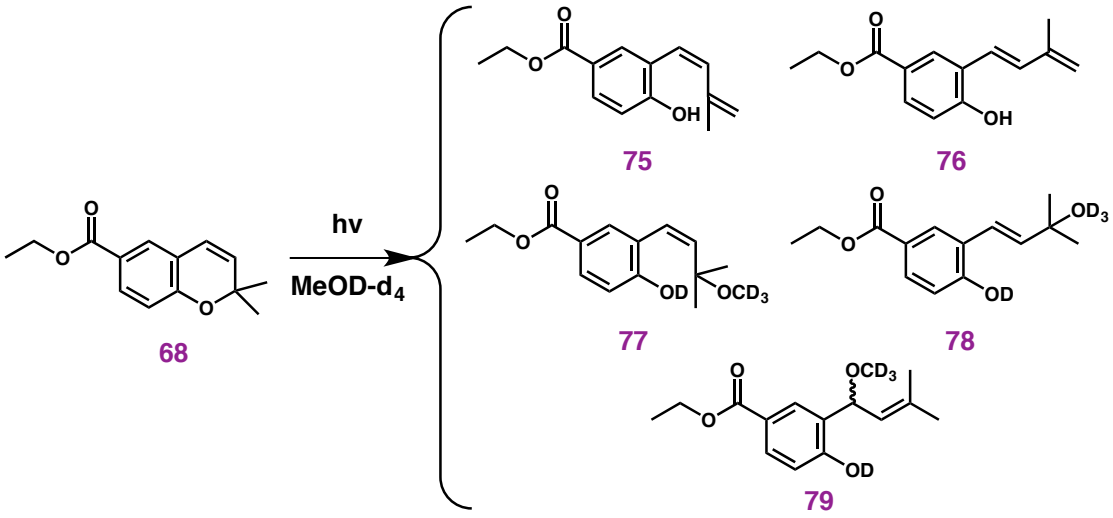
Table 3.3.4 Qualitative photochemical analysis of chromene **68** in methanol-d_4 with a photosensitizer, xanthone (P)



Entry	Time (mins)	[X] (M)	Solvent	Wavelength (nm)	% Ratios via ^{1}H-NMR 68 - 77:78 - 79
1	240	0.05	Methanol-d_4	300	0 - 50:31 - 19
2	240	0.05	Methanol-d_4 + P	300	0 - 56:44 - 0

In this study, two solutions with and without xanthone were irradiated until complete consumption of chromene **68**. Comparing these two studies, no apparent changes in the reactivity or selectivity for the products were apparent, however, the xanthone-sensitized reaction showed no 1,4 methanol addition **79**. We then pursued a study of the the effect of an acid catalyst on the photoreaction of the chromene (Scheme 3.3.5). The addition of TFA to the reaction (Entry 2) increased the rate of conversion to the methanol adduct compared to the reaction without TFA (Table 3.3.5, Entry 1) with complete conversion of chromene **68** within the hour yielding only the 1,6 methanol addition isomers, **77** and **78**. Under acidic conditions, the *o*-quinomethide can act as a Lewis base and accept a proton, rendering the electrophilic enone more electrophilic and more susceptible to nucleophilic attack. Unfortunately, upon additional irradiation of the acid-catalyzed reaction (Table 3.3.5, Entry 2) provided no observed selectivity between the *cis* and *trans* isomers.

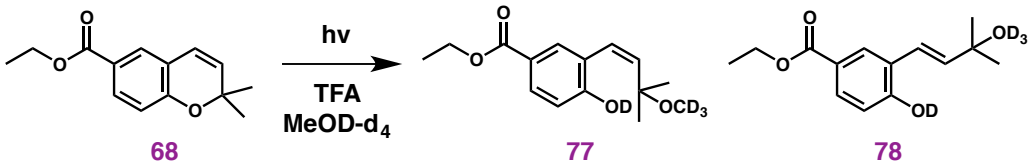
Table 3.3.5 Qualitative photochemical analysis of chromene **69** in methanol-d_4 with 2*u*L (1.5*eq*.) trifluoroacetic acid (H^+).



Entry	Time (mins)	[X] (M)	Solvent	Wavelength (nm)	% Ratios via ^{1}H-NMR 68 - 75:76 - 77:78 - 79
1	60	0.020	Methanol-d_4	300	14 - 14:0 - 24:24 - 24
	180	0.020	Methanol-d_4	300	3 - 5:0 - 40:23 - 28
2	60	0.020	Methanol-d_4 + H^+	300	0 - 0:0 - 53:47 - 0
	180	0.020	Methanol-d_4 + H^+	300	0 - 0:0 - 48:52 - 0

Finally, the effects on TFA stoichiometry has on the outcome of the chromene photoreaction at two different chromene concentrations was studied (Table 3.3.6). Each of these reactions were irradiated until complete conversion of chromene **68**, as determined by ^{1}H-NMR. At 0.017 molar concentration, Entry 1, the *trans* methanol addition isomer **78** was favored at increased acid stoichiometry. At 0.14 molar concentration, Entry 2, the trans methanol addition isomer **78** was also favored at increased acid stoichiometry.

Table 3.3.6 Qualitative acid-dependent photochemical analysis of chromene **67** in methanol-d_4 at different concentrations.

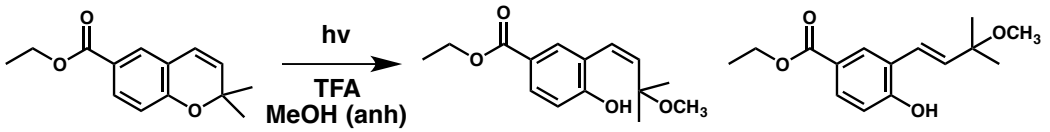


68 77 78

Entry	Time (mins)	[X] (M)	TFA (eq)	Solvent	Wavelength (nm)	% Ratios via ^{1}H-NMR 68 - 77:78
1	60	0.017	0.2	Methanol-d_4	300	0 - 54:46
		0.017	2.0	Methanol-d_4	300	0 - 30:70
2	60	0.14	0.2	Methanol-d_4	300	52 - 1:47
		0.14	2.0	Methanol-d_4	300	59 - 0:41
	240	0.14	0.2	Methanol-d_4	300	0 - 24:76
		0.14	2.0	Methanol-d_4	300	0 - 0:100

Reactions run with a low acid concentration were selective for the *trans* isomer **77** and increasing the acid concentration resulted in exclusive conversion to the trans isomer **77**. The trans isomer **81** was selectively produced on preparative scale by irradiating chromene **68** in anhydrous methanol (0.1 M) at 300 nm with 1 equivalent of TFA in 3.5 hours. Upon complete conversion of the chromene, the crude mixture was recrystallized in hexanes at moderate yields (70%). The cis isomer **80** was found in the mother liquor and purified via column chromatography (5%).

Table 3.3.7 Large-scale production of trans isomer **81**

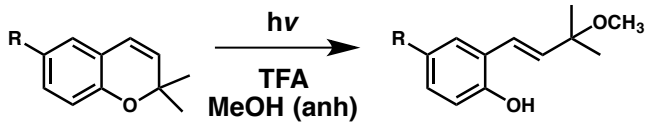


68 **80, 5%** **81, 70%**

Entry	Time (mins)	[X] (M)	TFA (eq)	Solvent	Wavelength (nm)	% Ratios via ^{1}H-NMR 68 - 80:81
1	210	0.1	1	Methanol (anh)	300	0 - 5:95
2	180	0.1	2	Methanol (anh)	300	0 - 5:95

Acid chromene **69** and bromo-chromene **71** were dissolved in anhydrous methanol (0.1M) with 1 equivalent of TFA and irradiated at 300 nm for 3.5 hours. By NMR, the acid chromene **69** was 90% consumed, however the bromo-chromene **71** was only 66% consumed. The bromo-chromene was subjected to an additional hour of irradiation, which gave 75% consumption of the starting material. The acid chromene and bromo-chromene reaction provided their corresponding trans methanol adduct **82** & **83**, respectively, at moderate to high yields, with some unreactive chromene and trace amounts of cis methanol adduct in both cases (Table 3.3.8). The bromo-chromene **53** with the geranyl side chain was also irradiated under the same conditions as described above. After four hours of irradiation, **53** provided its desired trans methanol adduct with some unreactive (20%) and trace amounts of the cis methanol adduct (Table 3.3.9).

Table 3.3.8 Large-scale production of trans isomer **82** & **83**

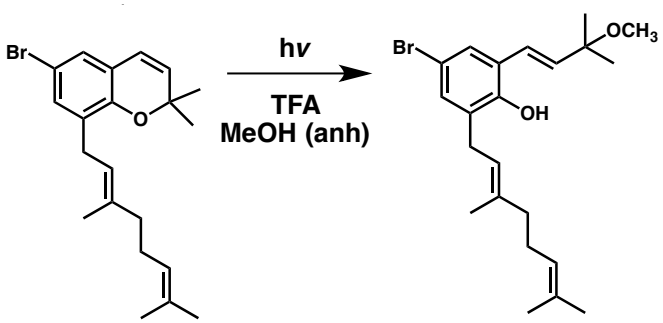


69, R = COOH
71, R = Br

82, R = COOH, 75%
83, R = Br, 58%

Entry	Time (mins)	% Ratios via ^{1}H-NMR SM - Pdt
1, R = COOH	210	10 - 90
2, R = Br	240	75 - 25

Table 3.3.9 Large-scale production of trans isomer **84**



53

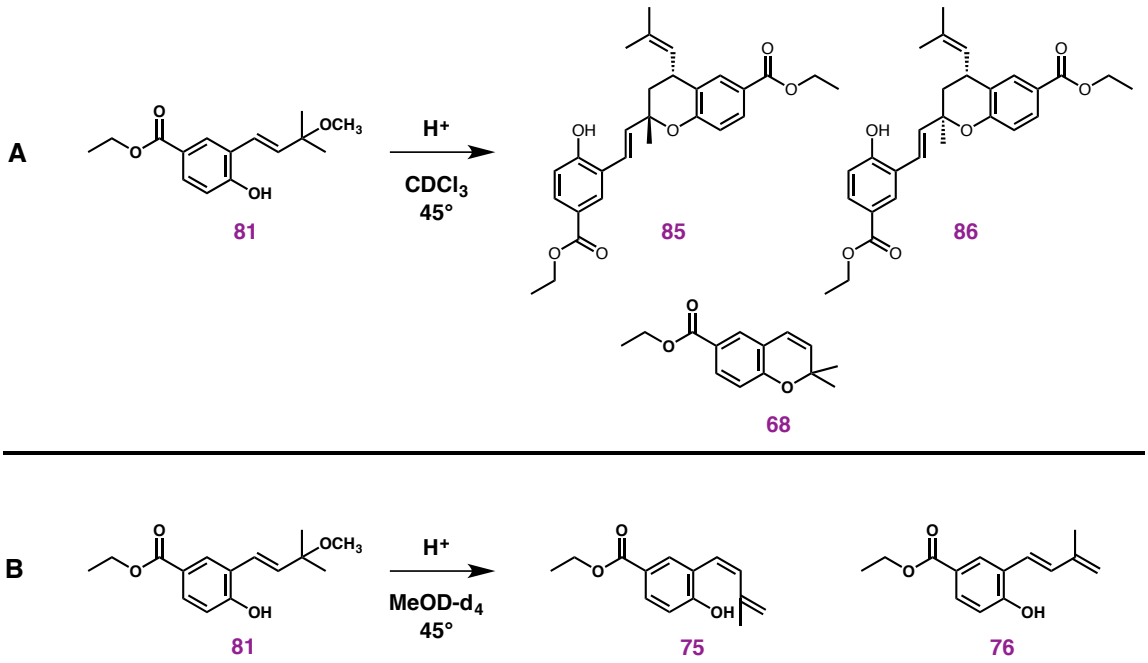
84, 88%

Entry	Time (mins)	% Ratios via ^{1}H-NMR 53 - 84
1	240	20 - 80

3.5 Dimerization studies

We envisioned that the *trans* methanol adduct would provide access to the *o*-quinone methide intermediate through an acid-catalyzed elimination of methanol. To study this conversion, we treated the methanol adduct **81** to a variety of reaction

conditions, surveying acid-catalysts, solvents and temperature. Dimerization was found to occur using both pyridinium p-toluenesulfonate (PPTS, 1 eq) and trifluoroacetic acid (TFA, 1 eq) in $CDCl_3$. Both diastereomers **85** and **86** of the dimer was observed (1:1) by ^{1}H-NMR analysis, in addition to reversion to chromene **68** at 45° C (~40%) (Scheme 3.5.1, Panel **A**). When changing the solvent to deuterated methanol, under the same conditions, no dimerization was observed, but instead the cis and trans dienes **75** and **76** were observed (Scheme 3.4.1, Panel **B**). In the attempts to control the diastereoselectivity in dimer **82** and **83**, we surveyed the reaction at three different temperatures.

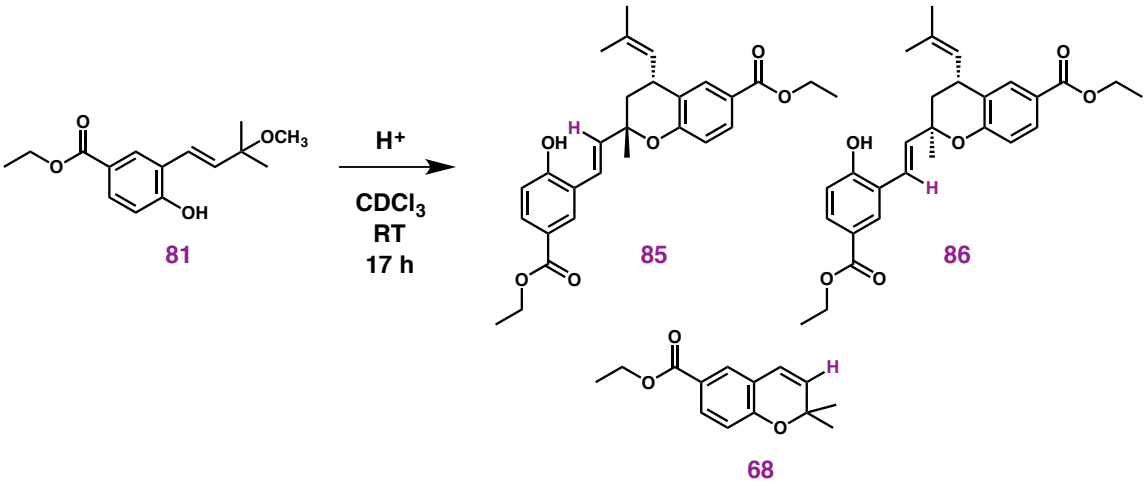


Scheme 3.4.1 Thermal dimerization of trans isomer **81**.

In each study, we dissolved the trans methanol adduct **81** in *d*-chloroform, and 1 equivalent of PPTS was added after each reaction was cooled to their desired

temperatures (Table 3.4.1). From these observations, reversion to the parent chromene **68** was slower in colder temperature conditions compared to the reaction at the elevated temperature (45 °C). There was an increase in preference for the diastereoisomer **85** (2:1) when a substoichiometric amount of PPTS (0.5 equivalent) was used, however reversion to the parent chromene **68** also increased.

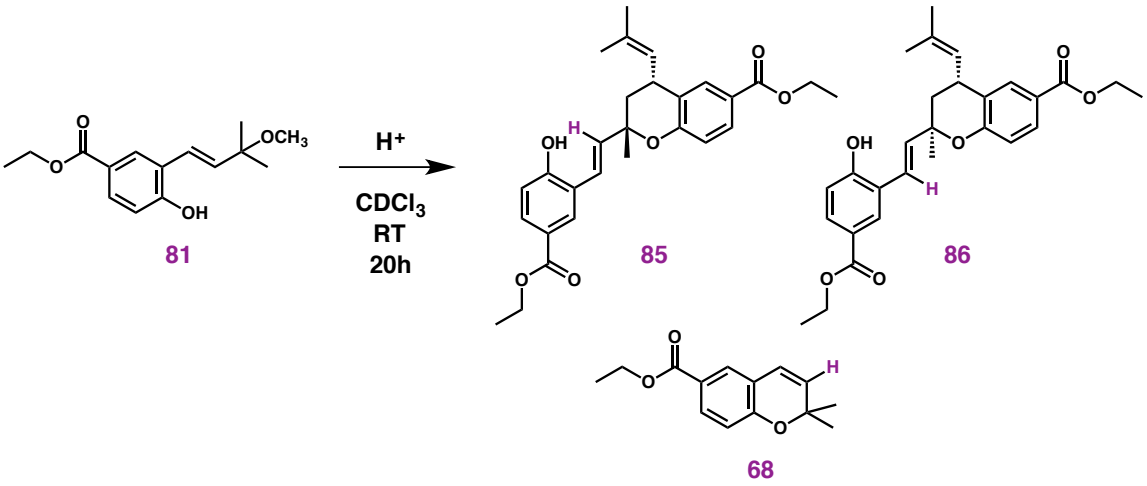
Table 3.4.1 Dimerization of **81** at different temperatures and different acid stoichiometry



Entry	Concentration (M)	PPTS (eq)	Temperature (°C)	% Ratios via ^{1}H-NMR 81 - 85:86:68
1	0.1	1	RT	0 - 50:41:9
	0.1	0.5	RT	0 - 46:25:29
2	0.1	1	0	0 - 53:36:11
	0.1	0.5	0	0 - 50:29:21
3	0.1	1	-78	0 - 55:35:10
	0.1	0.5	-78	0 - 47:34:19

Eight other acids[12] were surveyed to evaluate their effectiveness for producing the dimer of **81** (Table 3.4.2). From these experiments we observed no apparent trend in the stereoselectivity of the diastereomers using different acids.

Table 3.4.2 Dimerization of **81** with different acids



Entry	Concentration (M)	Acid (1 eq)/pKa	%Ratios via ^{1}H-NMR 81 - 85:86:68
1	0.1	PPTS/5.21[a]	0 - 50:40:10
2	0.1	Cyclohexanecarboxylic acid/4.90[b]	80 - 0:0:20
3	0.1	Propionic Acid/4.86[b]	90 - 0:0:10
4	0.1	Acetic Acid/4.76[a]	90 - 0:0:10
5	0.1	Monochloroacetic acid/2.86[a]	58 - 0:0:42
6	0.1	TsOH/-0.06[c]	0 - 33:64:3
7	0.1	TFA/-0.25[a]	0 - 50:10:40
8	0.1	HCl/-8.0[a]	0 - 50:40:10

The use of trifluoroacetic acid, TFA Entry 7, provided selectivity towards the major dimer diastereoisomer **85** (4:1), unfortunately reversion to parent chromene **68** also

increased. On the contrary, a preference for the minor diastereoisomer of the dimer was observed when *p*-toluene sulfonic acid, TsOH Entry 6, was used as the acid catalyst. From these studies, dimerization occurs best with non-nucleophilic acids (e.g. PPTS, TsOH, TFA and HCl) dissolved in non-polar solvents (e.g. chloroform). Reversion to the chromene **68** starting material was slower at lower temperatures (e.g. room temperature) and at higher acid stoichiometry. Upon purification, the dimer was isolated as a diastereomeric ratio (4:1 via ^{1}H-NMR). Current efforts are being developed to separate the dimeric products and to continue method development in improving stereoselectivity of the diastereomers.

3.6 Discussion

Chromenes **68-71** were easily prepared and were used as model systems to study their photochromic properties and dimerization. We found that chromene containing compounds under direct exposure to UV-irradiation provided no signs of dimerization. For dimerization to occur, the chromene core must ring open to an *o*-quinomethide intermediate followed by isomerization to a diene through a [1,7]-hydride shift, or tautomerization. Evidence of a photoproduced *o*-quinone methide intermediate was provided through low temperature UV-Vis studies, where irradiation of chromene **68** resulted in the production of a new absorption band at 465 nm that was assigned to the *o*-quinone methide intermediate. This observation provided us with the first empirical evidence in generation of an *o*-quinomethide intermediate from naturally occurring chromenes.

Reactivity studies were conducted using the model chromene. Irradiation under a variety of aprotic conditions provided both cis and trans diene isomers. The rate of conversion to these dienes increased when the chromene was irradiated in acetone as opposed to reactions dissolved in acetonitrile. Unfortunately, no signs of dimerization upon direct irradiation of the chromene was observed. Irradiating chromene **68** in methanol, however, provides five photoproducts: two diene isomers **75-76** and three methanol adducts **77-79**. As described in this chapter, we were able to control selectivity of these photoproducts. Specifically, we found that addition of xanthone, a photosensitizer, or TFA, an acid, selectively provides the two methanol adducts, **77-78**. In these two studies, selectivity for methanol adduct **78** was slightly increased in the presence of TFA. Therefore, by increasing the acid stoichiometry in the photoreaction gave an increase in selectivity towards the trans isomer **78**. As expected, the cis isomer is protonated by the acid to a stable benzylic carbocation and rotates to the stable *s*-trans conformer and generation of the trans isomer **78** via E_2 mechanism. These studies gave access to a single photoproduct for model chromenes **68-71** and PBA chromene precursor **54**, providing trans methanol adducts **81-83** and **84**, respectively, at moderate to high yields.

The methanol adduct **81** was found to produce the dimer under acid catalyzed conditions. Reactions conducted at 45 °C, provides 1:1 diastereomeric ratio of dimers **85** and **86** in addition to reversion to the parent chromene starting material **68**. Lowering the temperature to room temperature, decreases the reversion to the parent chromene starting material, which suggest that the parent chromene starting material is the thermodynamically stable compound. Decreasing the reaction temperature to -78 °C

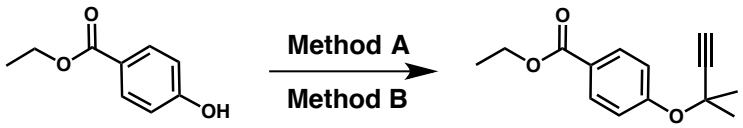
provided subtle stereoselectivity for diastereomers **85** and **86**, favoring **85**. Concluding that dimerization occurs best in non-nucleophilic acids as described in section 3.4. Future studies will be focused on enhancing stereoselectivity of these dimerization reactions.

3.7 Experimental Procedures

Reactions were carried out under inert atmosphere of nitrogen (N_2) gas in clean, oven-dried glassware (Pyrex) with magnetic stirring, unless otherwise specified. All reagents and solvents were purchased from Sigma-Aldrich Chemical Company and used without further purifications.[13] TLC was recorded and performed on Silicycle glass 60 F254 plates[14] and were observed under UV shortwave light or developed by staining with $KMnO_4$ or CAM. Reactions were purified using flash chromatography using Silicycle Siliaflash® P60 (230-400 mesh).[15] Photolysis was conducted on a Rayonet photoreactor, model RPR-200, using 2537Å, 3000Å, or 3500Å light bulbs. ^{1}H-NMR spectra were measured on Varian 400 (400 MHz)[16a] or Varian 500 (500 MHz) spectrometers[16a] and are reported in ppm (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad);[16] integration; coupling constant(s) in Hz, using *d*-$CDCl_3$ (7.26 ppm, with 0.01% TMS at 0.00 ppm) or in d_4-MeOD (3.31 ppm) or d_3-ACN (1.94 ppm) as locking solvents.[17] ^{13}C-NMR spectra were measured on Varian 400 (101 MHz) or Varian 500 (126 MHz) spectrometers[16a] and are reported in ppm using *d*-$CDCl_3$ (77.16 ppm, with 0.01% TMS at 0.00 ppm) or in d_4-MeOD (49.00 ppm) or d_3-ACN (118.26 and 1.32 ppm) as locking solvents. Infrared (IR) spectra were recorded on a Nicolet 6700 FT-IR with a diamond ATR[16a] and the bands reported in cm^{-1} (br = broad, st = strong).[16] High-resolution mass spectra were obtained using an Agilent 6230 TOF LC/MS with an

atmospheric pressure photo-ionization (APPI, with C60 and anthracene internal standards) or electrospray (ESI, with purine and HP-0921 internal standards).[16]

Ethyl 4-(1,1-dimethyl-2-propynyloxy)benzoate (67):

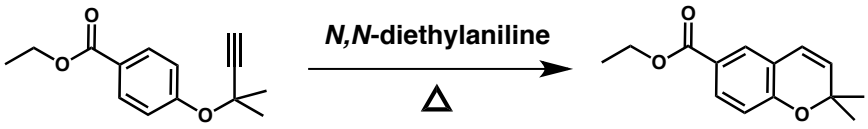


Method A: Ethyl *p*-hydroxybenzoate (0.5 g, 4.0 mmol), KI (1.0 g, 6.0 mmol), K_2CO_3 (7.2 mmol) and 3-chloro-3-methyl-1-butyne (1.1 mL, 10. mmol) was dissolved in acetone (5.0 mL, 0.8M) and heated at 50°C. After 48 hours, the mixture was cooled to room temperature, diluted with Et_2O (20 mL) and washed with 1.0 M NaOH (2x20 mL). The organic layer was dried in Na_2SO_4 (anh.) and the solvent was removed under reduced pressure[16a] providing a white solid (0.75g, 97%) without further purification.

Method B: DBU (1.34 mL, 9.0 mmol) was added to a solution of 2-methyl-3-butyn-2-ol (0.67 mL, 6.9 mmol) in anhydrous CH_3CN (3.5 mL, 2M) and cooled in an ice-salt bath (0° C). Trifluoroacetic anhydride (1.0 mL, 7.0 mmol) was added drop-wise and stirred for an additional 30 minutes to produce the propargyl-trifluoroacetate derivative *in situ*. In a second reaction, DBU (1.16 mL, mmol) and $CuCl_2 \cdot 2H_2O$ (1.5 mg, mmol) was added to a solution of ethyl *p*-hydroxybenzoate (1.0 g, mmol) dissolved in anhydrous CH_3CN (10 mL, 0.3M), cooled in an ice-salt bath under nitrogen, and the propargyl-trifluoroacetate solution was added drop-wise via cannulation addition. After 5 hours at 0°C, the mixture was concentrated at reduced pressure.[16a] The crude mixture was dissolved in toluene (50 mL) and washed with 1 M HCl (3x25mL), aqueous $NaHCO_3$ (2x25mL) and saturated aqueous brine (2x25 mL) and dried with anhydrous Na_2SO_4. The

solvent was removed at reduced pressure[16a] to give a crude product that was purified by column chromatography using 4:1 hexanes:ethyl acetate to give the desired white solid product (1.24 g, 90%); The experimental melting point was determined to be 50.2-51.6 °C;[16b] The R_f was calculated = 0.6 using 4:1 hexanes:ethyl acetate; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.99 (d, *J* = 9.0 Hz, 1H), 7.25 (d, *J* = 9.0 Hz, 1H), 4.36 (q, *J* = 7.1 Hz, 2H), 2.63 (s, 1H), 1.70 (s, 6H), 1.39 (t, *J* = 7.1 Hz, 3H),[16b] ^{13}C NMR (126 MHz, chloroform-*d*) δ 166.35, 159.68, 130.86, 124.19, 119.40, 85.26, 74.59, 72.32, 60.65, 29.57, 14.37;[16b] IR (neat): 3290.95, 3075.10, 2986.96, 2938.09, 2899.83, 2111.28, 1709.52 (st), 1603.16, 1505,46, 1271.98, 1245.64, 1098.99; HR-MS (ESI) calcd' for $C_{14}H_{16}O_3$ $(M+H)^+$ 232.1099, observed 232.1103.

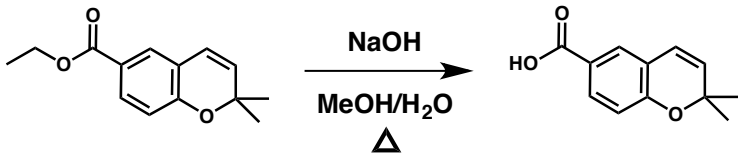
6-ethoxycarbonyl-2,2-dimethyl-2*H*-chromene (68):



Compound **67** (1.1 g, 4.7 mmol) was dissolved in *N*, *N*-diethylaniline (5 mL, 1 M) and stirred at reflux (210°C) for two hours. Upon completion, the reaction mixture was cooled to room temperature and diluted with Et_2O (50 mL), washed (with caution; highly exothermic) with 6 M aqueous HCl (4x25 mL), followed by saturated $NaHCO_3$ solution (2x20 mL), saturated NaCl solution (2x20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed giving crude amber oil that was flushed through a plug of silica yielding a light yellow oil (0.98 g, 90%). R_f = 0.6 (4:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.82 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.69 (d, *J* = 2.1 Hz, 1H), 6.78 (dd, *J* = 8.6, 0.6 Hz, 1H), 6.36 (d, *J* = 9.8 Hz, 0H), 5.65 (d, *J* = 9.9 Hz, 1H), 4.34 (q, *J* =

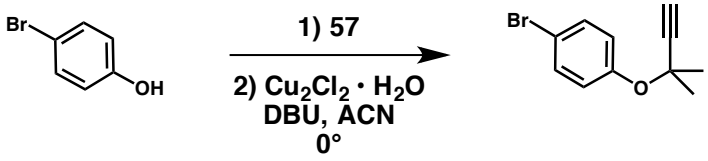
7.1 Hz, 2H), 1.46 (s, 6H), 1.38 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 166.31, 157.05, 131.01, 130.95, 127.99, 122.86, 121.72, 120.59, 116.08, 77.29, 60.57, 28.27, 14.37; IR (neat): 3025.10, 2972.06, 2924.16, 2850.93,1715.83 (st), 1433.31, 1310.90, 1245.72, 1195.77, 1163.39; HR-MS (ESI) calcd' for $C_{14}H_{16}O_3$ $(M+H)^+$ 232.1099, observed 232.1099.

2,2-dimethyl-2*H*-chromene-6-Carboxylic acid (69):



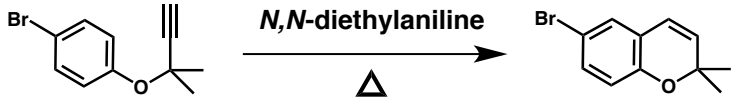
Compound **68** (1.25 g, 5.39 mmol) and NaOH (2.16 g, 53.9 mmol) was dissolved in methanol:DI-water mixture (0.3 M, 9:1 v:v) and heated at 80°C for 1 hour. Upon completion (by TLC), the reaction was acidified with 1M HCl (pH = 3.4). The desired compound precipitated and separated via Buchner funnel as a white powder without further purification. MP = 156.4-157.6 °C; R_f = 0.077 (4:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, methanol-d_4) δ 7.78 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.68 (d, *J* = 2.1 Hz, 1H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.42 (d, *J* = 9.9 Hz, 1H), 5.76 (d, *J* = 9.9 Hz, 1H), 1.44 (s, 6H); ^{13}C NMR (126 MHz, cd_3od) δ 168.28, 157.13, 131.00, 130.85, 127.85, 122.77, 121.16, 120.68, 115.64, 77.12, 27.11; IR (neat): 3056.46, 2974.14, 2863.32, 2812.66, 2546.70 (br), 1666.49 (st), 1596.83, 1596.83, 1444.85, 1270.71, 1128.23; HR-MS (ESI) calcd' $C_{12}H_{12}O_3$ $(M+H)^-$ 204.07864, observed 204.07834

***p*-bromo-(1,1-dimethyl-2-propynyloxy)benzene (70):**



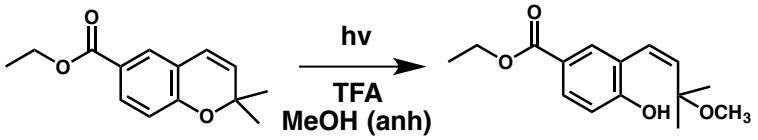
DBU (1.3 mL, 8.7 mmol) was added to a solution of 2-methyl-3-butyn-2-ol (0.64 mL, 6.6 mmol) in anhydrous CH_3CN (3.3 mL, 2M) and cooled in an ice-salt bath (0° C). Trifluoroacetic anhydride (0.95 mL, 6.7 mmol) was added drop-wise and stirred for an additional 30 minutes to produce the propargyl-trifluoroacetate derivative *in situ*. In a second reaction, DBU (1.12 mL, 7.5 mmol) and $CuCl_2{\cdot}2H_2O$ (1.0 mg, 0.006 mmol) was added to a solution of ethyl *p*-bromophenol (1.0 g, 5.8 mmol) dissolved in anhydrous CH_3CN (20 mL, 0.3M), cooled in an ice-salt bath under nitrogen, and the propargyl-trifluoroacetate solution was added drop-wise via cannulation addition. After 5 hours at 0°C, the mixture was concentrated at reduced pressure.[16a] The crude mixture was dissolved in toluene (50 mL) and washed with 1 M HCl (3x25mL), aqueous $NaHCO_3$ (2x25mL) and saturated aqueous brine (2x25 mL) and dried with anhydrous Na_2SO_4. The solvent was removed at reduced pressure[16a] to give the desired product as a light brown oil (0.96 g, 70%) without further purification. R_f = 0.62 (4:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.39 – 7.36 (m, 2H), 7.12 – 7.07 (m, 2H), 2.57 (d, *J* = 0.6 Hz, 1H), 1.63 (d, *J* = 0.7 Hz, 6H); ^{13}C NMR (126 MHz, chloroform-*d*) δ 154.65, 131.84, 123.21, 115.62, 85.65, 74.24, 72.74, 29.49. IR (neat): 3293.32, 3045.95, 2988.20, 2935.98, 2863.15, 2111.28, 1484.85 (st), 1381.61, 1235.11, 1133.78;

6-bromo-1,1-dimethyl-2*H*-chromene (71):



Compound **70** (0.96 g, 4.0 mmol) was dissolved in *N*, *N*-diethylaniline (5 mL, 1 M) and stirred at reflux (210°C) for two hours. Upon completion, the reaction mixture was diluted with Et_2O (50 mL), washed (with caution; highly exothermic) with 6 M aqueous HCl (4x25 mL), followed by saturated $NaHCO_3$ (2x20 mL), saturated NaCl (2x20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed giving crude amber oil that was flushed through a plug of silica yielding light brown oil (0.87%, 91%) without further purification. R_f = 0.62 (4:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.17 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.08 (d, *J* = 2.4 Hz, 1H), 6.65 (dd, *J* = 8.5, 0.7 Hz, 1H), 6.26 – 6.23 (m, 1H), 5.64 (d, *J* = 9.8 Hz, 1H), 1.42 (s, 6H); ^{13}C NMR (126 MHz, chloroform-*d*) δ 151.98, 131.91, 131.52, 128.71, 123.12, 121.30, 118.07, 112.56, 76.53, 27.89. IR (neat): 3043.48, 2974.78, 2926.38, 2866.21, 1477.22, 1360.89, 1260.99, 1195.37, 1163.86, 1127.29;

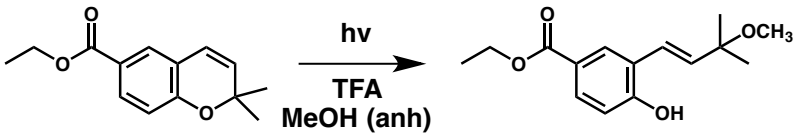
4-ethoxycarbonyl-2-(3-d$_3$-methoxy-cis-isopenten-1-yl)phenol (80):



Compound **68** (1.0 g, 4.3 mmol) was dissolved in methanol (0.1 M) and TFA (0.40 mL, 5.2 mmol) was added. The solution was purged under nitrogen and irradiated

at 300 nm for 3.5 hours. The solution was neutralized with aqueous sodium bicarbonate and methanol was removed under reduced pressure.[16a] The resulting solution was extracted with ethyl acetate (4x25mL), dried with anhydrous sodium sulfate and concentrated. The crude mixture was recrystallized using hexanes providing compound **81** (trans isomer) and compound **80** was found in the mother liquor. The solution was concentrated and purified via column chromatography (4:1 hexanes:ethyl acetate) providing an eggshell white solid (58 mg, 5.0 %). MP = 54.3-56.2 °C; R_f = 0.23 (4:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.69 (s, 1H), 7.86 (ddd, *J* = 8.5, 2.2, 0.6 Hz, 1H), 7.81 (dd, *J* = 2.2, 0.6 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.41 (dd, *J* = 12.8, 0.7 Hz, 1H), 5.91 (d, *J* = 12.8 Hz, 1H), 4.34 (q, *J* = 7.1 Hz, 2H), 3.25 (s, 3H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.29 (s, 6H); ^{13}C NMR (126 MHz, chloroform-*d*) δ 166.43, 157.39, 138.92, 132.37, 130.71, 126.35, 124.50, 122.18, 117.54, 75.08, 60.64, 50.15, 25.56, 14.36; IR (neat): 3354.09, 3002.64, 2970.98, 2926.65, 2904.49, 2815.83, 1675.99 (st), 1596.83, 1498.68, 1359.37, 1273.88, 1074.41; HR-MS (ESI) calcd' $C_{15}H_{20}O_4$ $(M+H)^-$ 264.13616, observed 264.13605

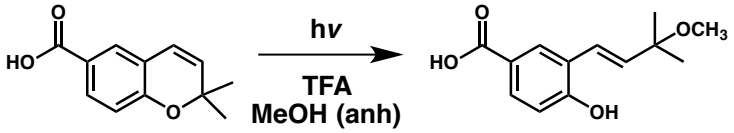
4-ethoxycarbonyl-2-(3-methoxy-trans-isopenten-1-yl)phenol (81):



Compound **68** (1.0 g, 4.3 mmol) was dissolved in methanol (0.1 M) and TFA (0.40 mL, 5.2 mmol) was added. The solution was purged under nitrogen and irradiated at 300 nm for 3.5 hours. The solution was neutralized with aqueous sodium bicarbonate

and methanol was removed under reduced pressure.[16a] The resulting solution was extracted with ethyl acetate (4x25mL), dried with anhydrous sodium sulfate and concentrated. The crude mixture was recrystallized using hexanes provided an eggshell white solid (0.80 g, 70 %). MP = 137.3-139.1 °C; R_f = 0.20 (4:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, methanol-d_4) δ 8.06 (d, J = 2.2 Hz, 1H), 7.75 (dd, J = 8.5, 2.2 Hz, 1H), 6.87 – 6.79 (m, 2H), 6.26 (d, J = 16.5 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 3.22 (s, 3H), 1.39 – 1.35 (m, 9H); ^{13}C NMR (126 MHz, methanol-d_4) δ 166.80, 159.13, 135.12, 129.79, 128.19, 123.85, 123.67, 121.28, 114.85, 75.49, 60.37, 49.33, 24.87, 24.86; HR-MS (ESI) calcd' $C_{15}H_{20}O_4$ $(M+H)^-$ 264.1362, observed 264.1367

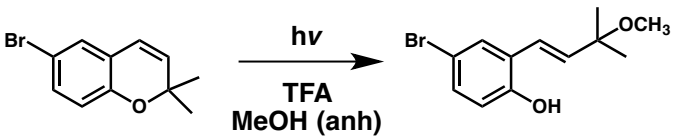
4-hydroxy-3-(3-methoxy-trans-isopenten-1-yl)benzoic acid (82)



Compound **69** (0.330 g, 1.62 mmol) was dissolved in methanol (0.1 M) and TFA (0.12 mL, 1.6 mmol) was added. The solution was purged under nitrogen and irradiated at 300 nm for 3.5 hours. The desired product instantly crystalized upon cooling. The solid was recrystallized using methanol provided an eggshell white solid (0.288 g, 75 %). MP = 135.3-136.3 °C; R_f = 0.1 (4:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, methanol-d_4) δ 8.09 (d, J = 2.2 Hz, 1H), 7.77 (dd, J = 8.5, 2.2 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.84 (d, J = 16.4 Hz, 1H), 6.27 (d, J = 16.5 Hz, 1H), 3.23 (s, 3H), 1.39 (s, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 168.59, 159.04, 135.02, 130.11, 128.52, 123.78, 123.71, 121.48, 114.78, 75.49, 49.32, 24.85; IR (neat): 3176.78 (br), 3046.97, 2986.81, 2958.31, 2515.04

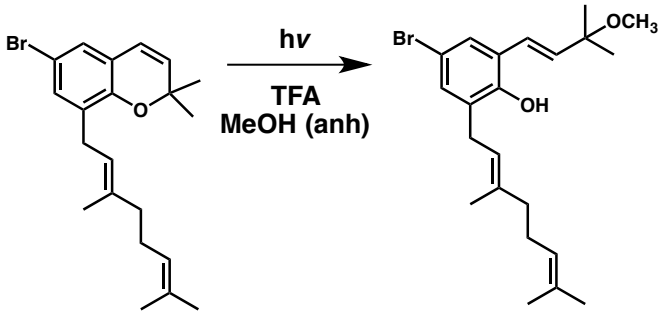
(br), 1672.82, 1600.00, 1498.68, 1372.03, 1273.88, 1137.73,1049.08; HR-MS (ESI) calcd' $C_{14}H_{16}O_4$ $(M+H)^-$ 236.10486, observed 236.10536.

4-bromo-2-(3-methoxy-trans-isopenten-1-yl)phenol (83)



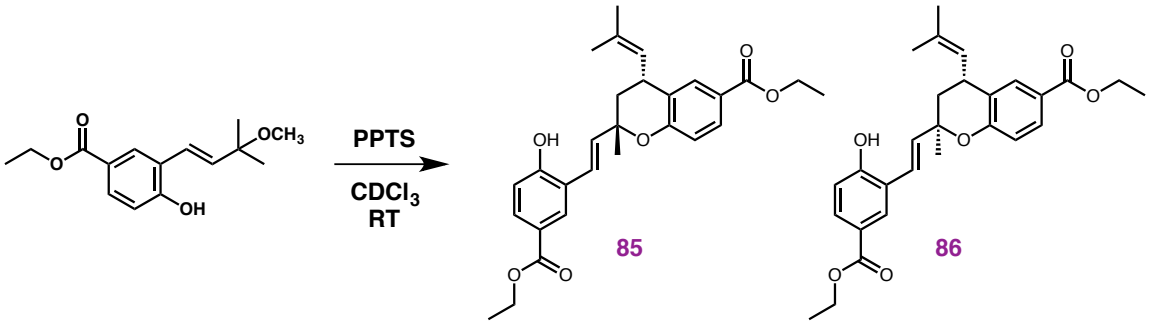
Compound **71** (0.503 g, 2.10 mmol) was dissolved in methanol (0.1 M) and TFA (0.16 mL, 2.1 mmol) was added. The solution was purged under nitrogen and irradiated at 300 nm for 4 hours. The solution was neutralized with sodium bicarbonate and the methanol was removed under reduced pressure.[16a] The crude solid was recrystallized using DI-water provided an eggshell white solid (0.33 g, 58 %). MP = 135.5-139.8 °C; R_f = 0.26 (4:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, methanol-d_4) δ 7.49 (d, J = 2.5 Hz, 1H), 7.16 (dd, J = 8.6, 2.5 Hz, 1H), 6.77 (d, J = 16.5 Hz, 1H), 6.71 (d, J = 8.6 Hz, 1H), 6.20 (d, J = 16.5 Hz, 1H), 3.22 (s, 3H), 1.37 (s, 6H); ^{13}C NMR (126 MHz, cd_3od) δ 153.88, 135.18, 130.53, 128.70, 126.10, 123.32, 116.90, 111.01, 75.47, 49.33, 24.82; IR (neat): 3245.25 (br), 3047.52, 2985.31, 2961.99, 2930.89, 1585.75, 1484,67, 1403.02, 1270.84, 1053.13; HR-MS (ESI) calcd' $C_{12}H_{15}BrO_2$ $(M+H)^-$ 270.02554, observed 270.02622.

4-bromo-2-[(2*E*)-3,7-dimethyl-2,6-octadiene-1-yl]-5-(3-methoxy-trans-isopenten-1-yl)phenol (84)



Compound **53** was dissolved in methanol (0.1 M) and TFA (0.16 mL, 2.1 mmol) was added. The solution was purged under nitrogen and irradiated at 300 nm for 4 hours. The solution was neutralized with sodium bicarbonate and the methanol was removed under reduced pressure.[16a] The crude liquid was purified via column chromatography (0.49 g, 88 %). R_f = 0.46 (4:1 hexanes:ethyl acetate); ^{1}H NMR (500 MHz, methanol-d_4) δ 7.36 (d, *J* = 2.0 Hz, 1H), 7.06 (d, *J* = 2.5 Hz, 1H), 6.84 (d, *J* = 16.3 Hz, 1H), 6.12 (d, *J* = 16.3 Hz, 1H), 5.29 (ddp, *J* = 8.7, 6.1, 1.4 Hz, 1H), 5.11 (dddt, *J* = 7.0, 5.6, 2.9, 1.4 Hz, 1H), 3.31 (d, *J* = 7.5 Hz, 2H), 3.22 (s, 3H), 2.16 - 2.11 (m, 2H), 2.10 - 2.07 (m, 2H), 1.70 (d, *J* = 1.6, 3H), 1.68 (d, *J* = 1.3 Hz, 3H), 1.60 (s, 3H), 1.38 (s, 6H); ^{13}C NMR (126 MHz, cd_3od) δ 150.94, 136.86, 135.72, 131.75, 130.99, 130.46, 127.20, 126.08, 123.81, 123.44, 121.33, 112.12, 75.47, 49.37, 39.37, 27.72, 26.12, 24.78, 24.57, 16.39, 14.77; IR (neat): 3284.67 (br), 3051.40, 2977.54, 2919.22, 2845.36, 1659.61, 1453.56, 1375.81, 1247.52, 1189.20, 1156.87, 1076.46, 1049.24; HR-MS (ESI) calcd' $C_{22}H_{31}BrO_2$ $(M+H)^-$ 406.14687, observed 406.14770

Dimer (85:86, 4:1):



Compound **81** (10 mg, 0.038 mmol) was dissolved in $CDCl_3$ (0.1 M) and PPTS (5 mg, 0.019 mmol) was added. The solution was stirred at RT monitored via ^1H NMR. After 15 minutes, the solution was concentrated under reduced pressure[16a] and purified via prep TLC (4:1 DCM:hexanes) providing the diasteroisomers **82:83** (6 mg, 60 %, 4:1 d.r). R_f = 0.31 (4:1 DCM:hexanes); ^1H NMR (500 MHz, chloroform-*d*) δ 8.11 (d, *J* = 2.1 Hz, 1H), 7.87 - 7.80 (m, 3H), 6.92 (d, *J* = 16.3 Hz, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.48 (d, *J* = 16.2 Hz, 1H), 5.79 (s, 1H), 5.12 (dq, *J* = 9.0, 1.4 Hz, 1H), 4.41 - 4.32 (m, 4H), 3.82 (ddd, *J* = 11.3, 9.5, 6.0 Hz, 1H), 2.04 (dd, *J* = 13.7, 6.0 Hz, 1H), 1.83 (d, *J* = 1.4 Hz, 3H), 1.82 (d, *J* = 1.6 Hz, 3H), 1.53 (s, 3H), 1.44 – 1.34 (m, 7H); ^{13}C NMR (126 MHz, $cdcl_3$) δ 166.78, 166.35, 157.43, 156.84, 137.21, 134.07, 131.29, 130.41, 129.40, 129.38, 126.56, 124.44, 123.85, 123.29, 122.37, 121.44, 117.25, 115.65, 77.11, 60.81, 60.58, 39.05, 31.62, 25.85, 24.17, 18.06, 14.40, 14.38; HR-MS (ESI) calcd' $C_{28}H_{32}O_6$ $(M+H)^-$ 464.21989, observed 464.21947

3.8 References

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13) Purchased from Sigma-Aldrich Chemical Company (http://www.sigmaaldrich.com/united-states.html)

14) Purchased from VWR International - Chemicals and Laboratory Scientific (https://en.wikipedia.org/wiki/VWR_International)

15) Purchased from SiliaFlash – Silicycle (http://www.silicycle.com/products/siliaflash-irregular-silica-gels)

16) a) Instrumentation and use provided by the department of chemistry, UNR. b) Data and values are tabulated from the University instrumentation.

17) Cambridge Isotope Labortories, Inc. (http://www.isotope.com/applications/subapplication.cfm?sid=Deuterated%20NMR%20Solvents_18)

Chapter 4: Dienogest Photo-transformation Products as Potential Endocrine Disruptors

4.1 Introduction

Trenbolone acetate (TBA) is a widely used synthetic growth hormone that is injected into cattle in a number of countries including the United States to increase muscle growth and appetite of the cattle.[1-3] Cattle at feed lots are implanted with slow-releasing steroidal hormones that can contain both natural and semi-synthetic steroids to promote growth, such as 17β –TBOH **33** (40-200 mg) doped with estradiol **30** (8-20 mg)[4] (Figure 4.1.1).

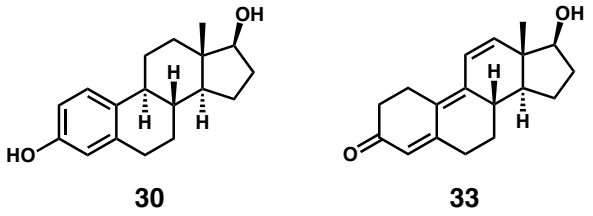
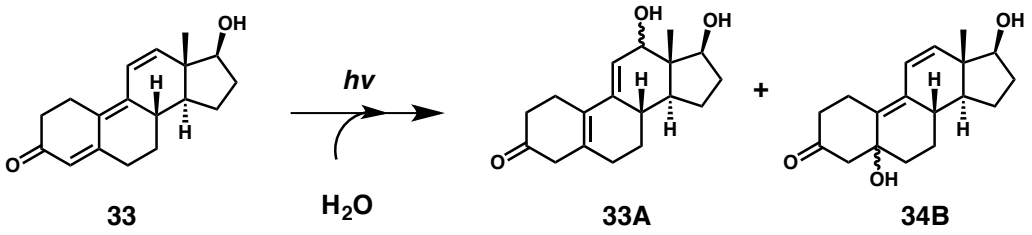


Figure 4.1.1 Natural hormone, estradiol, and semi-synthetic hormone, 17β –TBOH.

The FDA requires information and/or toxicological testing in laboratory animals to determine safe levels in the animal products that we eat (edible tissues). In addition, the FDA required that the manufacturers determine the amount of hormones left in each edible tissue after treatment is below the appropriate safe level.[5] According to the FDA, a safe level for human consumption is a level of drug in the meat that would be expected to have no harmful effect in humans based on extensive scientific study and review.[5] The hormone concentrations within these implants are considered to be a safe level to have no

harmful effects in humans;[5] however, they did not consider what effects these hormones could have on aquatic organisms when excreted into natural water systems. Although only trace amounts of the added hormones were found to remain in edible tissue, these metabolites were found in the cattle's manure at concentrations between 5-75 ng TBA/g manure.[6] When these metabolites reach into streams or ponds, they can disrupt the endocrine systems of aquatic life at concentrations as low as 10 ng/L effecting sex ratios and fecundity in fish.[3,7-8] Industrial studies for manufacture approvals specifically states minimal ecosystem risks of TBA metabolites due to their rapid photodegradation to their hydroxylated analogs, **33A** and **33B** (Scheme 4.1.2).[3]



Scheme 4.1.2 Proposed aqueous photodegradation of 17β –TBOH to their hydroxylated analogs.

In 2013 Qu and coworkers studied the stability of these photoproducts and found that they regenerate to the parent compound in the absence of light upon dehydration.[3] This mode of reactivity was found to occur under neutral conditions at ambient temperature.[3] Recently, similar reactivity characteristics were found to occur in dienogest (DIE) (Figure 4.1.2), a potent, steroidal progestin used as an oral contraceptive.

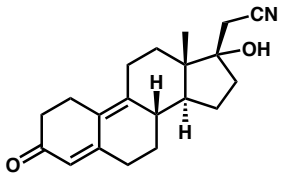


Figure 4.1.2 Dienogest

DIE is structurally similar to trenbolone acetate (TBA) and has been found to photo-transform of an unstable product, which reverts to the starting material in the absence of light.[3] Unfortunately, photoproducts of dienogest have yet been isolated and characterized. Our group has generated preliminary data on the photo-transformation of DIE. The consumption and reversion of DIE was monitored using modern spectroscopy instrumentation (e.g. ^{1}H-NMR and/or High Performance Liquid Chromatography). We were able to monitor the formation of DIE's photoproducts as well as the reversion back to the parent DIE molecule when left in the dark by ^{1}H-NMR using cyclooctadiene (COD) as a reference peak.

4.2 Preliminary dienogest photo-transformation study

In this study, DIE was dissolved in ACN/D_2O solvent system and the photodegradation of the parent compound was observed via ^{1}H-NMR. To determine the half-life of DIE's photodegradation, we dissolved DIE in d_3-ACN/D_2O solvent mixture (5 μM, 9:1 v:v), the sample was irradiated in a NMR tube, and a time series of ^{1}H-NMR spectra were obtained over 45 minutes of total irradiation. Quantitative values were

obtained by integration of key proton against cyclooctadiene (COD) internal standard (Figure 4.2.1).

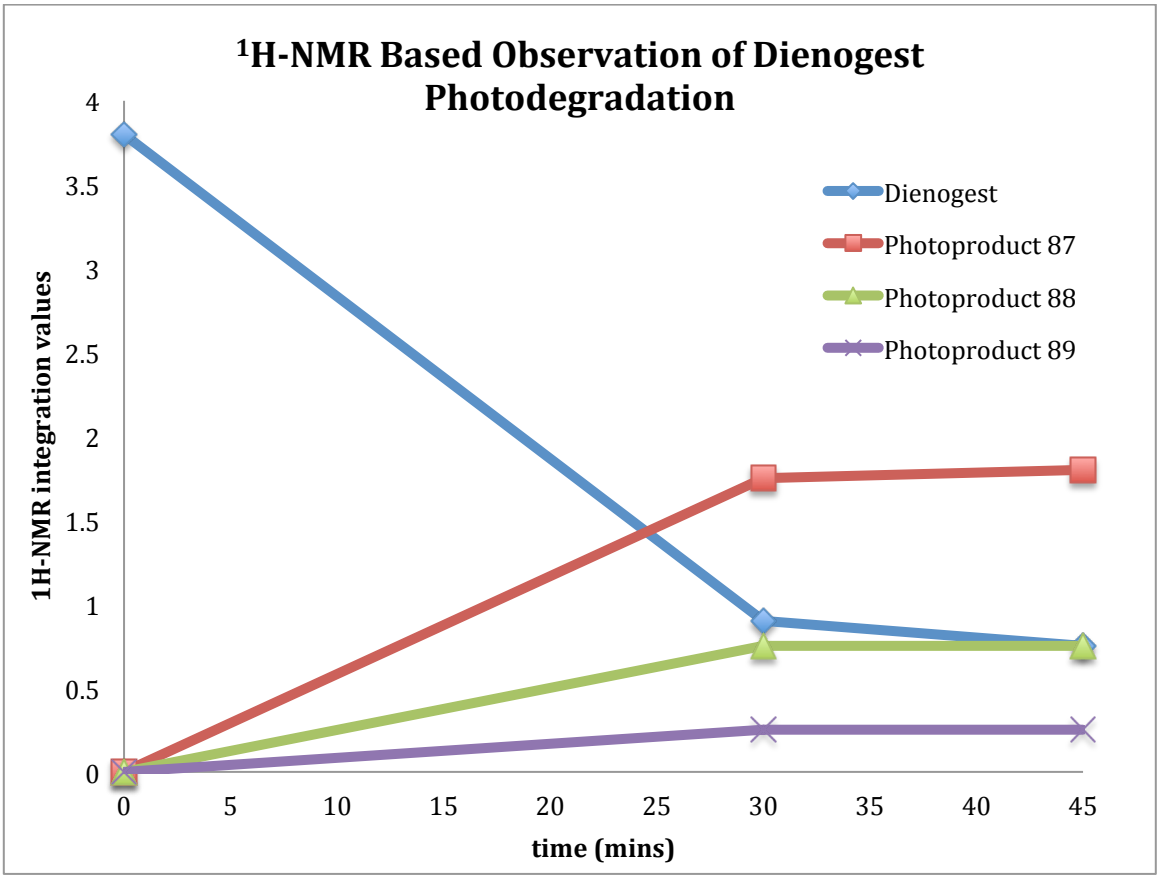


Figure 4.2.1 Dienogest photodegradation after 45 minutes of irradiation.

From this initial observation, DIE photodegrades into two major products, photoproduct **87** and photoproduct **88**, in the first 30 minutes, in addition to a minor photoproduct **89**. DIE photodegradation plateaus at % 80 conversion, and upon additional light exposure, the compounds within the mixture photodegrade. After 45 minutes of irradiation, the mixture was left in the dark and ^{1}H-NMR spectra were recorded periodically to observe

for any signs of DIE's recovery. Similar to DIE's photodegradation study, ^{1}H NMR spectra were obtained at various time points when the mixture was left in the dark for two days (Figure 4.2.2).

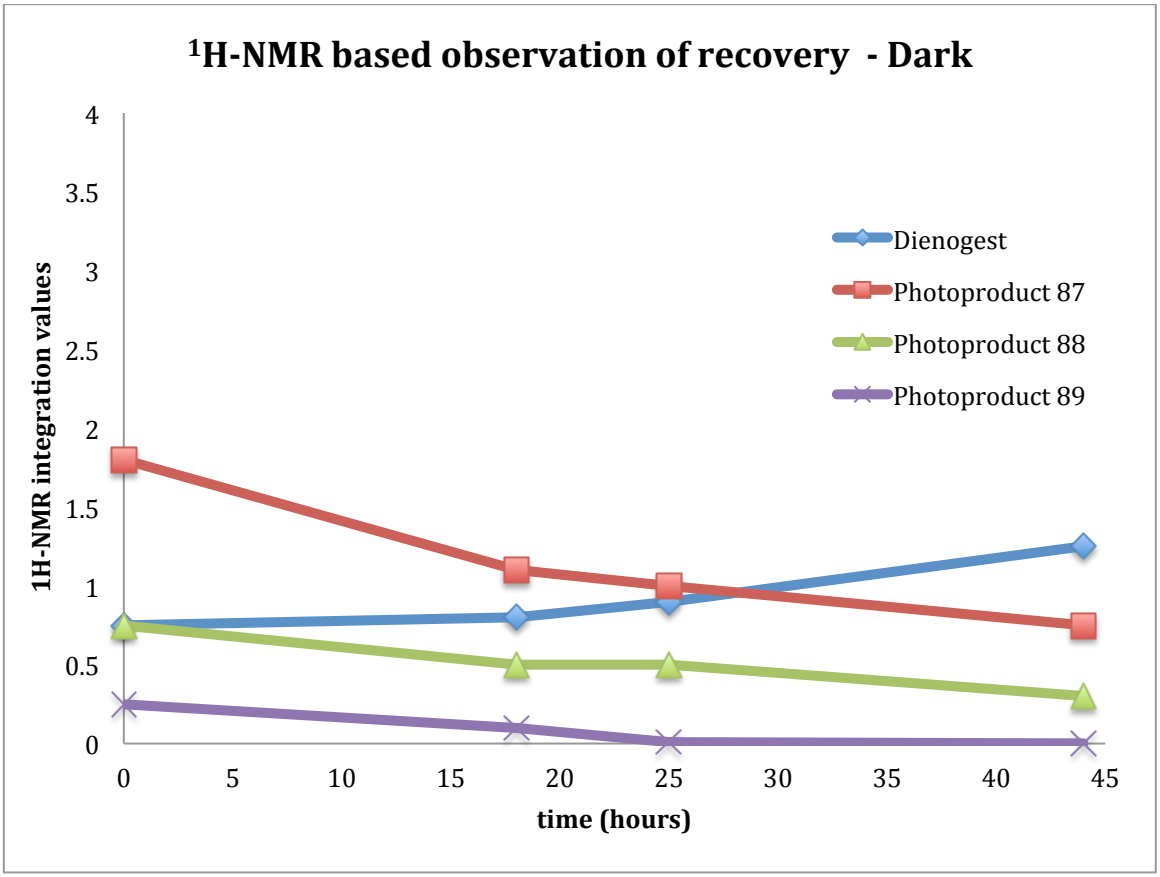


Figure 4.2.2 Observation of dienogest recovery in the dark.

In this study, integration of the ^{1}H NMR spectra show an increase of DIE's alkene proton, indicating that DIE is regenerating in the dark. From this study and NMR spectra, we speculate a dimer photoproduct **87** via [2+2] cycloaddition or another reversibly formed intermediate, in addition to two aromatic A photoproducts **88** and **89** (Figure

4.2.3). Although these assignments are tentative, they correlate well with literature steroidal compounds.[10-11] From these results, we believe that the dimerization or hydration of the steroid is the major photoproduct. This mode of dimerization via photolysis is not uncommon and has been established previously by Williams and coworkers in their photochemical study of estr-4-en-3-ones, a steroidal analog.[11] They provided evidence of thermal ring opening of the dimer to regenerate the monomer steroid at room temperature.[11]

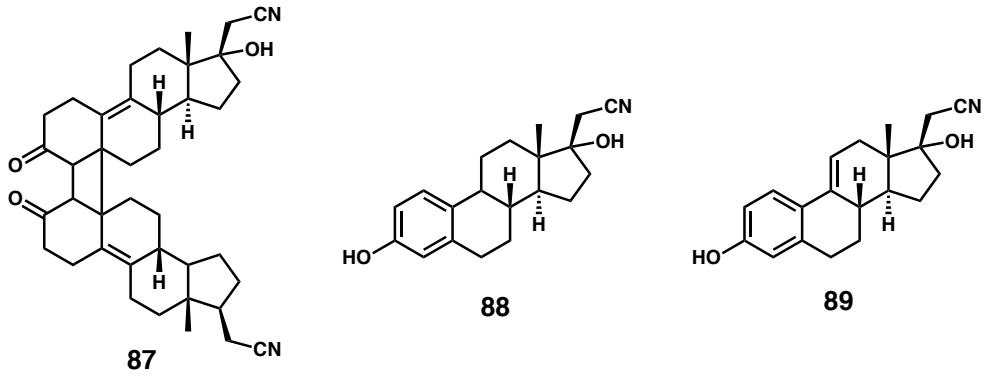


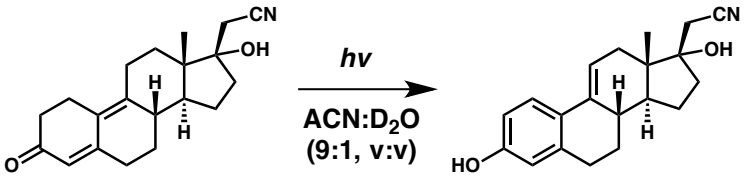
Figure 4.2.3 Predicted dienogest photoproducts.

This project is still ongoing and current efforts are being applied towards the isolation, separation and characterization of these photoproducts. Unfortunately, due to the instability of the products, isolation and separation has been proven to be quite difficult and only evidence of photoproduct **86** has been isolated. We plan on continuing with this NMR based study of the photodegradation and regeneration of DIE, in addition to isolating all photoproducts.

4.3 Experimental Procedures

Reactions were carried out under inert atmosphere of nitrogen (N_2) gas in clean, oven-dried glassware (Pyrex) with magnetic stirring, unless otherwise specified. All reagents and solvents were purchased from Sigma-Aldrich Chemical Company and used without further purifications.[12] TLC was recorded and performed on Silicycle glass 60 F254 plates[13] and were observed under UV shortwave light or developed by staining with $KMnO_4$ or CAM. Reactions were purified using flash chromatography with Silicycle Siliaflash® P60 (230-400 mesh).[14] Photolysis was conducted on a Rayonet photoreactor, model RPR-200, using 2537Å, 3000Å, or 3500Å light bulbs. ^{1}H-NMR spectra were measured on Varian 400 (400 MHz)[15a] or Varian 500 (500 MHz) spectrometers[15a] and are reported in ppm (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad);[15b] integration; coupling constant(s) in Hz, using *d*-$CDCl_3$ (7.26 ppm, with 0.01% TMS at 0.00 ppm) or in d_4-MeOD (3.31 ppm) or d_3-CAN (1.94 ppm) as locking solvents. ^{13}C-NMR spectra were measured on Varian (101 MHz)[15a] or Varian 500 (126 MHz) spectrometers[15a] and are reported in ppm using *d*-$CDCl_3$ (77.16 ppm, with 0.01% TMS at 0.00 ppm) or in d_4-MeOD (49.00 ppm) or d_3-ACN (118.26 and 1.32 ppm) as locking solvents. Infrared (IR) spectra were recorded on a Nicolet 6700 FT-IR with a diamond ATR[15a] and the bands reported in cm^{-1} (br = broad, st = strong).[15] High-resolution mass spectra were obtained using an Agilent 6230 TOF LC/MS with an atmospheric pressure photo-ionization (APPI, with C60 and anthracene internal standards) or electrospray (ESI, with purine and HP-0921 internal standards).[15a]

Photoproduct 86:



Dienogest (10 mg, 0.032 mmol) was dissolved in d-ACN/D_2O (0.04 M, 0.8 mL 7:1 v:v) and irradiated at 300 nm for 6 hours. The reaction was extracted with ethyl acetate (4x2 mL) dried with anhydrous sodium sulfate and concentrated at reduced pressure.[15a] The crude product was purified via column chromatography (3:1 ethyl acetate:hexanes) providing the aromatic A ring product (1.5 mg, 15 %). R_f = 0.7 (3:1 ethyl acetate:hexanes); ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.46 (d, *J* = 8.7 Hz, 1H), 6.62 (dd, *J* = 8.6, 2.9 Hz, 1H), 6.53 (d, *J* = 2.7 Hz, 1H), 6.15 – 6.04 (m, 1H), 4.67 (s, 1H), 2.89 – 2.45 (m, 4H), 2.25 – 2.14 (m, 2H), 2.10 – 2.02 (m, 1H), 2.04 – 1.85 (m, 4H), 1.56 – 1.25 (m, 4H), 0.95 (d, *J* = 1.0 Hz, 3H).

4.4 References

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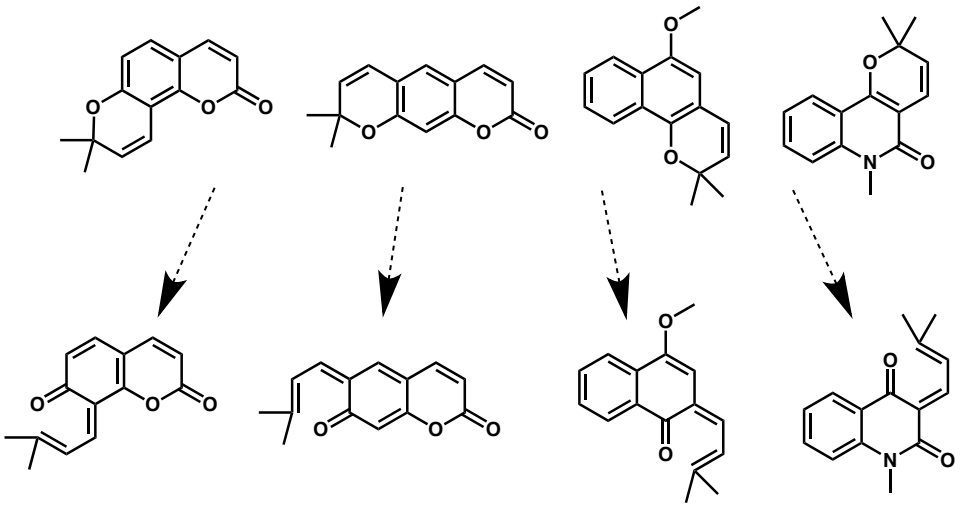
11. Williams, J.R.; Mattei, P.L.; Abdel-Magid, A., Blount, J.F. *J. Org. Chem.* **1986**, *51*(6), 769-773.
12. Purchased from Sigma-Aldrich Chemical Company (http://www.sigmaaldrich.com/united-states.html)
13. Purchased from VWR International - Chemicals and Laboratory Scientific (https://en.wikipedia.org/wiki/VWR_International)
14. Purchased from SiliaFlash – Silicycle (http://www.silicycle.com/products/siliaflash-irregular-silica-gels)
15. a) Instrumentation and use provided by the department of chemistry, UNR. b) Data and values are tabulated from the University instrumentation.
16. Cambridge Isotope Labortories, Inc. (http://www.isotope.com/applications/subapplication.cfm?sid=Deuterated%20NMR%20Solvents_18)

Chapter 5: Conclusions and Future Work

5.1 Conclusion and Future Work

Over the past twenty years natural products from the Genus *Piper* family have been extensively studied due to their wide range of biologically activity and unique structures.[1-2] A plethora of these natural products or plants secondary metabolites have been isolated from *Piper* and have been found to have defensive properties towards their herbivores and the herbivore's associated predators.[3-5].[6-8] Our group has recently isolated three acid derivatives found in the methanolic extract of the leaves of *Piper kelleyi*, a newly described species of *Piper* that grow in the Andes cloud forests.[6 5] We have devised a method to prepare these biosynthetically related natural products in order to comprehensively study their biological activity. Initially, the bromo phenol **36** was oxidized and the chromene **37** was produced. Unfortunately, this method lacked regioselectivity and oxidative cyclization occurred on both the prenyl and geranyl side chains. After additional studies we developed a scalable synthesis of the chromene **37** in 5 steps with an overall yield of 30%. Our next goal, was synthesizing the dimer chromane **38**, however, we found that direct irradiation of the chromene failed to provide the desired dimeric product. Further studies of a model chromene revealed that an *o*-quinone methide was produced upon irradiation with UV light. Preparative studies demonstrated that the *o*-quinomethide can be trapped as a methanol adduct. This methanol adduct can serve as a precursor to the *o*-quinone methide and can undergo subsequent dimerization under using an acid catalyst in non-polar solvents (e.g. chloroform). This mode of dimerization is proposed to occur through a hetero [4+2] cycloaddition between the *o*-quinomethide and its tautomeric diene, which provides both dimer diastereomers as low

as a 4:1 ratio. Future studies will involve controlling diastereoselectivity of the dimer and applying these methods towards our desired chromene **37** to produce the chromane dimer **38**. This new photoinitiated method of generating the *o*-quinomethide intermediate enables the further utility as a method for the preparation of a variety of natural products libraries (Scheme 5.1.1).



Scheme 5.1.1. Chromene containing compounds for studying photochromic properties

Recent studies show that steroidal analogs, such as TBA, found in our water systems were regenerating to the dark and directly affecting aquatic organisms.[8] Our study of the aquatic photochemistry of DIE demonstrated that DIE photodegrades rapidly into three potential photoproducts. In addition, we've provided evidence of regeneration of DIE in the dark via ^{1}H NMR. Due to the sensitivity of these photoproducts, isolation has proven to be difficult and only tentative evidence of photoproduct **86** has been established. Future goals of this project are directed toward the isolation and full

characterization all photoproducts of DIE and evaluate the estrogenic and androgenic properties of all stable products.

5.2 References

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Appendix

A.1 ^{1}H and ^{13}C NMR spectra

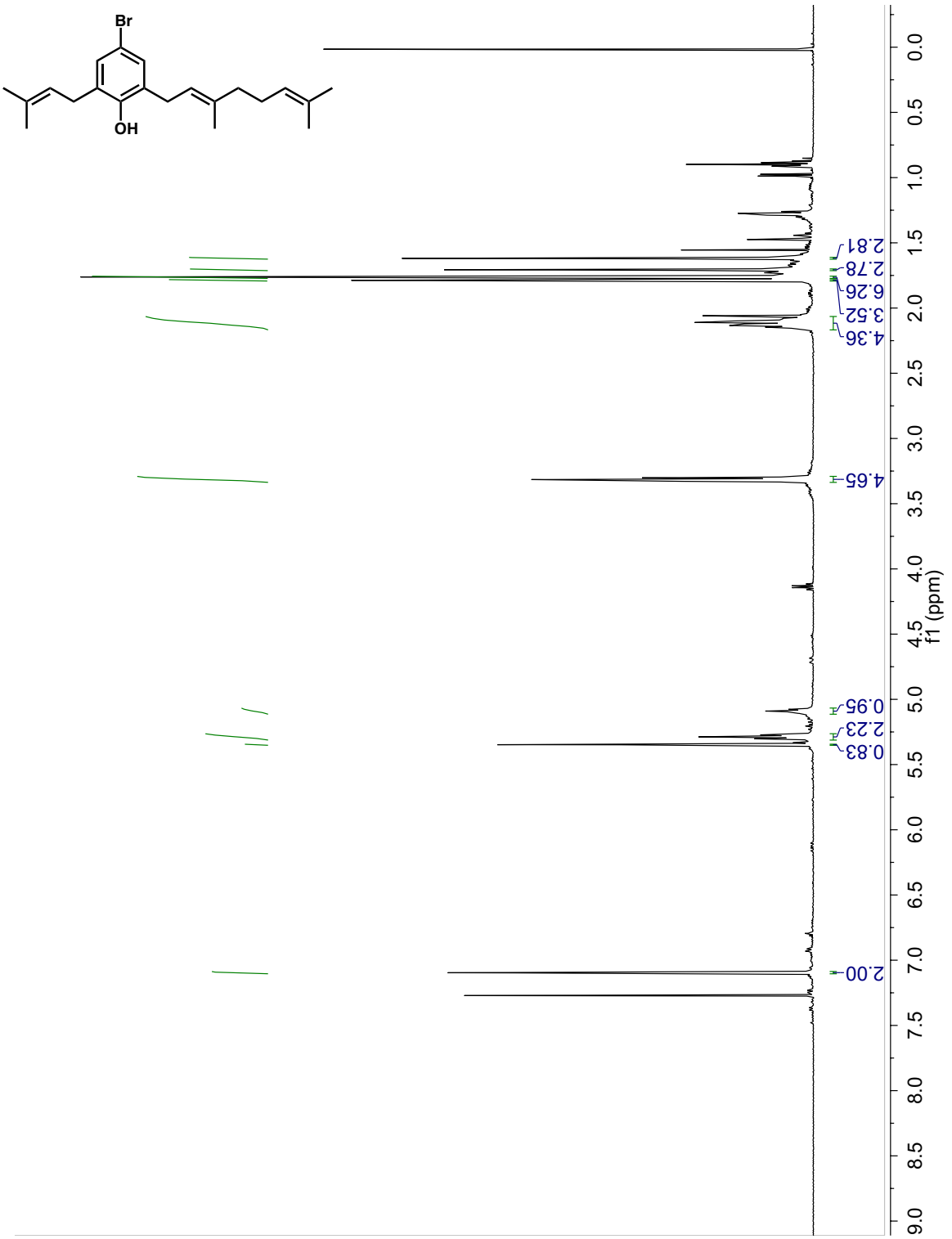

Figure A.1.1 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **48**

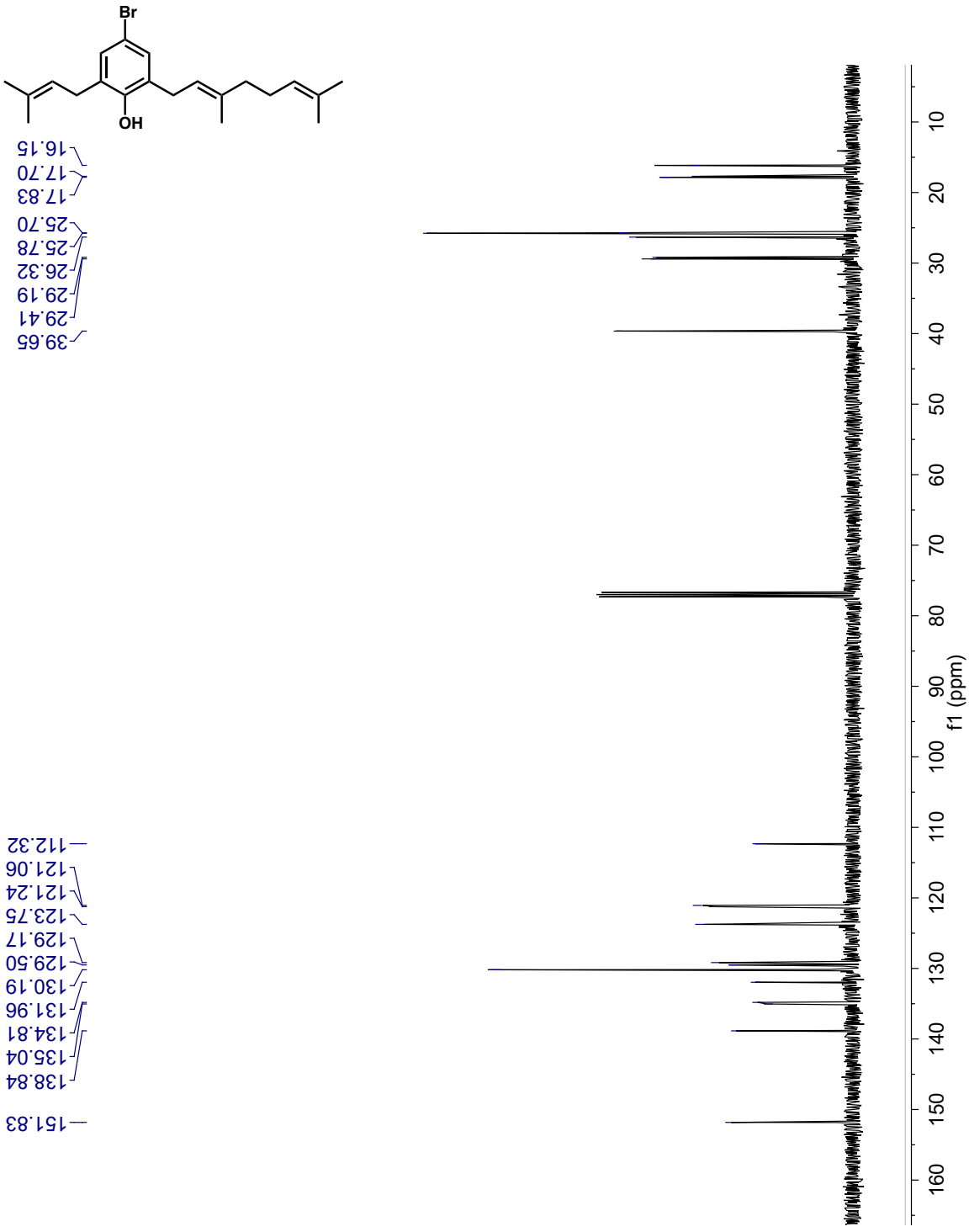

Figure A.1.2 ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **48**

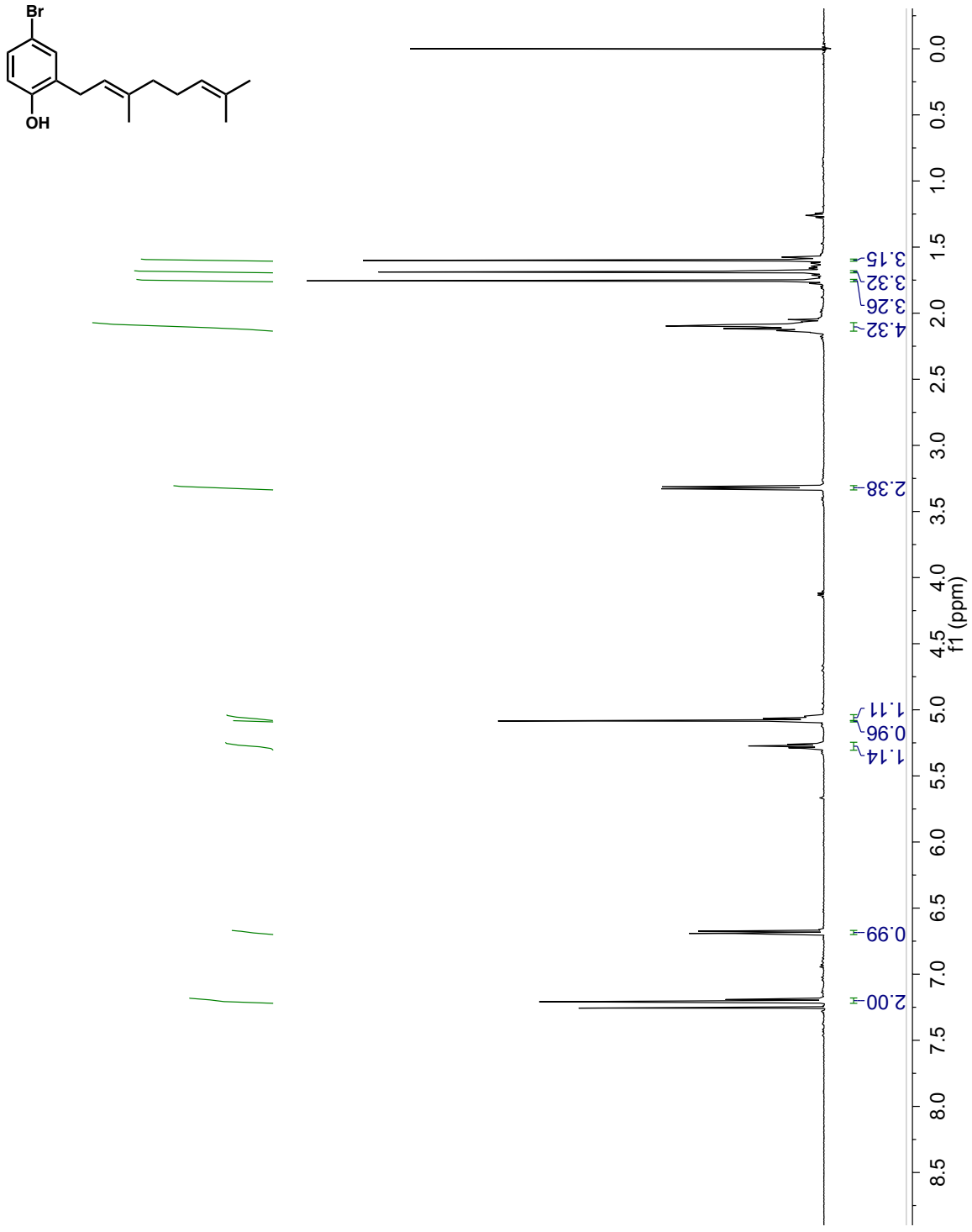

Figure A.1.3 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **51**

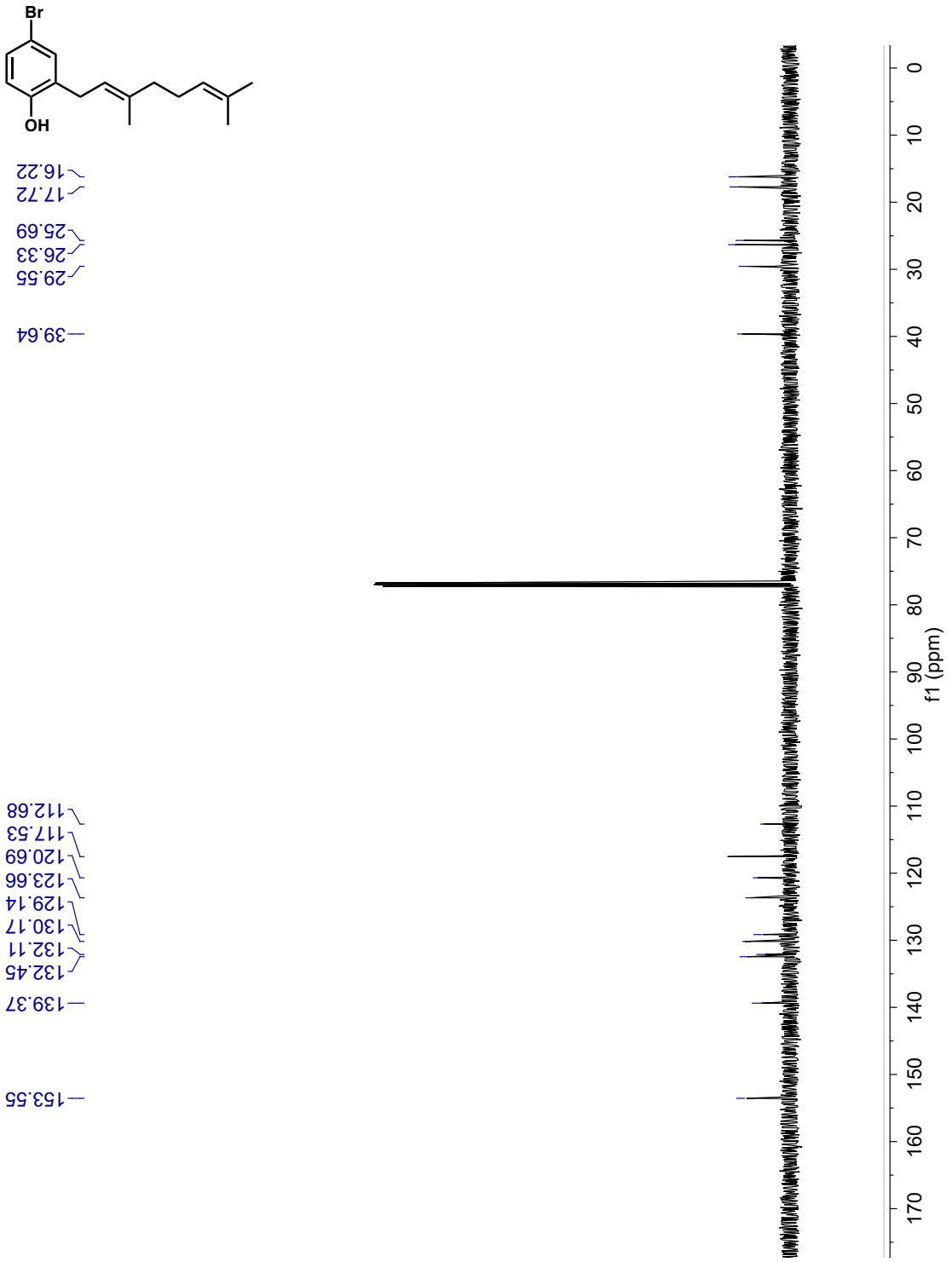

Figure A.1.4 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **51**

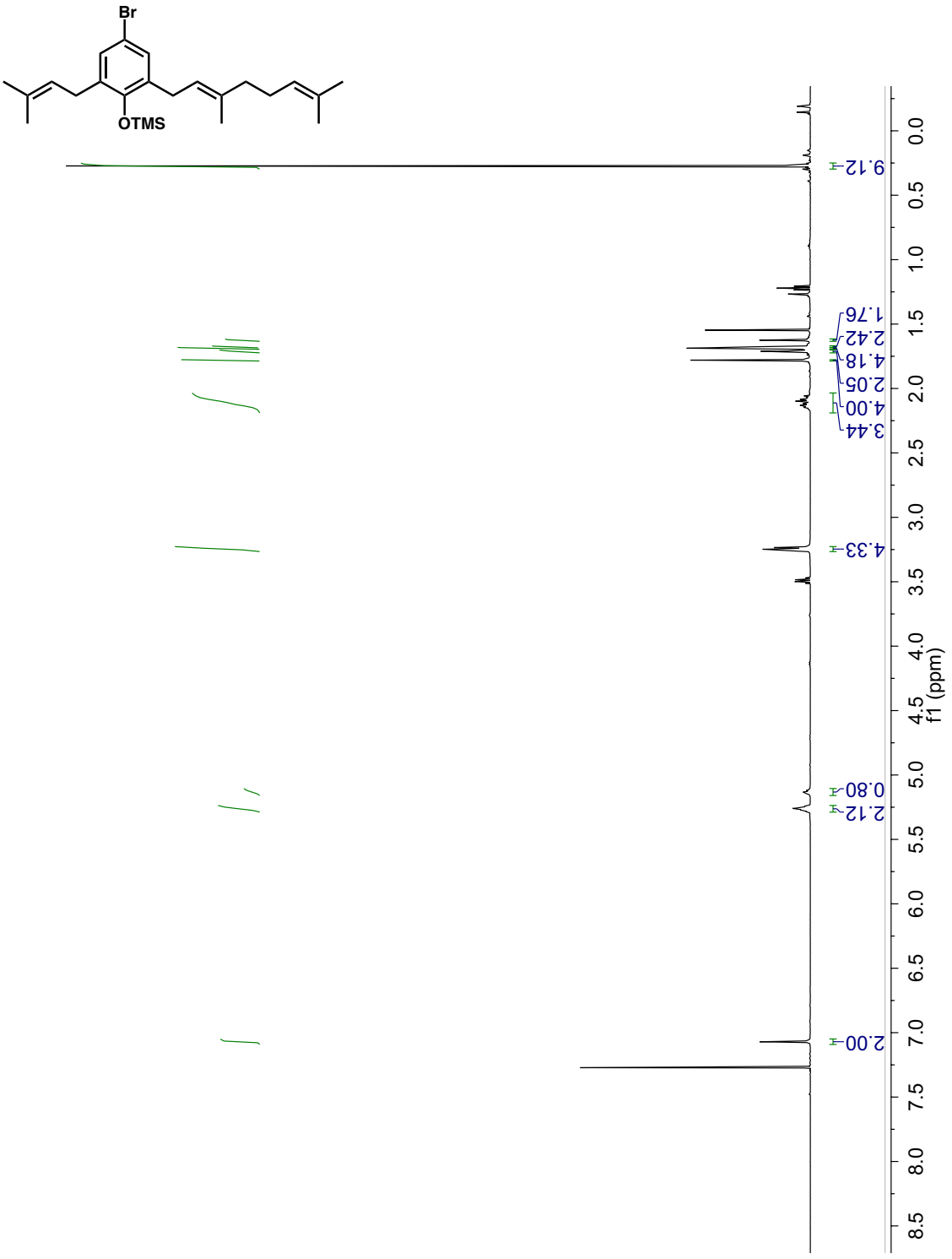

Figure A.1.5 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **54**

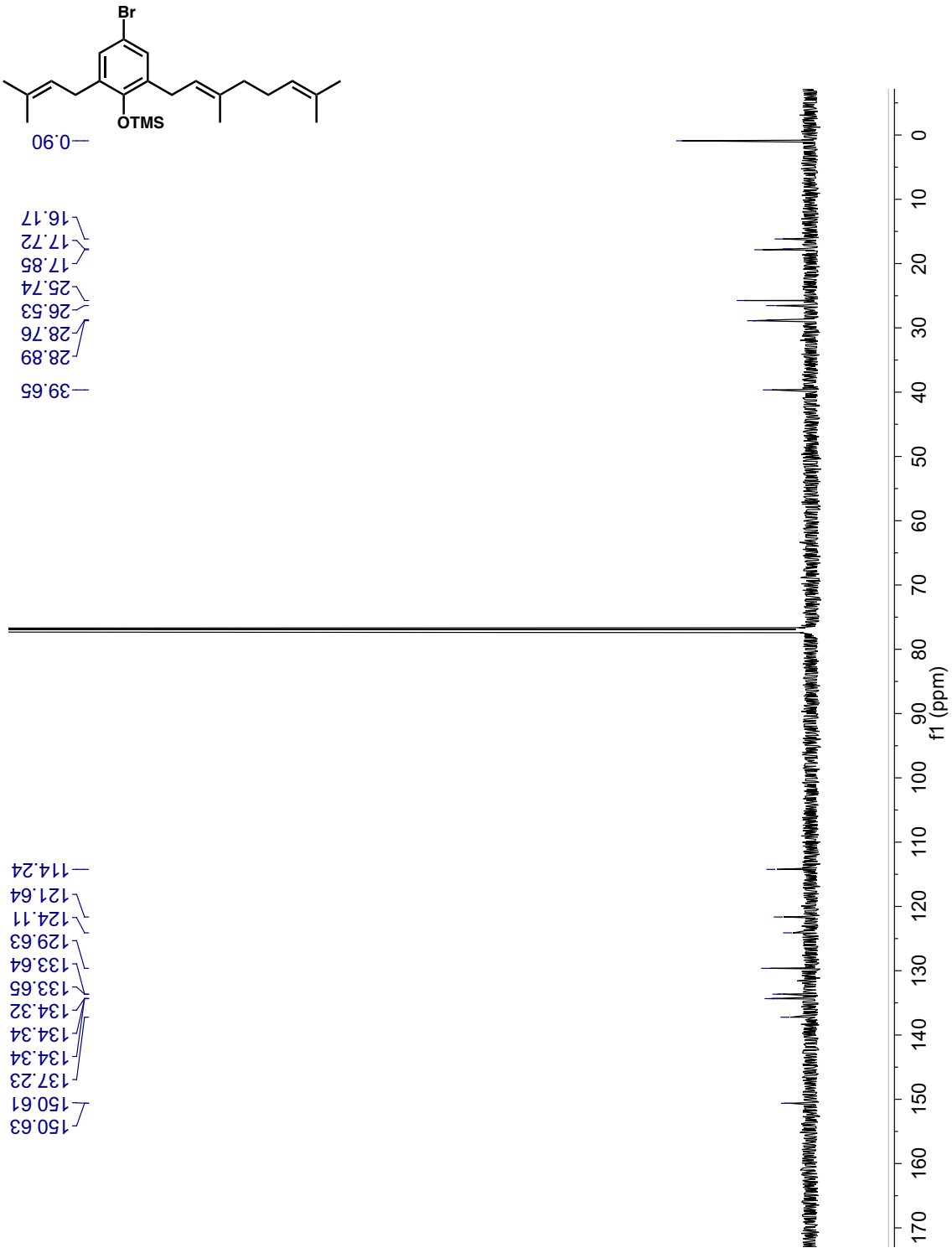

Figure A.1.6 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **54**

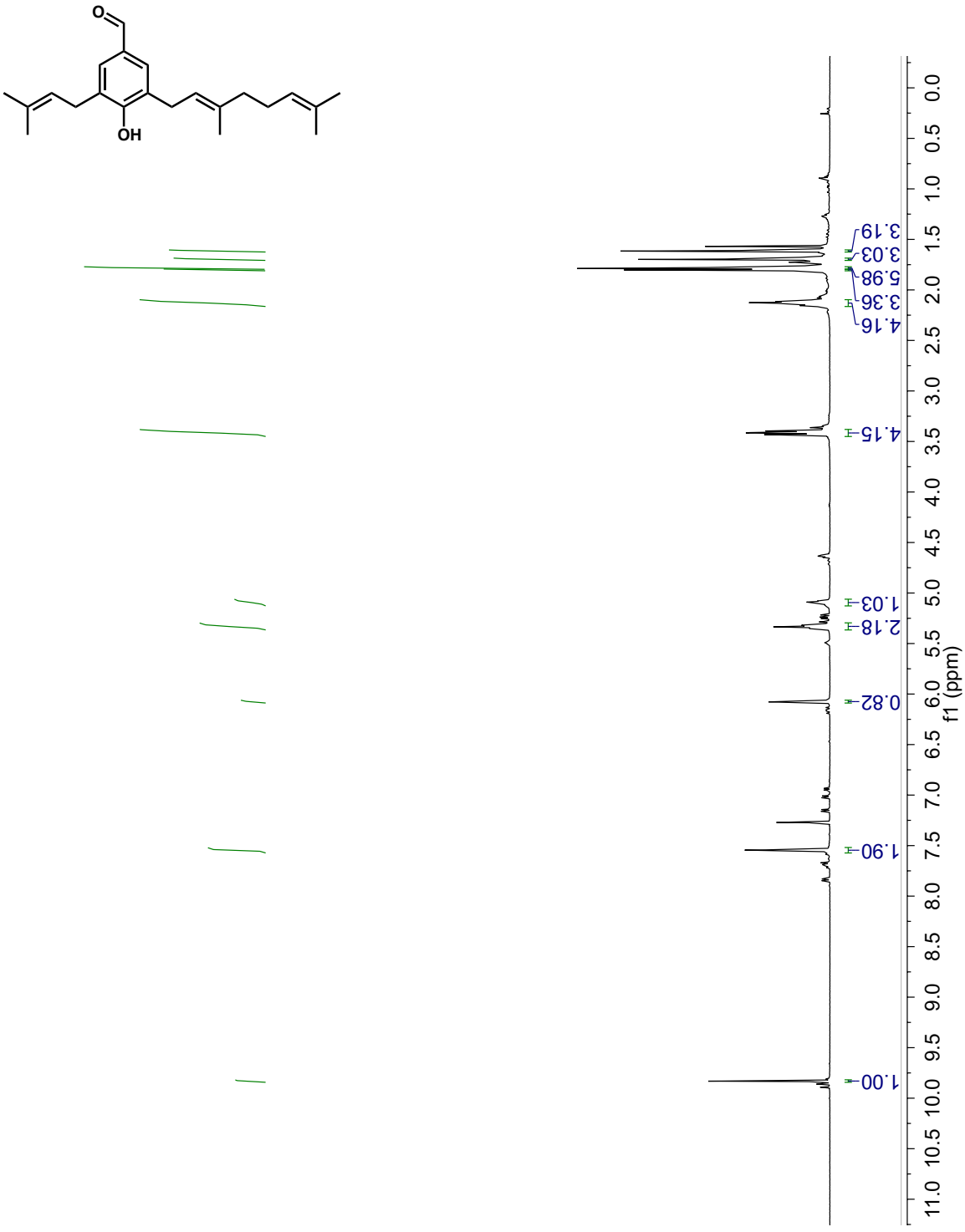

Figure A.1.7 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **56**

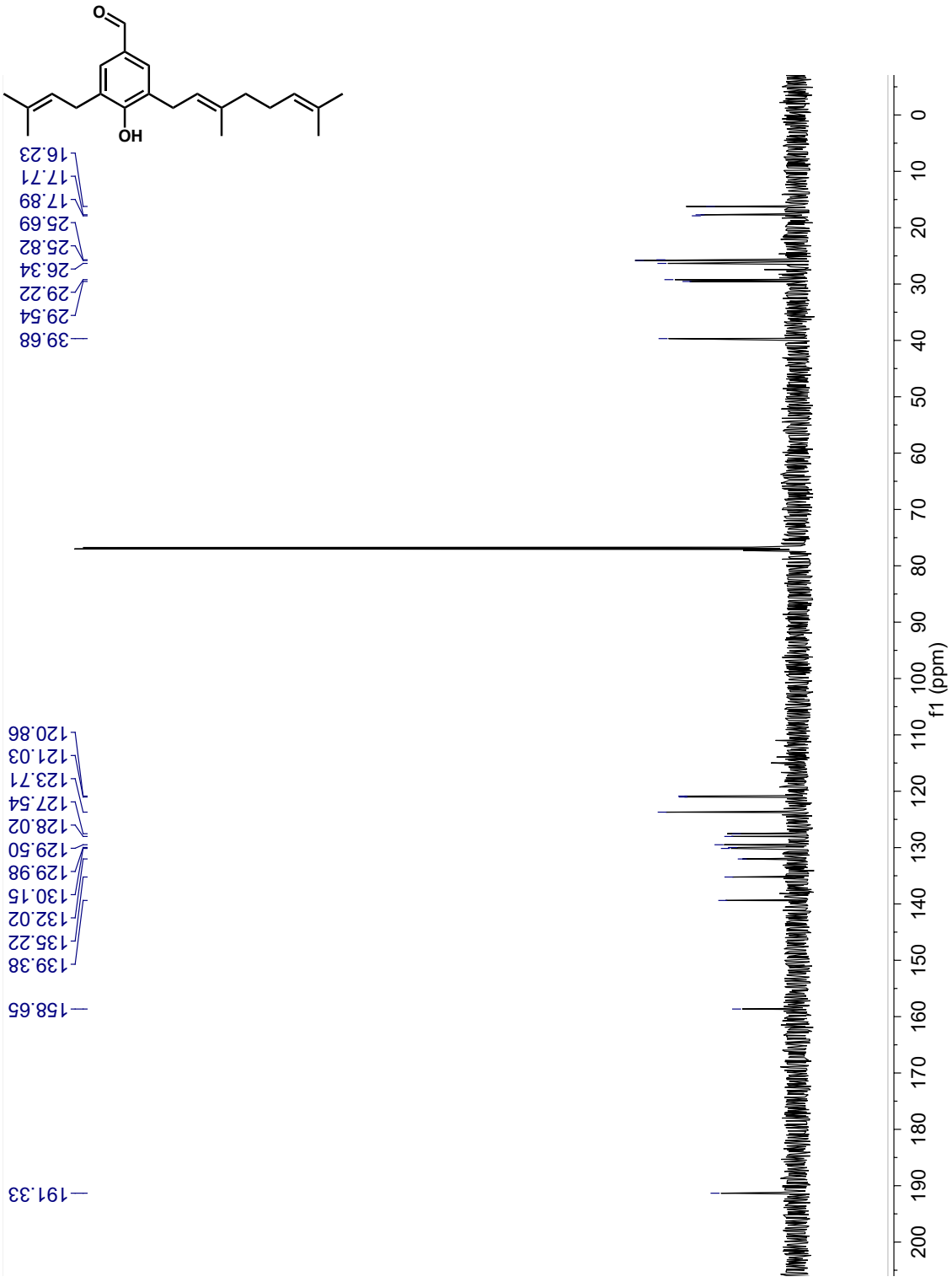

Figure A.1.8 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **56**

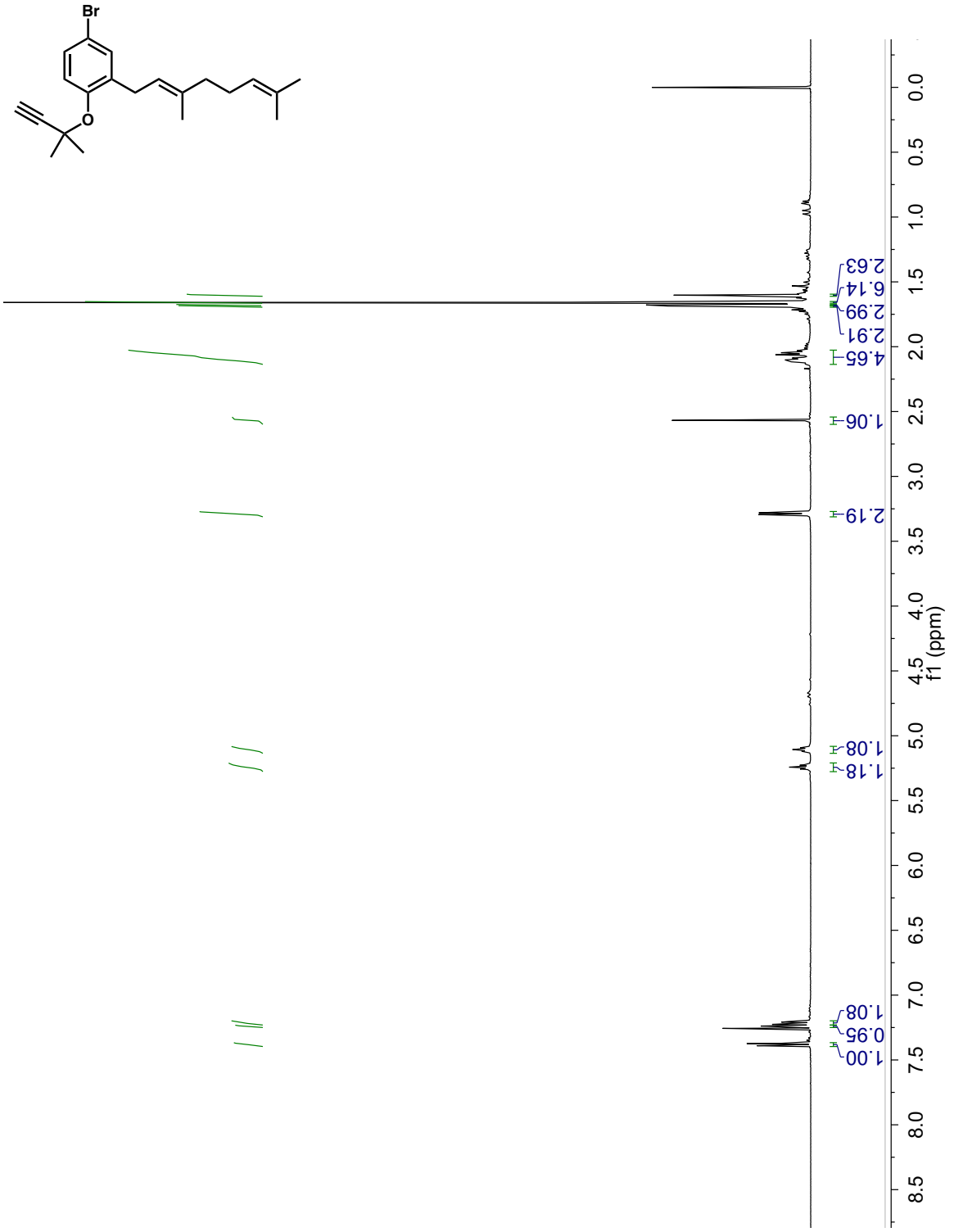

Figure A.1.9 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **58**

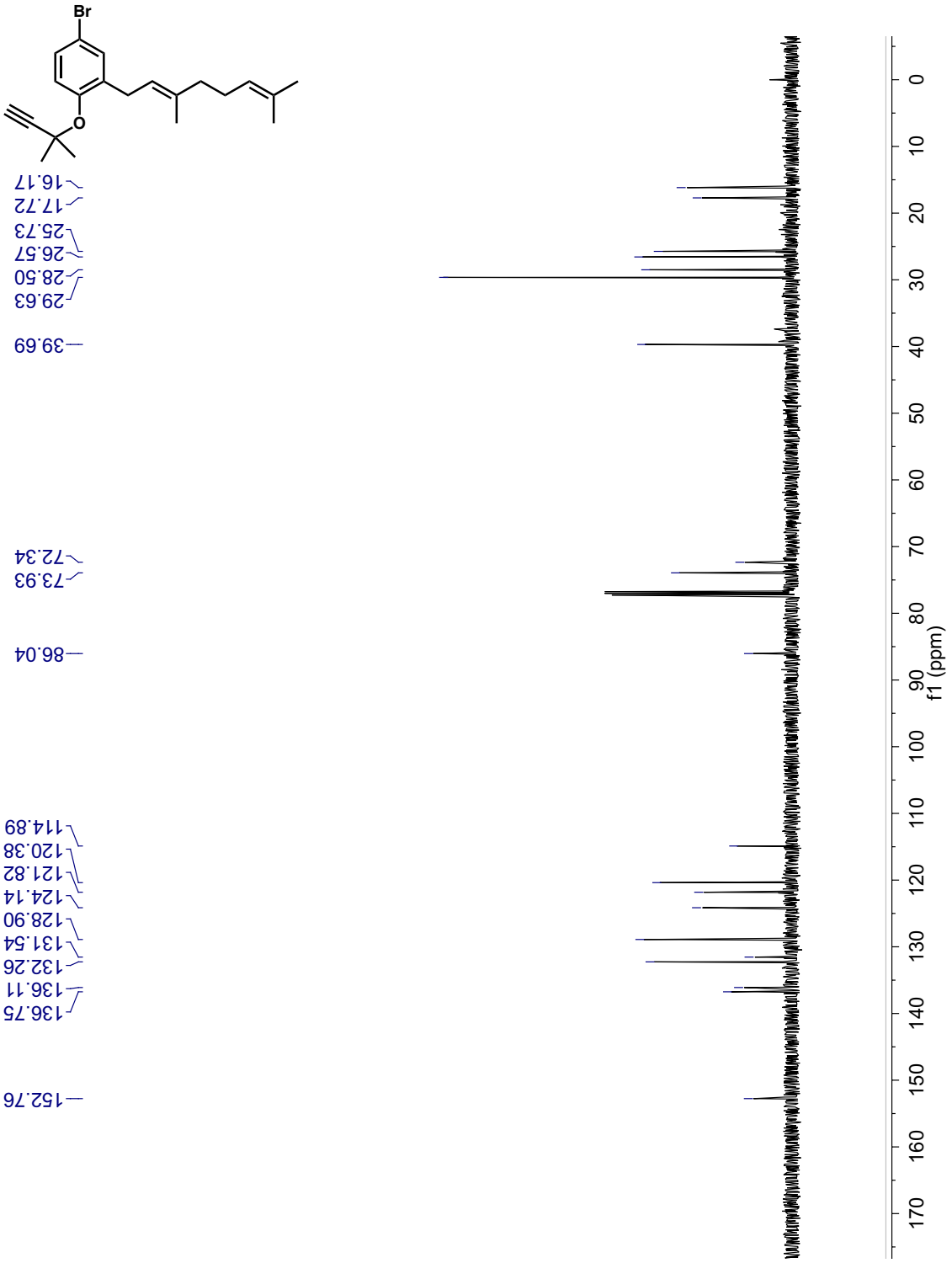

Figure A.1.10 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **58**

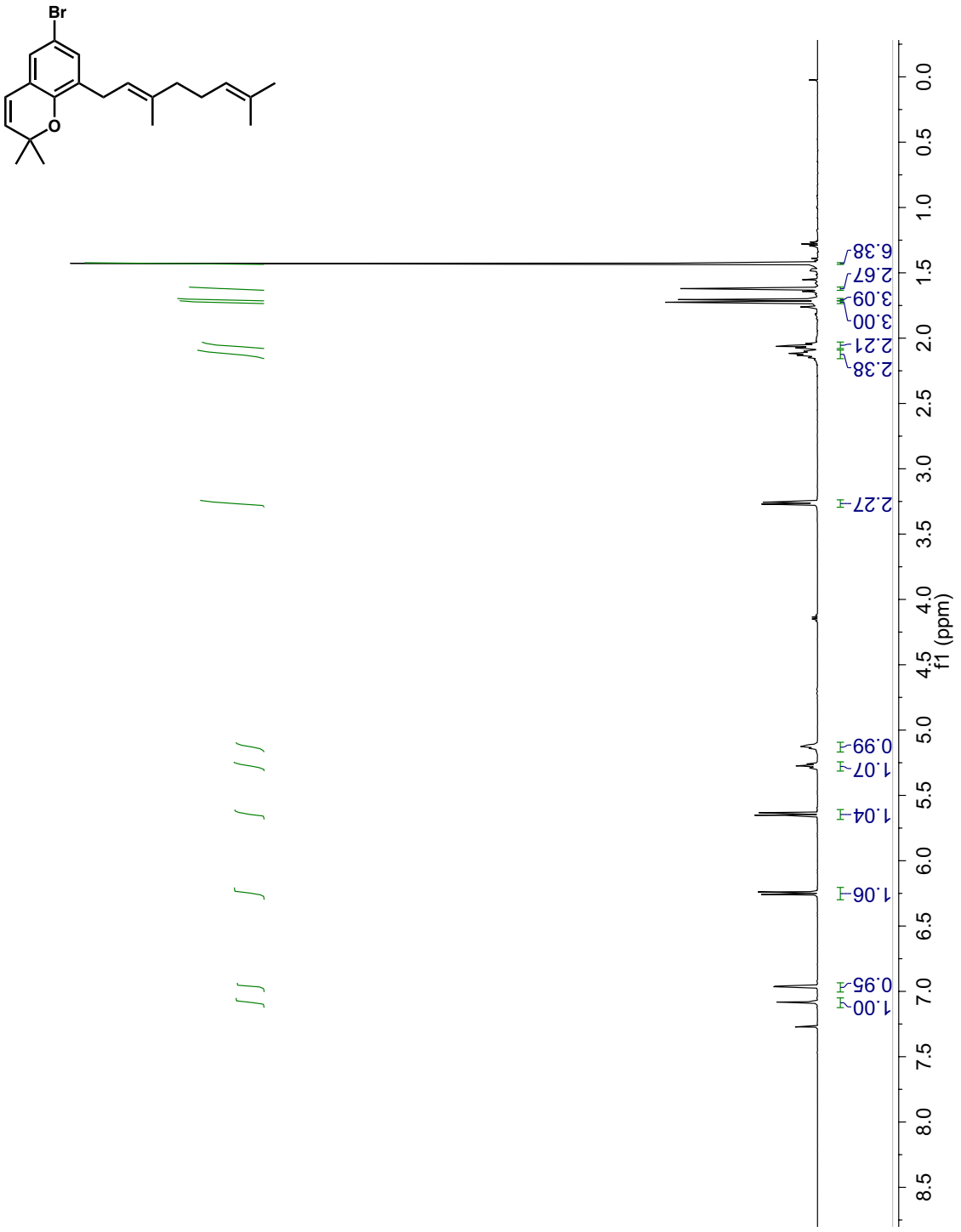

Figure A.1.11 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **53**

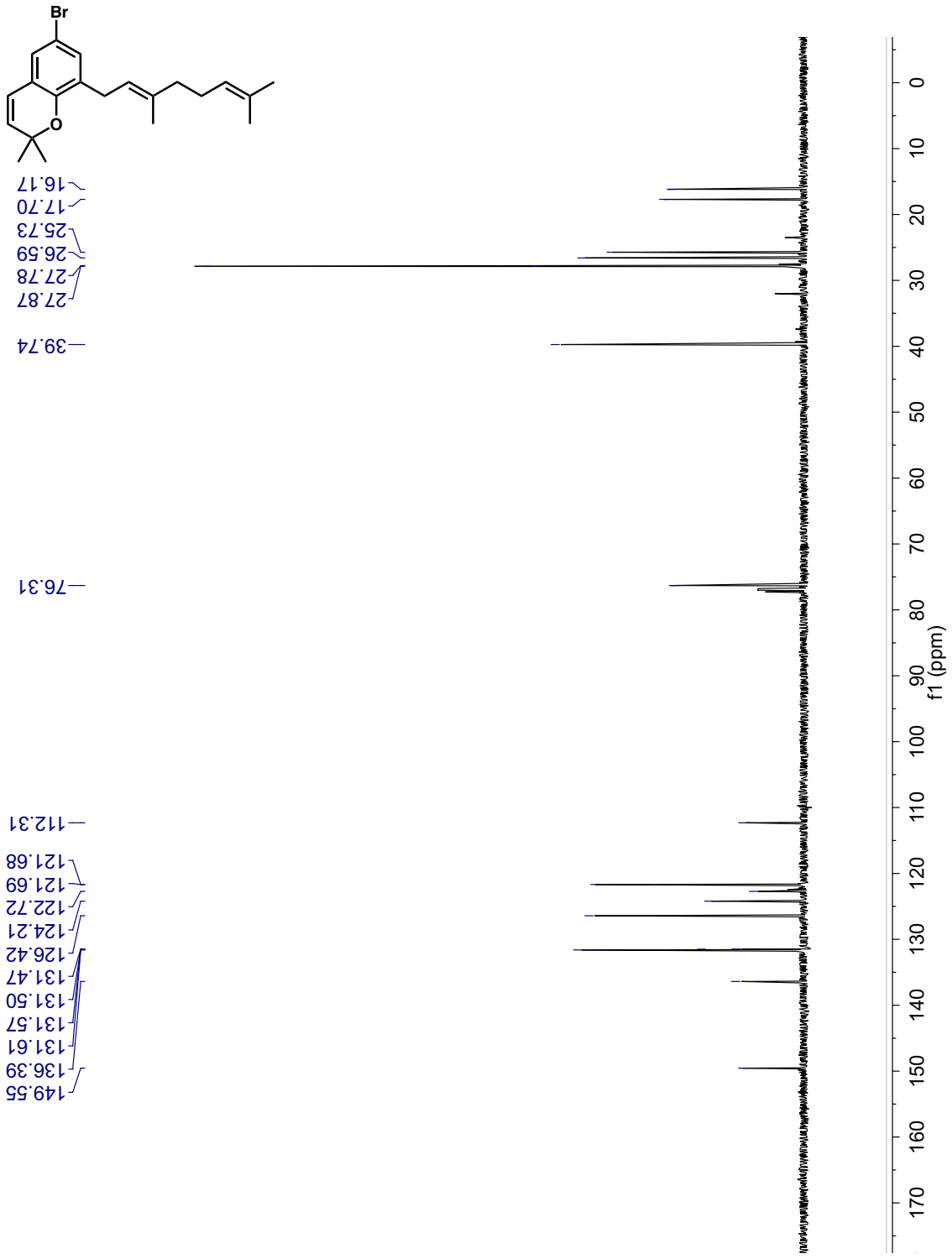

Figure A.1.12 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **53**

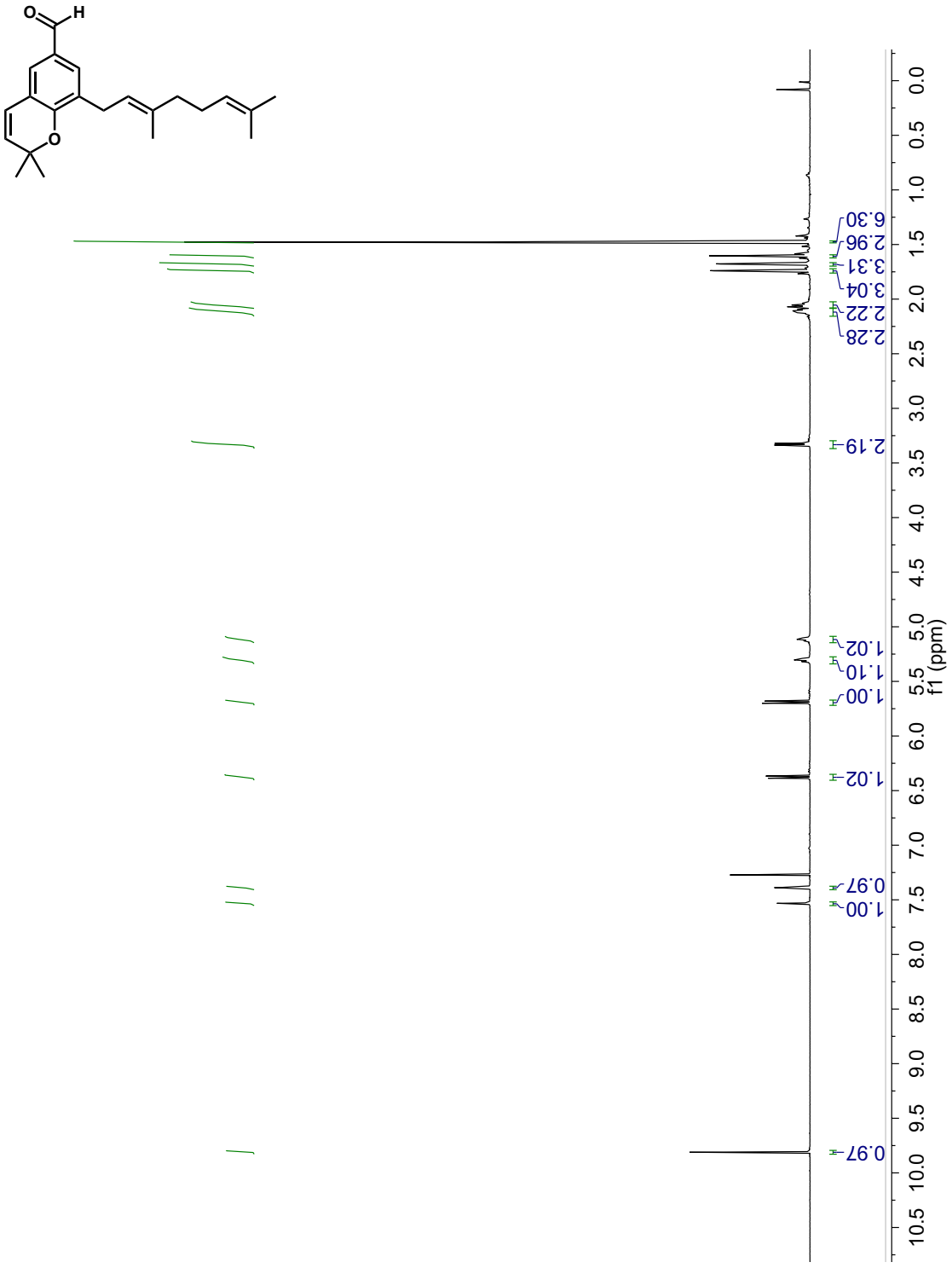

Figure A.1.13 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **60**

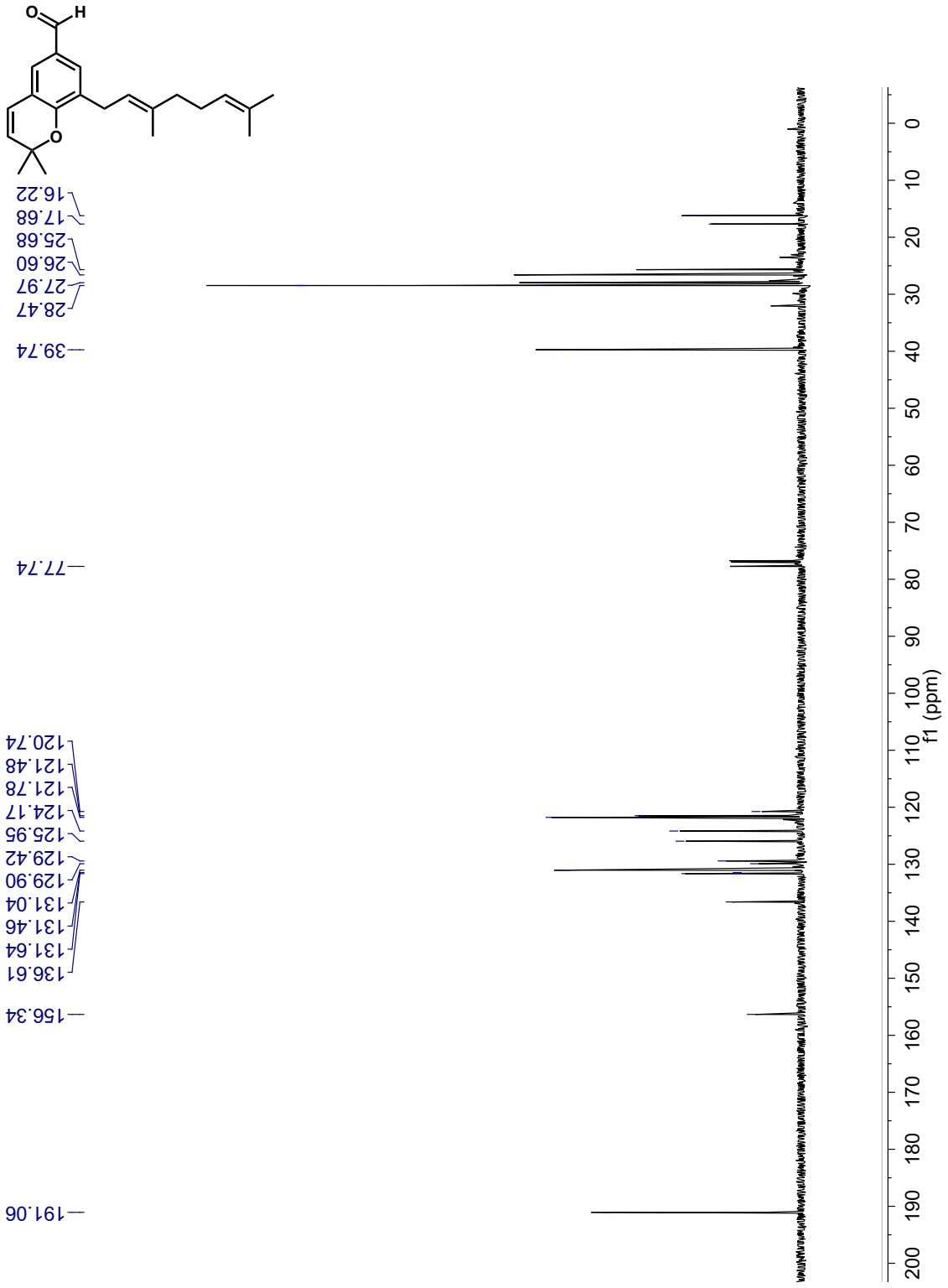

Figure A.1.14 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **60**

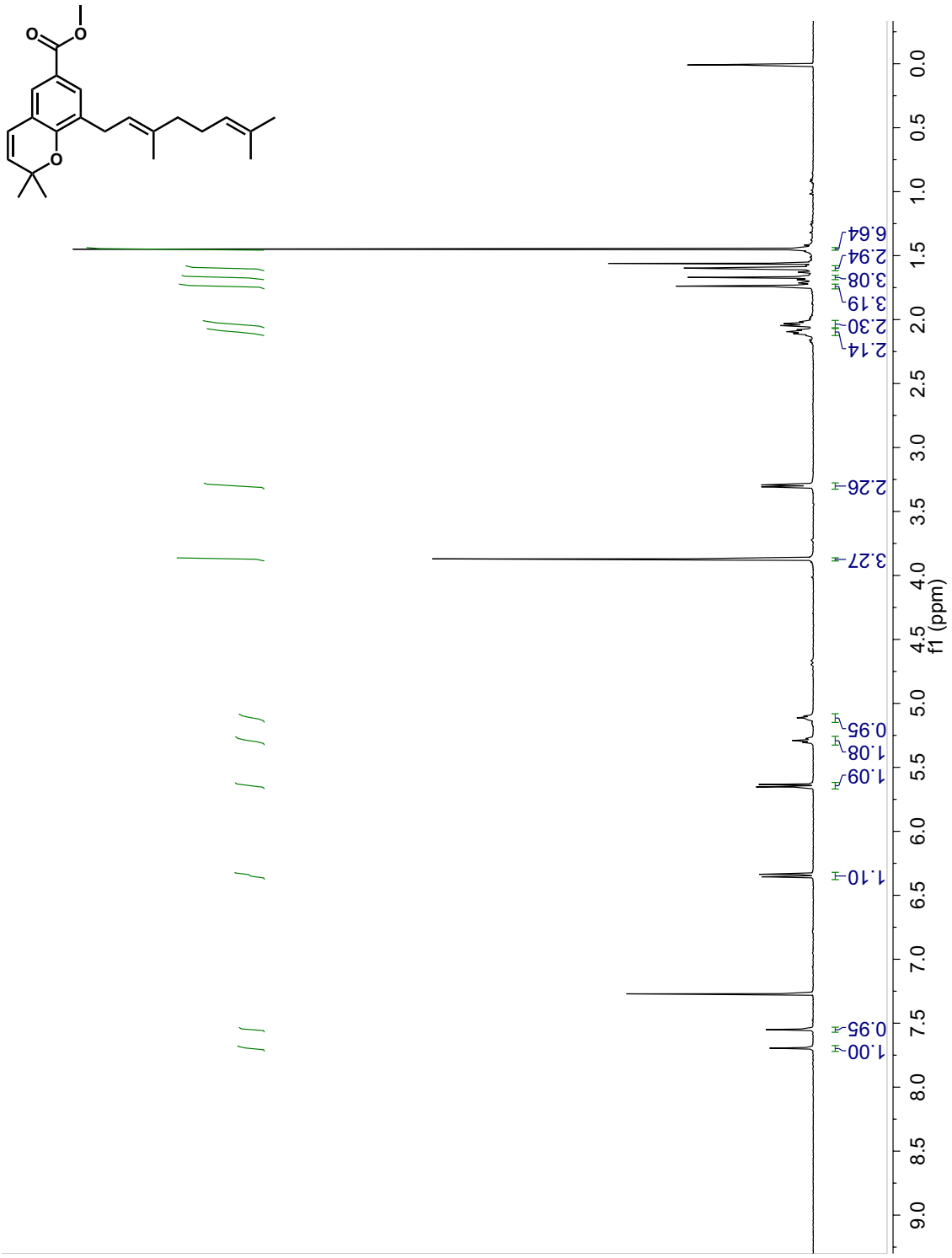

Figure A.1.15 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **61**

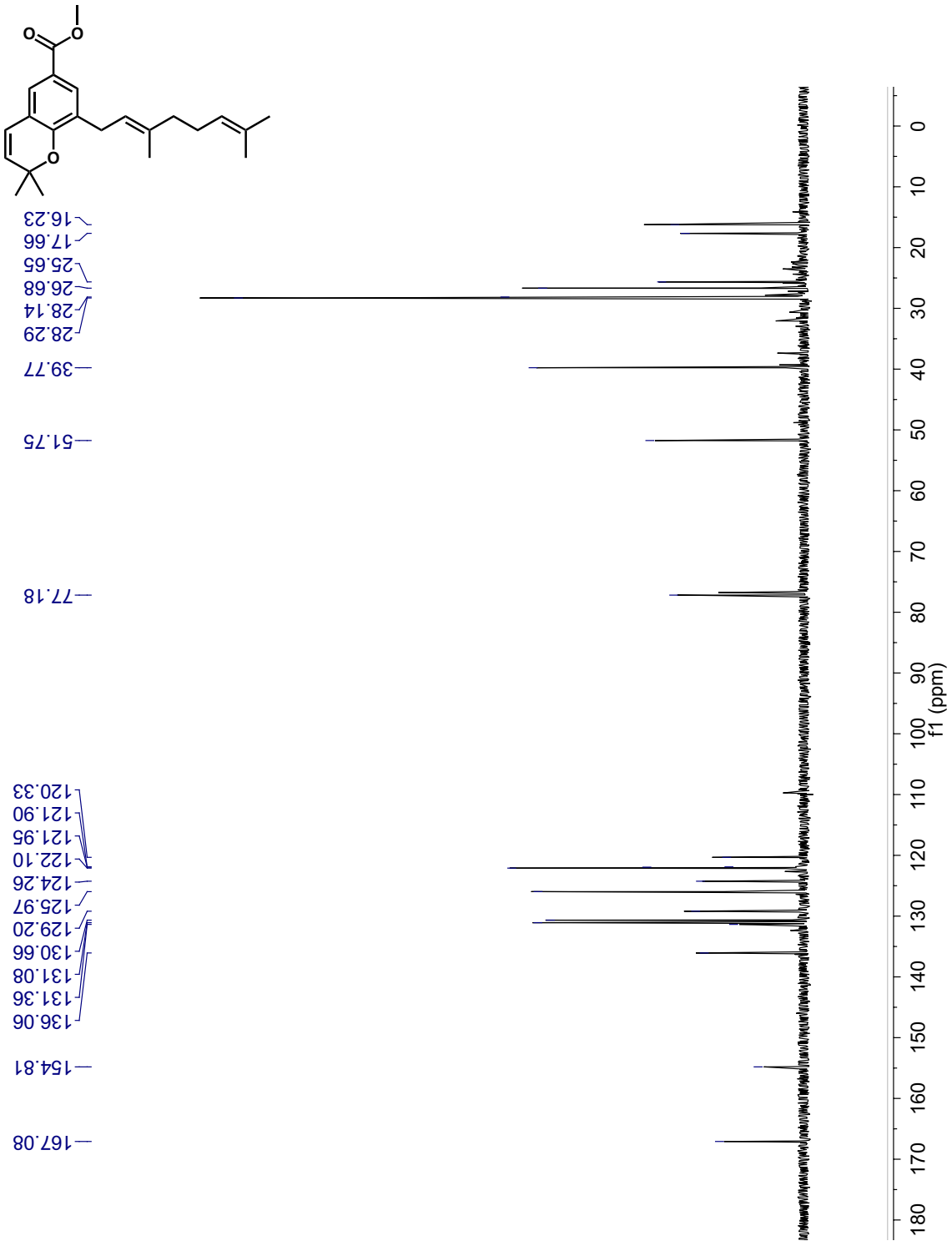

Figure A.1.16 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **61**

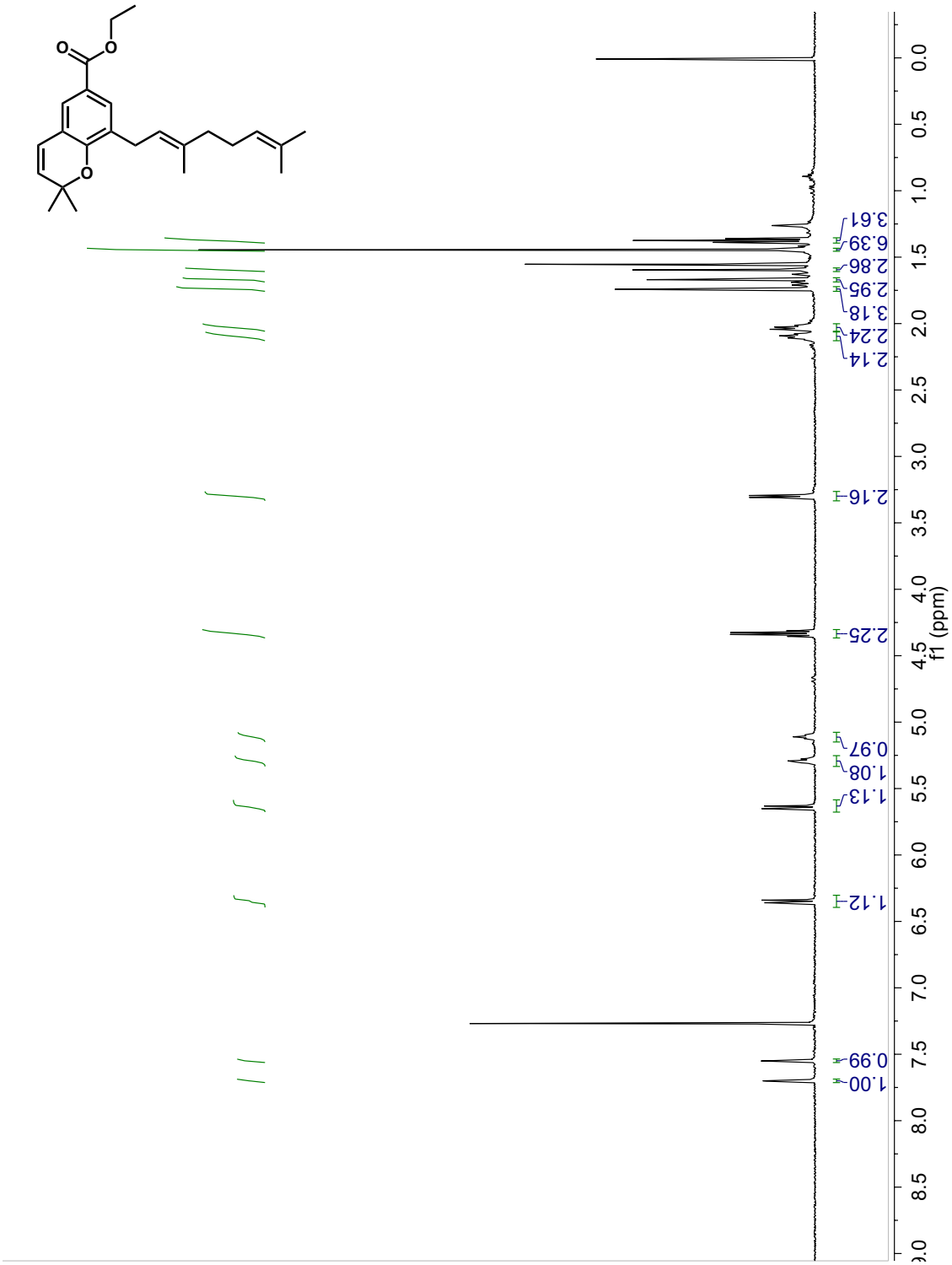

Figure A.1.17 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **62**

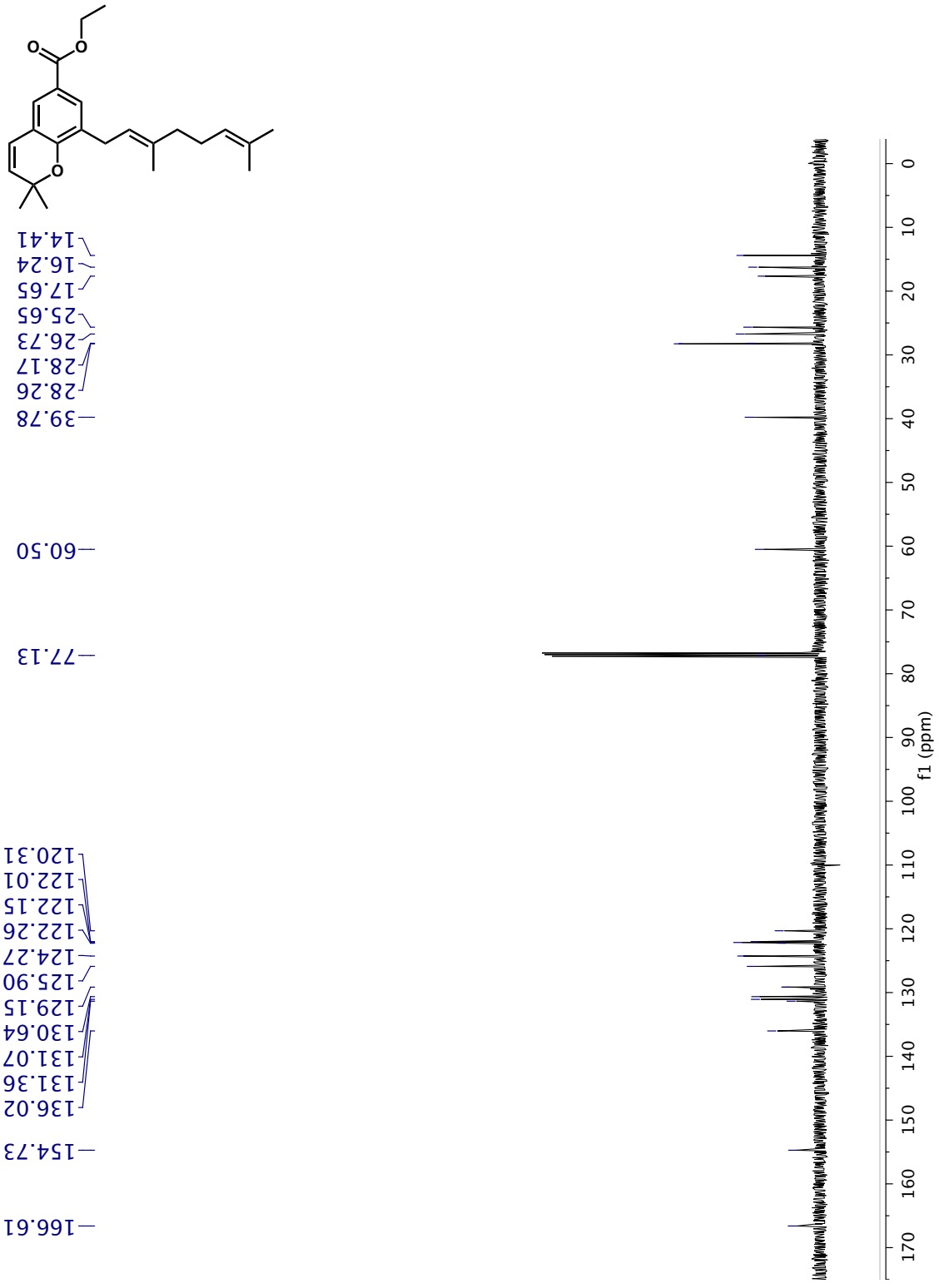

Figure A.1.18 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **62**

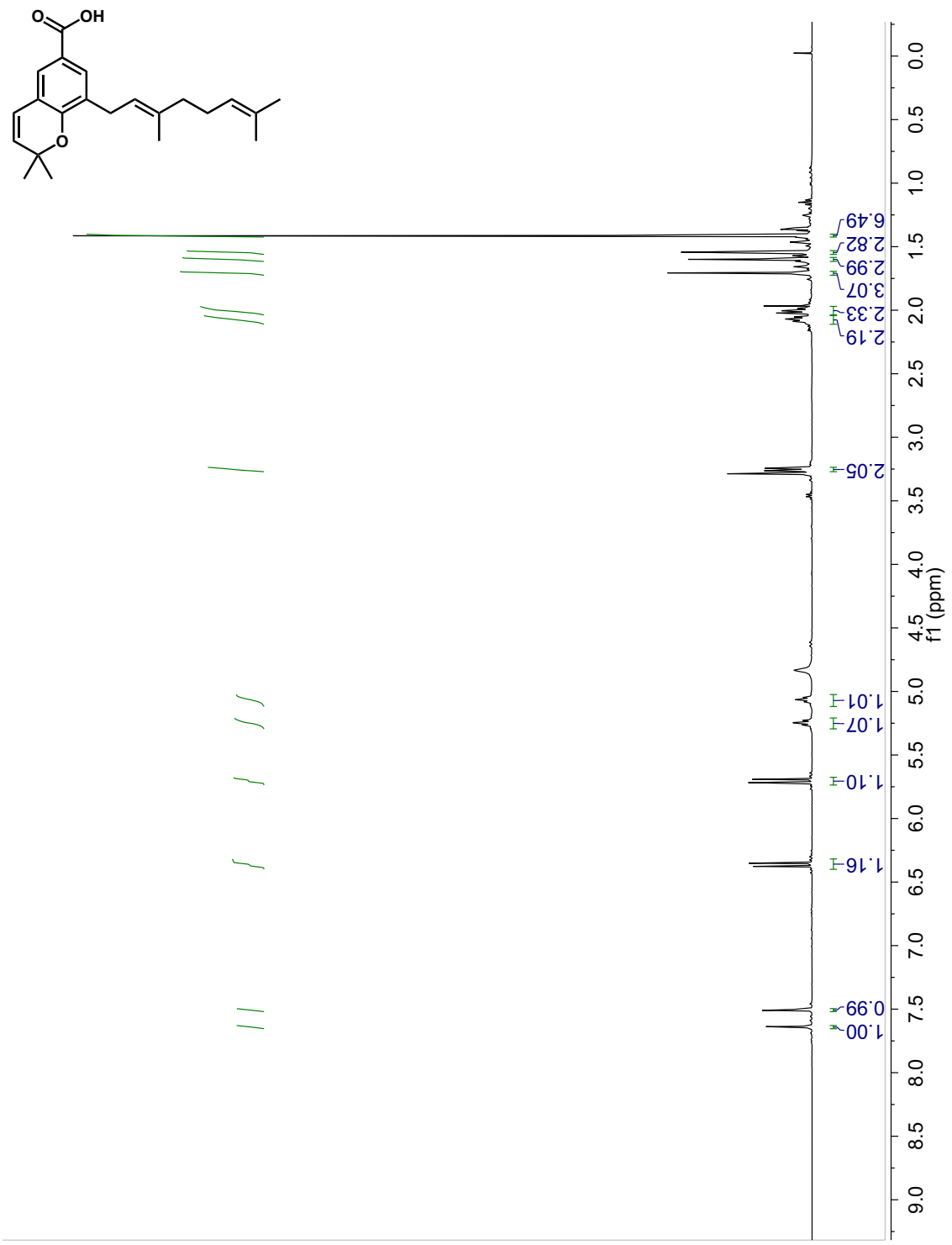

Figure A.1.19 ^{1}H NMR (400 MHz, CD_3OD) spectrum of **37**

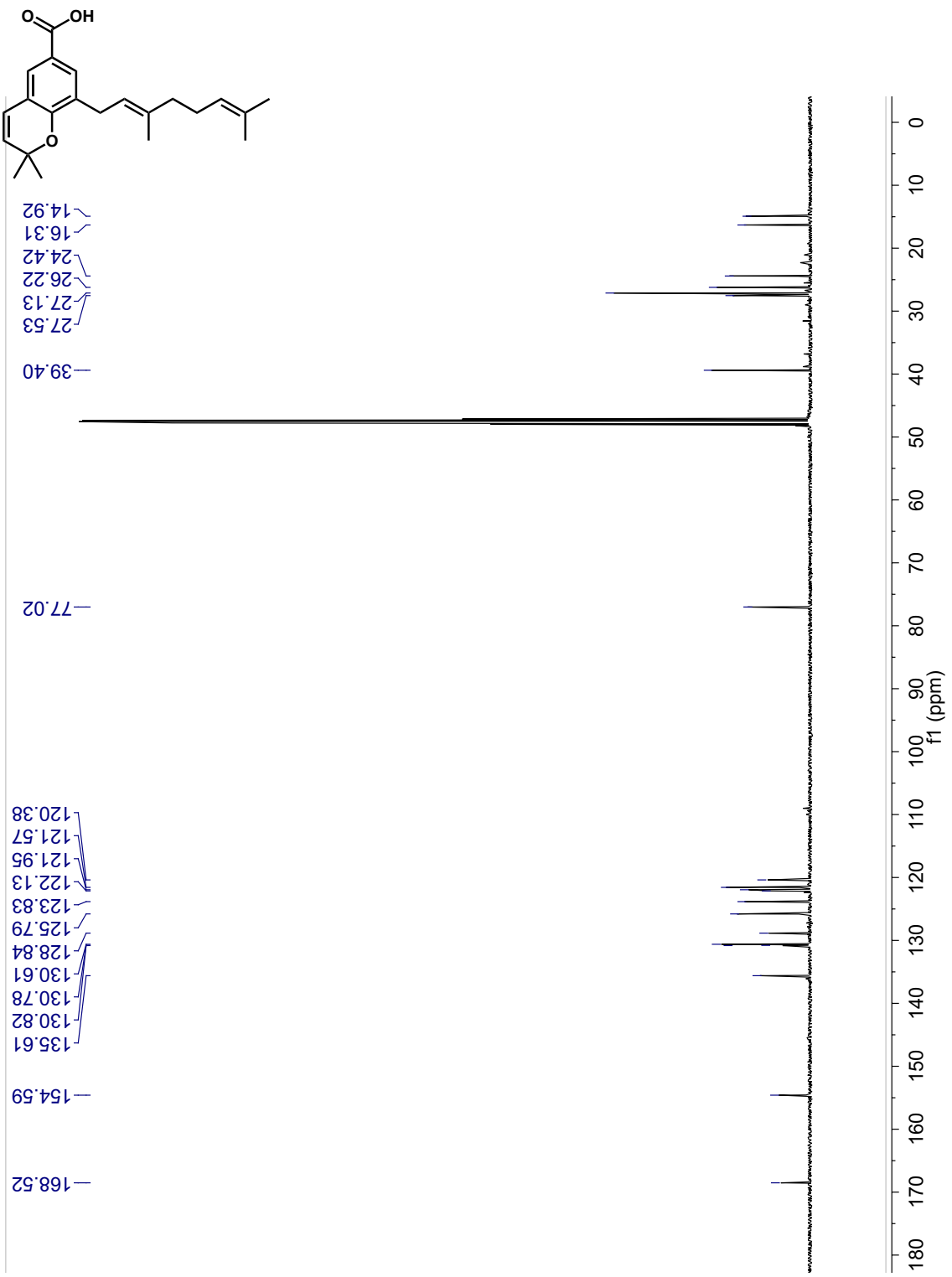

Figure A.1.20 ^{13}C NMR (101 MHz, CD_3OD) spectrum of **37**

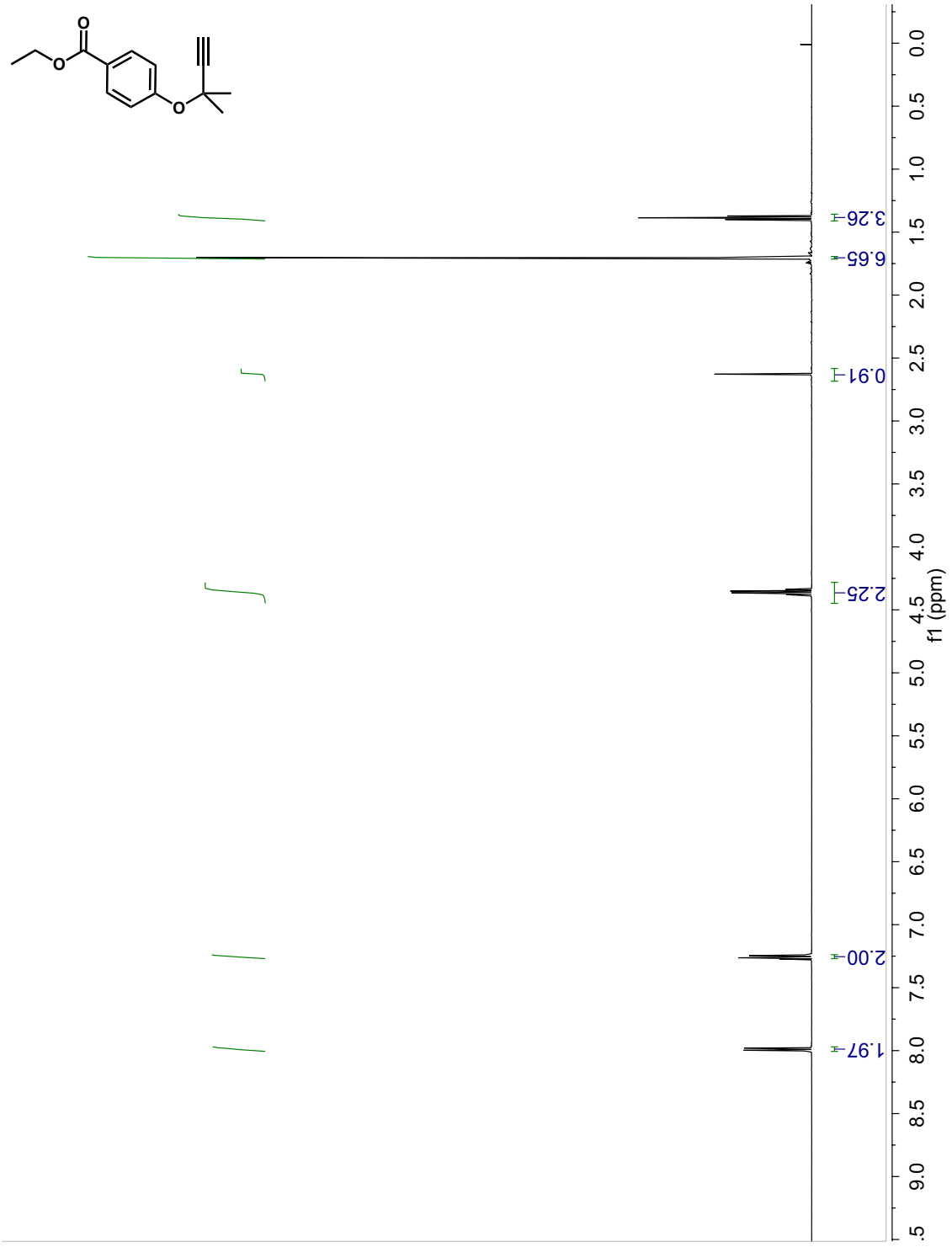

Figure A.1.21 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **67**

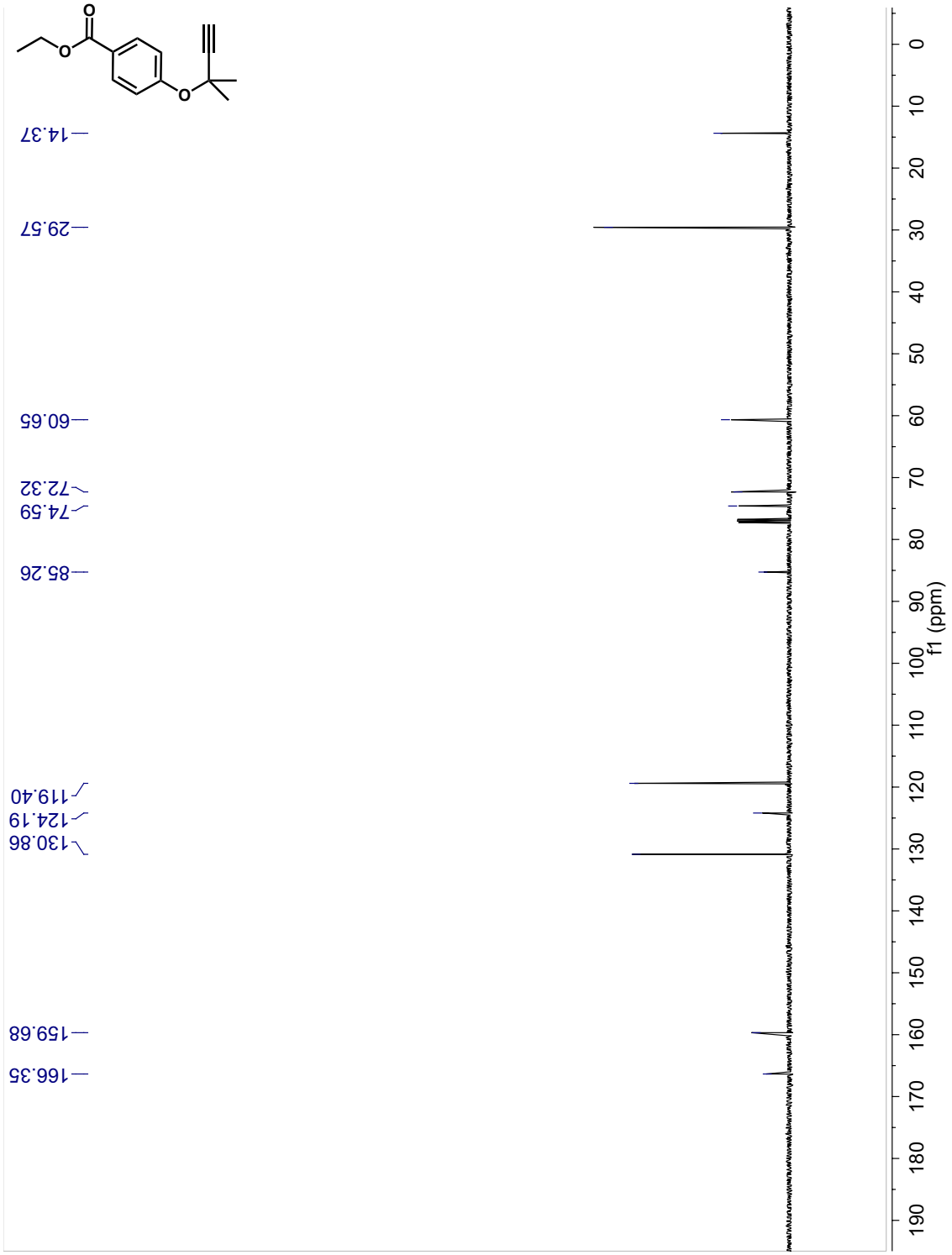

Figure A.1.22 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **67**

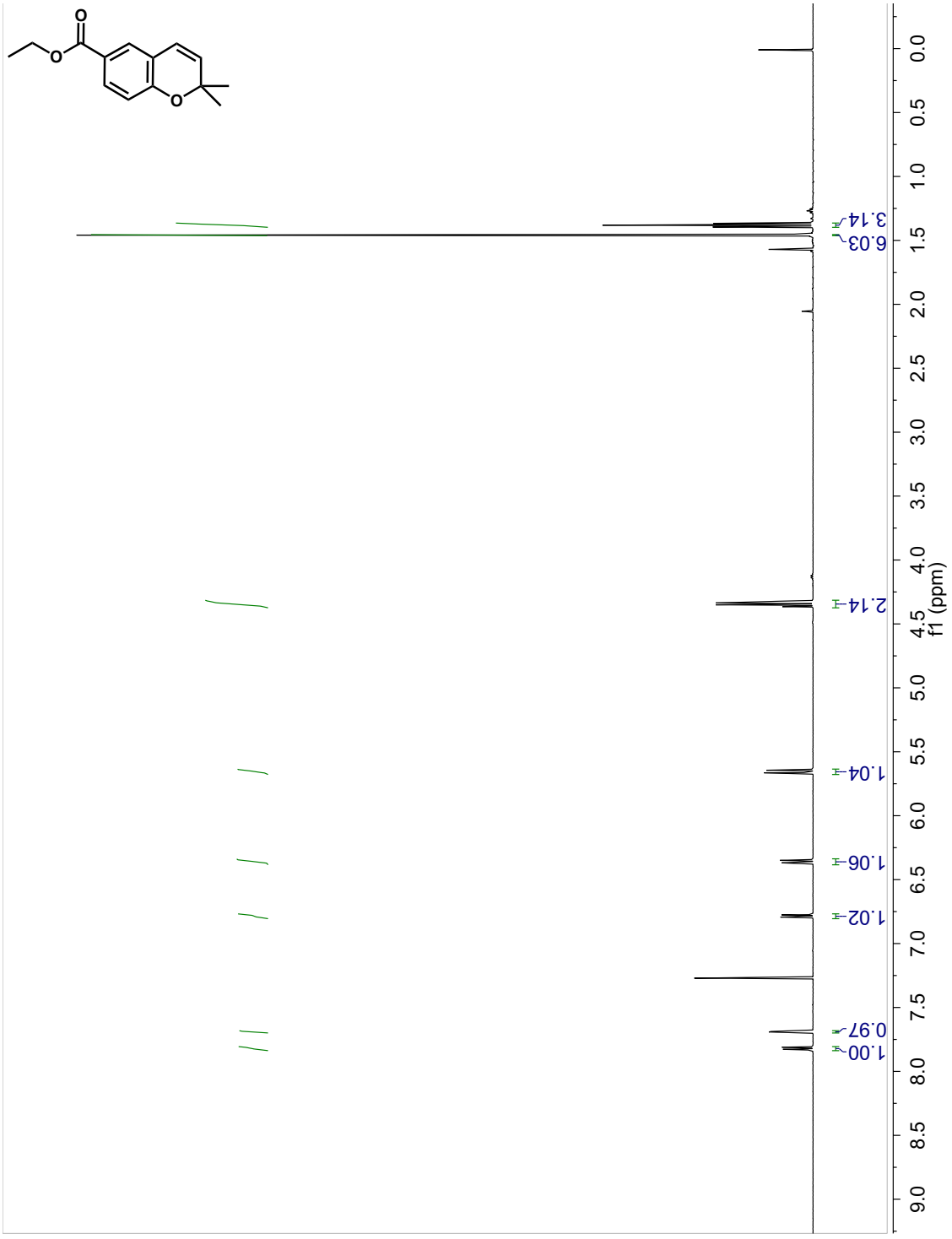

Figure A.1.23 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **68**

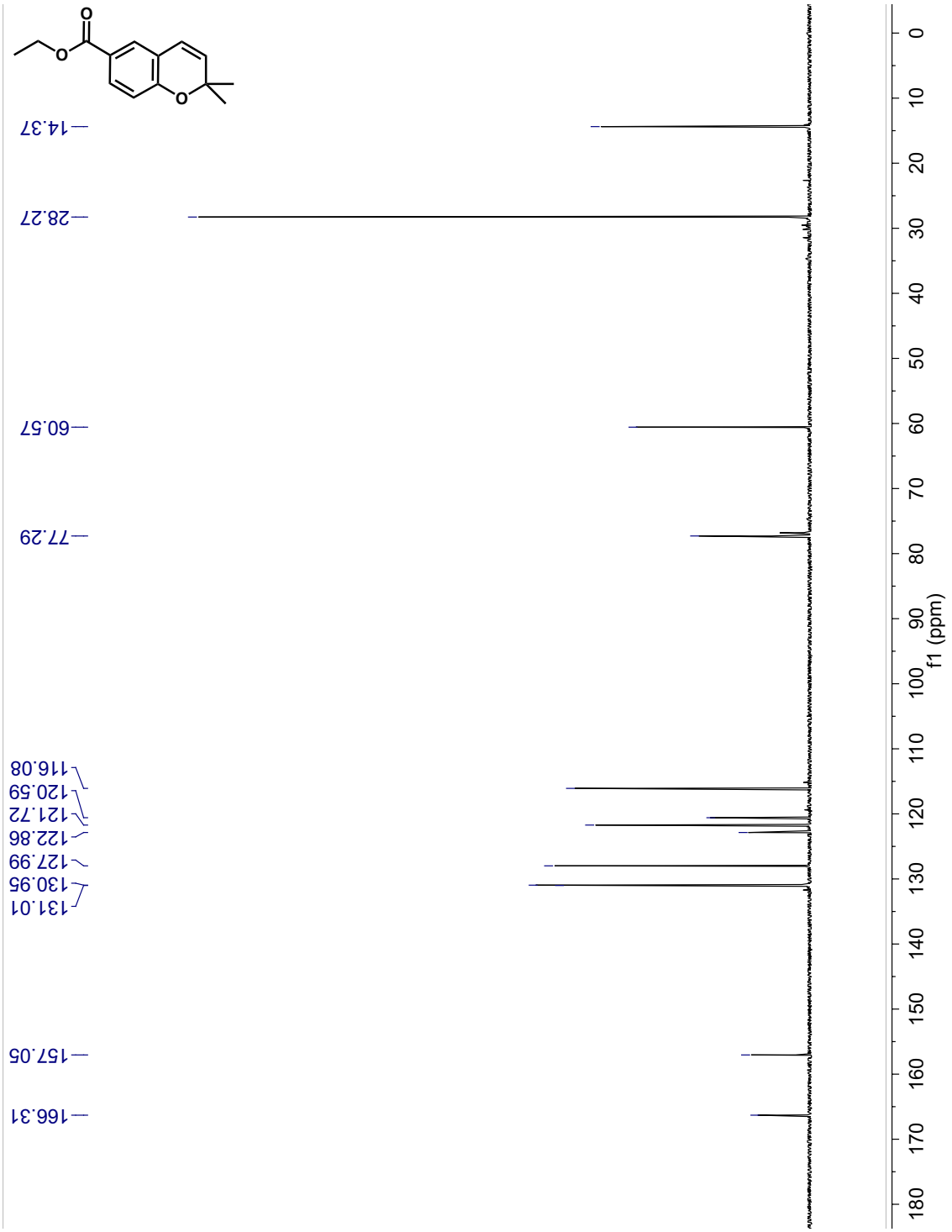

Figure A.1.24 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **68**

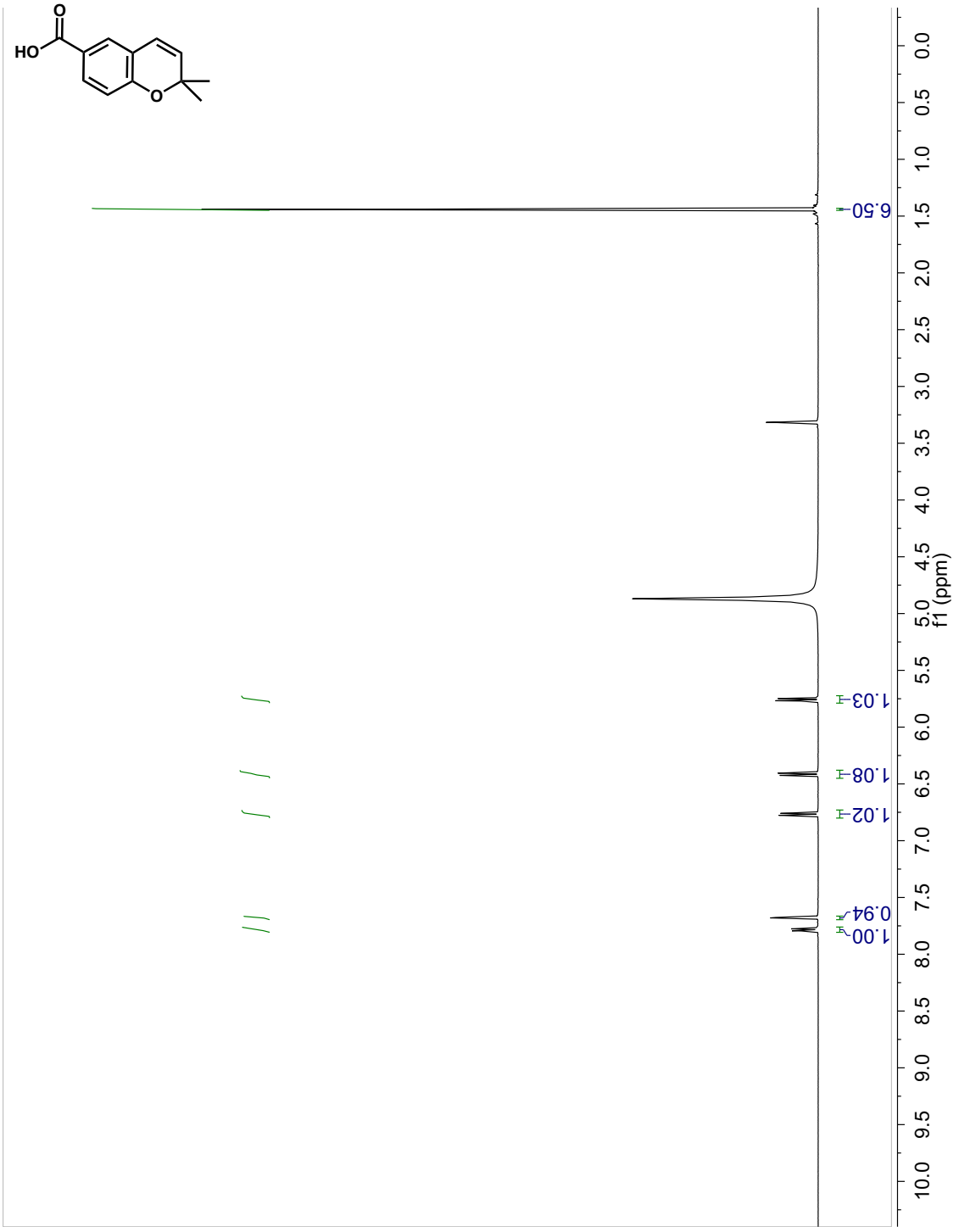

Figure A.1.25 ^{1}H NMR (500 MHz, CD_3OD) spectrum of **69**

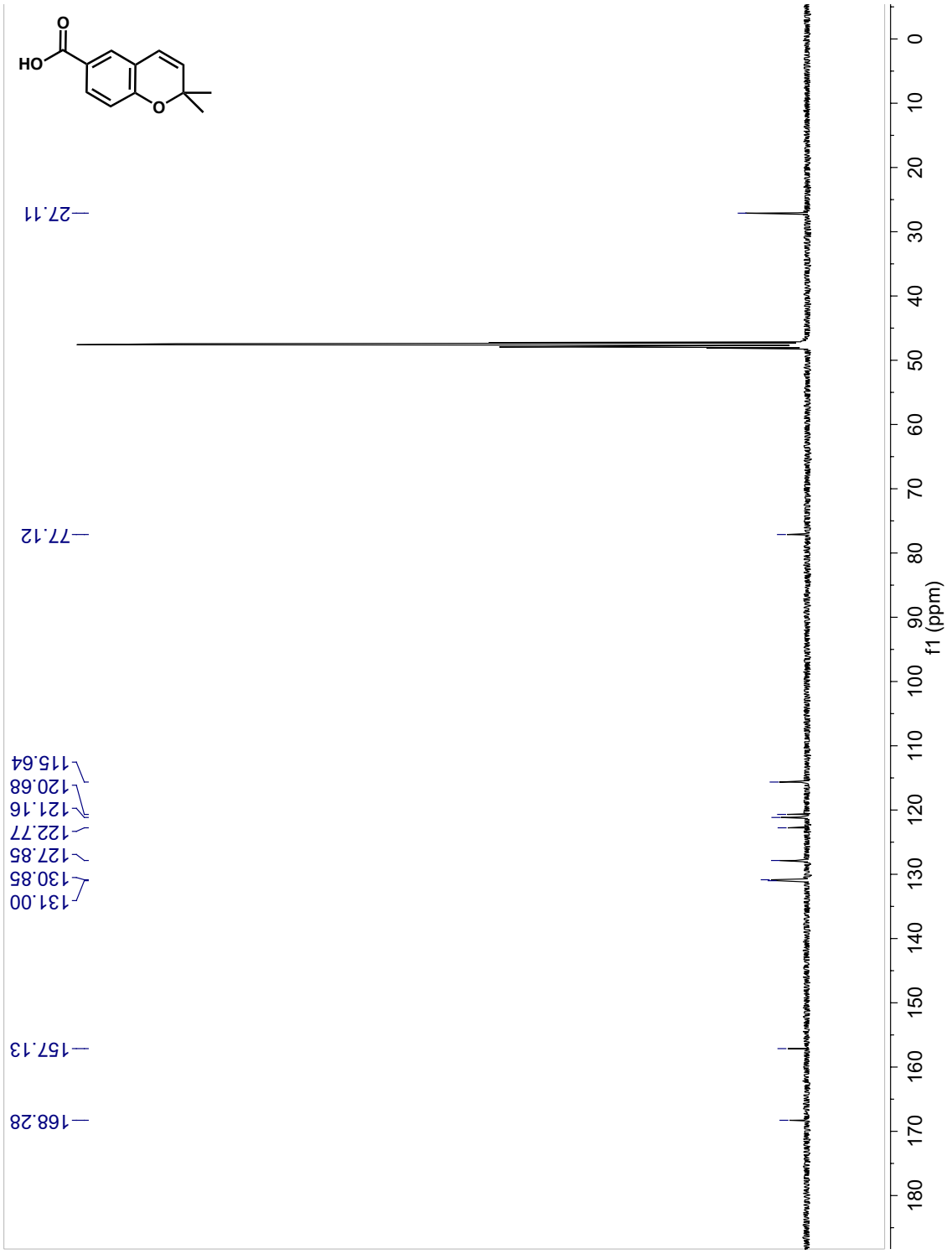

Figure A.1.26 ^{13}C NMR (126 MHz, CD_3OD) spectrum of **69**

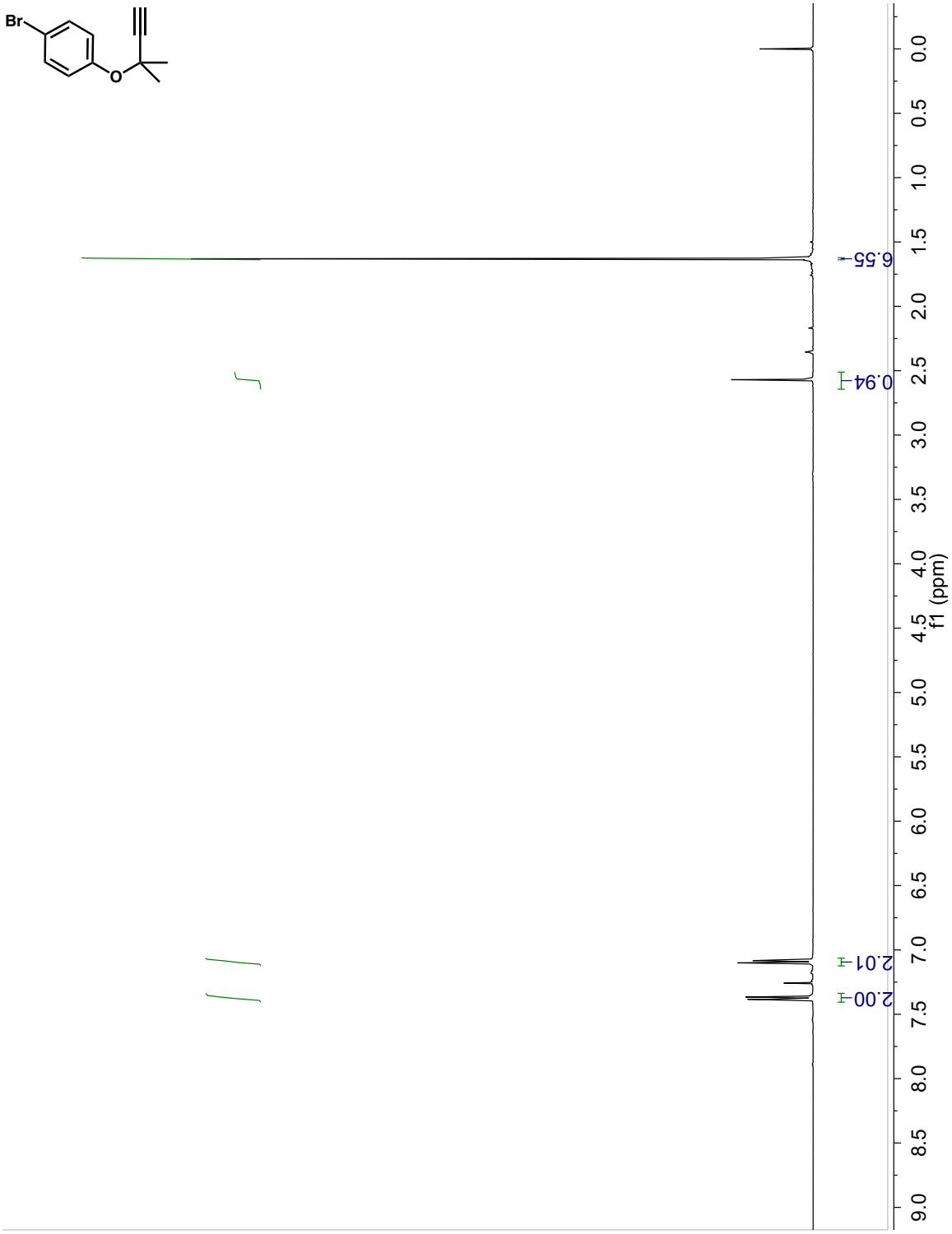

Figure A.1.27 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **70**

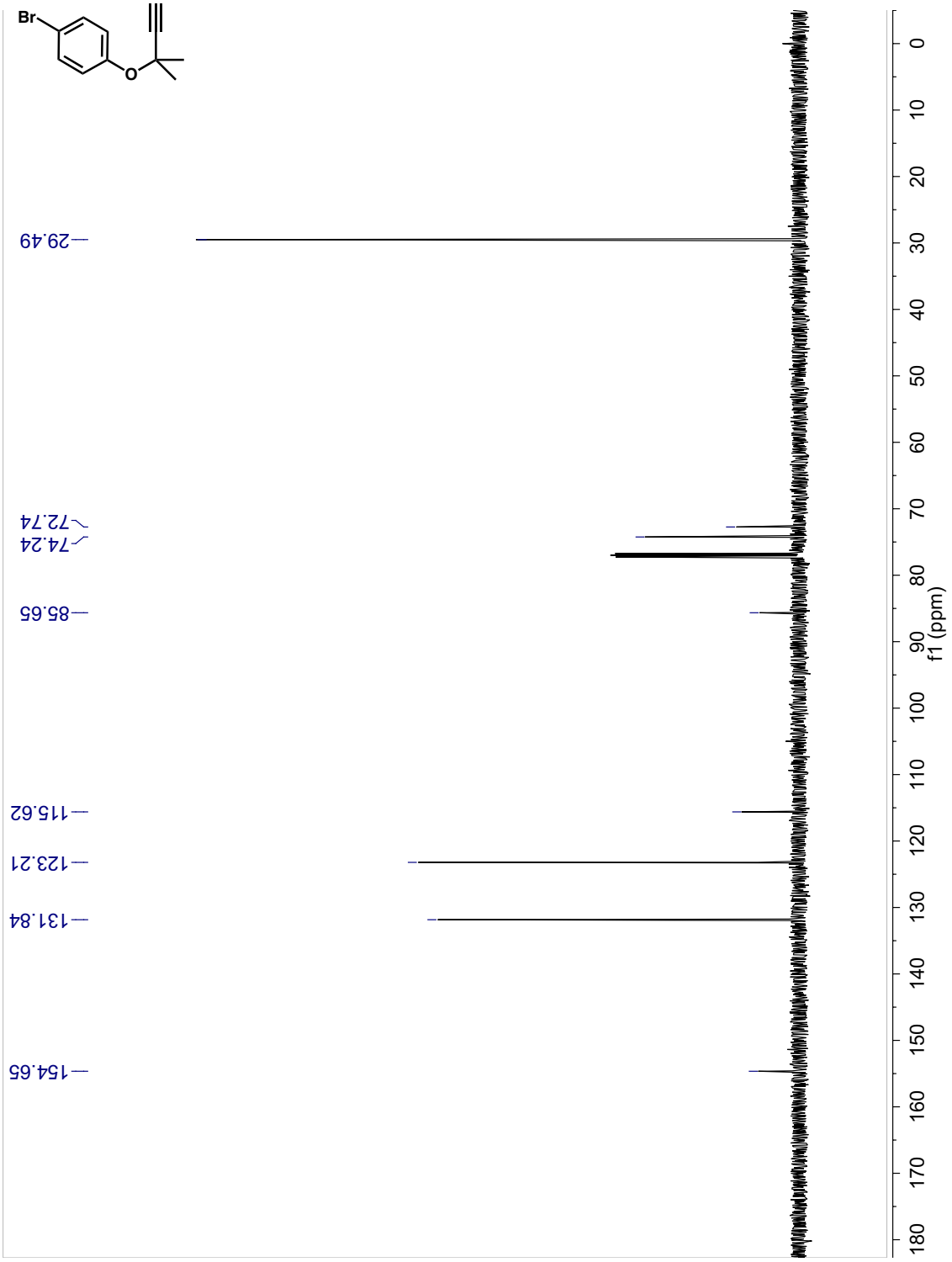

Figure A.1.28 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **70**

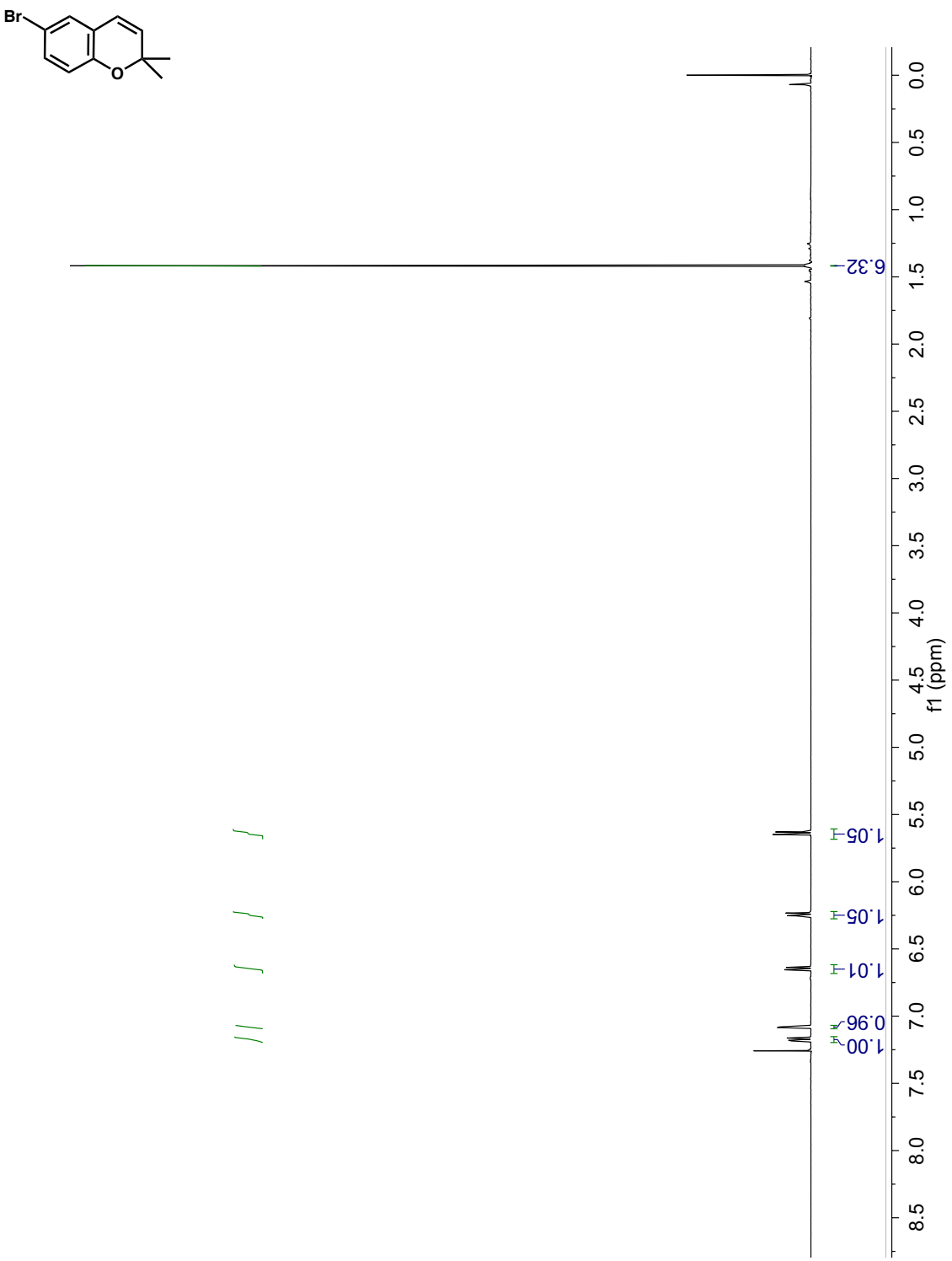

Figure A.1.29 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **71**

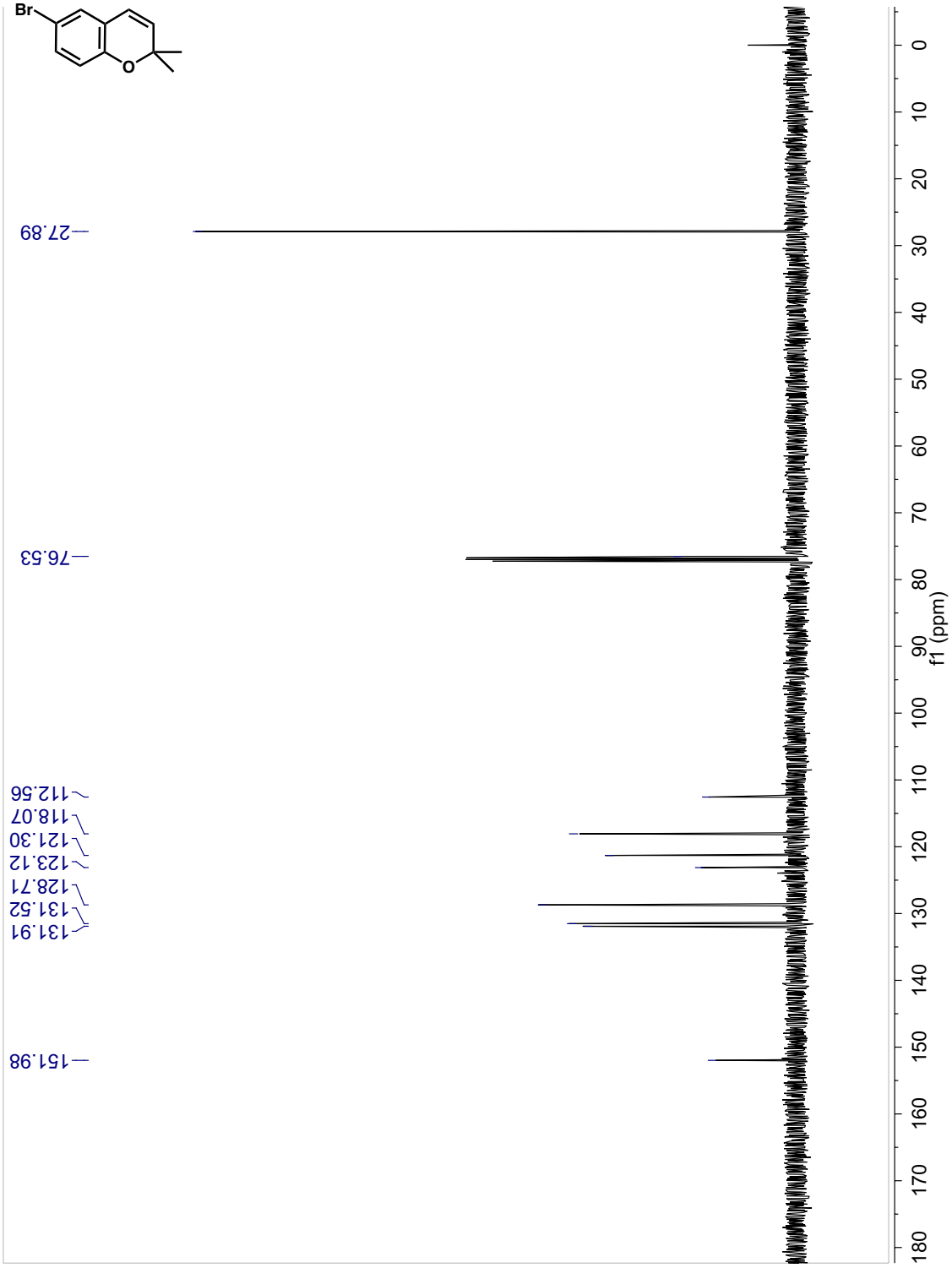

Figure A.1.30 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **71**

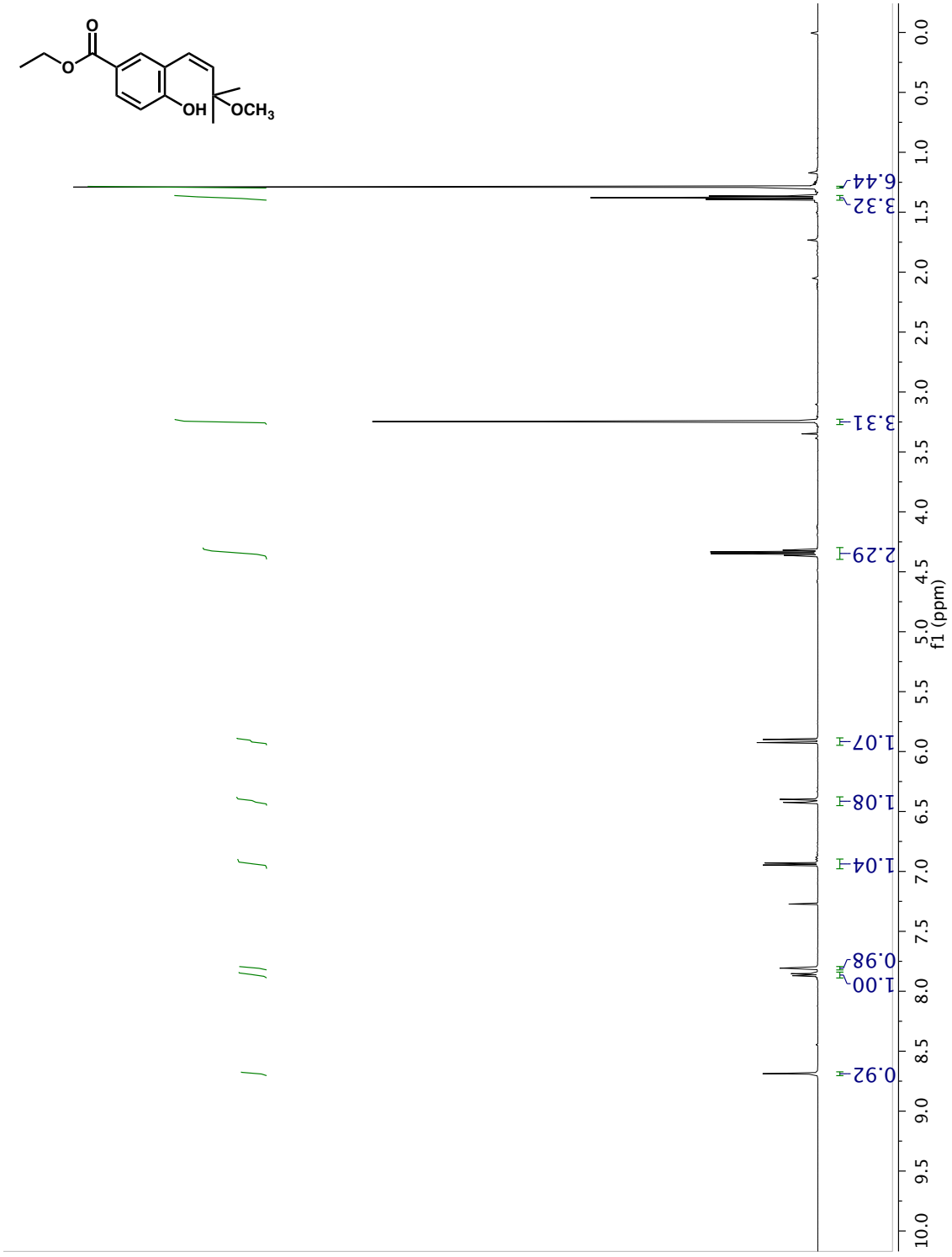

Figure A.1.31 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **80**

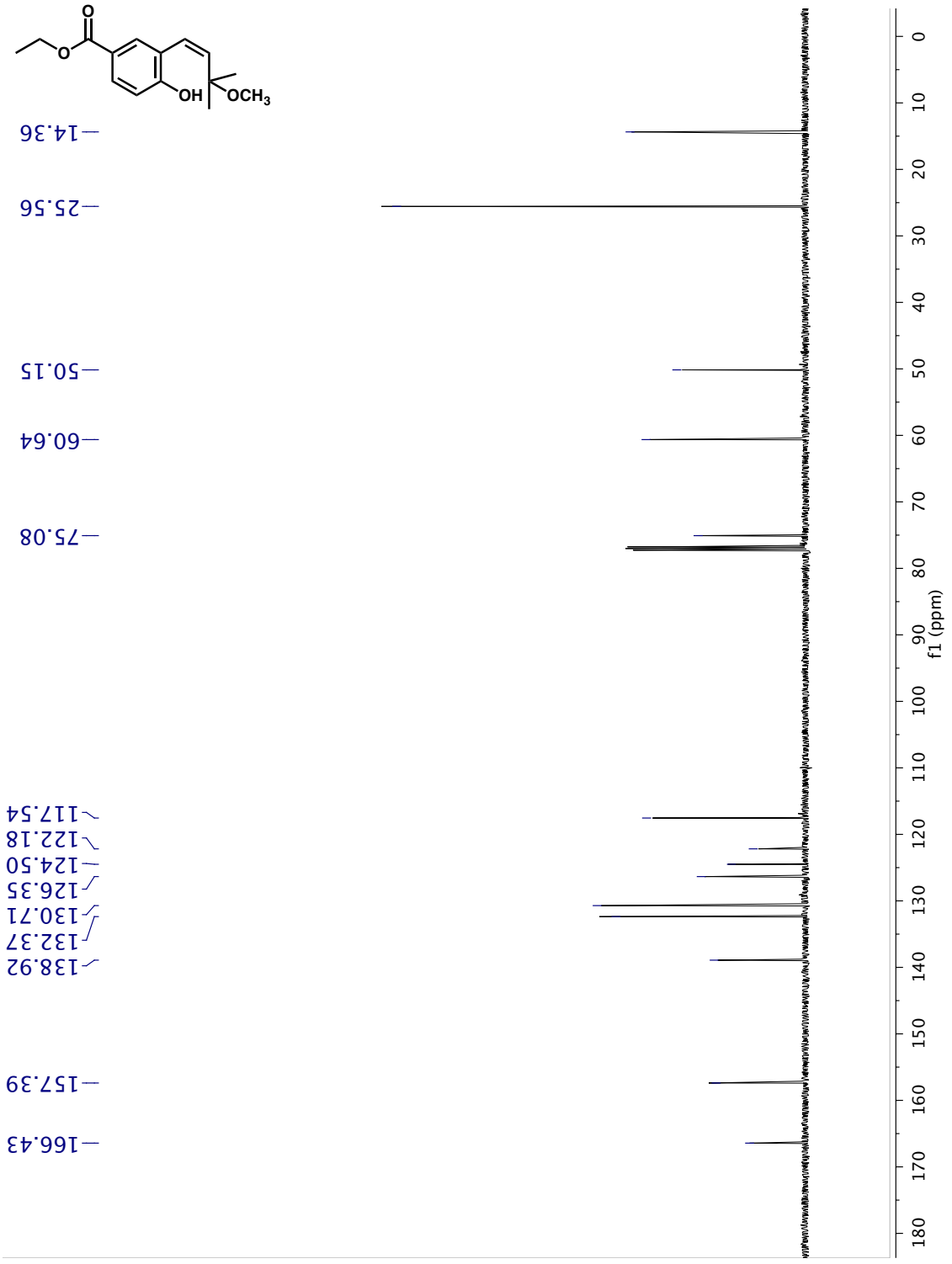

Figure A.1.32 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **80**

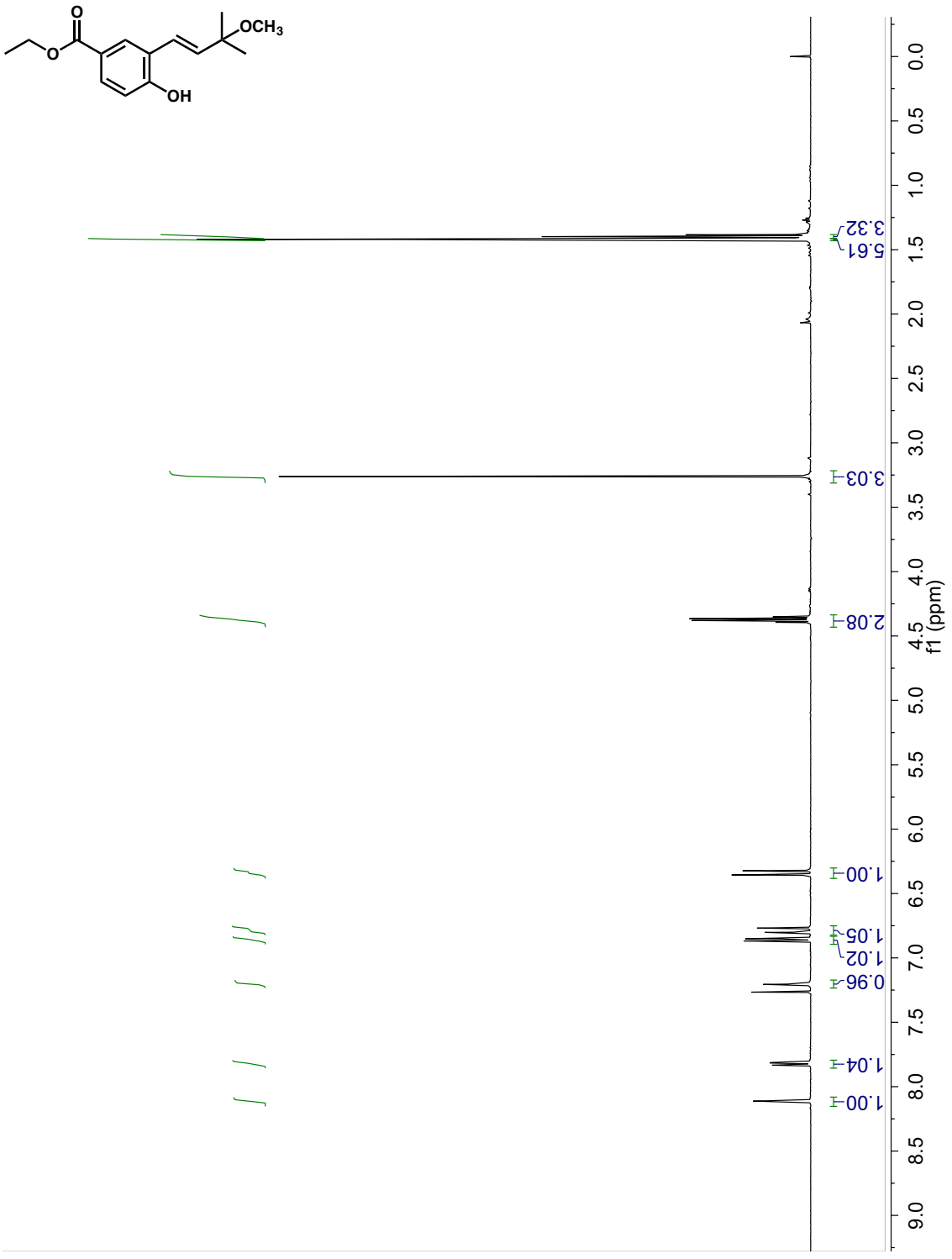

Figure A.1.33 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **81**

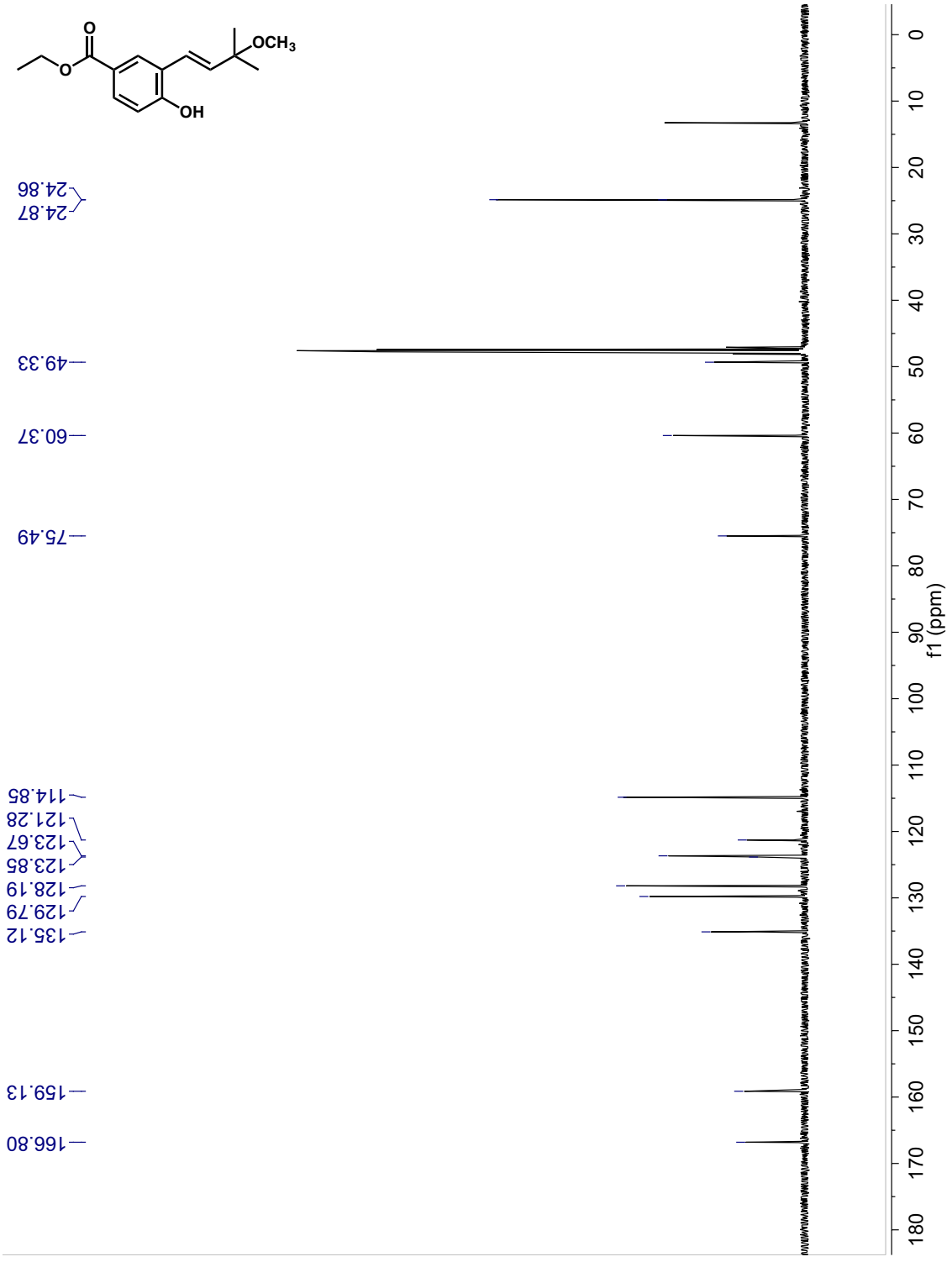

Figure A.1.34 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **81**

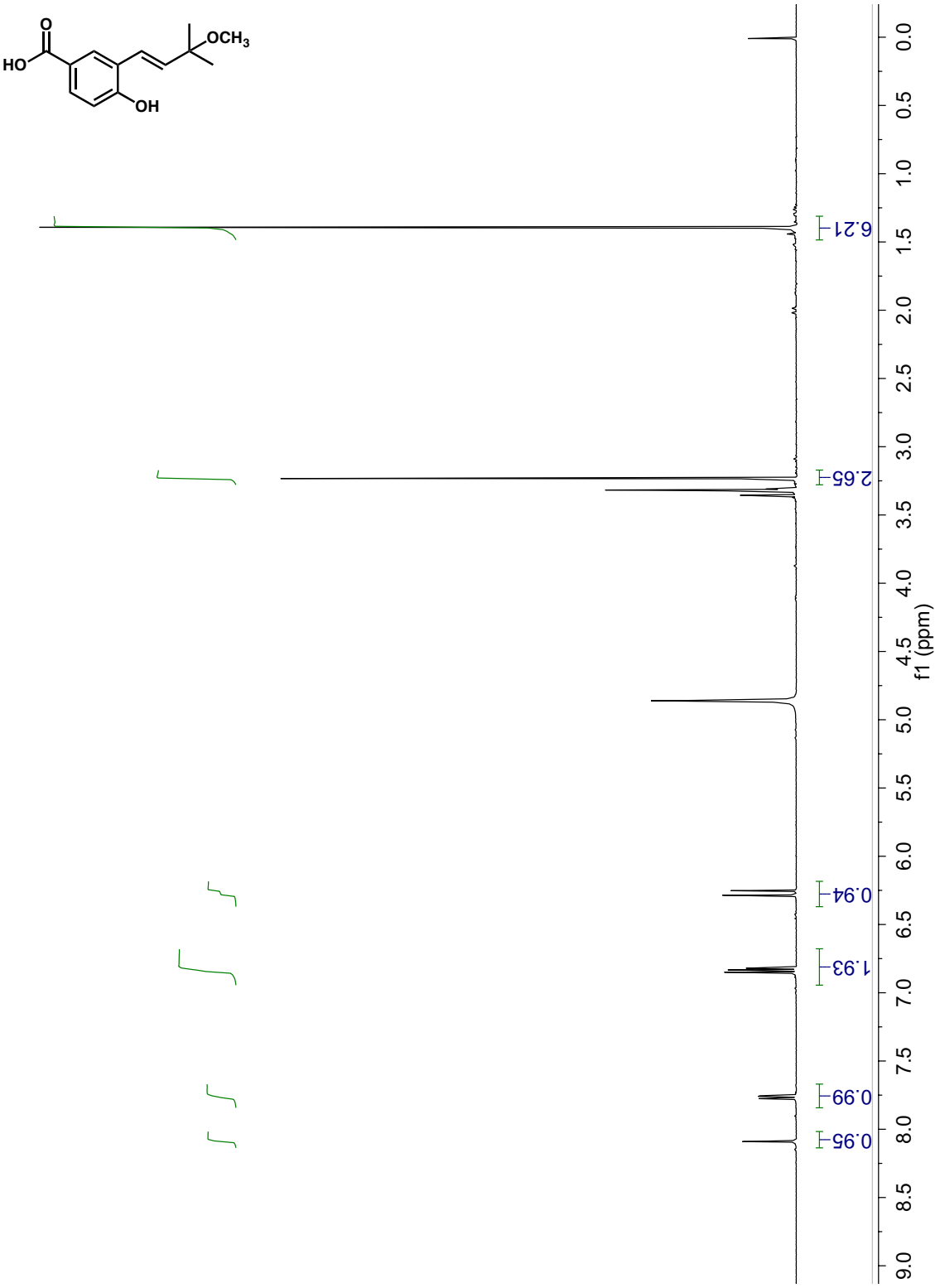

Figure A.1.35 ^{1}H NMR (500 MHz, CD_3OD) spectrum of **82**

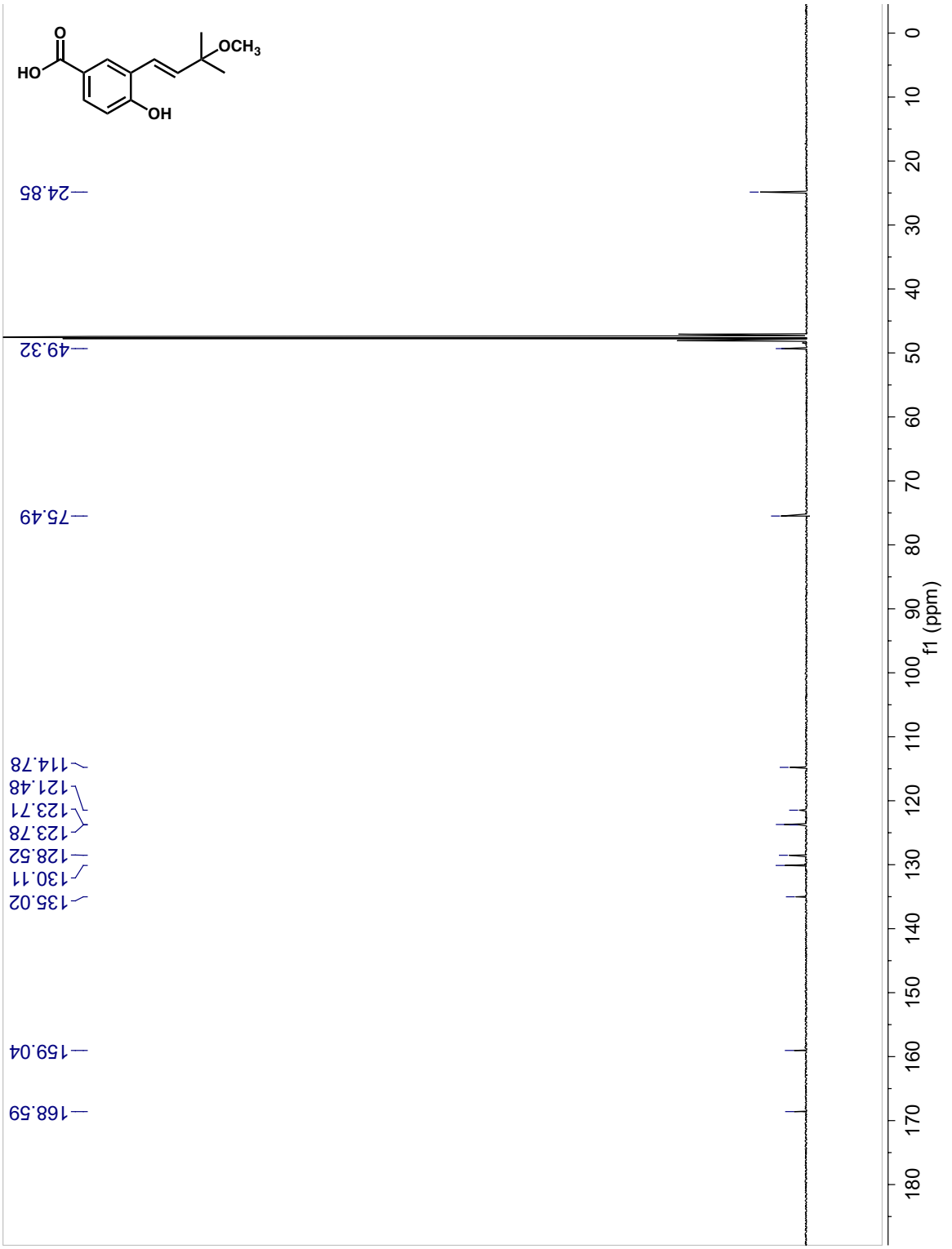

Figure A.1.36 ^{13}C NMR (500 MHz, CD_3OD) spectrum of **82**

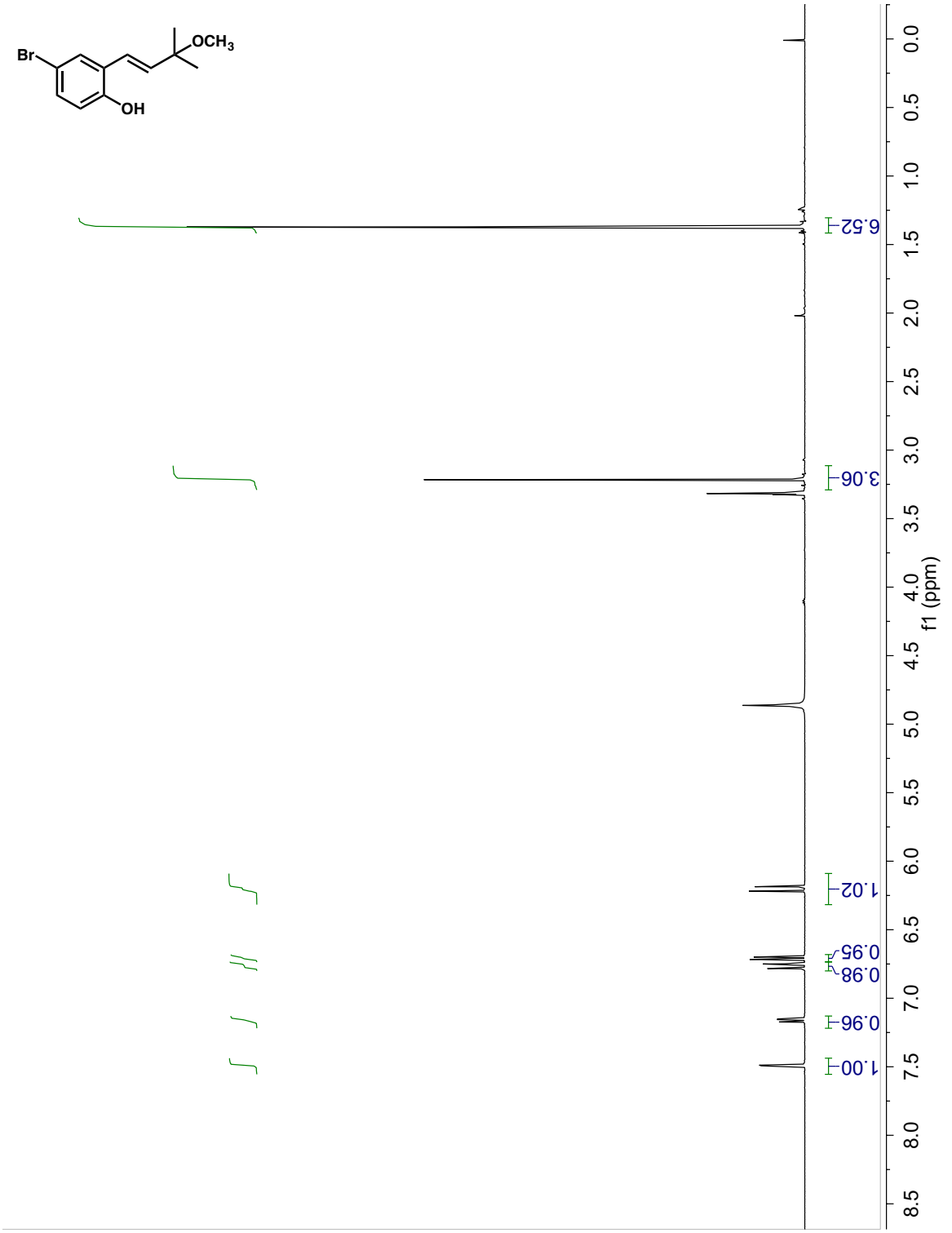

Figure A.1.37 ^{1}H NMR (500 MHz, CD_3OD) spectrum of **83**

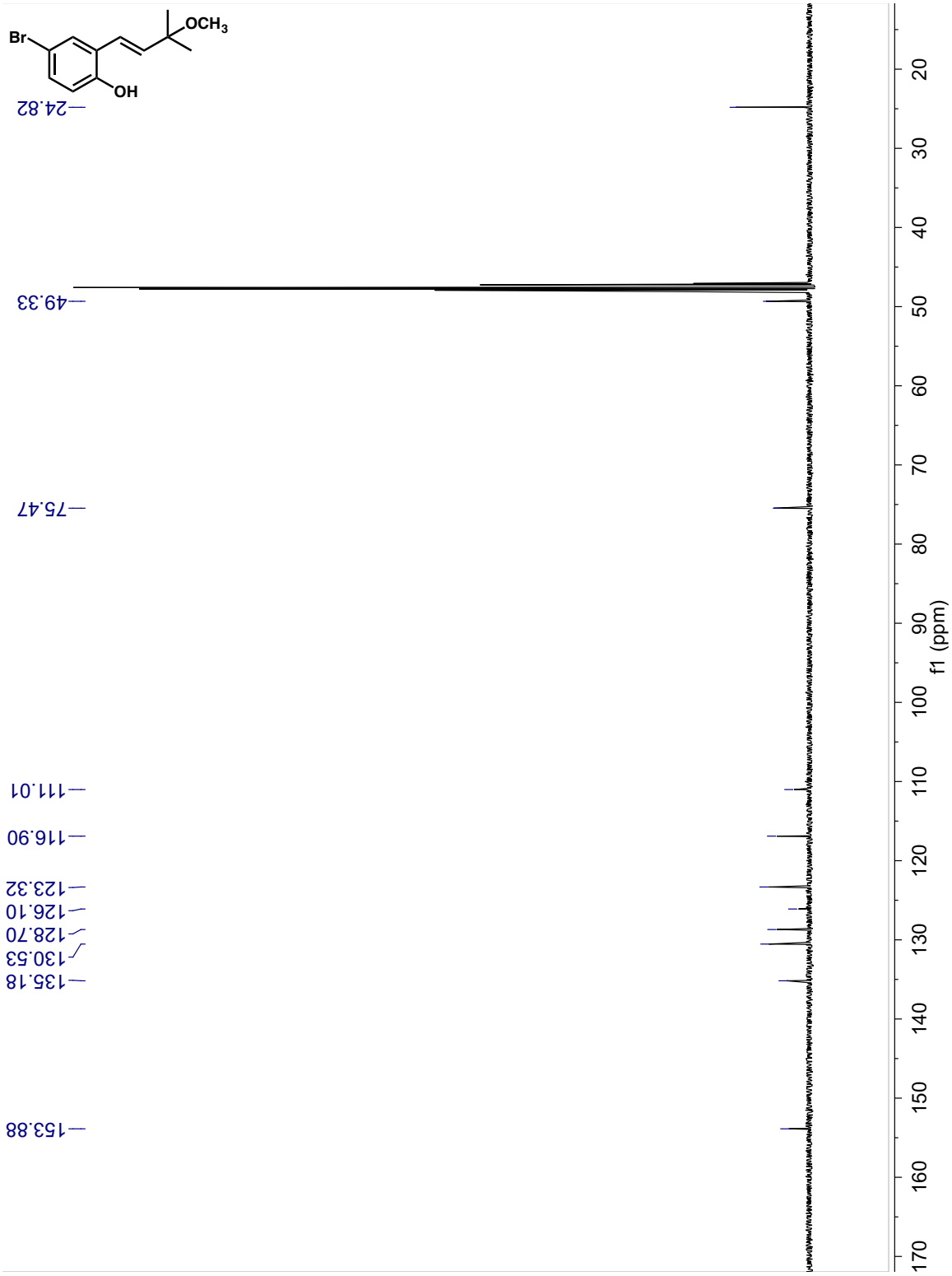

Figure A.1.38 ^{13}C NMR (500 MHz, CD_3OD) spectrum of **83**

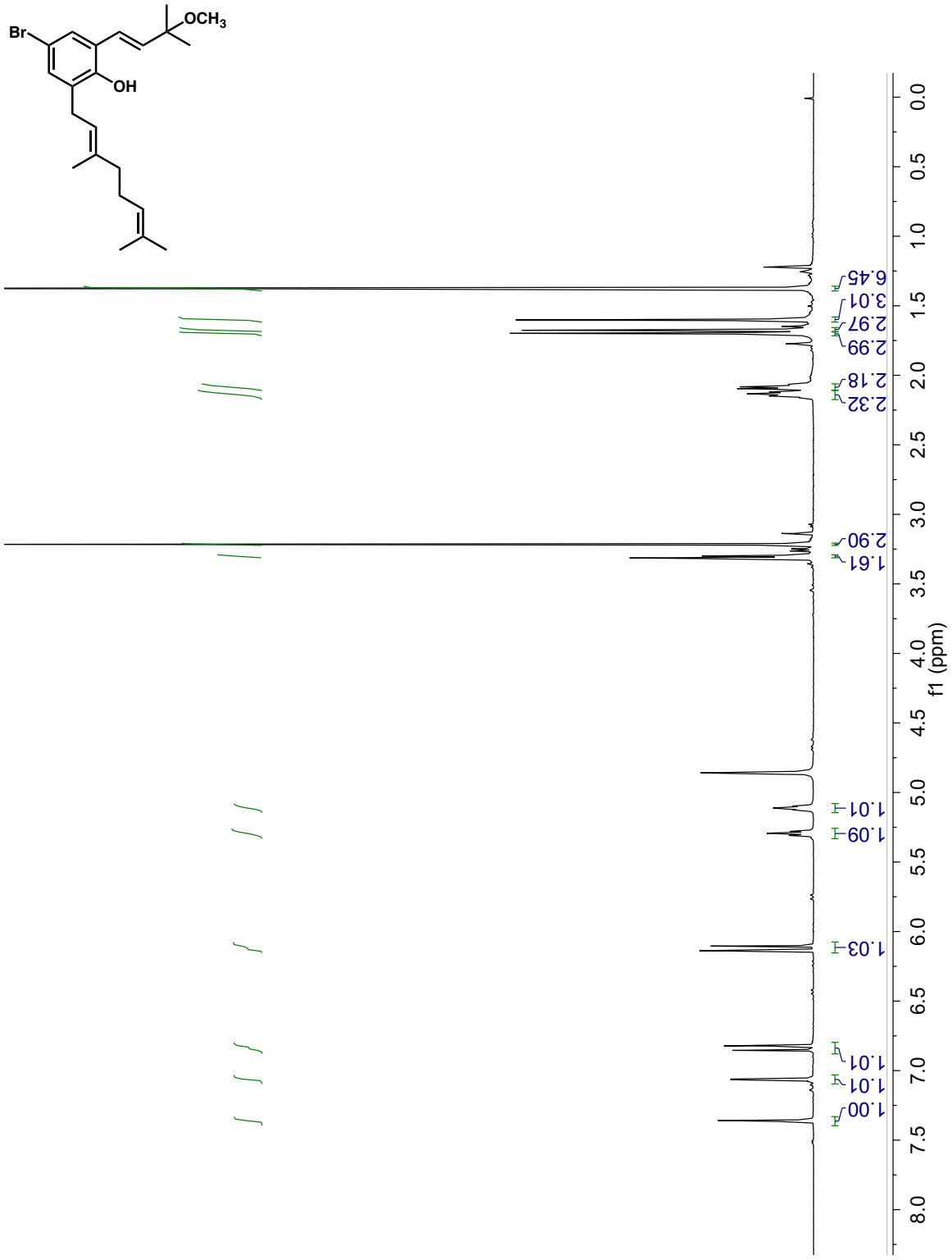

Figure A.1.39 ^{1}H NMR (500 MHz, CD_3OD) spectrum of **84**

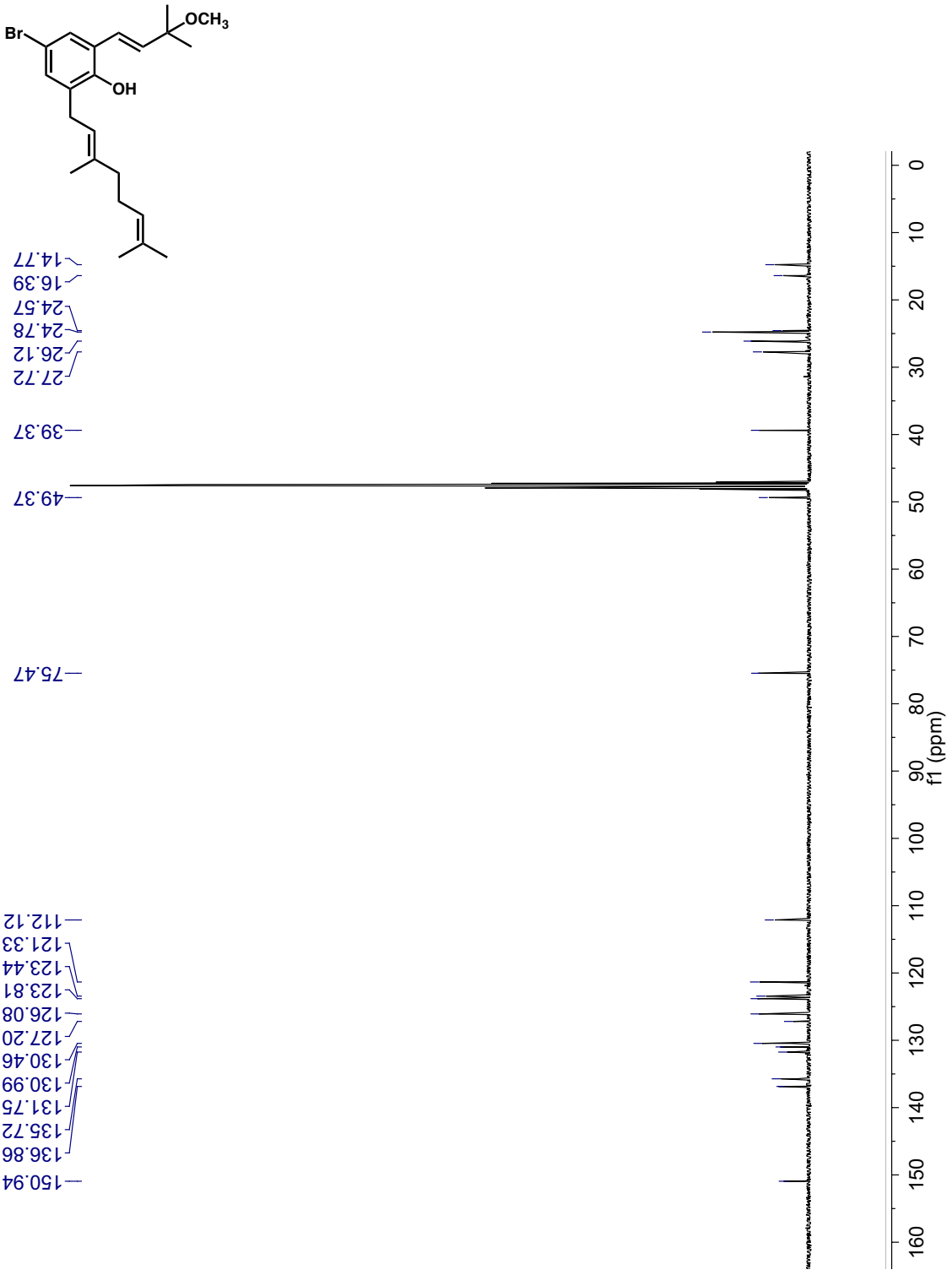

Figure A.1.40 ^{13}C NMR (500 MHz, CD_3OD) spectrum of **84**

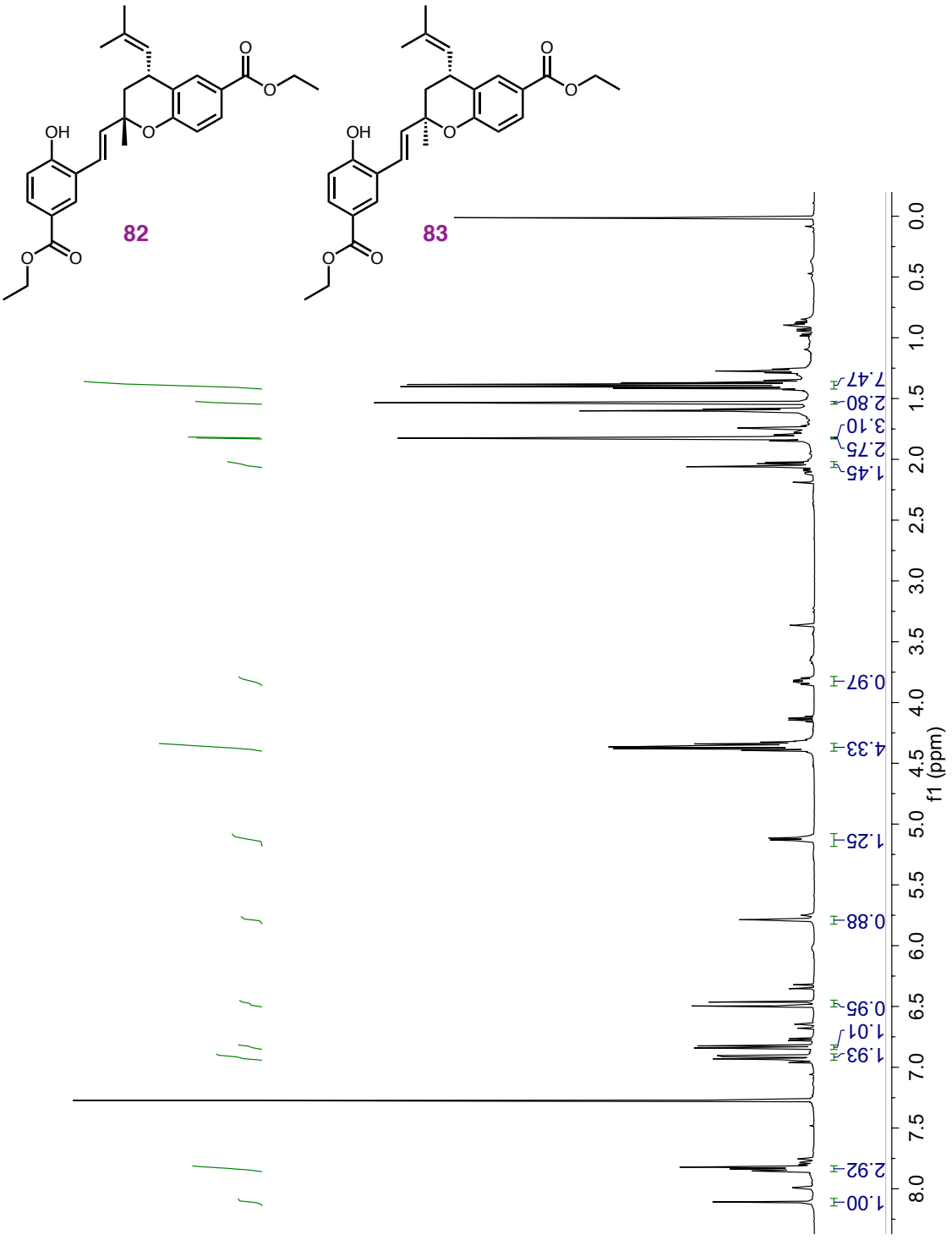

Figure A.1.41 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **85** & **86** (4:1 d.r.).

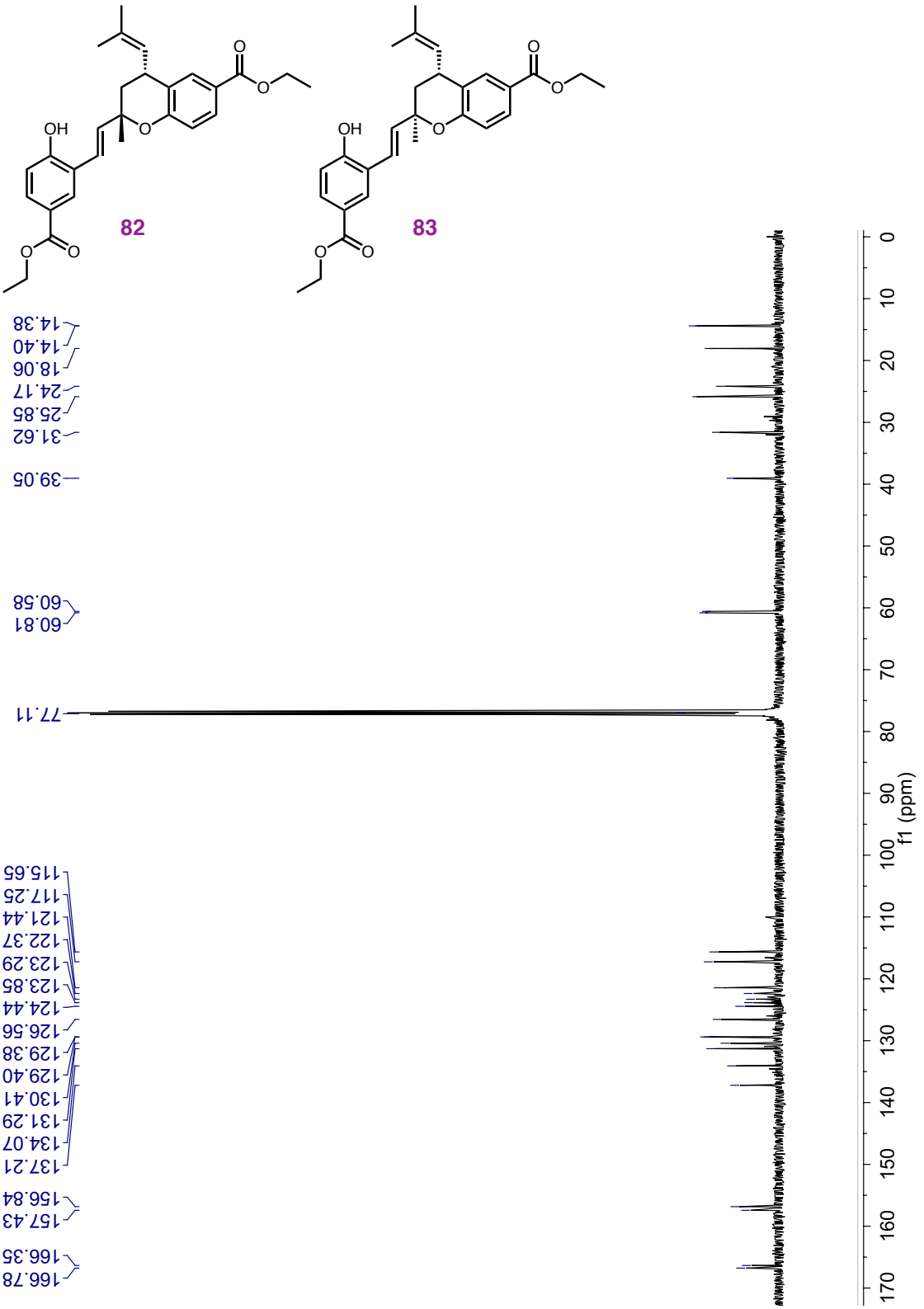

Figure A.1.42 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **85** & **86** (4:1 d.r.)

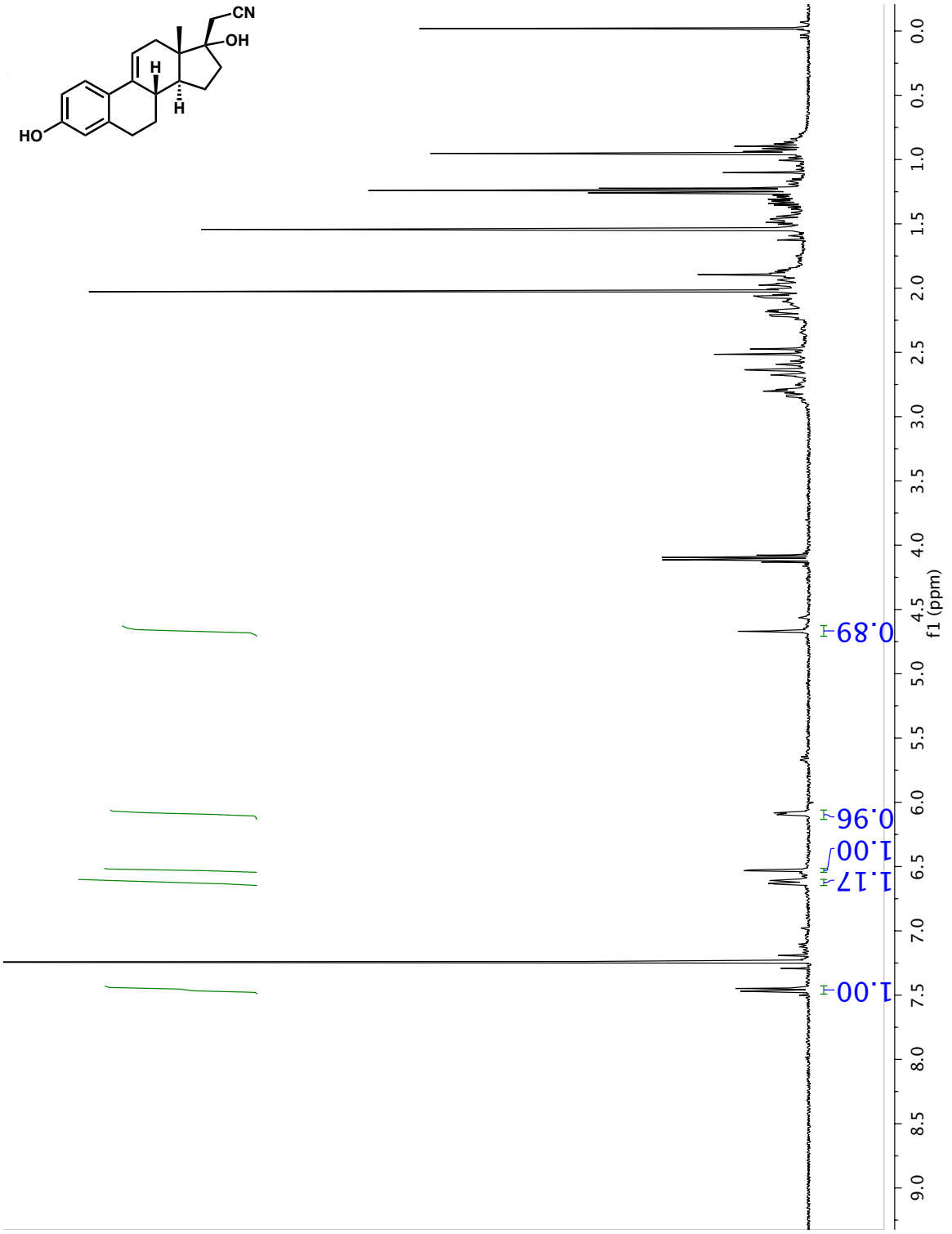

Figure A.1.43 ^{1}H NMR (500 MHz, CDCl) spectrum of **89**